EVALUATION OF A TRITIUM IRRIGATION SITE: A FIELD STUDY AND LABORATORY EXPOSURE STUDY IN MICE WITH LOW-LEVEL, ORAL EXPOSURE TO TRITIUM

by

ANGEL KELSEY WALL

(Under the Direction of Charles Jagoe)

ABSTRACT

Tritium, a radioactive isotope of hydrogen, is a contaminant of concern at the Savannah River Site (SRS) and at other nuclear production facilities. New methods are being employed on SRS to dispose of tritium, including the irrigation of forests with tritiated water. A field study was performed to determine the tritium concentrations of rodents living within the irrigation site, and to examine the tritium dynamics within. In addition, a laboratory experiment was designed to determine the biological-half life of tritium in mice, mimicking environmental exposure. The possibility of metabolic stress resulting from exposure to low-level β-radiation was examined with oxidative-stress induced enzymes: catalase, glutathione peroxidase, and superoxide dismutase. The biological half-life of tritium was determined to be 2.26 days and, based on enzyme activities, there were no indications of oxidative stress induction. This study concludes that land application is an effective and low-risk method for the disposal of tritium.

INDEX WORDS: Tritium, Tritiated water, Rodents, Cotton mice, Biological half-life, Oxidative stress, β-radiation, Catalase, Glutathione peroxidase, Superoxide dismutase

EVALUATION OF A TRITIUM IRRIGATION SITE: A FIELD STUDY AND LABORATORY EXPOSURE STUDY IN MICE WITH LOW-LEVEL, ORAL EXPOSURE TO TRITIUM

by

ANGEL KELSEY WALL

B.S., Augusta State University, 2001

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2004

EVALUATION OF A TRITIUM IRRIGATION SITE: A FIELD STUDY AND LABORATORY EXPOSURE STUDY IN MICE WITH LOW-LEVEL, ORAL EXPOSURE TO TRITIUM

by

ANGEL KELSEY WALL

Major Professor: Charles Jagoe

Committee: John Seaman
Cham Dallas

Electronic Version Approved:

Maureen Grasso
Dean of the Graduate School
The University of Georgia
August 2004

DEDICATION

I would like to dedicate this to my parents, Richard and Patricia Kelsey, who have always encouraged me to do my best at everything and have always supported me through every endeavor. Also, I dedicate this to my husband, David Wall, whose support, love, and devotion are immeasurable.

ACKNOWLEDGEMENTS

There are many people that I would like to acknowledge for their help during my graduate career, and for which I am eternally grateful. First, let me thank my family; this would not have been possible without their support and encouragement.

I'd like to thank my lab group for their support, including John Seaman, Jane Logan, Jennifer McIntosh, Mark Komoroski, Julian Singer, and Franta Majs. In addition, I'd like to acknowledge the many technicians and graduate students that have helped and befriended me over my years at SREL: Heather Brant, Joan Gariboldi, Jennifer Parikh, Cub Stephens, Karen Gaines, Diane Addis, Jason Unrine, Olga Tsyusko, Brad Reinhart, Greg O'Quinn, Morris Jones, Jay Cumbee, Surjeet Bhutani, Noelle Garvin, Lindy Paddock, Lindy Steadman, Steve Schaff, Brandon Staub, Virginia Jin and Momin Uddin.

Also, I'd like to thank my committee members, Chuck Jagoe, John Seaman and Cham Dallas. This research was supported by Westinghouse Savannah River Company--Soil & Groundwater Closure Projects and through Financial Assistance Award No. DE-FC09-96SR18546 from the U.S. Department of Energy to the University of Georgia Research Foundation, Inc.

TABLE OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER I

INTRODUCTION

Tritium

As a radioisotope of hydrogen, the tritium atom is composed of one electron, one proton and two neutrons, for an atomic mass of approximately 3 amu. Because of the relatively low binding energy of the three nuclear particles, the nucleus of the tritium atom is unstable (Feindendegen 1967). To become stable, the tritium atom releases radiation. Radiation is the spontaneous release of energy in the form of particles or electromagnetic rays; the process of emitting radiation can also be referred to as decay (N.C.R.P. 1985). Tritium decays to form helium by releasing a β-particle (an electron) and a neutrino (transforming a neutron to a proton). The energy released with the emitted β-particle can have an energy maximum of 18.6 keV (Jenks et al. 1949), with a mean energy of 5.685 keV. Reaction (1) is a summary of tritium decay:

$$^{3}H \rightarrow {}^{3}He + e^{-} \qquad (1)$$

The time it takes for half of a given amount of tritium to decay, or the half-life, of tritium is approximately 12.35 years. By comparison to the half-lives of other radioisotopes, the half-life of tritium is relatively short (N.C.R.P. 1985).

For some elements, there can be significant differences in the physical and chemical properties between isotopes. As an isotope of hydrogen, tritium has physical properties very similar to that of hydrogen. For example, the vapor pressure of tritiated water is about 90% of

natural water (Murphy Jr. 1993). Jacobs hypothesized that the physical similarities between tritium-containing compounds and hydrogen-containing compounds may be attributed to the small contribution of these constituents to the overall mass of a molecule (Jacobs 1968). Though alike physically, the chemical properties of tritium and hydrogen differ. Tritium is three times heavier than hydrogen; thus reactions involving tritium are generally slower than those involving only hydrogen. However, even though tritium reactions usually take longer, an isotopic effect cannot easily be detected against most sources of environmental variability (Murphy Jr. 1993).

Although the discovery of tritium was attributable to a man-made reaction, it has been found to exist in the natural environment. Sources of natural tritium have been found in minimal concentrations from cosmic rays in the atmosphere (Hartek and Faltings 1950) and nuclear reactions in the Earth's crust (Kaufman and Libby 1954). However, the major source of tritium is from its man-made production for use in a variety of applications.

Tritium Production

Initially produced by Lord Rutherford in 1932, the actual discovery of tritium is credited to Alvarez and Cornog in 1939 (Moghissi and Carter 1973). Alvarez and Cornog produced tritium, and its decay product He^3, by reacting two deuterium atoms (stable isotope of hydrogen with an atomic mass of 2 amu) in a cyclotron. Within the cyclotron, a magnetic field induced the atoms into a circular orbit and an oscillating electric field accelerated the particles; in this environment reactions are initiated. The nuclear reactions that occurred inside the cyclotron are seen in Reactions 2 and 3, where η is a neutron:

$$H^2 + H^2 \rightarrow He^3 + \eta^1 + 4.04 \text{ MeV} \quad (2)$$

$$H^2 + H^2 \rightarrow H^3 + H^1 + 3.26 \text{ MeV} \quad (3)$$

The tritium produced in these reactions can undergo another reaction with a deuterium atom to

produce the result seen in Reaction 4:

$$H^3 + H^2 \rightarrow He^4 \quad (4)$$

The process is completed when a steady state is reached between the tritium formation in Reaction 3 and helium formation, for both 3 and 4 amu isotopic products seen in Reactions 2 and 4 (Alvarez and Cornog 1939).

Nuclear reactions producing tritium have been studied thoroughly to determine which reactions yield greater quantities of tritium. Some nuclear processes produce very small amounts of tritium, far too small to be considered practical in production facilities (Feinendegen 1967). The most commonly used large-scale reaction involves bombarding a lithium atom with a neutron, causing the lithium nucleus to split and form a tritium atom and a helium molecule, seen in Reaction 5:

$$Li^6 + \eta^1 \rightarrow H^3 + He_2^4 \quad (5)$$

There are many techniques in which the above reaction can be carried out. One example includes bombarding a lithium fluoride crystal with neutrons and extracting the produced tritium from the gaseous radiation products (Jenks et al. 1963). As with most manufacturing processes, optimal conditions have been determined to produce a maximum yield of product, which is followed by the purification of the product. In this case, the neutron flux and energy are optimized to produce the maximum yield of tritium (Feindendegen 1967).

Tritium Use

The production of tritium, for the most part, can be accounted for by its manufacture for consumer products by tritium production plants and nuclear facilities, such as nuclear power plants and nuclear weapon processing plants.

There are two major uses of tritium in consumer products: self-luminous light and tritium-labeled tracers. Worldwide, tritium has been incorporated into plastics and paint for use as a self-luminous light source in products such as the face of a timepiece or the sights of a rifle (N.C.R.P. 1977, Hill and Johnson 1993, Okada and Momoshima 1993). The second consumer use is in the utilization of tritium in biomedical research and clinical medicine, first used in 1951. The widespread use of tritium as a tracer is largely due to the stability and specificity of tritium when used under experimental conditions. Since its adoption as a tracer, laboratories have been using tritium in studies by labeling proteins, macromolecules, and DNA. Tritium has also been used to evaluate cellular processes, such as metabolic hydrogen transfer reactions and programmed cell death, or apoptosis (Moghissi and Carter 1973).

Other than consumer products, nuclear facilities are another major contributor to the production of tritium. Along with the production of energy, nuclear power plants generate various radionuclides, including tritium, during routine operations (Hoffman et al. 2003). Commercially, in 1999 the United States had approximately 104 nuclear power plants operating in 31 states. There were also another 37 non-power reactors operating in 24 states for research and educational purposes (U.S.NRC 2000).

Nuclear weapons processing plants in the United States are operated by the Department of Energy (DOE) to produce weapons grade tritium for its use as one component of a nuclear weapon. There are three main locations for the nuclear weapons processing of tritium: the Hansford Reservation, Hansford, Washington; the Idaho National Engineering Laboratory, Idaho Falls, Idaho; and the Savannah River Site, Aiken, South Carolina (Hoffman et al. 2003).

Savannah River Site

The Savannah River Site (SRS) is a former nuclear weapons manufacturing plant operated by the U.S. DOE. It is an approximately 310 square mile facility in South Carolina, bordered by the Savannah River, covering an estimated 1% of the state of South Carolina (Figure 1-1). SRS was established in 1952 with a mission to produce weapons-grade nuclear materials, mostly tritium and plutonium. By March 1955, all five reactors on SRS were operating, with the levels of manufactured products closely monitored. Most of the reactors remained in operation until August 1988, when the reactors were shutdown. Even though the production of tritium was halted, SRS operates the only tritium recycling facility in the nation, allowing the US to use its supply of tritium more efficiently (Murphy Jr. et al. 1991, Arnett 1997).

All five reactors on SRS produced tritium by bombarding lithium with a neutron, causing the lithium nucleus to split and form a tritium atom and a helium molecule (Reaction 5). Along with the two products formed, energy was also released as heat. To cool off reactions inside the reactors, thousand of gallons of water per minute were used as a heat exchanger. After tritium production, the tritium is extracted, separated, purified and packaged for shipment to other DOE facilities (Murphy Jr. et al. 1991, Arnett 1997).

With the shutdown of the reactors, the mission of SRS has shifted to the environmental cleanup of the site, in addition to its responsibility of purifying and maintaining the existing inventories of tritium. Initiatives were taken to dispose of tritiated waste derived from nuclear processes to prevent and reduce the amount of environmental contamination. Although these are no longer considered acceptable, seepage basins, tank farms and burial grounds were disposal methods employed by the site. Routine monitoring of these disposal methods have been

performed to better understand the fate of tritiated waste within these systems and to determine the effectiveness of each (Murphy Jr. et al. 1991, Arnett 1997).

Tritium Releases in the Environment

As mentioned earlier, small concentrations of tritium are found in the natural environment (Hartek and Faltings 1950, Kaufman and Libby 1954). However, natural processes are not the only source for the tritium concentrations that exist in the environment. Tritium has been released into the environment from the nuclear bomb testing in the 1950s and 1960s and from nuclear production facilities. Forms of tritium that have been released into the environment include gaseous tritium, tritiated water or a mixture of the two (Okada and Momoshima 1993).

From 1954 until 1963, hydrogen bomb testing was being performed in the US. These tests released large quantities of tritium into the earth's atmosphere, estimated to be 185-240 times greater than the natural tritium concentrations (UNSCEAR 1977). With the release of tritium in the atmosphere, the oxidation of gaseous tritium occurs, allowing rain to act as a removal mechanism (Okada and Momoshima 1993). Thus, the concentration of tritium in rainwater is indicative of tritium levels in the atmosphere (Murphy Jr. et al. 1991). Based upon radioactive decay and rainwater monitoring, in 1990, the background tritium concentration was estimated to be about 40-52 times higher than estimates for natural tritium production (Okada and Momoshima 1993).

Nuclear production facilities release tritium into the environment in the form of gas, $^{3}H_2$, and water, $^{3}H_2O$. Gaseous tritium is released into the atmosphere from the stacks of reactors; tritiated water is released through cooling effluent and seepage (Murphy Jr. et al. 1991, Okada and Momoshima 1993). Most releases of tritium into the environment are incidental, occurring during the normal operation of nuclear facilities, such as SRS. Others are purely accidental, such

as disastrous Chernobyl incident (April 26 - May 6, 1987), which released concentrations of tritium into the air that were about 100 times higher than background levels (Salonen 1987).

SRS has released tritium in the gaseous form, inadvertently, from the stacks of operating reactors or in the process of waste disposal. When the seepage basins were an acceptable method of disposal, approximately 30% of the tritium in the tritiated wastewater was released into the environment by evaporation. Another minor contributor to the gaseous release of tritium on SRS was the evaporation of tritium from the radioactive wastes stored in the waste tank farm. Monitoring these tritium releases includes the analysis of rainwater, vegetation, and soil (Murphy Jr. et al. 1991).

Tritiated water has also been released by SRS into the environment from coolant processes and waste disposal. Leaks between the primary reactor vessel and the coolant reservoirs inside reactors enabled tritium to mix with the cooling water, which was then discharged into various water sources, including streams, such as Fourmile Branch and Upper Three Runs Creek, or man-made bodies of water on site. Discharge from the seepage basins and burial ground has released contaminated water to the underlying aquifer, forming two plumes, and outcropping into either Fourmile Branch or Upper Three Runs Creek. Monitoring the aqueous tritium waste released includes the analysis of groundwater, stream water and Savannah River water (Murphy Jr. et al. 1991, Arnett 1997).

Reducing the release of tritium by SRS into the watershed and surrounding streams, such as Fourmile Branch, is a difficult environmental problem and has given rise to new methods of disposal. One of the current remediation strategies on SRS involves the construction of a dam to contain tritiated water at the seepline feeding Fourmile Branch and to use this tritiated water to irrigate a nearby hardwood forest. Preliminary estimates indicated that such an approach reduces

the amount of tritiated water going into Fourmile Branch approximately 60 %. Since implementation, routine monitoring has indicated that 56 to 87 % of the applied tritium has been lost to evapotranspiration (Blake 2000, Hitchcock et al. 2004, Rebel et al. 2004). This new disposal method is continually monitored and has operated for several years with a high efficiency (Seaman and Aburime 2002, Seaman et al. 2003). Although the reduction of tritiated waste migrating into Fourmile Branch has been accomplished with this new disposal method, there are still several areas of research needed to make a complete assessment of the system: (1) monitoring of wildlife inhabiting the irrigation site to determine the level of tritium exposure, (2) determining the biological half-life of tritium in exposed organisms in an ecologically relevant manner, and (3) evaluating the risk of exposure to tritium by examining biochemical endpoints that may be altered with radiation exposure.

Works Cited

Alvarez LW, Cornog R. Helium and Hydrogen of Mass 3. Physical Review 56: 613; 1939.

Arnett M. Savannah River Site Environmental Report for 1997. Aiken, SC: Westinghouse Savannah River Company; WSRC-TR-97-00322; WSRC-TR-97-00322; 1997.

Blake JI. Effectiveness monitoring plan for the tritiated water management facilility southwest plume interim measures. Aiken, SC: Westinghouse Savannah River Company; SRI 00-10-R; SRI 00-10-R; 2000.

Feindendegen LE. Tritium-Labeled Molecules in Biology and Medicine. New York, NY: Academic Press; 1967.

Hartek P, Faltings V. The helium-3 problem of the atmosphere. Nature 166: 1109; 1950.

Hill RL, Johnson JR. Metabolism and dosimetry of tritium. Health Physics 65: 628-647; 1993.

Hitchcock DR, Barton CD, Rebel KT, Singer JH, Seaman JC, Strawbridge JD, Riha SJ, Blake JI. A containment and disposition stategy for the management of tritium-contaminated groundwater at the Savannah River Site, SC USA. Environmental Geosciences submitted; 2004.

Hoffman DJ, Rattner BA, Burton Jr. GA, Cairns Jr. J. Handbook of Ecotoxicology. Second ed. Boca Raton, FL: Lewis Publishers; 2003.

Jacobs DG. Sources of tritium and its behavior upon release to the environment. Oak Ridge, TN: USAEC; 1968.

Jenks GH, Ghormley JA, Sweaton FH. Measurement of the Half-Life and Average Energy of Tritium Decay. Physical Review 75: 701; 1949.

Jenks GH, Shapiro EM, Elliott N, Cannon CV. Production of tritium. 1963.

Kaufman S, Libby WF. The natural distribution of tritium. Physical Review 93: 1337-1344; 1954.

Moghissi AA, Carter MW. Tritium. Phoenix, AZ: Messenger Graphics; 1973.

Murphy Jr. CE. Tritium transport and cycling in the environment. Health Physics 65: 683-712; 1993.

Murphy Jr. CE, Bauer LR, Hayes DW, Marter WL, Zeigler CC, Stephenson DE, Hoel DD, Hamby DM. Tritium in the Savannah River Site Environment. Aiken, SC: Westinghouse Savannah River Company; WSRC-RP-90-424-1; WSRC-RP-90-424-1; 1991.

N.C.R.P. Radiation exposure from consumer products and miscellaneous sources. National Council on Radiation Protection and Measurements; 56; 56; 1977.

N.C.R.P. A handbook of radioactivity measurements procedures. National Council on Radiation Protection and Measurements; 58; 58; 1985.

Okada S, Momoshima N. Overview of tritium: Characteristics, sources and problems. Health Physics 65: 595-609; 1993.

Rebel KT, Riha SJ, Seaman JC, Barton CD. The use of dynamic modeling in assessing tritium phytoremediation. Environmental Geosciences submitted; 2004.

Salonen L. Carbon-14 and tritium in the air in Finland after the Chernobyl accident. Radiochima Acta 41: 145-148; 1987.

Seaman JC, Aburime S. Estimating evapotranspiration losses for tritium at the MWMF. Aiken, SC: Savannah River Ecology Lab; 2002.

Seaman JC, Singer JH, Aburime SA. Estimating evapotranspiration losses for tritium at the MWMF: 2002 data. Aiken, SC: Savannah River Ecology Laboratory; 2003.

U.S.NRC. Information Digest 2000 Edition. 12; 2000.

UNSCEAR. Sources and effects of ionizing radiation. New York, United Nations: UNSCEAR; 1977a; 1977a; 1977.

Figure 1-1 – The Savannah River Site (SRS) is a former nuclear weapons manufacturing plant in South Carolina with current missions including environmental restoration of the site and the maintenance of existing inventories of tritium.

CHAPTER II

RODENTS AS RECEPTOR SPECIES AT A TRITIUM IRRIGATION SITE

Introduction

Site Background

The Savannah River Site (SRS) is a former nuclear weapons production facility with a current mission that includes the environmental restoration of the site from previous contamination (Figure 1-1). In addition, SRS is the only tritium recycling facility in the US, allowing the supply of tritium to be used more efficiently by the purification and maintenance of existing inventories. Throughout the history of SRS, tritium has been released into the environment during normal operations, as well as during waste disposal activities in the environmental restoration process (Murphy Jr. et al. 1991, Arnett 1997).

Many of the methods employed by SRS to prevent or reduce the release of tritiated waste into the environment are no longer acceptable forms of waste disposal, such as seepage basins, tank farms and burial grounds. Tritiated wastewater was disposed of in seepage basins and tank farms, and radioactive solid waste was disposed of in burial grounds. Each disposal method holds the potential to spread tritium contamination to the surrounding environment. For example, these disposal practices have resulted in contamination of the underlying groundwater. Once reaching the water table, the tritiated wastewater outcrops into one of the streams on SRS, such as Fourmile Branch, a tributary to the Savannah River. Routine monitoring of groundwater and the surface waters, including the Savannah River, has been done in the past and are currently

being performed to better understand the fate and effectiveness of waste disposal (Murphy Jr. et al. 1991, Arnett 1997).

In 1997, an environmental assessment was performed based on tritium concentrations outcropping in Fourmile Branch, the tritium plume location and outcrop history for the previous decade. The impact of remediation efforts on local geology and hydrology was taken into consideration during the evaluation, with the conclusion being that tritium would continue to migrate into Fourmile Branch, but hopefully decrease over the next 20-25 years. In spite of predictions that tritium concentrations would decrease due to radioactive decay, the reduction of tritium migration was considered part of the SRS mission because of the Resource Conservation and Recovery Act (RCRA). A corrective action plan was initiated with the goal of employing new technology for the reduction of tritium migration (Arnett 1997).

Irrigation Site

The elevated levels of tritium in Fourmile Branch were largely attributed to solid waste disposal, in the form of burial grounds. The suspected burial ground responsible for the elevated tritium concentrations was the Old Radiological Waste Burial Ground (ORWBG). Nuclear material processing activities on SRS derived a mixture of wastes, including tritiated waste that is now contained in the ORWBG. Seepage from the ORWBG contains tritiated water, which has leached from the ORWBG into the water-table aquifer and outcropped within a watershed that feeds Fourmile Branch (Arnett 1997).

To reduce the concentration of tritium reaching Fourmile Branch, a dam was constructed at the base of the seepline at the Mixed Waste Management Facility (MWMF) to intercept the contaminant plume. The resulting pond, with an average tritium concentration around 14,000 pCi/mL, is being used to irrigate a neighboring 22-acre hardwood/pine forest (Figure 2-1). The

initial operation of the irrigation facility has, from preliminary estimates, reduced the concentration of tritium being released into Fourmile Branch by 60 % (Blake 2000, Hitchcock et al. 2004).

Field Monitoring

To evaluate the efficiency of the remediation effort, several aspects of the tritium levels in the irrigation site have been monitored on a monthly basis, including: irrigation pond, soil profile, and vegetation. Also, the irrigation schedule is thoroughly documented and the precipitation is monitored. Soil monitoring includes the taking of soil cores, in addition to monthly lysimeter and soil vapor tube sampling to survey the tritium concentrations found in porewater at various depths within the soil profile. Vegetation sampling includes determining tritium concentrations in leaf litter samples, also the determination of vegetation height, and species composition for the site. Evapotranspiration of tritium by trees is monitored for various tree species at multiple canopy heights (Blake 2000, Rebel et al. 2004).

Most of the current monitoring is used to calculate evapotranspiration efficiencies for the management of the remediation effort. Prior to the current study, there has been no monitoring of the wildlife inhabiting the irrigation site. In this study, tritium concentrations in wildlife exposed to the irrigation water are used to evaluate the tritium dynamics within this disposal system (Blake 2000; Seaman and Gaines 2002).

Receptor Species for Tritium Exposure

The uptake of radionuclides by biota has been studied at sites with a history of radiation contamination and nuclear facilities (Hoffman et al. 2003). For example, following the Chernobyl accident, the 30 km environment surrounding the reactor has since been used to study the response of wildlife to radiological contamination. In Chernobyl, studies have evaluated the

effect of radiation on various levels of biota, including terrestrial (i.e., plants, invertebrates, small mammals, avian populations) and aquatic species (i.e., fish, invertebrates, and plants) (Golley et al. 1965, Krivolutzki and Pokarzhevski 1992, Baker and Chessler 2000).

Since the use of wildlife has not yet been employed at the tritium irrigation site described above, an evaluation of tritium concentrations in terrestrial biota inhabiting the site would provide useful information about the tritium dynamics in animals living among the irrigation. However, the appropriate receptor species must meet several criteria including: the inhabitation of the irrigation site, high reproductive capacity and sensitivity to the contaminant of interest. In addition, the life history of the receptor species must be fully understood. Because the above criterions are met, small mammals are an excellent choice for the receptor species at the tritium irrigation site (Hoffman et al. 2003).

Small mammals, especially mice, have been used for decades in ecological research to determine the fate and transport of contaminants (Johnson et al. 1978, Anthony and Kozlowski 1982, Hunter et al. 1987). Within the southeast, one of the most common small mammal species is the cotton mouse (*Peromyscus gossypinus*) and within SRS, the ecology of cotton mice, as well as contaminant fate and transport, have been studied for decades (Golley et al. 1965, Gentry et al. 1971, Smith et al. 1971, Reinhart 2003).

For the above reasons, in this field study, small mammals, more specifically the cotton mouse, were chosen as the receptor species inside the tritium irrigation site. Once captured, whole body tritium concentrations of the small mammals were determined, enabling the examination of tritium dynamics inside the irrigation site. Whole body tritium concentrations of the captured animals were correlated with the irrigation site facility management practices, more

specifically tritium application records and local meteorological conditions for the site (Seaman et al. 2002).

Materials and Methods

Field Methods

Two sites were chosen for rodent trapping: the tritium irrigation site and a control location, Boggy Gut, which is located off of the SRS (Figure 2-2).

Irrigation Site:

Trapping was performed using baited Sherman rodent-traps. The traps were deployed in four evenly spaced (75 ft) trap-lines comprised of 35 Sherman rodent-traps at 20 ft spacing placed across plots 13-19, and 23. Two additional trap-lines comprised of 20 Sherman rodent-traps placed in the plots 20 and 21, for a total of 180 traps (Figure 2-3).

All traps were checked daily and re-baited as necessary. In the fall season, traps were checked from September 30 until November 22, 2002. Traps were initially baited using sunflower seed, with poor trapping success, until October 22, when the bait was switched to a combination of peanut butter and oatmeal. The spring season lasted from March 31 until May 26, 2003. The peanut butter and oatmeal bait was used again for the spring season. Captured animals were euthanized in the field using cervical dislocation before transport to the laboratory for processing.

Boggy Gut:

Trapping at Boggy Gut also used baited Sherman rodent-traps. The traps were deployed in four evenly spaced lines approximately 75 feet apart, with approximately 30 feet between traps. Three of the lines contained 19 Sherman rodent-traps, with the fourth line only containing 18 traps because of landscape restraints, for a total of 75 traps. All traps were checked daily and

re-baited as necessary. Boggy Gut was only trapped for one season, fall 2002, lasting from September 8 until December 3, 2002. Traps were baited with sunflower seeds for the entire season due to high trapping success. Captured animals were euthanized in the field using cervical dislocation before transport to the laboratory for processing.

Laboratory Analysis

Once at the laboratory, the body weight and sex of captured animals were recorded; until analysis was performed whole bodies of mice were freezer-stored. The whole body of captured rodents was used to extract body fluid for analysis by sublimation (i.e., freeze drying) using the procedure described by Westinghouse Savannah River Company (WSRC) for removing "free" tritium from foodstuffs (WSRC 2001). Body fluid was analyzed for tritium by liquid scintillation with an estimated detection limit of 0.20 pCi/L and a counting error below 2% for elevated tritium concentrations (Minaxi Tri-Carb 4000, Packard Instru. Co), using a procedure outlined by WSRC (WSRC 2001), with the exception of adjusting the sample and scintillation cocktail ratios to reduce the amount of waste in a manner consistent with the QA/QC protocols.

Calculations

Savannah River Site-Forestry Service (SRS-FS) is responsible for the operational aspect of the irrigation site. With this responsibility, SRS-FS maintains weekly records of the volume of tritiated water applied to each plot. For daily estimates for the volume of irrigation, the weekly volume was divided by seven.

In addition, SRS maintains daily records of meteorological data around the entire site, including the area in which the irrigation site is located. Using this precipitation information, along with the area of individual plots, an estimate of rain volume for individual plots was calculated. For each animal, irrigation and precipitation data for the plot where the animal was

captured in was used to calculate an estimated dose of tritium, as seen in Equation 1:

$$\text{Dose}_{\text{estimate}} = \frac{(\text{Tritium Concentration})_{\text{pond}} \times \text{Volume}_{\text{applied}}}{(\text{Volume}_{\text{rain}} + \text{Volume}_{\text{applied}})} \quad (1)$$

The tritium concentration of the pond used in the above equation was an average of the monthly pond concentrations over the first and second year of operation data, which was calculated to be approximately 14,700 pCi/mL.

Statistical Analysis

Most statistical analyses were performed using SAS (version 9.0; SAS Institute). Body weights and whole body tritium concentrations were tested for normality using the Shapiro-Wilk statistic (PROC UNIVARIATE). Analysis of variance models (ANOVA; PROC GLM), along with post-ANOVA tests (Tukey, Scheffe and Dunnett), were used to examine relationships between whole body tritium concentrations, capture season, body weight, sex, and location. For correlation analyses, regression models (PROC REG) were used to examine any significant relationships between whole body tritium concentrations to irrigation levels and precipitation. For all tested models, F-statistics were used to provide an estimate of the predictive value of the model. All statistical tests were considered significant at the $p \leq 0.05$ level. For a below detection value, the instrumental detection limit was entered for that sample. The statistical program Uncensor (version 4.0; Dr. Mike Newman) was used to obtain a better estimate of the mean whole body tritium concentration for Boggy Gut mice (regression method).

Results

Field Data

For the irrigation site, in the fall season, there were a total of 9,720 trapping days. There were 19 captured animals, which included 14 *Peromyscus gossypinus* (cotton mouse), 3 *Blarina carolinensis* (Southern short-tailed shrew), and 2 *Sigmodon hispidus* (cotton rat). The spring

trapping season consisted of 7,020 trapping days. There were a total of 16 captured animals: 11 cotton mice, 4 Southern short-tailed shrews, and 1 *Rattus norvegicus* (Norwegian rat). At the control site, Boggy Gut, the fall season had a total of 6,525 trapping days. There were 17 captured animals, which included 16 cotton mice and 1 Southern short-tailed shrew.

A summary of the data for the captured animals from the irrigation site, including date of capture, location, species, sex, weight, and whole body fluid tritium concentration for both seasons are shown in Table (2-1). Boggy Gut data, shown in Table (2-2), includes the date of capture, species, sex, body weight and whole body fluid tritium concentration. Calculated data, including volume of tritium applied, volume of precipitation, and the estimated tritium dose for seven days prior to capture, are shown for each cotton mouse captured at the tritium-irrigated site in Table (2-3).

As seen in the above tables, at the irrigation site, the lowest whole body tritium concentration in the captured animals was below detection, which was assigned the value of 0.20 pCi/mL, and the highest concentration was 3,204.3 pCi/mL. The animal with the lowest whole body tritium concentration was captured in the fall 2002 trapping season and the animal with the highest value was captured in the spring 2003 trapping season. The average whole body tritium concentrations were 1337.8 ± 240.0 pCi/mL and 802.4 ± 276.9 pCi/mL for the fall 2002 and spring 2003 trapping seasons, respectively. The lowest whole body tritium concentration at Boggy Gut was also below detection and assigned a value of 0.20 pCi/mL, and the highest whole body tritium concentration was 5.0 pCi/mL. Boggy Gut had an average whole body tritium concentration of 0.6 ± 2.2 pCi/mL.

Statistical Results

Due to low sample sizes of most small mammal species captured, only one species, *Peromyscus gossypinus* (cotton mouse), was used for statistical analyses. For the irrigation site, there was a significant difference between body weight for season ($F = 7.00$; $p = 0.0026$), with the smaller mice being caught in the spring season (Figure 2-4). However, there were no significant differences between seasons for whole body tritium concentration ($F = 2.16$; $p = 0.1560$), even when weight was used as a cofactor ($F = 2.45$; $p = 0.1101$). Because no difference was found between the two seasons for whole body tritium concentration, the data were pooled for remaining statistical analyses (Figure 2-5). For mice captured within the irrigation site, there was no significant difference between whole body tritium concentration and sex ($F = 0.89$; $p = 0.4260$).

Comparisons between the irrigation site and Boggy Gut showed that there were no significant differences between the body weights of captured animals (Figure 2-4). Mice captured at Boggy Gut did not differ significantly from either irrigation field season, which was determined using both Tukey and Scheffe post-ANOVA tests. As expected, a statistically significant difference was found for whole body tritium concentrations in mice from the irrigation site and Boggy Gut (Figure 2-6; $F = 20.55$; $p < 0.0001$).

The irrigation and precipitation data for the individual plots, along with the estimated dose for animals, seven days prior to capture were used to look for correlations with whole body tritium concentration. There was a significant, positive, linear regression between whole body tritium concentration of mice and the volume of tritium applied within the plot captured (Figure 2-7; $F = 4.89$; $p = 0.0376$; $r = 0.4266$; $r^2 = 0.1820$). A significant, negative, linear regression was found between whole body tritium concentration of mice and the volume of precipitation within

the plot captured ($F = 7.67$; $p = 0.0112$; $r = -0.5084$; $r^2 = 0.2585$). A significant, positive, linear regression was also found between whole body tritium concentration of mice and the estimated dose prior to capture (Figure 2-8; $F = 6.97$; $p = 0.0149$; $r = 0.4905$; $r^2 = 0.2406$).

Discussion

Results from this field study found significant differences between whole body tritium concentrations in mice from the tritium irrigation site and the control site, Boggy Gut. Increases in whole body tritium concentrations of mice on the irrigation site were related to increases in tritium irrigation levels and increases in tritium dose. Decreases in whole body tritium concentrations of mice on the irrigation site were related to increases in precipitation.

The use of rodents from the irrigation site has allowed a closer look into the tritium dynamics that occur within this system. Detectable concentrations of tritium were found in almost all mice captured at the irrigation site, with the exception of one animal. In this system, tritium is available to mice by several pathways: directly by ingestion as a water source, absorption through the skin, or inhalation of the vapor, and indirectly by the incorporation of tritium into their food source with exposure occurring with digestion. Thus it can be concluded that if tritium is made available in the terrestrial environment, such as at the irrigation field site, the biota that live within that environment will experience a quantifiable exposure, as opposed to locations with only background levels of tritium, such as Boggy Gut (Figure 2-6; $F = 20.55$; $p < 0.0001$). In addition, the data from the tritium irrigation site also indicated that there were no significant differences between the whole body tritium concentrations of male and female mice ($F = 0.89$; $p = 0.4260$).

Because tritium is an isotope of hydrogen, the pathway in which tritium is metabolized in an organism is the same pathway in which hydrogen is metabolized. It has been theorized that

because hydrogen is encountered constantly in the environment and sufficient processes exist to metabolize hydrogen, organisms do not have a method to bioconcentrate hydrogen, or therefore tritium. Thus, bioaccumulation and biomagnification are not encountered with tritium as it is with other contaminants (Murphy Jr. 1993). The statistical data presented earlier support this hypothesis.

Mice captured in the spring season were significantly smaller than those captured in the fall season ($F = 7.00$; $p = 0.0026$), suggesting that there may be more juveniles available for capture in the spring season (Figure 2-4). However, whole body tritium concentrations in mice for the two seasons were not significantly different (Figure 2-5; $F = 2.16$; $p = 0.1560$). If bioconcentration occurred in this system, the larger animals, those captured in the fall, which are presumably older and with more exposure, would have higher whole body tritium concentrations than those who are younger and smaller. In addition, the highest whole body tritium concentration found in the captured mice was only approximately 25 % of the pond tritium concentration. If tritium was able to bioconcetrate, the whole body tritium concentration of exposed mice could potentially to surpass that of the source, i.e. the pond. This field data supports the hypothesis that tritium does not bioconcentrate (Murphy Jr. 1993, Klaassen 2001).

Intuitively, a prediction of the relationship between whole body tritium concentrations of exposed mice and amount of tritium made bioavailable can be made. With an increase in bioavailabilty, in this case tritium irrigation, the whole body tritium concentration of exposed mice should be predicted to rise. The data from this field study supports this prediction with a significant, positive regression between volume of tritiated water applied and mouse whole body tritium concentration (Figure 2-7; $F = 4.89$; $p = 0.0376$; $r = 0.4266$; $r^2 = 0.1820$).

In the same respect, a prediction can also be made for whole body tritium concentrations of exposed mice and the amount of precipitation. With an increase in precipitation, the whole body tritium concentration of an exposed mouse should be predicted to decrease, due to the dilution of the tritiated water by rainwater. This field study supports this prediction with a significant, negative regression between volume of precipitation and mouse whole body tritium concentration ($F = 7.67$; $p = 0.0112$; $r = -0.5084$; $r^2 = 0.2585$).

Perhaps the most useful correlation, from a toxicological standpoint, examines the relationship between mouse whole body tritium concentration and an estimated tritium dose. As mentioned earlier, the estimated dose takes into consideration the concentration of the irrigation water, the volume of the tritiated water applied and any dilution of this water with rain water (Equation 1). As with most other toxicants, a prediction should expect an increased whole body tritium concentration with an increased dose. The data from this field study support this prediction with a significant, positive regression between the estimated dose and mouse whole body tritium concentration (Figure 2-8; $F = 6.97$; $p = 0.0149$; $r = 0.4905$; $r^2 = 0.2406$).

This field study, performed within a tritium irrigation site, has allowed for better understanding of the factors that influence the tritium dynamics within the terrestrial environment in this system. However, more information is needed to better evaluate this form of hazardous waste disposal for tritium. The determination of the biological half-life of tritium within a mouse, measured in an ecologically relevant manner, would evaluate tritium concentrations in the case of animal migration from the irrigation site to other areas after exposure. Also, biochemical effects resulting from exposure to low-level β-radiation should be examined to better evaluate risks associated with exposures to tritium similar to that seen at the irrigation site.

Works Cited

Anthony RG, Kozlowski R. Heavy-metals in tissues of small mammlas inhabiting waste-water irrigatied habitats. Journal of Environmental Quality 11: 20-22; 1982.

Arnett M. Savannah River Site Environmental Report for 1997. Aiken, SC: Westinghouse Savannah River Company; WSRC-TR-97-00322; WSRC-TR-97-00322; 1997.

Baker RJ, Chessler RK. The Chernobyl nuclear disaster and subsequent creation of a wildlife preserve. Environmental Toxicology and Chemistry 19: 1231; 2000.

Blake JI. Effectiveness monitoring plan for the tritiated water management facilility southwest plume interim measures. Aiken, SC: Westinghouse Savannah River Company; SRI 00-10-R; SRI 00-10-R; 2000.

Gentry JB, Golley FB, Smith MH. Yearly fluctuations in small mammal populations in a southeastern United States hardwood forest. Acta Theriologica 12: 179-190; 1971.

Golley FB, Gentry JB, Caldwell L, Davenport Jr. L. Numbers and variety of small mammals on the AEC Savannah River Plant. Journal of Mammology 46: 1-18; 1965.

Golley FB, Gentry JB, Menhinic EF, Carmon JL. Response of wild rodents to acute gamma radiation. Radiation Research 24: 350-356; 1965.

Hitchcock DR, Barton CD, Rebel KT, Singer JH, Seaman JC, Strawbridge JD, Riha SJ, Blake JI. A containment and disposition stategy for the management of tritium-contaminated groundwater at the Savannah River Site, SC USA. Environmental Geosciences submitted; 2004.

Hoffman DJ, Rattner BA, Burton Jr. GA, Cairns Jr. J. Handbook of Ecotoxicology. Second ed. Boca Raton, FL: Lewis Publishers; 2003.

Hunter BA, Johnson MS, Thompson DJ. Ecotoxicology of copper and cadmium in a contaminated grassland ecosystem. 3. Small mammals. Journal of Applied Ecology 24: 601-614; 1987.

Johnson MS, Roberts RD, Hutton M, Inskip MJ. Distibution of lead, zinc and cadmium in small mammals from polluted environments. Oikos 30: 153-159; 1978.

Klaassen CD. Cassarett and Doull's Toxicology: The Basic Science of Poisons. New York, NY: McGraw-Hill; 2001.

Krivolutzki DA, Pokarzhevski AD. Effects of radioactive fallout on soil animal populations in the 30km zone of the Chernobyl atomic power station. Science and the Total Environment 112: 69; 1992.

Murphy Jr. CE. Tritium transport and cycling in the environment. Health Physics 65: 683-712; 1993.

Murphy Jr. CE, Bauer LR, Hayes DW, Marter WL, Zeigler CC, Stephenson DE, Hoel DD, Hamby DM. Tritium in the Savannah River Site Environment. Aiken, SC: Westinghouse Savannah River Company; WSRC-RP-90-424-1; WSRC-RP-90-424-1; 1991.

Rebel KT, Riha SJ, Seaman JC, Barton CD. The use of dynamic modeling in assessing tritium phytoremediation. Environmental Geosciences submitted; 2004.

Reinhart BD. The use of small mammals as indicators of heavy metal bioavailability in a contaminated riparian zone. In: Athens, GA: University of Georgia; 2003.

Seaman JC, Gaines KF, Kelsey-Wall A. Tritium Burden in Rodents at the MWMF Irrigation Site. Aiken, SC: Savannah River Ecology Laboratory; 2002.

Smith MH, Blessing R, Chelton JG, Gentry JB, Golley FB, McGinnis JT. Determining density for small mammal populations using a grid and assessment lines. Acta Theriologica 16: 105-125; 1971.

WSRC. Determination of tritium (HTO) in food. Aiken, SC: Westinghouse Savannah River Company; WSRC-3Z1-4-2001-2240; WSRC-3Z1-4-2001-2240; 2001.

WSRC. Determination of tritium (HTO) in water. Aiken, SC: Westinghouse Savannah River Company; WSRC-3Q1-4-2001-2760; WSRC-3Q1-4-2001-2760; 2001.

Figure 2-1 – The Mixed Waste Phytoremediation Site is utilizing a new disposal technique that consists of irrigating a hardwood/pine forest with tritiated water to reduce the concentration of tritium flowing from the Burial Grounds into Fourmile Creek.

Figure 2-2 – Locations for the field study included: a control site, Boggy Gut, located off of the SRS, and the tritium irrigation site on SRS. Rodents captured from each location were used for the determination of whole body tritium concentrations.

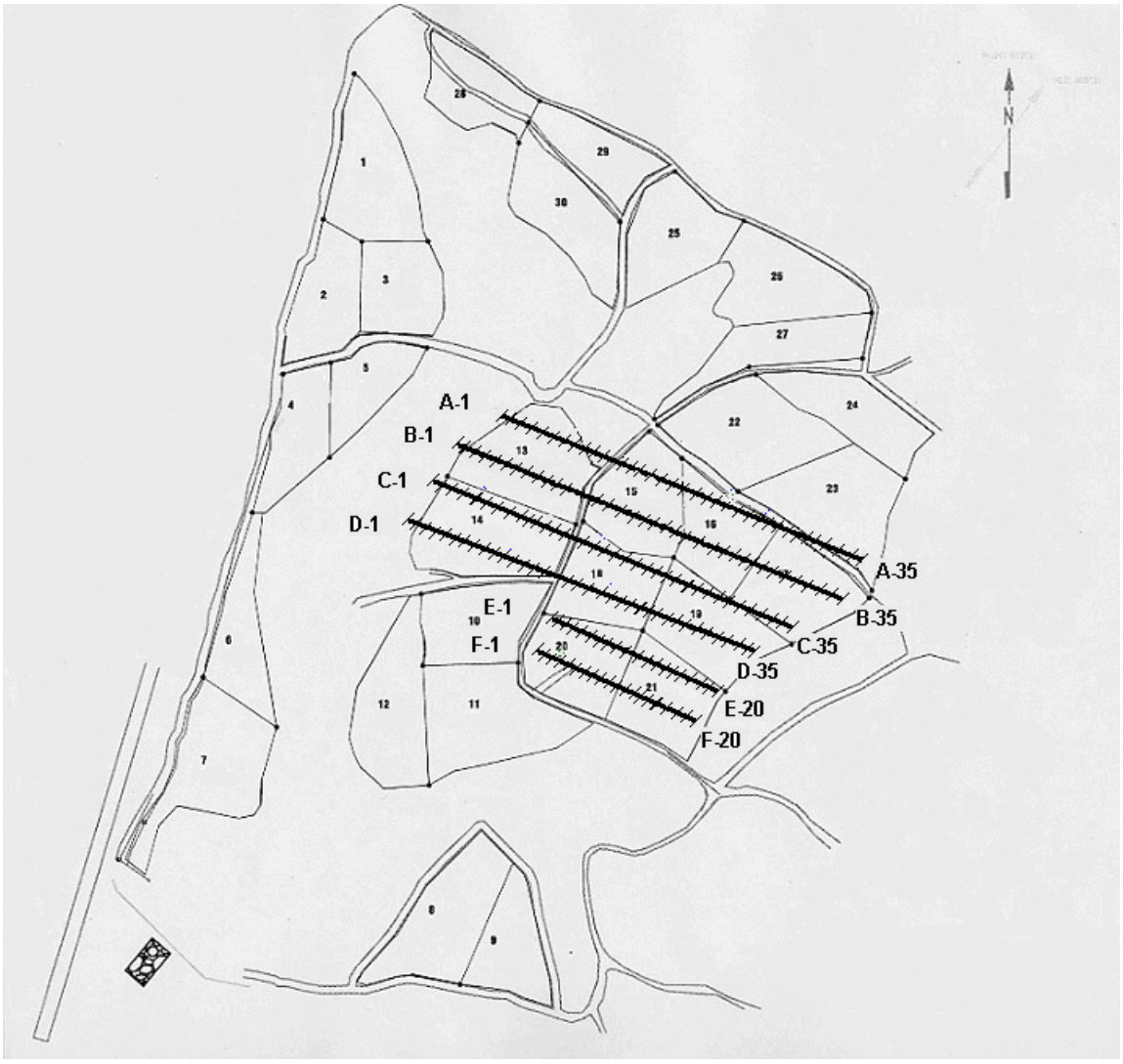

Figure 2-3 – Rodent trapping lines used in the tritium irrigation site on SRS included 4 trapping lines containing 35 Sherman traps and 2 trapping lines containing 20 Sherman traps, for a total of 180 traps distributed among 10 irrigation plots.

Table 2-1 – Field Data for Rodents Captured in fall 2002 and spring 2003 at the tritium irrigation site.

Sample ID	Date	Trap #	Species	Weight (g)	Sex	Tritium (pCi/mL)
F01	10/1/2002	C30	*Peramyscus gossypinus*	28.99	M	3204.28
F02	10/3/2002	D16	*Peramyscus gossypinus*	31.93	M	1764.03
F03	10/3/2002	D21	*Peramyscus gossypinus*	22.73	F	2623.66
F04	10/8/2002	A08	*Peramyscus gossypinus*	30.48	M	1127.41
F05	10/23/2002	C07	*Peramyscus gossypinus*	33.75	F	859.30
F06	10/24/2002	A14	*Peramyscus gossypinus*	20.91	F	791.59
F07	10/29/2002	B24	*Sigmadon hispidus*	110.01	M	1285.14
F08	10/30/2002	A29	*Peramyscus gossypinus*	32.96	F	1962.47
F09	11/1/2002	D21	*Peramyscus gossypinus*	24.52	M	1091.24
F10	11/1/2002	A16	*Peramyscus gossypinus*	31.19	F	1695.88
F11	11/1/2002	C15	*Sigmadon hispidus*	95.22	F	561.32
F12	11/4/2002	A08	*Peramyscus gossypinus*	35.09	M	283.94
F13	11/4/2002	D15	*Blarina carolinensis*	11.27	M	1611.63
F14	11/5/2002	A33	*Peramyscus gossypinus*	29.85	F	748.76
F15	11/6/2002	B6	*Peramyscus gossypinus*	28.59	M	736.20
C01	11/8/2002	E6	*Blarina carolinensis*	7.73	M	204.38
C02	11/10/2002	E6	*Blarina carolinensis*	6.65	M	2178.79
F16	11/20/2002	D8	*Peramyscus gossypinus*	36.58	F	502.85
F16-2	4/1/2003	C5	*Peramyscus gossypinus*	19.95	M	1608.33
F17	4/2/2003	C16	*Peramyscus gossypinus*	20.05	M	410.78
F18	4/3/2003	A5	*Peramyscus gossypinus*	28.51	M	369.99
F19	4/6/2003	A11	*Peramyscus gossypinus*	18.75	F	763.11
F20	4/10/2003	A22	*Blarina carolinensis*	10.47	M	197.06
F21	4/10/2003	A24	*Peramyscus gossypinus*	37.27	F	15.53
F22	4/10/2003	B31	*Peramyscus gossypinus*	25.52	F	110.69
F23	4/10/2003	B35	*Peramyscus gossypinus*	18.12	F	9.70
F24	4/10/2003	D28	*Blarina carolinensis*	11.4	F	171.10
F25	4/10/2003	D19	*Peramyscus gossypinus*	20.05	F	< 0.20
F26	4/18/2003	A22	*Peramyscus gossypinus*	20.15	F	78.67
F27	4/19/2003	B21	*Blarina carolinensis*	11.01	F	1052.23
F28	4/24/2003	D11	*Peramyscus gossypinus*	17.43	F	859.53
F29	5/6/2003	A25	*Peramyscus gossypinus*	19.28	F	1644.36
C03	5/15/2003	F03	*Blarina carolinensis*	9.14	F	165.05
F30	5/16/2003	D19	*Rattus norvegicus*	263.76	M	832.11

Table 2-2 - Field Data for Rodents Captured in fall 2002 at the control site, Boggy Gut.

Sample ID	Date	Species	Weight (g)	Sex	Tritium (pCi/mL)
BG1-15/10-1-02	10/1/2002	*Peramyscus gossypinus*	28.88	M	< 0.20
BG4-16/10-15-02	10/15/2002	*Peramyscus gossypinus*	31.36	M	< 0.20
BG1-11/10-16-02	10/16/2002	*Peramyscus gossypinus*	31.51	M	< 0.20
BG2-16/10-16-02	10/16/2002	*Peramyscus gossypinus*	26.33	M	2.91
BG2-19/10-16-02	10/16/2002	*Peramyscus gossypinus*	30.53	F	< 0.20
BG1-10/10-17-02	10/17/2002	*Peramyscus gossypinus*	19.38	M	< 0.20
BG4-16/10-17-02	10/17/2002	*Peramyscus gossypinus*	25.94	M	< 0.20
BG1-1/10-23-02	10/23/2002	*Peramyscus gossypinus*	29.13	F	< 0.20
BG2-7/10-25-02	10/25/2002	*Peramyscus gossypinus*	22.37	F	5.03
BG4-13/10-25-02	10/25/2002	*Peramyscus gossypinus*	24.18	F	2.38
BG1-19/10-29-02	10/29/2002	*Peramyscus gossypinus*	26.86	M	2.58
BG4-9/11-1-02	11/1/2002	*Peramyscus gossypinus*	16.07	M	< 0.20
BG1-2/11-5-02	11/5/2002	*Peramyscus gossypinus*	27.29	F	< 0.20
BG4-17/11-5-02	11/5/2002	*Peramyscus gossypinus*	24.72	F	2.38
BG2-18/11-6-02	11/6/2002	*Peramyscus gossypinus*	29.30	M	< 0.20
BG1-2/11-7-02	11/7/2002	*Blarina carolinensis*	7.94	M	< 0.20
BG1-2/12-3-02	12/3/2002	*Peramyscus gossypinus*	27.21	M	< 0.20

Table 2-3 – Calculated data included volume of tritium applied, volume of precipitation and an estimated dose for rodents captured both seasons at the tritium irrigation site, for 7 days prior to capture.

Sample ID	Tritium Applied (10^{11} mL)	Rain (10^{6}mL)	Estimated Dose (pCi/mL)
F01	77.73	4.77	13467.16
F02	84.85	5.07	13496.24
F03	46.03	3.56	13182.02
F04	66.51	0.00	14679.07
F05	43.51	3.04	13313.69
F06	1.14	2.86	10736.91
F08	4.45	2.69	7771.46
F09	8.42	2.94	9705.37
F10	6.48	3.15	8571.05
F12	11.36	3.57	10040.42
F14	22.19	1.60	13274.74
F15	12.70	3.10	10808.59
F16	22.72	9.69	9027.91
F16-2	27.31	2.41	12997.78
F17	40.52	2.41	13501.98
F18	27.21	2.26	13081.69
F19	18.60	3.57	11450.06
F21	19.01	27.54	4694.08
F22	24.53	28.27	5453.62
F23	24.53	28.27	5453.62
F25	2.69	28.99	871.28
F26	48.10	0.47	14473.59
F28	61.29	2.15	13959.23
F29	40.64	0.76	14284.73

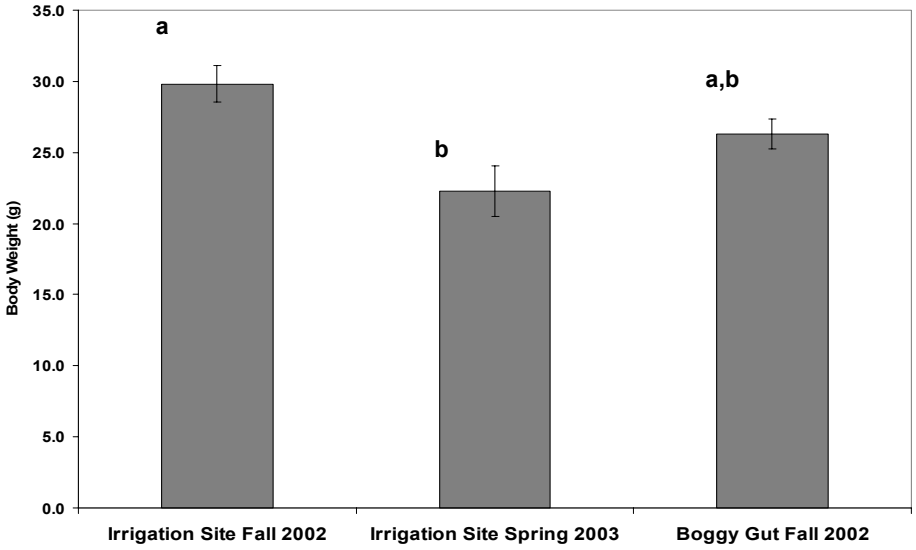

Figure 2-4 – *Peromyscus gossypinus* (cotton mouse) body weight for location and season captured. Locations and seasons with the different letters are statistically significant at the $\alpha = 0.05$ level of significance.

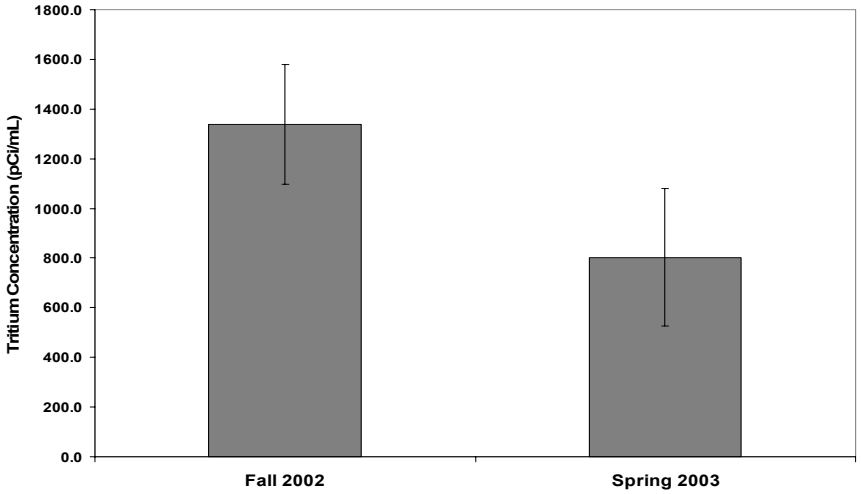

Figure 2-5 – Whole body tritium concentrations of rodents captured fall 2002 and spring 2003 in the tritium-irrigated hazardous waste site. The tritium concentrations for each season were not significantly different.

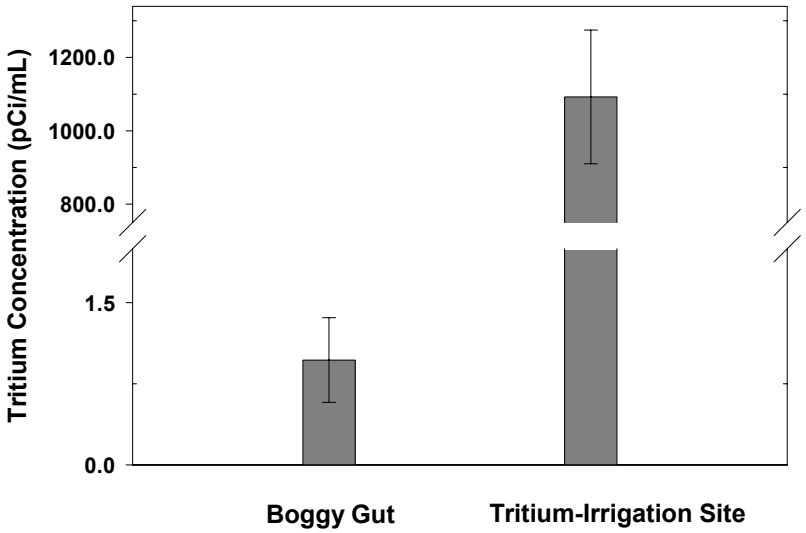

Figure 2-6 – Whole body tritium concentrations of mice captured at the tritium irrigation site and the control site, Boggy Gut, are significantly different ($p < 0.0001$).

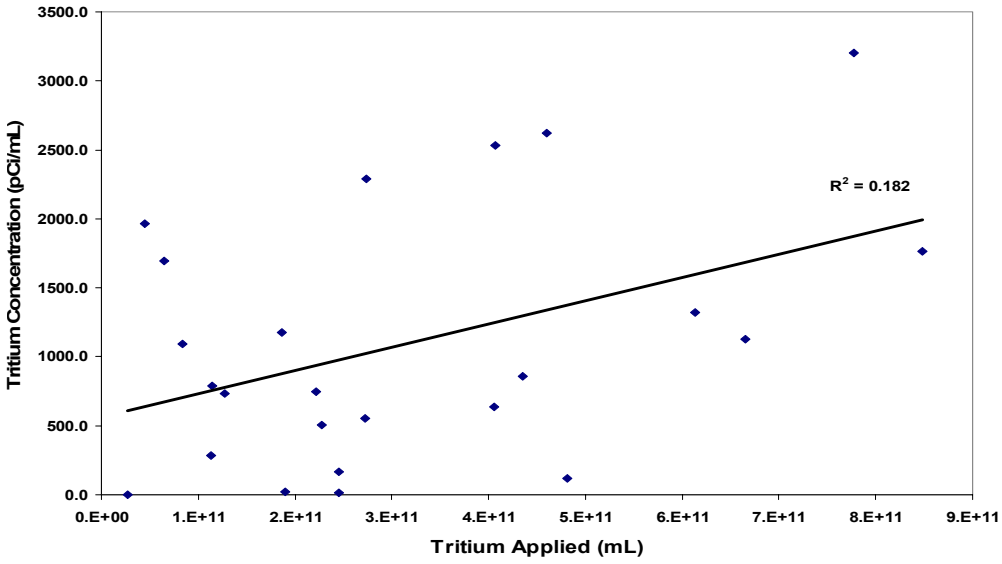

Figure 2-7 – Whole body tritium concentrations of mice captured at the tritium irrigation site increased with an increase in the amount of tritium applied ($p = 0.0376$).

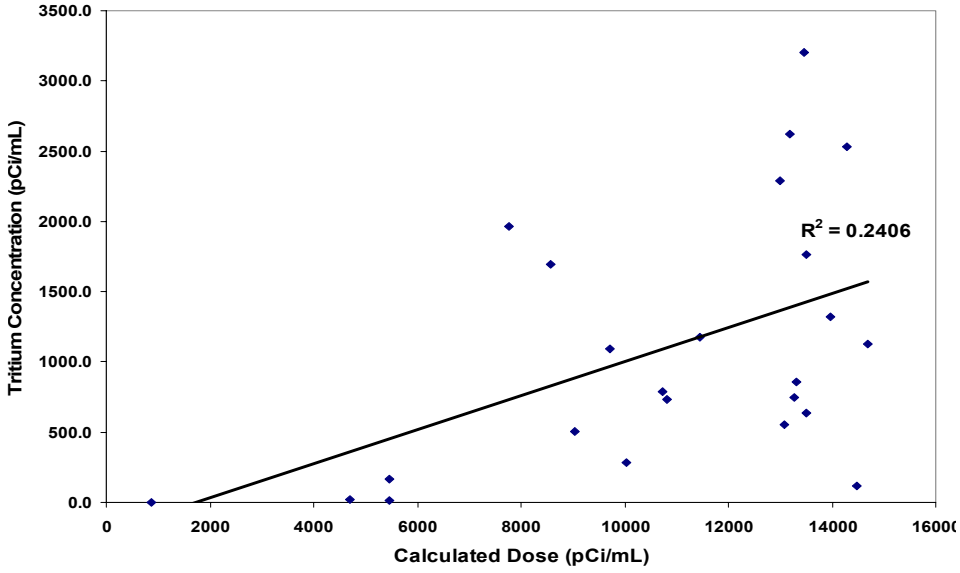

Figure 2-8 – Whole body tritium concentrations of mice captured at the tritium irrigation site increased with an increase in the estimated tritium dose prior to capture ($p = 0.0149$).

CHAPTER III

BIOLOGICAL HALF-LIFE OF TRITIUM IN RODENTS WITH LOW-LEVEL, ORAL EXPOSURE

Introduction

Tritium Exposure in the Environment

Tritium is found at background levels throughout the world due to its own natural production in the environment (Hartek and Faltings 1950, Kaufman and Libby 1954). However, tritium is also made by man during nuclear reactions for a variety of uses ranging from its role in the nuclear bomb, a self-luminous light source, or for use as a tracer for virtually any type of molecule (Moghissi and Carter 1973, N.C.R.P. 1977, Hill and Johnson 1993, Okada and Momoshima 1993). The environmental impact of tritium as a contaminant has become a topic of concern at nuclear facilities, such as Savannah River Site (SRS), who were responsible for the production of radioisotopes, including weapons-grade tritium. The production of tritium has generated a substantial amount of tritiated waste, mostly in the form of tritiated water (HTO), and with strategies shifting from the production of radioisotopes to that of environmental cleanup, these facilities are now responsible for the disposal of the generated tritiated waste (Murphy Jr. et al. 1991, Arnett 1997).

With the task of waste disposal, the potential for environmental contamination always follows; the potential for contamination during disposal of tritiated waste on SRS has become an actuality. The first initiatives by SRS to dispose of tritiated waste incorporated seepage basins and tank farms for the disposal of HTO, and burial grounds for the disposal of solid radioactive

waste (Murphy Jr. et al. 1991). However, these methods were proven an ineffective means of disposal when seepage from these systems leaked into the groundwater, and eventually traveled into the SRS streams, such as Fourmile Branch (Arnett 1997). Beginning in 1997, initiatives were taken to reduce the amount of tritium released into the environment from the burial ground, which included constructing a dam to intercept the contamination outcropping into Fourmile Branch. The resulting pond is being used to irrigate a neighboring 22-acre hardwood/pine forest (Figure 2-1) and has been reduced the tritium concentrations discharged into Fourmile Branch by approximately 60 % (Blake 2000, Hitchcock et al. 2004).

A field study evaluated biota in the terrestrial environment of the tritium irrigation site and found that small mammals did incorporate tritium into their bodies when exposed to the HTO used for irrigation (Kelsey-Wall et al. 2003, Kelsey-Wall et al. 2003). The next step in the evaluation of this disposal system is to evaluate the biological residence time of tritium in small mammals, more specifically mice, should their exposure to tritium cease with migration away from the irrigation site or the termination of the irrigation system (Seaman et al. 2002).

Metabolism of Tritium in the Body

Even though tritium can be present in several forms, such as a gas or organically bound, it is far more abundant in the oxide form, more commonly referred to as HTO (Okada and Momoshima 1993). In the case of the tritium irrigation site, most of the tritium exposure is from HTO. Therefore, in this discussion of tritium metabolism, the only form of tritium considered will be HTO.

HTO can be incorporated into the body by several routes including: respiration by the inhalation of vapor, absorption through the skin, and ingestion by drinking or eating (Pinson and Langham 1957, Murphy Jr. 1993, Okada and Momoshima 1993), with the major path of entrance

being drinking HTO (Murphy Jr. 1993). Once inside the body, HTO essentially follows the pathway of water, by exchanging with body water, with relatively small fractions being incorporated into various body components, i.e. liver, kidney, brain, muscle, fat, etc. The major pathway of elimination in the body is excretion through urine and feces. With continuous exposure to HTO, the body concentration of tritium forms a steady-state with the concentration of the exposure, and is evenly distributed throughout the body. Removal of the source of HTO, allows for the body to excrete tritium, thus lowering the concentration of tritium in the body (Richmond et al. 1962, Feindendegen 1967, Moghissi and Carter 1973, van den Hoek 1986, Moghissi et al. 1987, Murphy Jr. 1993).

The ability of HTO to move across barriers at the same rate as ordinary water, along with the overall stability of tritium in experimental settings, has allowed for its use as a tracer (Pinson 1952, Moghissi and Carter 1973). HTO has been used as a tracer to study the movement of water in biological systems for decades (Thompson 1952, Feindendegen 1967, Moghissi and Carter 1973). Because of its use as a tracer, a question may arise concerning any isotopic differences between the kinetics of HTO versus regular water. In general, reactions involving tritium–labeled compounds are generally slower than their hydrogen counterparts (Murphy Jr. 1993). However, in the case of HTO, a kinetic effect of radiobiological significance should not occur unless the specific activity of the system in question undergoes a very high increase (Moghissi and Carter 1973).

Biological Half-Life

The biological half-life of a compound is defined as the time required for half the concentration of a compound deposited within a living organism to be eliminated by normal biological processes (Hoffman et al. 2003). The biological half-life of HTO has been determined

in several species, including humans (Thompson 1952, Richmond et al. 1962, Takeda and Kasida 1979, Takeda et al. 1982, van den Hoek 1986).

From experimental data, it has been determined that the biological half-life of tritium is composed of at least three stages, possibly more. The first stage is essentially water metabolism, the elimination of HTO by excretion in urine and feces; approximately 90 % of the total dose of tritium will be eliminated in this fashion. The remaining 10 % of the dose is eliminated in two steps, from fractions of tritium stored inside the body and incorporated into various components, such as proteins, carbohydrates and lipids. The second and third stages in the elimination process of tritium from the body is dependent on the metabolism of the compound in which tritium is incorporated. The second stage of the elimination is generally related to tritium bound to carbohydrates and protein, while the third stage is generally related to tritium incorporated into lipids. In humans, the biological half-life of these three components are estimated to be 9 days, 30 days and 450 days, respectively (Thompson and Ballou 1954, N.C.R.P. 1979, Murphy Jr. 1993, Okada and Momoshima 1993).

Several studies have calculated the biological half-life of HTO in rodent species (Table 4) (Thompson 1952, Thompson and Ballou 1954, Richmond et al. 1962, Takeda and Kasida 1979, Takeda et al. 1982). However, these studies are performed using an acute dose of a large concentration of tritium using an intraperitoneal injection as the route of administration. Intraperitoneal administration involves the injection of a known amount of a compound into the peritoneal cavity of an animal, essentially bypassing gastric emptying, and allowing the compound to immediately pass through the liver (Klaassen 2001). Although this route of administration is appropriate under toxicological circumstances, it holds very little ecological relevance.

The evaluation of the biological residence time of tritium in small mammals, in the case of environmental exposure, can not be effectively estimated based on the previous studies for several reasons. These studies use an acute dose, or a single exposure. Once a contaminant enters the environment, it is a persistent problem, therefore biota living around the contamination can be continually exposed over a long periods of time (Klaassen 2001). In addition, as mentioned earlier, the route of administration used for the HTO exposure in these studies, intraperitoneal injection, is not as relevant as a study looking at the major pathway of exposure, drinking HTO (Murphy Jr. 1993). Finally, the experimental methods of the previous studies involve estimation of tritium concentrations in mice based on blood tritium concentrations (Richmond et al. 1962) and whole body tritium concentrations following a benzene extraction (Thompson 1952). This laboratory experiment was performed by monitoring whole body tritium concentrations of exposed mice and under conditions more representative of those for rodents living in the irrigation site.

Materials and Methods

Laboratory Experiment Methods

The solution used for tritium exposure was prepared by diluting of an approximately 1.0 μCi/mL stock solution (Amersham Inc.) with tap water. After dilution, the solution was analyzed using liquid scintillation, outlined in a procedure by Westinghouse Savannah River Company (WSRC) (WSRC 2001), with the exception of adjusting the sample and scintillation cocktail ratios to reduce the amount of waste that was produced in a manner that is consistent with QA/QC protocols. Analysis was performed with an estimated detection limit of 0.20 pCi/L and a counting error below 2% for elevated tritium concentrations (Minaxi Tri-Carb 4000, Packard Instru. Co). The final concentration of HTO for use in the exposure was determined to

be approximately 8,200 pCi/mL. For comparison, the current drinking water limit for HTO is 20 pCi/mL.

All animal research conducted was performed with the approval of the University of Georgia Institutional Animal Care and Use Committee (IACUC# A2004-10015-0). Mouse husbandry was performed in accordance with University of Georgia Standard Operating Procedure (SOP 01.04). Mice were housed in standard shoebox cages, with a maximum of four mice residing in each cage; cage cleaning was performed once weekly. Bed-O-Cob bedding, composed of variable corn cob pieces, was used along with Purina Mouse Chow 5001 for bedding and food, respectively. Mice were housed at a constant temperature and humidity level of 72.3 ± 0.7 ° F and 64.8 ± 3.3 %, respectively. A twelve-hour light period, beginning at 6:00 A.M., and a twelve-hour dark period, beginning at 6:00 P.M, was also observed. The use of cage cards and a room log were employed throughout the experiment.

Sixty-one male BALB/C inbred mice age 39-49 days, weighing between 19-21 g, were purchased from Charles River Laboratories (Wilmington, MA). Upon receipt, the mice were randomly assigned a cage, which were assigned a cage number ranging from 1-16; the end result being 14 cages with four mice, 1 cage with three mice, and 1 cage with two mice residing. Mice were allowed to acclimate in the laboratory environment for one week prior to the beginning of the exposure period, in which all mice received tap water as their drinking water.

After the one-week period of acclimation, cages were randomly assigned control or exposed. Nine mice in three different cages were randomly assigned to be control mice, receiving tap water throughout the duration of the experiment, and 52 mice in the remaining 13 cages were randomly assigned to be exposed mice, receiving HTO with a concentration of approximately 8,200 pCi/mL for two weeks. After the two-week exposure, the control mice,

along with 7 randomly picked exposed mice, referred to as "time 0" mice, were sacrificed. Time 0 mice represent the whole body fluid tritium concentration of mice in equilibrium with the HTO used for exposure. The 45 mice remaining were maintained on tap water. At 9 different time intervals, a group of 5 randomly chosen mice were sacrificed. The time intervals at which the mice were sacrificed were: 6 hours, 12 hours, 18 hours, 24 hours, 30 hours, 36 hours, 48 hours, 72 hours, and 96 hours (Figure 3-1). All mice were sacrificed using cervical dislocation.

Laboratory Analysis

Before extraction, body weight was recorded for each mouse; mice were freezer-stored prior to extraction. The whole body of captured rodents was used to extract body fluid for analysis by sublimation (i.e., freeze drying) using the procedure described by WSRC for removing "free" tritium from foodstuffs (WSRC 2001). Body fluid was analyzed by liquid scintillation in the same manner as stated above for the HTO used for exposure (Minaxi Tri-Carb 4000, Packard Instru. Co.).

Statistical Analysis

All statistical analyses were performed using SAS (version 9.0; SAS Institute). Body weights and whole body tritium concentrations were tested for normality using the Shapiro-Wilk statistic (PROC UNIVARIATE). Analysis of variance models (ANOVA; PROC GLM), along with post-ANOVA tests (Tukey, Scheffe and Dunnett), were used to examine relationships between body weight and time after exposure. For modeling analysis, whole body tritium concentrations were log-transformed and regressed (PROC REG) to fit an appropriate linear model to the data. For all tested models, F-statistics were used to provide an estimate of the predictive value of the model. All statistical tests were considered significant at the $p \leq 0.05$

level. For a below detection value, the detection limit for the instrument was entered for that sample.

Results

There were no significant differences between body weights for exposed mice based on time after exposure ($F = 1.84$; $p = 0.0773$) and control mice, which was verified by Tukey, Scheffe, and Dunnett post-ANOVA tests. The mice sacrificed at time 0 had an average whole body tritium concentration of 4,395 ± 68 pCi/mL; the control mice had an average whole body tritium concentration below detection, which was assigned to be 0.20 pCi/mL. The whole body tritium concentrations of mice decreased in an exponential fashion for all time intervals after exposure ended (Figure 3-2; Table 3-2). When the data was log transformed and fit to a linear model, there is a very significant, negative, linear relationship between whole body tritium concentration and time after exposure ($F = 789.27$; $p < 0.0001$; $r = -0.9696$, $r^2 = 0.9402$).

The log-transformed data was used to determine the biological half-life of tritium, based on the average concentration of the time 0 mice. Half of this concentration was considered to be the half-life concentration. Therefore, the biological half-life of tritium in mice is the time, after exposure ended, at which the average whole body mouse tritium concentration is equivalent to the half-life concentration. Using the statistical model that was derived by SAS and solving for the half-life concentration, the biological half-life of tritium in mice is calculated to be 54.15 hours, or 2.26 ± 0.04 days, with a 95 % confidence interval of 2.15 days to 2.36 days.

Discussion

The results from this study conclude that the biological half-life of HTO in exposed mice is approximately 2.26 ± 0.04 days, which is double the previous estimate of 1.1 days (Thompson 1952, Richmond et al. 1962). Differences in the route of administration and experimental

methods between this study and the previous studies may account for the underestimation of the previous estimate values.

Although the use of intravenous injection is the most ideal kinetic approach to estimate the biological half-life of a contaminant (Klaassen 2001), due to the very high uptake of tritium, it would not be the best route of exposure. In order to accurately evaluate the biological half-life of tritium in mice, and to make this laboratory study more environmentally realistic, the exposure was administered through drinking water. Mice were exposed for a period of time to allow equilibration with the HTO. The concentration of HTO used in the exposure is representative of concentrations found inside the tritium irrigation site, and the average equilibrated whole body tritium concentration from the laboratory experiment was comparable to whole body tritium concentrations found in the field study.

The concentration of the tritium used for exposure was roughly double the concentration observed in the most concentrated field mouse captured (Table 2-1) (Kelsey-Wall et al. 2003). This concentration was chosen because previous studies have found that mice will always have a lower concentration than the when in a steady state with the HTO used for the exposure. This is perhaps due to only approximately 58 % of total body water being available for exchange (Richmond et al. 1962, Feindendegen 1967, Moghissi and Carter 1973). For example, tissue concentrations of mice with continuous exposure to HTO throughout their life only had a concentration 56% of the HTO used for exposure (Brooks et al. 1976). In this laboratory experiment, the average concentration for the time 0 mice was approximately 53 % of the concentration of the HTO used in the exposure. Therefore, the steady state whole body tritium concentration for mice from the laboratory experiment (~ 4,395 pCi/mL) was comparable to the highest whole body tritium concentration from the field study (~ 3,204 pCi/mL).

In order to accurately determine the biological half-life of tritium in mice, a steady-state must be achieved. To allow mice to be in equilibrium with the HTO used in the exposure, mice were exposed for a period of two weeks. This value was chosen based on a study of mice continuously given HTO throughout their life which determined that a steady-state between the bodies of mice and the HTO used for exposure was achieved within 7 days of administering the HTO (Brooks et al. 1976). Thus, to ensure the mice were in complete equilibrium with the HTO used for exposure, in this laboratory exposure the 7-day time period was doubled. Although a steady state was achieved in this study, it should be noted that due to changes in precipitation and variation in the application of tritium, mice in the field will never be in complete equilibrium.

As seen in Table (3-1), previous estimates of the biological half-life of HTO in mice were around 1.1 days. This estimate is expected when compared to those of other species listed (which have significantly higher biological half-lives) due to the faster metabolism of mice (Richmond et al. 1962, Klaassen 2001). Because the biological half-life estimate is reasonably short, in the laboratory experiment, most mice were sacrificed during the first two days after exposure ceased (Figure 3-2; Table 3-2). With this sampling plan and the experimental design, it was determined that the biological half-life of tritium was approximately 2.26 days; this estimate was calculated as with a high degree of significance (Figure 3-2; $F = 789.27$; $p < 0.0001$; $r = -0.9696$, $r^2 = 0.9402$).

When compared to the earlier estimates, this laboratory study found a biological half-life that is essentially double those found in previous studies. One possible explanation may be a combination between the route of exposure, intraperitoneal injection, and the experimental methods used in the previous mouse studies. Richmond et al. (1962) used blood to monitor tritium concentrations in mice (Richmond et al. 1962). Using blood for monitoring tritium may

be problematic due to biphasic elimination. In biphasic elimination, there are distinct phases in elimination, which can include both redistribution and elimination phases (Klaassen 2001). Sampling during the redistribution phase may cause an underestimation in tritium concentrations since equilibrium has not yet been reached between the blood component and the body component. Thompson (1952) used a more representative monitoring method, whole body tritium concentration (Thompson 1952). However, these concentrations were determined following a crude benzene extraction procedure, which may not have been very successful at extracting the tritium from the whole body of the mouse. The methods used in this study are more reliable and efficient when compared to those used in previous studies.

With relation to the tritium irrigation site, if a mouse were to migrate away from the irrigation site or if the use of the irrigation system was terminated, allowing for exposure to HTO to cease, within 2.26 days after the exposure ended, the whole body tritium concentration of the mouse would expected to be half of its original value. Within the period of a month, based on the concentrations seen in mice from the field study, the mouse would have a whole body tritium concentration slightly above background levels. The data from the field study and this laboratory study suggest that the potential of tritium contamination spreading from the irrigation site to the surrounding areas by the terrestrial biota is minimal. However, in order to completely evaluate the tritium irrigation site, more data is needed to estimate the risk of exposure of mice to low-level β-radiation, such as examining for the potential of metabolic stress.

Works Cited

Arnett M. Savannah River Site Environmental Report for 1997. Aiken, SC: Westinghouse Savannah River Company; WSRC-TR-97-00322; WSRC-TR-97-00322; 1997.

Blake JI. Effectiveness monitoring plan for the tritiated water management facilility southwest plume interim measures. Aiken, SC: Westinghouse Savannah River Company; SRI 00-10-R; SRI 00-10-R; 2000.

Brooks AL, Carsten AL, Mead DK, Retherford JC, Crain CR. The effect of continuous intake of tritiated water (HTO) on the liver chromosomes of mice. Radiation Research 68: 480-489; 1976.

Butler HL, Leroy JH. Observation of biological half-life of tritium. Health Physics 11: 283-285; 1965.

Feindendegen LE. Tritium-Labeled Molecules in Biology and Medicine. New York, NY: Academic Press; 1967.

Hartek P, Faltings V. The helium-3 problem of the atmosphere. Nature 166: 1109; 1950.

Hill RL, Johnson JR. Metabolism and dosimetry of tritium. Health Physics 65: 628-647; 1993.

Hitchcock DR, Barton CD, Rebel KT, Singer JH, Seaman JC, Strawbridge JD, Riha SJ, Blake JI. A containment and disposition stategy for the management of tritium-contaminated groundwater at the Savannah River Site, SC USA. Environmental Geosciences submitted; 2004.

Hoffman DJ, Rattner BA, Burton Jr. GA, Cairns Jr. J. Handbook of Ecotoxicology. Second ed. Boca Raton, FL: Lewis Publishers; 2003.

Kaufman S, Libby WF. The natural distribution of tritium. Physical Review 93: 1337-1344; 1954.

Kelsey-Wall A, Komoroski MJ, Gaines KF, Seaman JC, Aburime S. Tritium burden in rodents captured at the MWMF: Annual progress report. Aiken, SC: Savannah River Ecology Laboratory; 2003.

Klaassen CD. Cassarett and Doull's Toxicology: The Basic Science of Poisons. New York, NY: McGraw-Hill; 2001.

Moghissi AA, Bretthauer EW, Patzer RG. Biological concentration of 3H. Health Physics 53: 385-388; 1987.

Murphy Jr. CE. Tritium transport and cycling in the environment. Health Physics 65: 683-712; 1993.

Murphy Jr. CE, Bauer LR, Hayes DW, Marter WL, Zeigler CC, Stephenson DE, Hoel DD, Hamby DM. Tritium in the Savannah River Site Environment. Aiken, SC: Westinghouse Savannah River Company; WSRC-RP-90-424-1; WSRC-RP-90-424-1; 1991.

N.C.R.P. Radiation exposure from consumer products and miscellaneous sources. National Council on Radiation Protection and Measurements; 56; 1977.

N.C.R.P. Tritium in the environment. Betheseda, MD: National Council on Radiation Protection and Measurements; 62; 1979.

Okada S, Momoshima N. Overview of tritium: Characteristics, sources and problems. Health Physics 65: 595-609; 1993.

Pinson EA. Water exchange and barriers as studied by the use of hydrogen isotopes. Physiolocical Reviews 32: 123-134; 1952.

Pinson EA, Langham WH. Physiology and toxicology of tritium in man. Journal of Applied Physiology 10: 108-126; 1957.

Richmond CR, Langham WH, Trujillo TT. Comparative metabolism of tritiated water by mammals. Journal of Comparitative Physiology 59: 45-53; 1962.

Seaman JC, Gaines KF, Kelsey-Wall A. Tritium Burden in Rodents at the MWMF Irrigation Site. Aiken, SC: Savannah River Ecology Laboratory; 2002.

Takeda H, Arai K, Iwakura T. Comparason of tritium metabolism in rat after single or continuous ingestion of tritium labeled wheat versus tritiated water. Journal of Radiation Research 26: 131-139; 1982.

Takeda H, Kasida Y. Biological behavior of tritium after administration of tritiated water in the rat. Journal of Radiation Research 20: 174-185; 1979.

Thompson RC. Studies of metabolic turnover with tritium as a tracer. I. Gross studies with the mouse. Journal of Biological Chemistry 197: 81-87; 1952.

Thompson RC, Ballou JH. Studies of metabolic turnover with tritium as a tracer. Journal of Biological Chemistry 208: 883-888; 1954.

van den Hoek J. Tritium metabolism in animals. Radiation Protection Dosimetry 16: 117-121; 1986.

WSRC. Determination of tritium (HTO) in food. Aiken, SC: Westinghouse Savannah River Company; WSRC-3Z1-4-2001-2240; WSRC-3Z1-4-2001-2240; 2001.

WSRC. Determination of tritium (HTO) in water. Aiken, SC: Westinghouse Savannah River Company; WSRC-3Q1-4-2001-2760; WSRC-3Q1-4-2001-2760; 2001.

Wylie KF, Bigler WA, Grove GR. Biological half-life of tritium. Health Physics 9: 911-914; 1963.

Table 3-1 – Biological half-life of tritium in various species, as determined by previous studies.

Species	Dose (mCi)	Route of Administration	Half-life (days)
Dog[a]	1.27	Oral	5.14
Horse[a]	142.10	Intravenous Injection	8.41
Kangaroo Rat[a]	0.85	Intraperitoneal Injection	11.82
Man[a]	2.00	Oral	9.46
Man[b]	> 20	Occupational Exposure	9.50
Man[c]	> 100	Occupational Exposure	8.50
Mouse[a]	0.71	Intraperitoneal Injection	1.13
Mouse[d]	1.2; 5.5	Intraperitoneal Injection	1.10
Rabbit[a]	7.10	Intraperitoneal Injection	3.87
Rat[a]	4.33	Intraperitoneal Injection	3.53
Rat[e]	6.00	Intraperitoneal Injection/ Gavage	3.50

a - (Richmond et al. 1962)
b - (Butler and Leroy 1965)
c - (Wylie et al. 1963)
d - (Thompson 1952)
e - (Takeda and Kasida 1979)

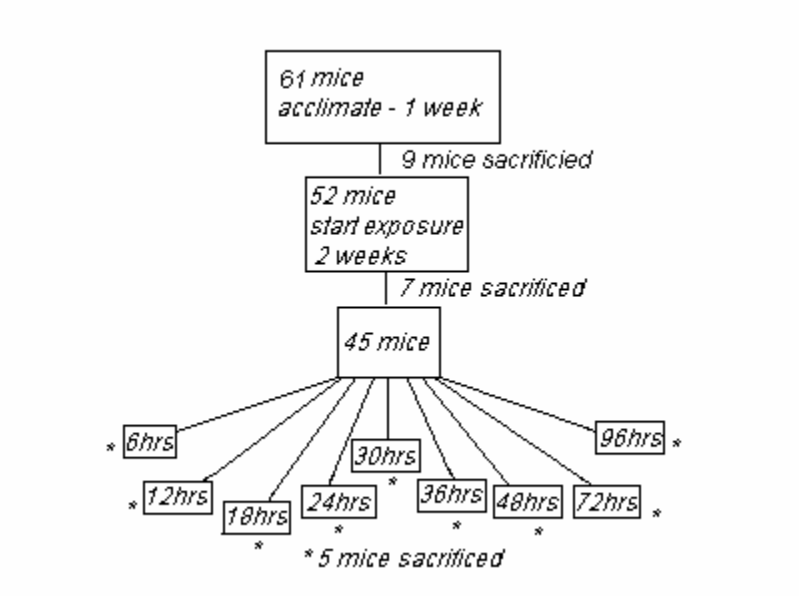

Figure 3-1 – Project outline for the determination of the biological half-life of tritium in mice.

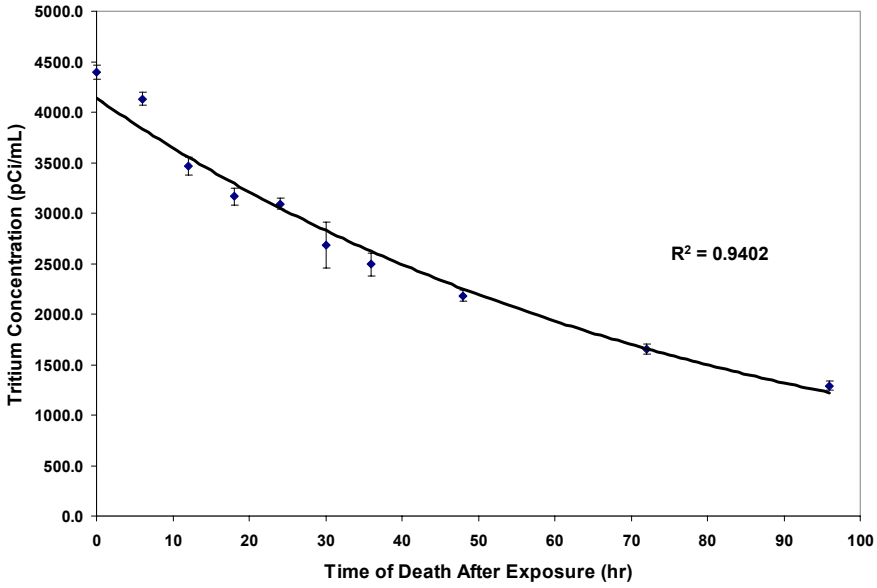

Figure 3-2 – Whole body tritium concentrations of tritium-exposed mice decreased in an exponential fashion after the exposure to tritiated water ended ($p < 0.0001$).

Table 3-2 – Laboratory exposure data used to determine the biological half-life of tritium in mice.

Sample ID	Sacrifice Time (hr)	Weight(g)	Tritium (pCi/mL)
1-1	6	24.57	4216.249
1-2	24	24.18	3276.612
1-3	48	23.51	2345.254
1-4	72	24.38	1816.435
2-1	6	22.36	4170.906
2-2	24	23.92	2973.542
2-3	48	23.16	2074.704
2-4	48	24.60	2062.997
3-1	12	24.46	3165.558
3-2	48	22.47	2165.473
3-3	72	24.13	1660.001
3-4	96	24.53	1234.740
4-1	6	22.41	3875.033
4-2	36	25.31	2365.307
4-3	48	23.47	2248.775
4-4	72	25.39	1590.839
5-1	0	19.54	4241.985
5-2	0	22.57	4511.632
5-3	12	23.99	3584.649
5-4	18	21.86	2947.712
6-1	6	23.12	4179.522
6-2	12	23.66	3360.692
6-3	24	22.90	3076.964
6-4	72	22.85	1517.166
9-1	0	25.07	4129.466
9-2	36	24.15	2484.525
9-3	36	24.87	2793.961
9-4	36	22.35	2678.275
10-1	0	23.33	4590.023
10-2	0	23.00	4324.908
10-3	18	25.20	3252.004
10-4	18	26.09	2985.317
11-1	24	24.01	3141.701
11-2	30	24.18	2905.184
11-3	72	25.25	1691.947
11-4	96	24.08	1351.356
12-1	12	25.18	3619.366
12-2	18	24.49	3389.411
12-3	30	24.23	3018.043
12-4	30	23.68	2552.893
13-1	0	22.26	4365.807
13-2	6	21.84	4206.788
13-3	12	25.08	3606.020
13-4	30	24.00	3076.618
14-1	0	23.72	4598.276
14-2	18	26.09	3252.162
14-3	24	26.18	2986.246
14-4	36	24.62	2141.332
15-1	30	24.92	1842.519
15-2	96	25.71	1387.011
15-3	96	24.41	1347.538
15-4	96	24.48	1138.203

CHAPTER IV

OXIDATIVE STRESS RESPONSE IN MICE WITH LOW-LEVEL ORAL EXPOSURE TO TRITIATED WATER

Introduction

Ionizing Radiation

The process of removing electrons from neutral atoms is referred to as ionization. When radiation from a radioactive material emits enough energy to cause ionization, it is referred to as ionizing radiation. In biological systems, ionizing radiation is the form of radiation that has the potential to cause harm. Tritium is a low-level β-emitter and once it has been internalized is considered a source of ionizing radiation. In the previous chapters, rodents were exposed to tritium in the form of tritiated water in both a field and laboratory setting. In these cases, as well as in the case of environmental tritium exposure, tritium is considered to be a source of ionizing radiation (N.C.R.P. 1979, N.C.R.P. 1985).

Ionizing radiation has been shown to cause damage in biological tissue through a mechanism involving highly reactive oxygen species. These reactive oxygen species (ROS) consist of free radicals (any atom or molecule containing one or more unpaired electrons) such as superoxide anion ($O_2^{\cdot -}$), as well as molecules including oxygen, such as hydrogen peroxide (H_2O_2). ROS are generated during normal cell functions; however, in the presence of certain chemicals or ionizing radiation, the production of ROS can be increased (Riley 1994, Hollan 1995, Anderson 1996, Sun et al. 1998). These highly reactive species can react with stable molecules within cells, causing damage that can disrupt cellular processes. The presence of

ROS has been linked to several diseases in humans, including atherosclerosis (Witzum 1994), respiratory tract disorders (Cross et al. 1994), cancer and aging (Ames 1989).

Cellular Response to ROS

Within the cell, defense mechanisms involving antioxidants have evolved to protect from ROS. Antioxidants are highly regulated enzymes that protect cells from the damaging effects of ROS; antioxidants can have general or more specific duties within a cell (Harris 1992, Anderson 1996, Sun et al. 1998). For example, a general role for an antioxidant may include converting ROS to less harmful products, but more specific roles can include destroying damaged proteins (Stadtman and Oliver 1991), repairing damage to DNA (Breimer 1991), or removing oxidized fatty acids from membranes (Maiorino et al. 1991). It has been hypothesized that, dependent on the regulation of ROS, a constant level of antioxidant enzyme activities are maintained within cells and any imbalance in the equilibrium between ROS and antioxidant levels may result in oxidative stress (Hollan 1995, Klaassen 2001).

Although several mechanisms exist which induce oxidative stress (Haimovitz-Friedman 1998), in general, with an exposure to a harmful agent, such as ionizing radiation, increasing concentrations of ROS can be generated. With an increase in ROS levels, the transcriptional regulators that monitor antioxidant levels within the cell manufacture more antioxidants in response (Harris 1992, Hardmeier et al. 1997). If concentrations of ROS within the cell overwhelm these antioxidant defenses, an organism may be forced into oxidative stress, after which normal cellular functions may be greatly impacted (Hollan 1995, Gate et al. 1999). Oxidative stress can be induced in an organism when exposed to any hazardous agent, even those which are commonly encountered in the environment such as cigarette smoke, polyunsaturated fats and air pollution (Hollan 1995).

Antioxidant Defenses

Three of the major antioxidant enzymes that exist in cells are catalase (CAT), glutathione peroxidase (GPX) and superoxide dismutase (SOD). These enzymes scavenge and detoxify ROS for the protection of cellular mechanisms and functions. Although the goal of these enzymes is the same, each uses different substrates and modes of action to fulfill their roles (Anderson 1996, Gate et al. 1999).

CAT is a heme enzyme and, within a cell, has the most activity within the peroxisome. Although it is found within all tissues of an organism, the highest levels of CAT are found within the liver, kidney, and erythrocytes. It is responsible for removing H_2O_2 by the NADPH-dependent detoxification mechanism seen in Equation (1):

$$H_2O_2 + H_2O_2 \rightarrow O_2 + 2H_2O \qquad (1)$$

CAT is believed to be responsible for the majority of the cellular decomposition of H_2O_2, which is produced mostly by peroxisomal oxidase enzymes (Aebi 1984, Kirkman et al. 1987, Harris 1992, Anderson 1996).

GPX is also involved in the detoxification of peroxides, including H_2O_2, within the cell. GPX is present in the cytoplasm, plasma and mitochondria of most organs. It is the key antioxidant in a number of cellular reactions, especially important in lipid peroxidation. Its mechanism of action involves the use of a cellular protein, glutathione, as seen in Reaction (2).

$$2GSH + H_2O_2 \rightarrow GSSG + 2H_2O \qquad (2)$$

GPX converts H_2O_2 into water by oxidizing reduced glutathione (GSH) into an oxidized form of glutathione (GSSG). Because GSH is required for the detoxification of peroxides by GPX, other NADPH-dependent enzyme systems work in collaboration with GPX in the reduction of GSSG to GSH (Harris 1992, Anderson 1996, Yamaoka et al. 1998, Gate et al. 1999).

Two forms of SOD exist, each using transition metals in the active site (MnSOD and CuZnSOD) to fulfill its role as an antioxidant. In eukaryotes, MnSOD is present within the mitochondria of a cell and CuZnSOD is present within the cytosol. Both of these forms of SOD are responsible for the conversion of $O_2^{\cdot -}$ into less harmful products, as seen in Reaction (3).

$$2O_2^{\cdot -} + 2H^+ \rightarrow H_2O_2 + O_2 \qquad (3)$$

In the process of converting $O_2^{\cdot -}$ into less toxic products, another form of ROS, H_2O_2, is generated; although the mechanism generates an ROS product, it is still recognized as detoxification due to the high toxicity of $O_2^{\cdot -}$ in comparison to H_2O_2. The H_2O_2 generated during SOD detoxification is degraded further by the aforementioned antioxidants GPX and CAT (Krizala et al. 1982, Harris 1992, Gate et al. 1999, Klaassen 2001).

Biomarkers as Indicators of Induced Oxidative Stress

As mentioned earlier, oxidative stress can be induced within an organism by exposure to harmful chemicals or ionizing radiation. The first lines of defense used to protect against oxidative stress are antioxidants, such as CAT, GPX and SOD. Because elevated ROS levels in cells can induce the production of these enzymes, their activities are expected to increase in the presence of increased ROS. Therefore, the measurement of enzyme activities for these enzymes can be used as a biomarker for exposure to harmful agents (Riley 1994, Hollan 1995, Anderson 1996, Sun et al. 1998).

CAT, GPX and SOD have been used as biomarkers for exposure to a wide range of agents including harmful chemicals (Bindhumol et al. 2003), stressful conditions (Xia et al. 1995), and ionizing radiation (Krizala et al. 1982, Yamaoka et al. 1998). These enzymes have been measured in the tissues of various species including rodents, fish, birds, and amphibians (Perez-Campo et al. 1993). In addition, they have been used as biomarkers of contaminant

exposure in both field (Hollowman et al. 2000) and laboratory exposure studies (Yamaoka et al. 1998). To date, there has not been an evaluation of oxidative stress enzymes in response to low levels of β-radiation resulting from tritium ingestion. In the current study, these enzymes were used as biomarkers to evaluate oxidative stress response in mice with low-level, oral exposure to tritium via tritiated water.

Materials and Methods

Tritium Exposure Methods

The solution used for tritium exposure was prepared by diluting of an approximately 1.0 μCi/mL stock solution (Amersham, Inc.) with tap water. After dilution, the solution was analyzed using liquid scintillation, with an estimated detection limit of 0.20 pCi/L and a counting error below 2% for elevated tritium concentrations (Minaxi Tri-Carb 4000, Packard Instru. Co). The final concentration of tritiated water for use in the exposure study was determined to be approximately 8,200 pCi/mL.

All animal research was performed with the approval of the University of Georgia Institutional Animal Care and Use Committee (IACUC# A2004-10015-0). Mouse husbandry was performed in accordance with University of Georgia Standard Operating Procedure (SOP 01.04). Mice were housed in standard shoebox cages, with a maximum of four mice residing in each cage; cage cleaning was performed once weekly. Bed-O-Cob bedding, composed of variable corncob pieces, was used along with Purina Mouse Chow 5001 for bedding and food. The animal facility was maintained at a constant temperature and humidity level of 72.3 ± 0.7 ° F and 64.8 ± 3.3 %, respectively. A twelve-hour light period, beginning at 6:00 A.M., and a twelve-hour dark period, beginning at 6:00 P.M, was also observed. The use of cage cards and a room log were employed throughout the experiment.

Sixty-one male BALB/C inbred mice age 39-49 days, weighing between 19-21 g, were purchased from Charles River Laboratories (Wilmington, MA). Upon receipt, the mice were randomly assigned a cage, which were assigned a cage number ranging from 1-16; the end result being 14 cages with four mice, 1 cage with three mice, and 1 cage with two mice residing. Mice were acclimated in the laboratory environment for one week, in which all mice received tap water as their drinking water, prior to the beginning of the exposure period.

After the one-week period of acclimation, cages were randomly assigned control or exposed. Nine mice in three different cages were randomly assigned to be control mice, receiving tap water throughout the duration of the experiment, and 52 mice in the remaining 13 cages were randomly assigned to be exposed mice, receiving tritiated water with a concentration of approximately 8,200 pCi/mL for two weeks. After the two-week exposure, the control mice, along with 7 randomly picked exposed mice, referred to as "time 0" mice, were sacrificed. The whole body fluid tritium concentration of Time 0 mice represent the equilibrium of the mice with the HTO used for exposure. The 45 mice remaining were subsequently maintained on tap water. At 9 different time intervals, a group of 5 randomly chosen mice were sacrificed. The time intervals at which the mice were sacrificed were: 6 hours, 12 hours, 18 hours, 24 hours, 30 hours, 36 hours, 48 hours, 72 hours, and 96 hours (Figure 3-1). All mice were sacrificed using cervical dislocation.

Positive Control Methods

Twelve male BALB/C inbred mice age 39-49 days, weighing between 19-21 g, were purchased from Charles River Laboratories (Wilmington, MA) and acclimated in the laboratory environment for one week prior to the beginning of the exposure period. All husbandry standards used were the same as mentioned in above experiment. After acclimation, four mice

were immediately sacrificed and used as a control, referred to as control-2. The remaining eight mice were irradiated using a Torrex model 150D cabinet X-ray unit (EG&G Astrophysics Corporation, Long Beach, CA) for a total dose of 5 Gy. Mice were irradiated by placing four mice in a 2 liter beaker, which was then placed in the center of the cabinet floor, approximately 18 inches from the source. After exposure, mice were sacrificed four hours post-irradiation using CO_2 gassing.

Enzyme Analysis

Prior to analysis, whole body mouse weight was recorded, followed by dissection. A portion of the liver, the median lobe, was removed and placed in a 10 % dimethylsulfoxide solution, prepared in phosphate buffered saline (PBS), before cryopreservation at -70 ° C. Before analysis, the samples were removed from the freezer and immediately thawed in a 37 ° C water-bath until all ice crystals disappeared. Once thawed, the liver sample was rinsed three times in PBS solution and ~ 50 mg of the liver tissue was weighed and recorded for use in the enzyme assays. The liver was homogenized gently using a glass mortar and pestle. Total mg protein in homogenate was determined using the Bradford protein assay, a colorimetric technique which employs Coomassie Blue, a protein-binding dye, along with bovine serum albumin for a standard (Bradford 1976).

For enzyme analyses, the homogenate was then transferred to a 2 mL centrifuge tube and centrifuged at 600 g for 10 minutes. A portion of the resulting supernatant was removed for use in the CAT assay. The remaining slurry was centrifuged further at 9,000 g for use in the GPX and SOD assays. The analyses for all three enzymes were performed using commercially available kits from Calbiochem, Inc. (San Diego, CA) using a Spectra Max Plus spectrophotometric platereader (Molecular Devices; Sunnyvale, CA).

Catalase:

CAT was measured in a two-step colorimetric procedure, based on research by Aebi (1984). The homogenized liver samples and diluted standards were allowed to react with a known concentration of H_2O_2 substrate for one minute, after which sodium azide was added to the sample mixture to terminate the reaction. Once the reaction has been stopped, the concentration of remaining H_2O_2 substrate was measured colorimetrically at 520 nm by the addition of a solution containing a quinoneimine dye, chromogen, and a catalyst, horseradish peroxidase. The activity of a sample was calculated using the calibration curve generated by dilution of a 160 U standard, where U was defined as μmol H_2O_2 consumed per minute per mg protein (Greenwald 1985).

Glutathione Peroxidase:

GPX was measured indirectly based upon the NADPH-dependent conversion of GSSG to GSH (seen in Reaction 2). This procedure is a modification of the method described by Paglia and Valentine (1967), in which the rate of the oxidation of NADPH to $NADPH^+$ was calculated and used to moinitor GPX activity (Paglia and Valentine 1967). This procedure does not consist of a calibration curve; GPX activity was determined based upon the kinetic rate of reaction. In the method, the organic peroxide, tert-butyl hydroperoxide was allowed to react with a mixture of the homogenized liver sample and NADPH reagent. The absorbance of this solution was recorded at 340 nm for 3 minutes to determine the slope of the line, or the reaction rate. This value was used to calculate GPX activity units, which is defined as nmol NADPH oxidized per minute per mg protein (Greenwald 1985).

Superoxide Dismutase:

Both Mn-SOD and ZnCu-SOD are measured in the assay based on Nebot (1993). SOD is measured based upon the alkaline autoxidation of a chromogenic reagent, 5,6,6a,11b-tetrahydro-3,9,10-trihydroxybenzo[c]fluorine, in the presence of SOD, into a chromophore, which is spectrophotometrically visible at 525 nm. The appearance of chromophore in the reaction mixture is measured over a period of 1 minute to determine the slope of the line, the reaction rate. The reaction rate is then used to calculate SOD activity units, which was defined as SOD-525 units per mg protein (Nebot et al. 1993).

Statistical Analysis

All statistical analyses were performed using SAS (version 9.0; SAS Institute). CAT, GPX, and SOD activities were tested for normality using the Shapiro-Wilk statistic (PROC UNIVARIATE); GPX activities were log-transformed for normality. Analysis of variance models (ANOVA; PROC GLM), along with the Tukey post-ANOVA test, were used to examine significant differences between enzyme activities and treatment groups. For all tested models, F-statistics were used to provide an estimate of the predictive value of the model. All statistical tests were considered significant at the $p \leq 0.05$ level.

Results

Enzyme activities were measured from livers of control, positive control, Time 0, Time 24 and Time 96 mice. A summary of CAT, GPX, and SOD activities for the various treatment groups are seen in Table 4-1. Average CAT activities for control, control-2, positive control, Time 0, Time 24 and Time 96 mice were 24.07, 27.79, 80.07, 19.79, 22.43, and 18.66 μmol H_2O_2 consumed per minute per mg protein, respectively (Figure 4-1). Average GPX activities for control, control-2, positive control, Time 0, Time 24 and Time 96 mice were 816.80,

1159.11, 2241.45, 932.79, 1224.34, and 1013.41 nmol NADPH oxidized per minute per mg protein, respectively (Figure 4-2). Average SOD activities for control, control-2, positive control, Time 0, Time 24 and Time 96 mice were 96.98, 95.41, 71.30, 87.84, 93.43, and 75.62 SOD-525 units per mg protein, respectively (Figure 4-3).

There were no significant differences between control and control-2 groups for CAT, GPX, or SOD activities; therefore, these two groups were combined for statistical analyses. For CAT activities, there were no significant differences between control, Time 0, Time 24 and Time 96 treatment groups. However, there was a significant difference between each of these groups and the positive control group ($p < 0.0001$; $F = 82.24$), which is seen in Figure 4-1. There were also no significant differences between control, Time 0, Time 24 and Time 96 treatment groups for GPX activities. Although, each of these groups were significantly different from the positive control group ($p < 0.0001$; $F = 13.88$), illustrated in Figure 4-2. SOD activities for Time 0, Time 24 and Time 96 treatment groups were not significantly different from the positive control group or the control group. However, SOD activities in the positive control group were significantly lower than the control group ($p = 0.0380$; $F = 2.86$), seen in Figure 4-3.

Discussion

Results from this study found significant differences in CAT, GPX and SOD activities between control and positive control mice. There were no significant differences in CAT, GPX and SOD activities between control mice and mice exposed to tritiated water, even 24 and 96 hours after exposure ended.

Ionizing radiation has been shown to induce oxidative stress through the formation of ROS, following exposure to harmful chemicals or ionizing radiation. Antioxidants, such as CAT, GPX and SOD, are the first line of defense against oxidative stress, and with increased

ROS, the transcriptional regulators increases production of these enzymes (Harris 1992, Anderson 1996). In this study, these enzymes were used as biomarkers of exposure to two forms of ionizing radiation: β-radiation and x-ray radiation. Enzyme activities were measured in liver tissue based on results from previous laboratory studies that found higher activities of antioxidant enzymes within the liver of rodents (Perez-Campo et al. 1993, Xia et al. 1995).

Several studies have used rodents in laboratory exposures to various forms of radiation, mostly x-rays and δ-rays, in order to evaluate antioxidant enzyme response. Studies have shown increases in antioxidant enzyme activities, even with exposure to low doses (Krizala et al. 1982, Yamaoka et al. 1998). On the other hand, similar studies have also shown decreases in antioxidant activities with exposure (Lipecka et al. 1978, Petkau 1978). Although inconclusive, any imbalance in the equilibrium that exists between ROS and antioxidant activities can result in oxidative stress, which has been shown to be detectable from cellular responses (Hollan 1995).

To assess the sensitivity of the enzyme assays used in this experiment, a positive control experiment was performed involving x-ray radiation. Yamaoka (1998) found low-level x-ray radiation exposure (20 cGy) in mice to induce oxidative stress in rodents by raising SOD and GPX levels, with the maximum activity occurring four hours post-irradiation (Yamaoka et al. 1998). To ensure the induction of oxidative stress, the positive controls in this experiment were irradiated at a much higher level, 5 Gy, and were sacrificed four hours post-irradiation. The results confirmed that control-2 group, from the positive control experiment, was not significantly different from the control group in the tritium experiment for CAT, GPX, or SOD. In comparison to the combined control group, the positive control group CAT and GPX activities were significantly higher (Figures 4-1 and 4-2) and the SOD activity was significantly lower (Figure 4-3).

Increased activities of CAT and GPX in the positive control group are expected results based upon findings in previous studies of increased antioxidant activities, and therefore oxidative stress induction, with exposure to ionizing radiation (Hardmeier et al. 1997, Yamaoka et al. 1998). The decrease in SOD activities in the positive control group has also been shown to occur with exposure to radiation (Lipecka et al. 1978, Petkau 1978). Overall, the positive control mice had increases in two oxidative stress inducible enzymes, CAT and GPX, and a decrease in another oxidative stress inducible enzyme, SOD.

CAT and GPX are both responsible for detoxifying peroxides within the cell (Aebi 1984, Harris 1992, Anderson 1996, Gate et al. 1999). SOD is responsible for the detoxification of superoxide radical, which produces peroxide in the process (Krizala et al. 1982, Harris 1992, Gate et al. 1999, Klaassen 2001). As seen in Figure 4-4, superoxide anion is produced from the ionizing radiation, which is converted by SOD into peroxide, with completion of detoxification finished by CAT and GPX (Anderson 1996, Klaassen 2001). The decline in activity of SOD may be due to increased degradation of SOD within the cell or decreased availability of necessary cofactors (Harris 1992). Increases in activities for antioxidant enzymes responsible for peroxide detoxification, CAT and GPX, suggest that peroxides being generated from the irradiation, in addition to SOD detoxification, are increasing the transcription of the enzymes (Harris 1992, Hardmeier et al. 1997). The use of x-ray irradiation was successful as a positive control to test the sensitivity of the enzyme assays.

In this study, the inducible antioxidant enzymes CAT, GPX and SOD were used to evaluate oxidative stress in mice with low-level exposure to tritium in the form of tritiated water. In the past, tritium has been studied in rodents to evaluate endpoints ranging from chromosome

mutations (Brooks et al. 1976, Carsten and Commerford 1976) and development effects (Gao et al. 2002), to fatality from tumor development (Yamamoto et al. 1995). These previous studies used exposure concentrations of tritium orders of magnitude higher than those that are often experienced at moderately contaminated hazardous waste sites.

Results from this study indicate that CAT and GPX activity levels in mice exposed to low-level tritiated water for a two-week period were not significantly different from control mice, even after exposure to the tritiated water had ceased for 24 and 96 hours (Figures 4-1 and 4-2). SOD activities in mice exposed to tritiated water were not significantly different from control mice or positive control mice. Although a significant trend was not found, mice sacrificed 96 hours after exposure to tritiated water had marginally lower SOD activities than control, Time 0, and Time 24 mice (Figure 4-3). This may suggest that even without direct exposure to tritiated water, concentrations of tritium found within mice may be able to induce an oxidative stress response before elimination has been completed. However, even though mice exposed to tritiated water were not different than positive control mice and because SOD activities in mice exposed to tritiated water were also not different from control mice, there is no evidence to support an oxidative stress response in mice exposed to tritiated water. Overall, based on CAT, GPX, and SOD activities, the results from this study indicate that exposure to low-level tritiated water does not induce an oxidative stress response in mice.

The failure of this study to induce an oxidative stress response may be due to an insufficiency of the concentration of tritiated water used in the exposure. Another explanation may involve the ability of mammalian cells to become adaptive to radiation (Wolff 1996). Previous studies by Oliveieri et al. (1984) found an adaptive response to x-ray exposure in cells that had previously been exposed to low levels of tritium; cells with tritium exposure had half as

many chromosome aberrations as those without tritium exposure (Oliveieri et al. 1984). Perhaps administration of tritium to mammalian cells may be beneficial at low-level exposures, with little risk of oxidative stress induction.

As a form of ionizing radiation, tritium may have been expected to cause oxidative stress, even at low-level exposures. This study found no significant differences between control mice and mice exposed to low-level tritiated water. Based on the results from this study, there is very little risk, from a biochemical standpoint, to rodent exposure to tritiated water at the tritium irrigation site.

Works Cited

Aebi H. Catalase in vitro. Methods in Enzymology 105: 121-126; 1984.

Ames BN. Endogenous DNA damage as related to cancer and aging. Mutation Research 214: 41-46; 1989.

Anderson D. Antioxidant defences against reactive oxygen species causing genetic and other damage. Mutation Research 350: 103-108; 1996.

Bindhumol V, Chitra KC, Mathur PP. Bisphenol A induces reactive oxygen species generation in the liver of male rates. Toxicology 188: 117-124; 2003.

Bradford M. A rapid and sensitive method for quantitation of microgram quantities of protein using the principle of protein-dye binding. Analytical Biochemistry 72: 248-254; 1976.

Breimer LH. Repair of DNA damage induced by reactive oxygen species. Free Radical Research Communications 14: 159-171; 1991.

Brooks AL, Carsten AL, Mead DK, Retherford JC, Crain CR. The effect of continuous intake of tritiated water (HTO) on the liver chromosomes of mice. Radiation Research 68: 480-489; 1976.

Carsten AL, Commerford SL. Dominant lethal mutations in mice resulting from chronic tritiated water (HTO) ingestion. Radiation Research 66: 609-614; 1976.

Cross CE, van der Vliet A, O'Neil CA, Eisenrich JP. Reactive oxygen species and the lung. Lancet 344: 930-932; 1994.

Gao W, Lu H, Dong J, Zhang W, Zhou X, Jenkins LW, Dixon CE. Postnatal growth, neurobehavoiral and neurophysiologic changes of prenatal low dose B-radiation from tritiated water in mice. Neurotoxicology and Teratology 24: 247-254; 2002.

Gate L, Paul J, Ba GN, Tew KD, Tapiero H. Oxidative stress induced in pathologies: The role of antioxidants. Biomedicine and Pharmacotherapy 53: 169-180; 1999.

Greenwald RA. CRC Handbook of Methods for Oxygen Radical Research. Boca Raton, FL: CRC Press, Inc.; 1985.

Haimovitz-Friedman A. Radiation-induced signal transduction and stress response. Radiation Research 150: S102-S108; 1998.

Hardmeier R, Hoeger H, Fang-Kircher S, Khoschsorus A, Lubec G. Transcription and activity of antioxidant enzymes after ionizing irradiation in radiation-resistant and radiation-sensitive mice. Proceedings of the National Academy of Sciences 94: 7572-7576; 1997.

Harris ED. Regulation of antioxidant enzymes. The FASEB Journal 6: 2675-2683; 1992.

Hollan S. Free radicals in health and disease. Haematologia 26: 177-189; 1995.

Hollowman KA, Dallas CE, Jagoe CH, Tackett R, Kind JA, Rollor EA. Interspecies differences in oxidative stress response and radiocesium concentrations in rodents inhabiting areas highly contaminated by Chernobyl nuclear disaster. Environmental Toxicology and Chemistry 19; 2000.

Kirkman HN, Galiano S, Gaetani GF. The function of catalase-bound NADPH. Journal of Biological Chemistry 262: 660-666; 1987.

Klaassen CD. Cassarett and Doull's Toxicology: The Basic Science of Poisons. New York, NY: McGraw-Hill; 2001.

Krizala J, Kovarova H, Stoklasova A, Ledvina M. Superoxide Dismutase levels in various radioresistant and radiosensitive tissues of irradiated rats. Physiologia Bohemoslovaca 31: 151-157; 1982.

Lipecka K, Lipinski S, Kanski M. The influence of gamma irradiation and cysteamine on superoxide dismutase activity in rabbit liver. Studies in Biophysics 68: 25-30; 1978.

Maiorino M, Chu FF, Ursini F, Doroshow K, Davies HJ, Esworthy RS. Phospholipid hydroperoxide glutathione peroxidase is the 18 kDa selenoprotein expressed in human tumor lines. Journal of Biological Chemistry 266: 7728-7732; 1991.

N.C.R.P. Tritium in the environment. Betheseda, MD: National Council on Radiation Protection and Measurements; 62; 1979.

N.C.R.P. A handbook of radioactivity measurements procedures. National Council on Radiation Protection and Measurements;58; 1985.

Nebot C, Moutet M, Huet P, Xu JZ, Yanden JC, Chaudiere J. Spectrophotometric assay of superoxide dismutase activity based on the activated autoxidation of a tetracyclic catechol. Analytical Biochemistry 214: 442-451; 1993.

Oliveieri G, Bodycote J, Wolff S. Adaptive response of human lymphocytes to low concentrations of radioactive thymidine. Science 223: 594-597; 1984.

Paglia DE, Valenitine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. Journal of Laboratory and Clinical Medicine 70: 158; 1967.

Perez-Campo R, Lopez-Torres M, Rojas C, Cadenas S, Barja G. A comparative study of free radicals in vertebrates - I. Antioxidant enzymes. Comparative Biochemistry and Physiology 105B: 749-755; 1993.

Petkau A. Radiation protection by superoxide dismutase. Photochemistry and Photobiology 28: 765-774; 1978.

Riley PA. Free radicals in biology: Oxidative stress and the effects of ionizing radiation. International Journal of Radiation Biology 65: 27-33; 1994.

Stadtman ER, Oliver CN. Metal-catalyzed oxidaton of proteins: Physiological consequences. Journal of Biological Chemistry 266: 159-171; 1991.

Sun J, Chen Y, Mingtao L, Zhongliang G. Role of antioxidant enzymes on ionizing radiation resistance. Free Radical Biology and Medicine 24: 586-593; 1998.

Witzum JL. The oxidative hypothesis of atherosclerosis. Lancet 344: 793-795; 1994.

Wolff S. Aspects of the adaptive response to very low doses of radiation and other agents. Mutation Research 358: 135-142; 1996.

Xia E, Rao G, van Remmen H, Heydari AR, Richardson A. Activities of antioxidant enzymes in various tissues of male Fischer 344 rats are altered by food restriction. The Journal of Nutrition 125: 195-201; 1995.

Yamamoto O, Seyama T, Jo T, Terato H, Saito T, Kinomura A. Oral administration of tritiated water (HTO) in mouse. II. Tumour development. International Journal of Radiation Biology 68: 47-54; 1995.

Yamaoka K, Kojima S, Takahashi M, Nomura T, Iriyama K. Change of glutathione peroxidase synthesis along with that of superoxide dismutase synthesis in mice spleens after low-dose X-ray irradiation. Biochimica et Biophysics Acta 1381: 265-270; 1998.

Table 4-1 – Catalase, superoxide dismutase, and glutathione peroxidase activities for mice in treatment groups.

Sample ID	Treatment	Cat*	SOD**	GPX***
7-1	Control1	27.886	100.216	784.46
7-2	Control1	21.253	102.645	1228.77
7-3	Control1	18.369	78.348	603.66
7-4	Control1	21.747	98.127	735.58
8-1	Control1	24.442	114.668	779.77
8-2	Control1	31.129	121.446	745.05
8-3	Control1	27.517	97.609	707.40
16-1	Control1	24.358	96.585	985.69
16-2	Control1	19.960	63.214	780.89
5-1	Time0	16.071	62.961	795.09
5-2	Time0	18.942	96.073	707.98
9-1	Time0	21.390	79.851	1081.84
10-1	Time0	14.269	55.643	469.04
10-2	Time0	25.518	118.704	896.51
13-1	Time0	22.165	104.769	1336.43
14-1	Time0	20.241	96.879	1242.65
1-2	Time24	19.346	60.859	1162.10
2-2	Time24	24.353	98.917	1256.45
6-3	Time24	18.552	88.193	965.41
11-1	Time24	26.233	122.312	1309.44
14-3	Time24	23.671	96.912	1428.32
3-4	Time96	21.009	80.097	713.91
11-4	Time96	9.804	40.863	481.96
15-2	Time96	18.769	80.672	1057.80
15-3	Time96	18.936	79.868	1272.60
15-4	Time96	24.810	96.579	1540.80
C01	Control2	21.384	90.393	1192.37
C02	Control2	32.151	68.023	1055.10
C03	Control2	27.112	113.095	1476.72
C04	Control2	31.946	108.14	959.027
C05	Control2	26.368	97.427	1112.33
X01	PosCon	67.647	73.529	2202.91
X02	PosCon	79.833	77.508	2653.58
X03	PosCon	64.157	77.074	2151.31
X04	PosCon	80.136	75.736	2393.40
X05	PosCon	110.577	88.218	2339.98
X06	PosCon	71.513	55.562	2059.02
X07	PosCon	72.194	41.762	1889.94
X08	PosCon	94.512	81.029	-

* μmol H_2O_2 consumed / minute*mg protein
** SOD-525 units / mg protein
*** nmol NADPH oxidized / minute*mg protein

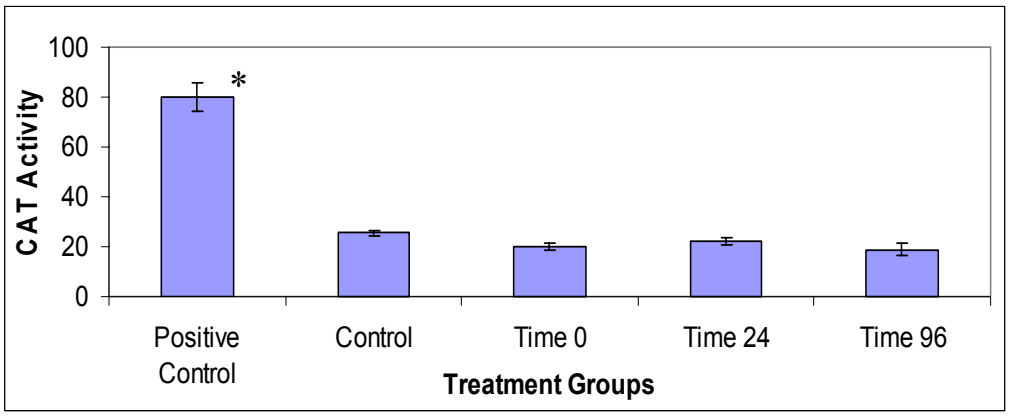

Figure 4-1 – Catalase activity (µmol H_2O_2 consumed / minute*mg protein) in the liver of exposed mice. There were no significant differences between control mice and mice exposed to low-level tritiated water, even 24 and 96 hours after exposure ceased. Mice exposed to x-ray radiation had significantly higher catalase activities than control mice and mice exposed to tritium (* denotes difference at $\alpha = 0.05$ level of significance).

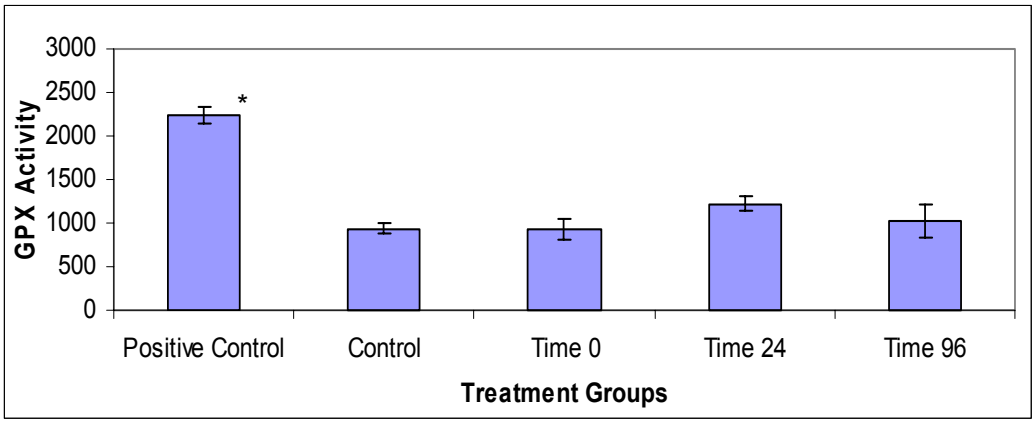

Figure 4-2 – Glutathione Peroxidase activity (nmol NADPH oxidized / minute*mg protein) in the liver of exposed mice. There were no significant differences between control mice and mice exposed to low-level tritiated water, even 24 and 96 hours after exposure ceased. Mice exposed to x-ray radiation had significantly higher glutathione peroxidase activities than control mice and mice exposed to tritium (* denotes difference at $\alpha = 0.05$ level of significance).

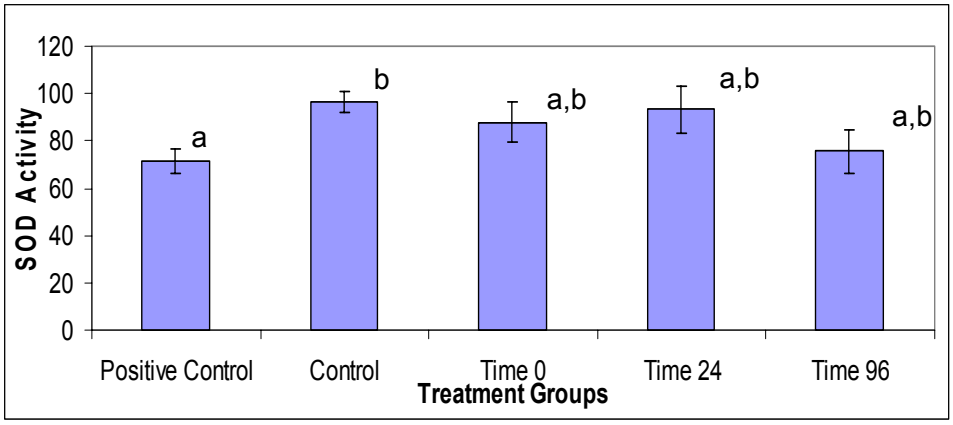

Figure 4-3 – Superoxide dismutase activity (SOD-525 units / mg protein) in the liver of exposed mice. Treatment groups with similar letters are not significantly different at the $\alpha = 0.05$ level of significance. Superoxide dismutase activities in mice exposed to x-ray radiation were significantly lower than control mice.

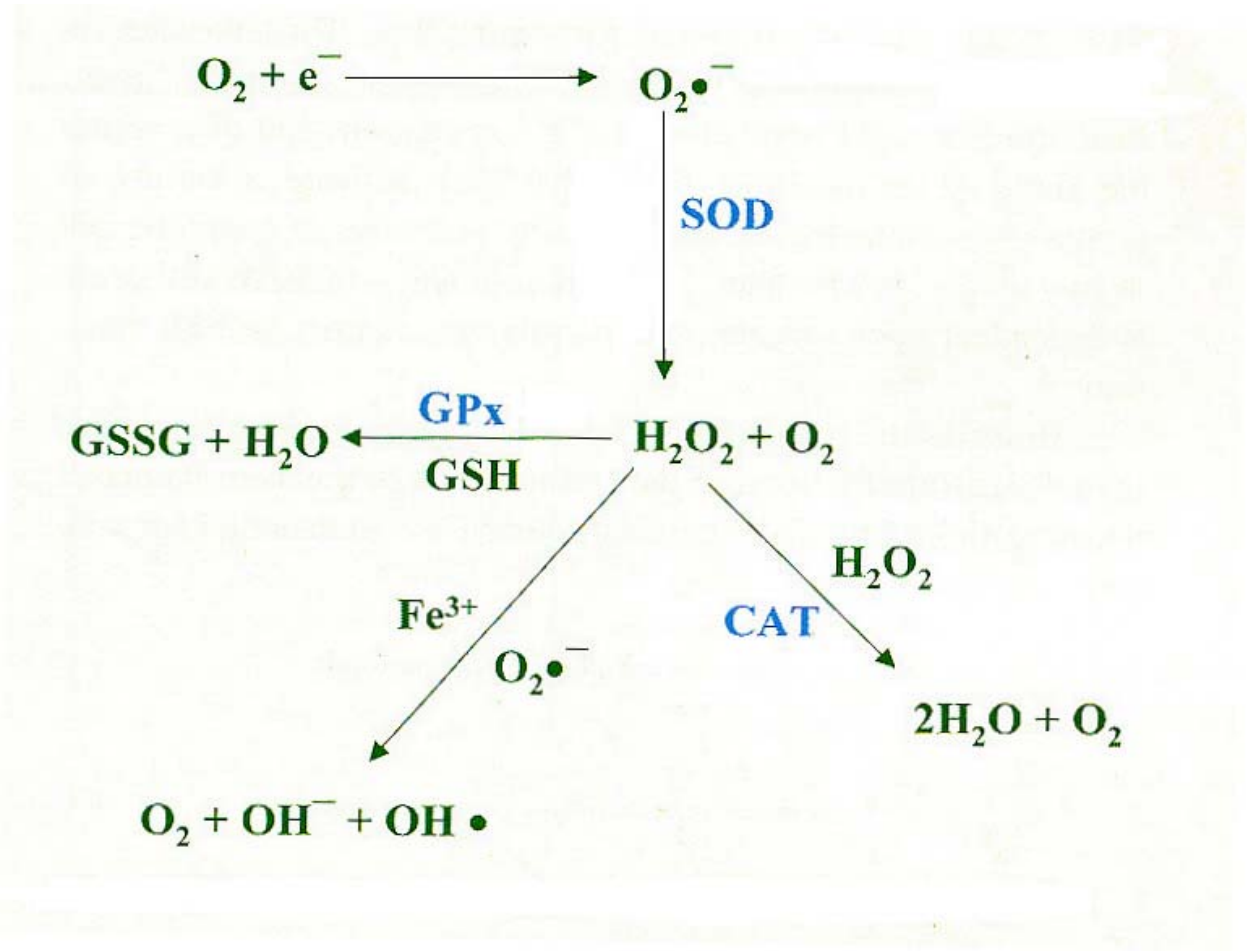

Figure 4-4 – Schematic representation of ROS formation and detoxification by antioxidant systems, diagram modified from (Klaassen 2001).

CHAPTER V

CONCLUSION

Tritium has become a contaminant of concern at nuclear production facilities, such as the Savannah River Site (SRS). In order to reduce the environmental release of tritiated waste to surrounding surface waters, new disposal methods are being employed on SRS, including the irrigation of a forest with tritiated water. Although this disposal method has proven to be an effective at reducing tritium release into SRS streams, the irrigation site is exposing vegetation and terrestrial organisms to tritiated water. As a form of ionizing radiation, exposure to tritium has the potential to cause cellular damage by inducing exposed organisms into oxidative stress. The purpose of this study was to evaluate rodent exposure in the tritium irrigation site, determine the biological half-life of tritium in mice in an ecologically relevant manner, and examine biochemical indicators of stress in mice that may be altered with exposure to tritiated water.

The field portion of this study examined tritium dynamics within rodents living within the tritium irrigation site. Detectable concentrations of tritium were found in almost all mice captured at the irrigation site. Relationships between whole body tritium concentrations in cotton mice (*Peromyscus gossypinus)* were correlated with tritium application records and local meteorological conditions for the site. In addition, based upon the capture location, dose estimates for individual mice were calculated based on irrigation and precipitation records. Volume of tritium applied and dose estimates within the plot captured were found to have significant, positive, linear correlations with whole body tritium concentrations of mice. Significant, negative linear correlations were found between whole body tritium concentrations

of mice and the volume of precipitation within the plot captured. Thus it can be concluded that if tritium is made available in the terrestrial environment, the biota that live within that environment will experience a quantifiable exposure, with significant influence from the precipitation within the tritium irrigation site.

The laboratory portion of this study examined the biological half-life of tritium and biochemical endpoints in mice with low-level, oral exposure to tritiated water. This study was designed to be as ecologically relevant as possible, based on the conditions at the tritium irrigation site. The mice were administered tritium in drinking water over a period of two weeks, allowing enough time for equilibration to occur between the mice and the tritiated water used in the exposure, which was reflective of concentrations found at the tritium irrigation site. Although mice at the field site will never be in complete equilibrium with the tritium in the environment due to the constant changes in precipitation and variation in the applied tritium, establishing a set starting point is required to accurately determine the biological half-life of tritium in mice.

The biological half-life of tritium in mice was determined to be 2.26 days, which is approximately double the value of previous estimates of 1.13 days (Thompson 1952, Richmond et al. 1962). A possible explanation for this difference may be a combination of the route of exposure, intraperitoneal injection, and experimental methods used in the previous mouse studies. Although this route of administration is appropriate under toxicological circumstances, it holds very little ecological relevance. Since the volume of tritium used in the dose was small in comparison to that of total body water and because tritiated water is excreted quite readily, much of the tritium used in these intraperitoneal doses could have been excreted before distribution throughout the body. This may explain the underestimation in predictions of

previous studies regarding the biological half-life of tritium (Feindendegen 1967). Based on the biological half-life estimation from this study and the concentrations seen in mice from the field study, a mouse migrating from the tritium irrigation site would have a whole body tritium concentration slightly above background levels within the period of a month after removal of the source.

To examine the possibility of biochemical effects in mice exposed to low-level tritiated water, the activities of antioxidant enzymes, catalase, glutathione peroxidase, and superoxide dismutase, were measured in exposed mice and compared to control mice. Tritium, as a form of ionizing radiation, may cause damage in biological tissue through a mechanism involving highly reactive oxygen species (ROS). Within the cell, defense mechanisms involving antioxidants have evolved to protect from ROS (Harris 1992, Anderson 1996, Sun et al. 1998). Any imbalance in the equilibrium between ROS and antioxidant levels may result in oxidative stress (Hollan 1995, Klaassen 2001). In the laboratory exposure, there were no significant differences between catalase, glutathione peroxidase, and superoxide dismutase activities in control mice and mice exposed to low-level tritiated water. The failure of this study to induce an oxidative stress response may be due to an insufficiency of the concentration of tritiated water used in the exposure or the ability of mammalian cells to become adaptive to radiation (Wolff 1996).

The conclusions from both the field and laboratory studies are: 1) with exposure, mice incorporated quantifiable concentrations of tritium within their bodies; 2) the biological half-life of tritium was found to be approximately 2.26 days; and 3) there was no indication of oxidative stress in mice with low-level exposure to tritiated water, based on catalase, glutathione peroxidase, and superoxide dismutase activities. This study has led to a better understanding of tritiated water in an ecologically relevant setting and has allowed for a better assessment of the

tritium irrigation site. Based on the results from this study, the land application of tritiated water at tritium irrigation site is an effective and low-risk method of disposal.

Works Cited

Anderson D. Antioxidant defences against reactive oxygen species causing genetic and other damage. Mutation Research 350: 103-108; 1996.

Feindendegen LE. Tritium-Labeled Molecules in Biology and Medicine. New York, NY: Academic Press; 1967.

Harris ED. Regulation of antioxidant enzymes. The FASEB Journal 6: 2675-2683; 1992.

Hollan S. Free radicals in health and disease. Haematologia 26: 177-189; 1995.

Klaassen CD. Cassarett and Doull's Toxicology: The Basic Science of Poisons. New York, NY: McGraw-Hill; 2001.

Richmond CR, Langham WH, Trujillo TT. Comparative metabolism of tritiated water by mammals. Journal of Comparitative Physiology 59: 45-53; 1962.

Sun J, Chen Y, Mingtao L, Zhongliang G. Role of antioxidant enzymes on ionizing radiation resistance. Free Radical Biology and Medicine 24: 586-593; 1998.

Thompson RC. Studies of metabolic turnover with tritium as a tracer. I. Gross studies with the mouse. Journal of Biological Chemistry 197: 81-87; 1952.

Wolff S. Aspects of the adaptive response to very low doses of radiation and other agents. Mutation Research 358: 135-142; 1996.