AN ABSTRACT OF THE THESIS OF

Hillary Kate Marlane Haskins for the degree of Master of Science in Radiation Health Physics presented on December 16, 2013.
Title: Dose Response of Zebrafish Exposed to Gamma Radiation

Abstract approved:

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Jack F. Higginbotham

Space radiation biology is an emerging field of research with a focus on improving the health and safety of astronauts in low-Earth orbit (LEO) and deep-space missions. Eye health is especially important to astronaut functionality. Recent studies have found an increased risk of cataract formation in astronauts with higher lens doses (> 8 mSv) of space radiation relative to astronauts with lower lens doses (< 8 mSv). In order to better understand cataract formation from ionizing radiation zebrafish embryos were exposed to various levels of γ-rays. Zebrafish embryos were exposed at 24 hour post fertilization (hpf) and examined shortly after irradiation and 120 hpf. At 120 hpf, mortality increased to over 80% at doses ≥ 30 Gy. Morbidity increased to over 75% at ≥ 15 Gy. New zebrafish embryos were then exposed to various levels of ionizing radiation at 4 days post fertilization (dpf) and showed no discernable differences in morbidity and mortality one day after irradiation. The next phase of research will expose adult zebrafish to Iodine-128 to examine the differences in damage from γ-ray exposure and β-particle exposure.

Dose Response of Zebrafish Exposed to Gamma Radiation

by
Hillary Kate Marlane Haskins

A THESIS

Submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

Presented December 16, 2013
Commencement June 2014

Master of Science thesis of Hillary Kate Marlane Haskins presented on December 16, 2013

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Hillary Kate Marlane Haskins, Author

ACKNOWLEDGEMENTS

Many people have helped me with this project and are deserving of thanks. First I would like to thank my advisor Dr. Jack Higginbotham for all of his guidance and giving me the opportunity to work on such an exciting project. I would also like to thank Dr. Kathryn Higley for all of her support and reassurances over the past three years. I would like to thank Dr. Adam Branscum for inspiring to make my love for statistics more than just a hobby. Finally, I would like thank Dr. Russell Turner for agreeing to be on my committee.

I would also like to thank the Oregon NASA Space Grant for funding my project. The SARL team, Dr. Robert Tanguay, Dr. Lisa Truong, and Chapell Miller, deserve a great deal of gratitude for all of their hours spent looking at zebrafish embryos under electron microscopes. Also a huge thanks to the Radiation Center staff, Dr. Scott Menn and Jim Darrough for their assistance with the Gammacell 220.

Finally I would like to thank my friends and family for their support and encouragement. Even though they are far away, my family has loved and encouraged me during my academic career. Thanks to Emily, Michelle, Jesse, Alison, and many others for their camaraderie and burning the midnight oil to finish lab reports. Last but not least I would like to thank Brian Bickford who has helped me stay sane during my trials and tribble-ations throughout graduate school.

TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

Page

LIST OF APPENDICES

Appendix Page

LIST OF FIGURES

LIST OF FIGURES (Continued)

LIST OF TABLES

LIST OF TABLES (Continued)

Chapter 1 Introduction

Exposure to galactic and solar radiation is an area of concern for human space travel. The various effects of space radiation exposure on humans are currently a heavily researched area for space programs across the globe. Hematopoietic, gastrointestinal, and neurovascular syndromes are well known acute radiation effects (Hall & Giaccia, 2006). Latent effects, such as cataracts, cancer, and genetic defects can be more difficult to discern the event of origin but are associated with radiation exposure (Hall & Giaccia, 2006). The research in this investigation was motivated by the increased risk of cataract formation in astronauts with higher lens doses of galactic cosmic radiation relative to astronauts with lower lens doses (Cucinotta, et al., 2001).

Dose and biological consequences vary with energy and particle type. Ionizing radiation can be classified into two categories, electromagnetic and particulate radiations. Electromagnetic radiation comes in the form of x- and γ-rays. The rays are streams of photons, or "packets" of energy. When an x- or γ- ray pass through living tissues, the packet of energy may be deposited into the cell. Particulate radiation may occur naturally or experimentally. The massive particles include electrons, protons, α-particles, neutrons, and heavy charged particles (HCP). Heavy charged particles are nuclei of elements that have been stripped of their electrons. In space, the HCP can have extremely high energy levels and are considered a major hazard of space travel. The lunar landing astronauts saw flashes of light when they were in dark spaces with their eyes closed. The flashes were caused by high-energy iron ions crossing the retina (Hall & Giaccia, 2006). The space radiation environment is composed of galactic cosmic rays (GCR), trapped protons and electrons in the magnetosphere, and solar-particle events (SPE).

Radiation may be either directly or indirectly ionizing caused by particulate and electromagnetic radiations respectively. Direct ionization occurs when a particle has enough kinetic energy to disrupt the atomic structure of the tissue it is passing through thereby causing chemical or biological changes. Indirect ionization occurs when an x- or γ-rays energy is absorbed into tissue which then produces a fast-moving charged particle that may cause damage (Hall & Giaccia, 2006). Figure 1.1 is the generally accepted sequence of events from the absorption of radiation to the expression of various forms of biological damage.

Cataract formation is a well-known effect of ionizing radiation exposure. It was once considered as a "biological dosimeter" since pathology is non-invasive and can be easily identified. Most human radiation induced cataract data is based on the work of Merriam and Focht (NCRP, 1999) in which they studied time-dose relationships (Table 1.1). They helped establish a lens dose limit of 150 mSv per year for a radiation worker. They did not consider what affect a low-dose, continuous exposure would have on the lens of the eye.

Other data on radiation formed cataracts come from atomic-bomb survivors (ABS). A recent study examined the incidence on clinically significant cataracts with lens radiation doses from 0-3 Gy on 6066 ABS who had cataract surgery between 1986 and 2005. They found vision-impairing cataracts at dose levels less than 1 Gy (Neriishi, et al., 2012).

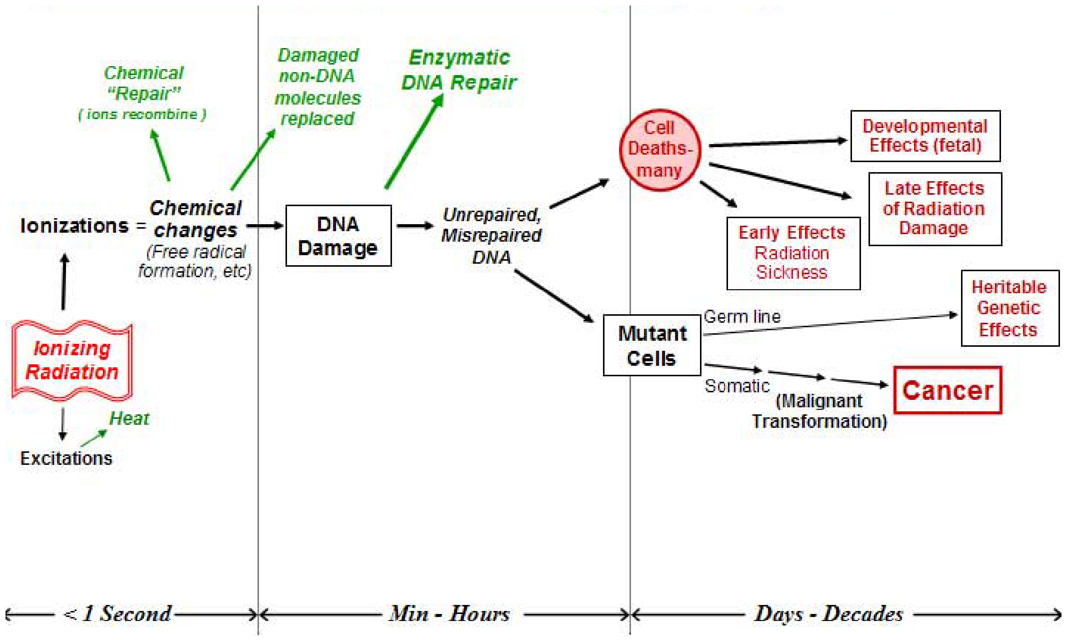


Figure 1.1 Classic paradigm of radiation injury (US DOE, 2013)

Table 1.1 Merriam and Focht cataract time-dose relationships from 1957 and 1962 (NCRP, 1999)

Duration of Treatment	Minimum cataractogenic dose (Gy)	Maximum non-cataractogenic dose (Gy)
Single	2.0	2.0
3 weeks to 3 months	4.0	10.0
Over 3 months	5.5	10.5

These data have resulted in the international community setting more restrictive limits on the irradiation of the lens of the eye relative to other organs. Both the National Council on Radiation Protection (NCRP) and the Nuclear Regulatory Commission (NRC) recommend a 150 mSv per year averaged over five years equivalent dose limit (NCRP, 1999) (ICRP, 2007). This is much lower than the equivalent dose limit of 500 mSv per year to the skin or an individual organ.

In 2011, The International Commission on Radiation Protection (ICRP) released a statement which lowered the threshold absorbed dose of 0.5 Gy to the lens of the eye (ICRP, 2011). The ICRP also lowered the recommended occupational "equivalent dose limit for the lens of the eye of 20 mSv in a year, averaged over five years, with no single year exceeding 50 mSv."

The intent of such limits is to provide assurance to individuals who are exposed to a radiation source so that they will suffer little to no biological consequences from that exposure. One group of workers who are exposed to environmental radiation hazards not considered when such limits were established are astronauts and cosmonauts who traveled outside the region known as near Earth orbits (NEO).

The Longitudinal Study of Astronaut Health (LSAH) began in 1992 and is an investigation into the incidence of acute and chronic morbidity and mortality of astronauts. Dr. Cucinotta's study, "Space Radiation and Cataracts in Astronauts," found an increased risk of cataract formation in astronauts with higher lens doses (> 8 mSv) of space radiation relative to astronauts with lower lens doses (< 8 mSv). The study included a total of 295 astronauts with a range of exposures to the lens of 0.2 – 91.0 mSv.

The two largest exposures include Skylab and the NASA-Mir space station with 87.0 and 91.0 mSv respectively (Cucinotta, et al., 2001).

In order to better understand the phenomenon of cataract formation a stepwise approach was used to examine the occurrence in zebrafish when exposed to various radiation types and energies. Stage one involved exposing zebrafish embryos to varying levels of gamma radiation in order to determine a dose response. Stage two will expose the fish to a beta and gamma emitter to discern a difference between particle types. Stage three will then move the fish to Brookhaven National Laboratory (BNL) where they can be exposed to heavy ion particles in the Relativistic Heavy Ion Collider (RHIC).

Chapter 2 Literature Review

Dose and Relative Biological Effectiveness

Absorbed dose is defined as the mean energy absorbed by an object or a person per unit mass. Its standard unit is the Gray (Gy) which is 1 Joule/kg. Biological dose equivalent is a measure of the biological damage to living tissue and is a product of the absorbed dose and a radiation weighting factor (W_R) which can be seen in Table 2.1. Its standard unit is the Sievert (Sv) which is also 1 Joule/kg. Relative biological effectiveness (RBE) is the ratio of biological effectiveness of ionizing radiation relative to another given the same amount of absorbed energy (Shultis & Faw, 2000).

When ionizing radiation passes through tissue the neighboring atoms may become excited or ionized. This in turn may damage the cell by altering the cell's DNA (deoxyribonucleic acid). The biological effect of radiation is not directly proportional to the energy deposited by radiation in an organism. It depends how the energy is deposited along the radiation path which is dependent upon radiation type and energy of the particle. Linear energy transfer (LET) is defined as the mean energy deposited per unit path length in the absorbing material (keV/μm). Therefore, for the same absorbed dose, the biological effect from high LET radiation such as HCP and α-particles is much greater than that of low LET radiation such as x- or γ-rays (Hall & Giaccia, 2006).

Table 2.1 Radiation weighting factors from ICRP (1991) and NCRP (1993) (Shultis & Faw, 2000)

Radiation type	Radiation weighting factor, W_R
X- and γ-rays of all energies	1
Electrons and muons of all energies	1
Protons (> 1 MeV, other than recoil)	5 – ICRP, 2 – NCRP
Alpha particles, fission fragments, heavy nuclei	20
Neutrons	Continuous function of neutron energy
<10 keV	5
10-100 keV	10
100 keV – 2 MeV	20
2 – 20 MeV	10
> 20 MeV	5

The two categories of radiation induced injury are deterministic and stochastic effects. Deterministic effects are generally associated with large absorbed doses on which severity is dependent on the dose and are characterized by a threshold. Examples include but are not limited to cataracts, skin erythema, and infertility. Stochastic effects have no threshold, severity is independent of dose, and the probability of occurrence is proportional to the absorbed dose. Examples of stochastic effects are caner and genetic effects (Hall & Giaccia, 2006).

Recently the ICRP recommended changing radiation effect categories to cancer and non-cancer effects. "These effects, previously called deterministic effects, are now referred to as tissue reactions because it is increasingly recognized that some of these effects are not determined solely at the time of irradiation but can be modified after radiation exposure (ICRP, 2011)."

Table 2.2 Comparison of 10CFR20 and ICRP 103 radiological limits for a radiation worker (ICRP, 2007), (NRC, 2013)

	10CFR20	**ICRP**
Total effective dose equivalent	50 mSv	50 mSv
Sum of the deep-dose equivalent and committed dose equivalent	500 mSv	500 mSv
Lens dose equivalent	150 mSv	20 mSv
Shallow dose equivalent	500 mSv	500 mSv

Space Radiation

Galactic cosmic rays originate outside of the Solar System and may have energies up to 10^{20} eV. The particles are 98% baryons and 2% electrons. The energetic heavier nuclei are termed HZE particles (high charge Z and high energy E). Galactic cosmic rays are over 80% of the effective dose to the International Space Station (ISS) crew due to their high penetration power to deep organs and large quality factors (Durante & Cucinotta, Physical Basis of Radiation Protection In Space Travel, 2011).

A solar-particle event (SPE) is an infrequent event that occurs when the surface of the Sun releases a large amount of energy in a sudden local outburst of hard (> 10 keV) and soft x-rays. The proposed method of radiation protection would be an accurate SPE prediction model and a "storm shelter" with specialized shielding for the astronauts. Trapped particle radiation refers to the protons and electrons trapped in the Van Allen belts (Figure 2.1) and contribute a significant dose to astronauts in low-Earth orbit (LEO). Trapped particles originate from the interaction of GCR and solar particles with the Earth's magnetosphere and atmosphere. Electrons may reach energies up to 7 MeV and protons up to 600 MeV (Durante & Cucinotta, Physical Basis of Radiation Protection In Space Travel, 2011).

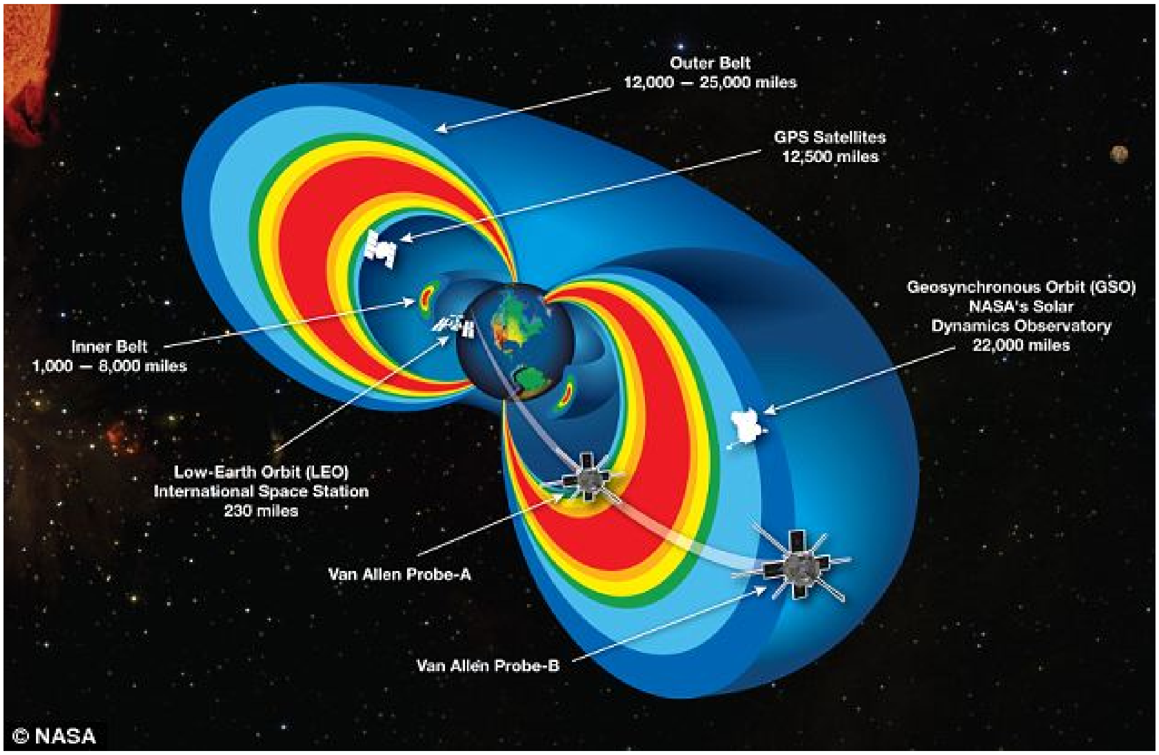


Figure 2.1 A cutaway model of the Earth's radiation belts (NASA, 2013)

The Eye

Anatomy

The eye is an important organ to consider when regarding risk from ionizing radiation. Its location, functional importance, and the difference in tissue sensitivity merits special consideration (NCRP, 1999). Figure 2.2 shows the basic structure of the human eye. The eye is composed of three layers: the sclera, the choroid, and the retina. The sclera is the white, tough outer layer that protects the eye. It becomes transparent at the front of the eye forming the cornea. The choroid is the middle layer and contains the network of blood vessels that oxygenate and nourish the eye. The innermost layer is the retina which contains light sensing cells. (Martini, 2006).

The lens of the eye is located behind the iris and pupil. It is a crystalline structure with no blood vessels which focuses light rays onto the retina. It has specialized cells called lens fiber which have lost all of their nuclei and other organelles. They are slender and elongated, filled with transparent proteins called crystallins which in turn are responsible for clarity and focusing power of the lens (Martini, 2006).

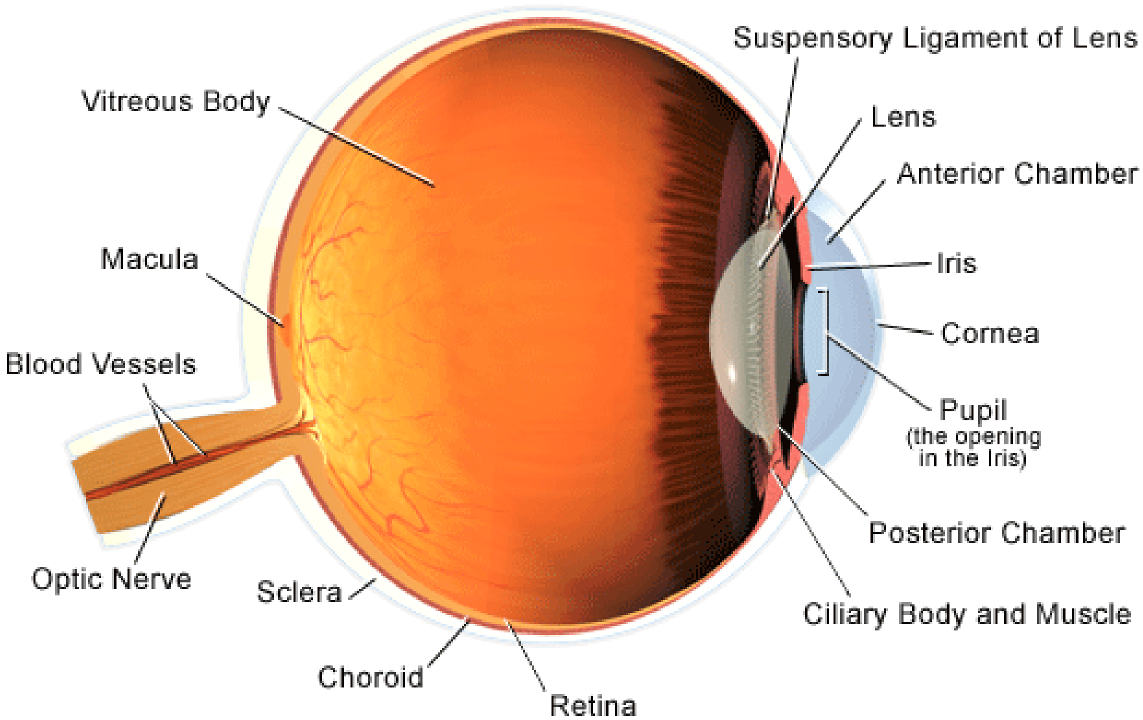


Figure 2.2 Cross section of human eye with major parts (URMC, 2013)

Cataract

A cataract is any cloudiness or opacity in the lens of the eye. It is the leading cause of blindness worldwide. Lens opacities can be found in 96% of the population over 60-years-old. The only treatment for cataract is surgical removal (Kleiman, 2012).

The World Health Organization (WHO) has developed a simplified cataract grading system in order to categorize the most common forms of cataract including severity:

- "Nuclear cataract (NUC), leading to a gradual opacification of the nucleus of the lens;
- Cortical cataract (COR), involving the cortex from the periphery towards more central opacification, often with typical, wedge-shaped 'spokes';
- Posterior subcapsular cataract (PSC), giving rise to usually distinct opacities centrally or paracentrally on the posterior capsule" (Thylefores, et al., 2002).

The clinical appearance of the radiation cataract is different from most age-related cataract formation. "Lens changes documented by a dilated slit-lamp examination include an initial formation of an opalescent sheen on the posterior lens capsule with an appearance of small vacuoles and diffuse punctuate opacities centered on the posterior lens suture" (Kleiman, 2012). The ICRP (2012) recently reported: "The precise mechanism of radiation cataractogenisis is not known, but genomic damage resulting in altered cell division, transcripted and/or abnormal lens fiber cell differentiation is considered to be the salient injury … heterozygosity for genes involved in cell cycle checkpoint control, DNA damage recognition, or DNA repair might also contribute to this phenomenon."

Figure 2.3 shows ionizing radiation striking the front of the eye damaging the epithelium cells which migrate to the rear of the eye gathering at the center. Eventually there will be enough cells covering the posterior pole blocking light from passing directly through forming a PSC. The epithelial cells are at the most risk from ionization since they are dividing cells (Radiation Effects Reseach Foundation, 2007).

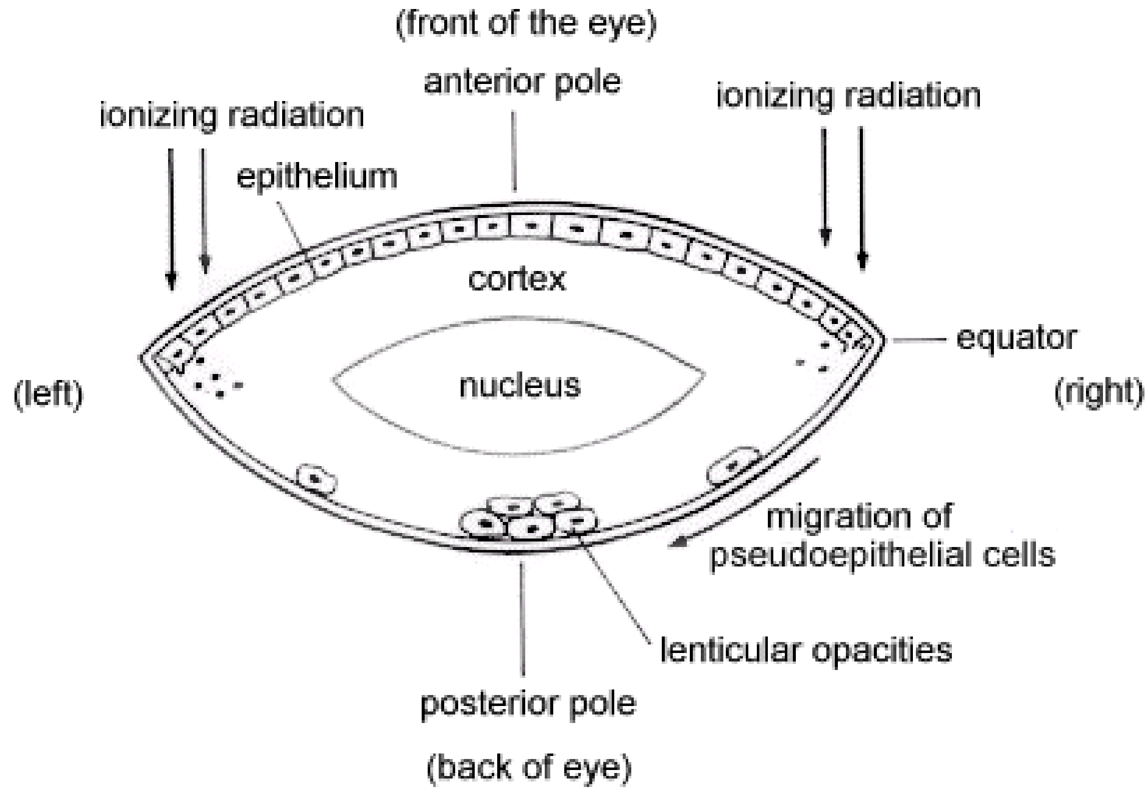


Figure 2.3 Lens opacity at the posterior subcapsular region caused by radiation (Shigematsu, Hibakusha, Kokusai, & Kyōgikai, 1995)

Zebrafish

The zebrafish, *Danio rerio,* is a teleost, ray-finned, fish. It is omnivorous, feeding mostly on zooplankton and phytoplankton. It lives in the rivers of northern India, northern Pakistan, Nepal, and Bhutan in South Asia (Hamilton-Buchanan, 1991). Due to its small size and ease of culture, the zebrafish has become a favorite model organism for biologists studying embryonic development.

Anatomy of the Zebrafish

The zebrafish is a vertebrate, freshwater, tropical fish. It is native to slow-moving and near-stagnant waters like rice paddies. At embryonic through larval stages the zebrafish is transparent as seen in Figure 2.4. When the zebrafish reaches adulthood it develops a silvery-gray dorsal side with a yellowish-white ventral side. Its sides are "Prussian blue" with four distinct golden strips on the males and silver stripes on the female spanning from head to caudal fin as seen in Figure 2.5 (Hamilton-Buchanan, 1991).

The zebrafish has a lifespan of approximately two years but can live up to five years in ideal conditions. Table 2.3 shows the time frame for each developmental stage. Of particular interest to this study is development of the lens. Lens cells begin to form as early as 19 hours post fertilization (hpf). The lens starts to form around 30-36 hours and should be fully formed at the time of hatching (Westerfield, 2000).

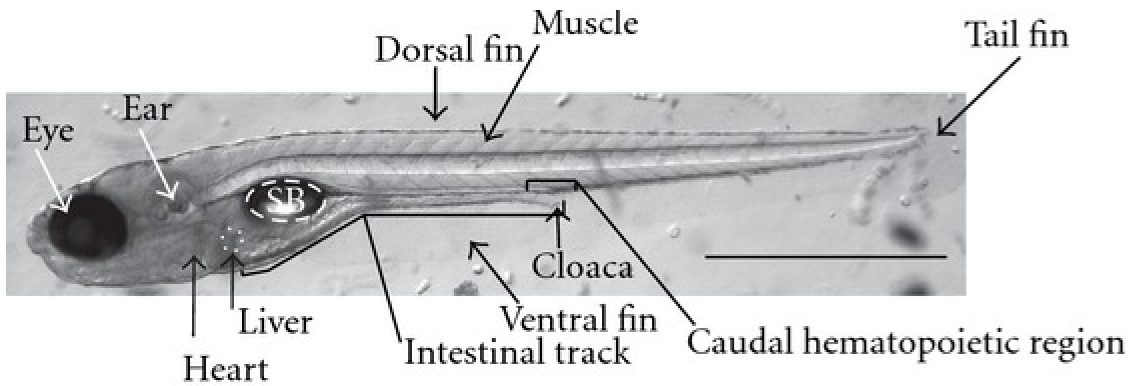


Figure 2.4 Diagram of zebrafish anatomy. A representative image of a transparent, 6 dpf larvae captured with brightfield microscopy. Organs and anatomical features are denoted in the figure. SB: swim bladder. Scale bar is 1 mm. (Goldsmith & Jobin, 2012)

Figure 2.5 Male (top right) and female (bottom left) adult zebrafish (Stoletov & Klemke, 2008)

Table 2.3 Developmental stages of the zebrafish (Westerfield, 2000), (Soules & Link, 2005)

Developmental stage	Time	Length (mm)	Lens growth
Embryo	0-48 hours	0.1- 2.9	Lens begins to develop ~ 30-36 hours
Hatching	48-72 hours	3.1-3.3	Loss of organelles within the lens fiber cells
Larva	6-29 days	3.5-7.8	Lens fiber cells begin compacting
Juvenile	30-89 days	10-14	Lens is fully developed
Adult	90 days-2 years	~ 40	

Zebrafish as a Human Model

Zebrafish have only recently started to gain recognition as a viable animal model of human disease and pathology. Many studies involve using higher mammalian systems like mice, rats and beagles. While these are well understood models they have several drawbacks such as high husbandry cost, slow gestation periods, low fecundity, and they need to be sacrificed in order to see internal cancerous growths (Goldsmith & Jobin, 2012).

On the other end of the spectrum, invertebrate species such as fruit flies may be used for human modeling. While fruit flies are low cost and allow for large-scale assays, they lack genetic similarities and disease pathologies (Lieschke & Currie, 2007). Zebrafish represent a good balance between higher level vertebrates and invertebrates (Goldsmith & Jobin, 2012).

Zebrafish have the physiological and anatomic characteristics of higher organisms but the ease of use of lesser organisms. They are highly fecund and breed rapidly with approximately 100 eggs per clutch with several clutches per week. They are transparent till about 7 days post fertilization (dpf) which can be extended up to 14 dpf. There has also been the introduction of the Casper line which is transparent throughout its life (Goldsmith & Jobin, 2012). This is especially important for observing disease progression without harming the specimen.

There are several areas of research that have been using zebrafish as study subjects. They are wide ranging fields including but not limited to: wound healing and restitution, gastrointestinal diseases, microbe-host interactions, genetic diseases, cancer, toxicology, pharmaceutical screening, genetics, and radiation effects (Goldsmith & Jobin, 2012), (Lieschke & Currie, 2007), (Stoletov & Klemke, 2008).

Since teleost fish can develop tumors spontaneously or from carcinogens they are useful when studying cancer generation and progression. There have even been researchers who were able to successfully xenograft human cancer cells onto zebrafish which allowed the propagation and visualization of the cancer cells through the transparent zebrafish (Stoletov & Klemke, 2008).

The zebrafish genome is fully mapped and can be found at http://www.sanger.ac.uk/Projects/D_rerio/ . It has significant homology with the human genome suggesting numerous human diseases could be matched to the zebrafish genome (Goldsmith & Jobin, 2012). Another benefit to using zebrafish is the inclusive community of researchers. There is a free exchange of materials and resources at the Zebrafish Model Organism Database (ZFIN) and the Zebrafish International Resource Center (Lieschke & Currie, 2007).

Some limitations in using zebrafish are that it is an emerging field of research. There are few validated cell lines and antibody reagents available to the research community. Another issue is the environmental conditions in which zebrafish is significantly different from humans. They need to be raised with specific ionic concentrations and some water-insoluble molecules cannot be administered. There are also limits on production facilities (Goldsmith & Jobin, 2012). Luckily there are two major zebrafish centers in Oregon at Oregon State University (OSU) and University of Oregon (UO).

Zebrafish Eye

The teleost eye shows many physiological and anatomical similarities to mammalian eyes. There are comparable cellular structures, signaling processes, and cognitive behaviors associated with the teleost and higher vertebrate eyes. Zebrafish have even shown similar eye diseases to mammalian eye diseases such as photoreceptor degeneration and cataract (Kitambi, Chandrasekar, & Addanki, 2011).

The lens of the zebrafish eye is relatively large and spherical (Figure 2.6) when compared to mammalian eyes which are ellipsoidal. They provide more focusing power due to the refractive index of water. The zebrafish eye is composed of the same cell types, epithelial and lens fiber cells. It is formed by the delamination of the lens placodal cells not invagination like the mammalian eye. By one month the fiber cells have fully compacted and the lens is suspended by zonules attaching the lens in place (Soules & Link, 2005).

Even though there are some differences between zebrafish and higher vertebrate eyes the structures have an overall likeness in anatomy and develop similarly (Soules & Link, 2005). Cataracts have been found in many teleost fish. Dietary cataracts are a well-known problem in farm raised salmon (Ersdal, Midtlyng, & Jarp, 2001), (Poston, Riis, Rumsey, & Ketola, 1978). Zebrafish have been found to have cataract and lens defects as well (Gestri, Link, & Neuhauss, 2012). Zebrafish should not be used to model all human eye diseases though. The trabecular meshwork of the zebrafish has morphological differences that make it unsuitable to compare it to glaucoma in humans (Chen, et al., 2008).

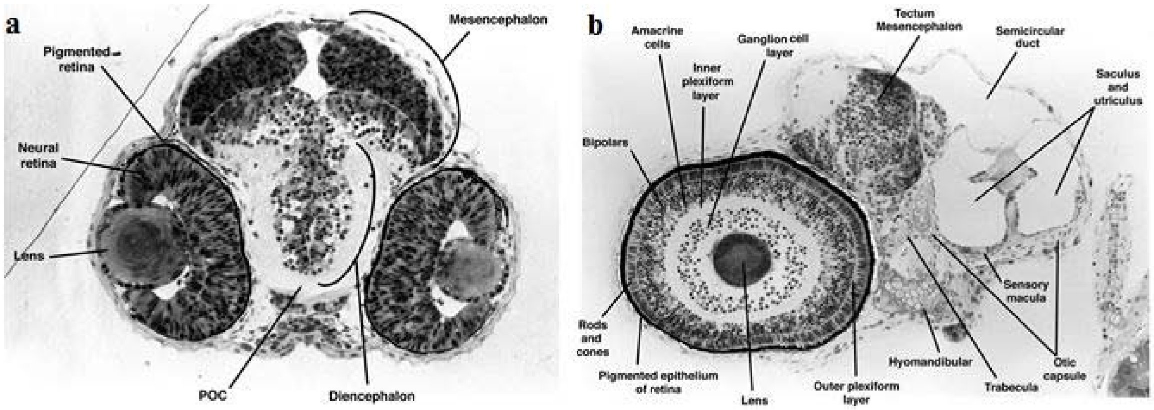


Figure 2.6 zebrafish fish eye of a 48-60 hour-old embryo (a) anterior view, (b) lateral view (Westerfield, 2000)

Zebrafish Irradiation

There have only been a few studies to investigate the radiosensitivity of zebrafish. The studies described in Table 2.4 generally used Cesium-137 (Cs-137) as a gamma-ray source and in one case Polonium-210 (Po-210) as an alpha-particle source. As to be expected, mortality increased with dose and embryos were more radiosensitive than adult zebrafish. The SNIFFER study attempted to establish an RBE standard for zebrafish regarding alpha-particle sensitivity (RBE_{α} = Dose rate of γ-radiation causing an effect / Dose rate of α-radiation causing the same effect). Of particular interest to the current investigation was the study performed by Geiger, et al. which found cataract formation in zebrafish embryos at doses as low as 10 Gy.

Table 2.4 Various studies regarding exposing zebrafish with ionizing radiation

Authors	**(Traver, et al., 2004)**	**(Geiger, et al., 2006)**	**(SNIFFER, 2002)**	**(Vala, et al., 2010)**
Title	Effects Of Lethal Irradiation In Zebrafish And Rescue By Hematopoietic Cell Transplantation	Zebrafish as a "Biosensor"? Effects of Ionizing Radiation and Amifostine on Embryonic Viability and Development	An Investigation into the Effects of Chronic Radiation on Fish	Low Doses of Ionizing Radiation Promote Tumor Growth and Metastasis by Enhancing Angiogenesis
Zebrafish Age	Wild-type Adult	Wild-type 2,4,6,8,or 14 hours post-fertilization (hpf)	Wild-type Adult	3 day post-fertilization
n	Control = 10 Exposed = 50	Total =4,439	Control = 50 γ-ray = 150 (50/group) α-particle = 200 (50/group)	Control =70 Exposed = 70
	Control = 65 Exposed = 650		10 mating pairs/dose	
Radiation Type	Gamma-ray: Cs-137 Gammacell 1000	Gamma-ray: Cs-137 Shepard-Mark I irradiator	Gamma-ray: Cs-137 sealed source	Not stated
			Alpha-particle: Po-210	
Radiation Dose	Minimum lethal dose(MLD): 0-50 Gy Groups of 10 fish per 5 Gy increments	Control 0 Gy Amifostine only (radio-protector) γ-ray at 5,10,20 Gy Amifostine and γ-ray at 5,10,20 Gy Survival assessed daily up to 144 hpf	External dose: 300, 1000, and 7400 μGy/h Cs-137 source continuous irradiation	0.5 Gy
	Survival curves: 0-50 Gy Groups of 65 fish per 5 Gy Increments		Internal dose: 8, 25, 185 and 740μGy/h Po-210 spiked food administered bi-weekly	

Results	0 - 25 Gy: ≥ 80% survival for up to 4 months ≥ 45 Gy: high mortality 1 day after irradiation MLD = 40 Gy (10% survival over 30 days)	Age of the embryos at the time of irradiation and radiation dose determine subsequent survival and morphology both can be modified by amifostine Ionizing radiation leads to: increased cell death in the eye and brain; lens opacification; increased caspase activation	7400 μGy/h γ-ray exposure resulted in sterilization of fish	low-dose irradiation accelerated angiogenic sprouting without causing excessive vessel formation and enhances the angiogenic response during fin regeneration
	Apparent threshold dose of 20 Gy for hematopoietic tissues		RBE_α not determined with precision Conservative upper limit $RBE_\alpha < 35$ using whole body dose $RBE_\alpha < 20$ and < 7 using dose to ovaries	

Zebrafish Exposures

As the first phase of the larger investigation, this work will develop the techniques for irradiating zebrafish to high energy gamma-rays from Cobalt-60. The gamma-ray exposure was conducted using a Gammacell 220. The second contribution of is work to develop the radiation protection and dosimetry protocols for zebrafish exposures to mixed gamma-ray and intermediate energy beta-particles from Iodine-128 and to heavy charged particles (HCP) in the Relativistic Heavy Ion Collider (RHIC) at Brookhaven National Laboratory (BNL).

Gammacell 220

Stage one of the study involved developing a dose rate response curve for embryonic zebrafish. In order to achieve this, the zebrafish were exposed to gamma-rays via the Gammacell 220.

The Gammacell 220 (Figure 2.7) is a self-contained irradiator which provides a field of high-intensity gamma rays. The lead shielded chamber has Cobalt-60 pencil sources with an initial loading of 23,000 curies (851 TBq) and can give a dose of up to 20 kGy/hour in the center of the chamber (Figure 2.8) (US AEC, 1968). The sample chamber can hold samples up to six inches in diameter and eight inches in height. After the timer is set the samples are placed into the chamber which is then mechanically lowered into the lower chamber (US AEC, 1968).

Figure 2.7 Gammacell 220 in the OSU Radiation Center

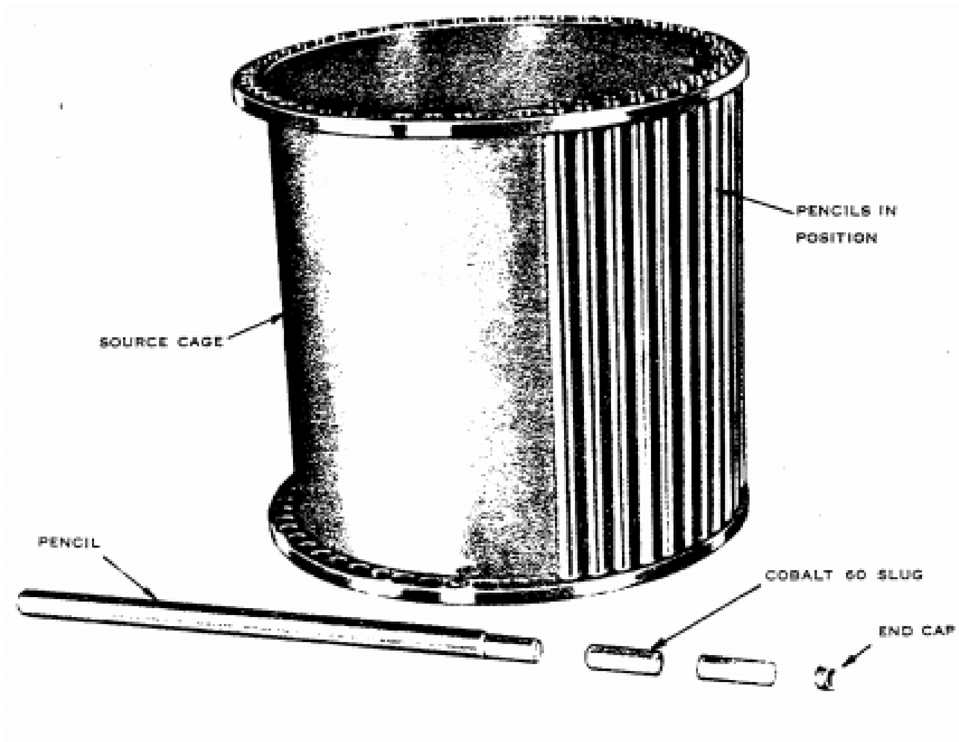


Figure 2.8 Gammacell 220 Cobalt-60 source pencils and cage (US AEC, 1968)

Cobalt-60 has a half-life of 5.27 years and beta decays into Nickel-60 (Figure 2.9). Co-60 has two characteristic gamma rays with energies 1173.237 and 1332.501 keV (KAERI, 2013). Since the Gammacell 220 is lined with lead the only radiation reaching the chamber will be from the gamma-rays and not the beta-particles.

The internal flux of the Gammacell 220 is depicted in Figures 2.10 and 2.11. Figure 2.11 represents the vertical cross section isodose curve. Figure 2.11 was developed from the center of the chamber between the heights of 3-5” (7.6-12.7 cm) in order estimate the dose delivered to the zebrafish.

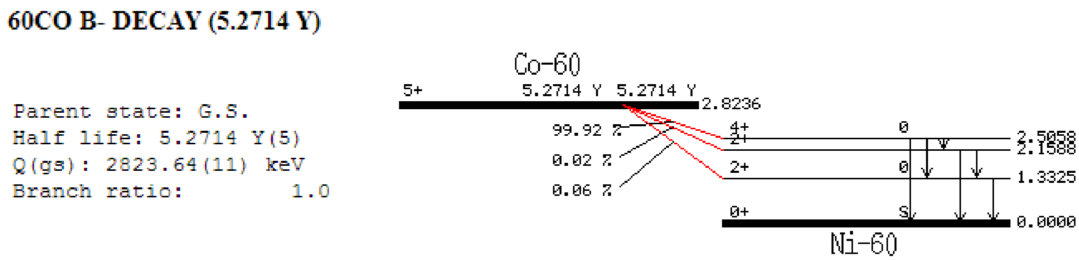
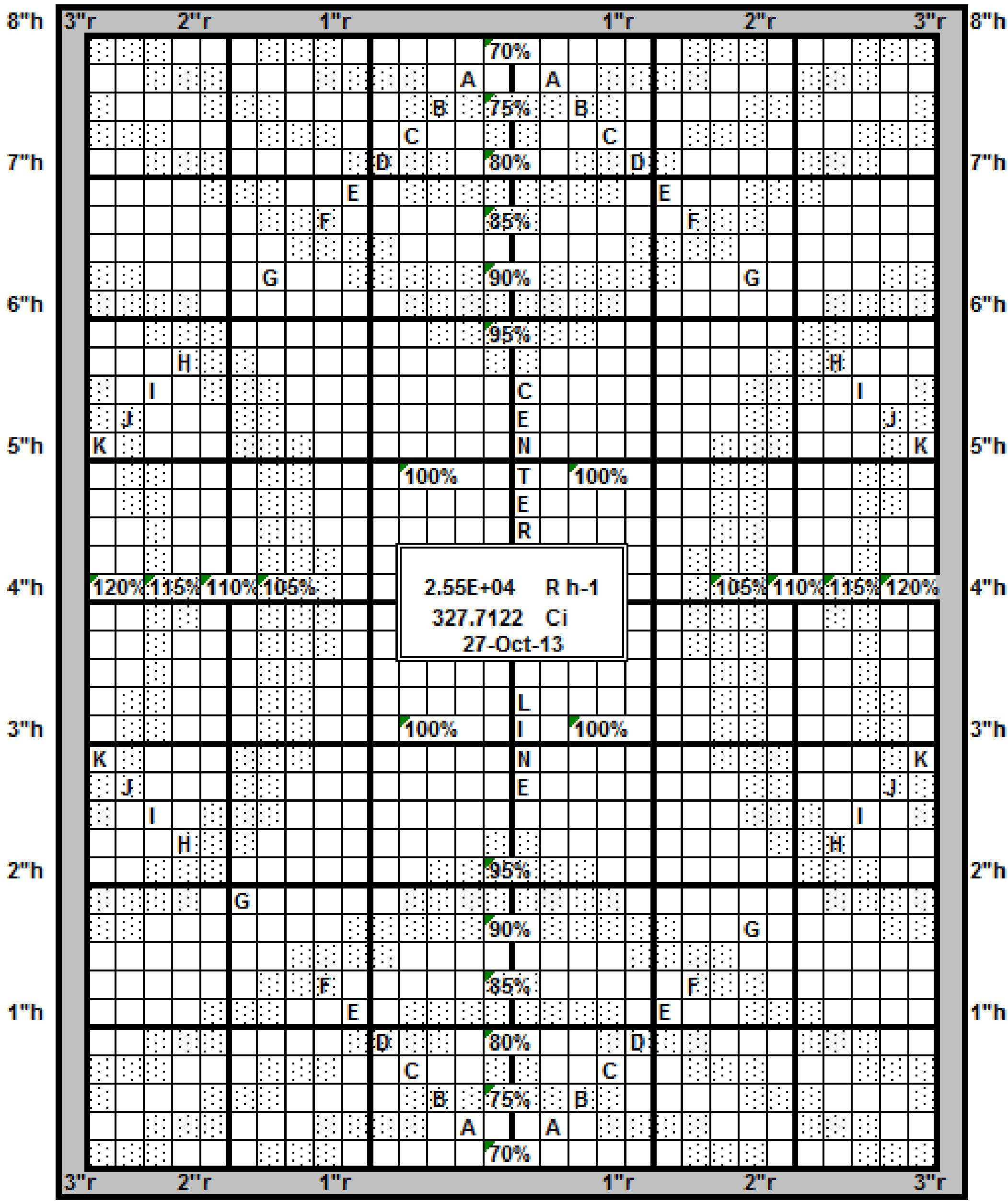


Figure 2.9 Co-60 decay scheme (KAERI, 2013)



REGION:								
A =	1.79E+04	R h-1	E =	2.30E+04	R h-1	I =	2.81E+04	R h-1
B =	1.92E+04	R h-1	F =	2.43E+04	R h-1	J =	2.94E+04	R h-1
C =	2.04E+04	R h-1	G =	2.55E+04	R h-1	K =	3.07E+04	R h-1
D =	2.17E+04	R h-1	H =	2.68E+04	R h-1			

Revised 2/04

Figure 2.10 Gammacell 220 chamber vertical cross section isodose curve

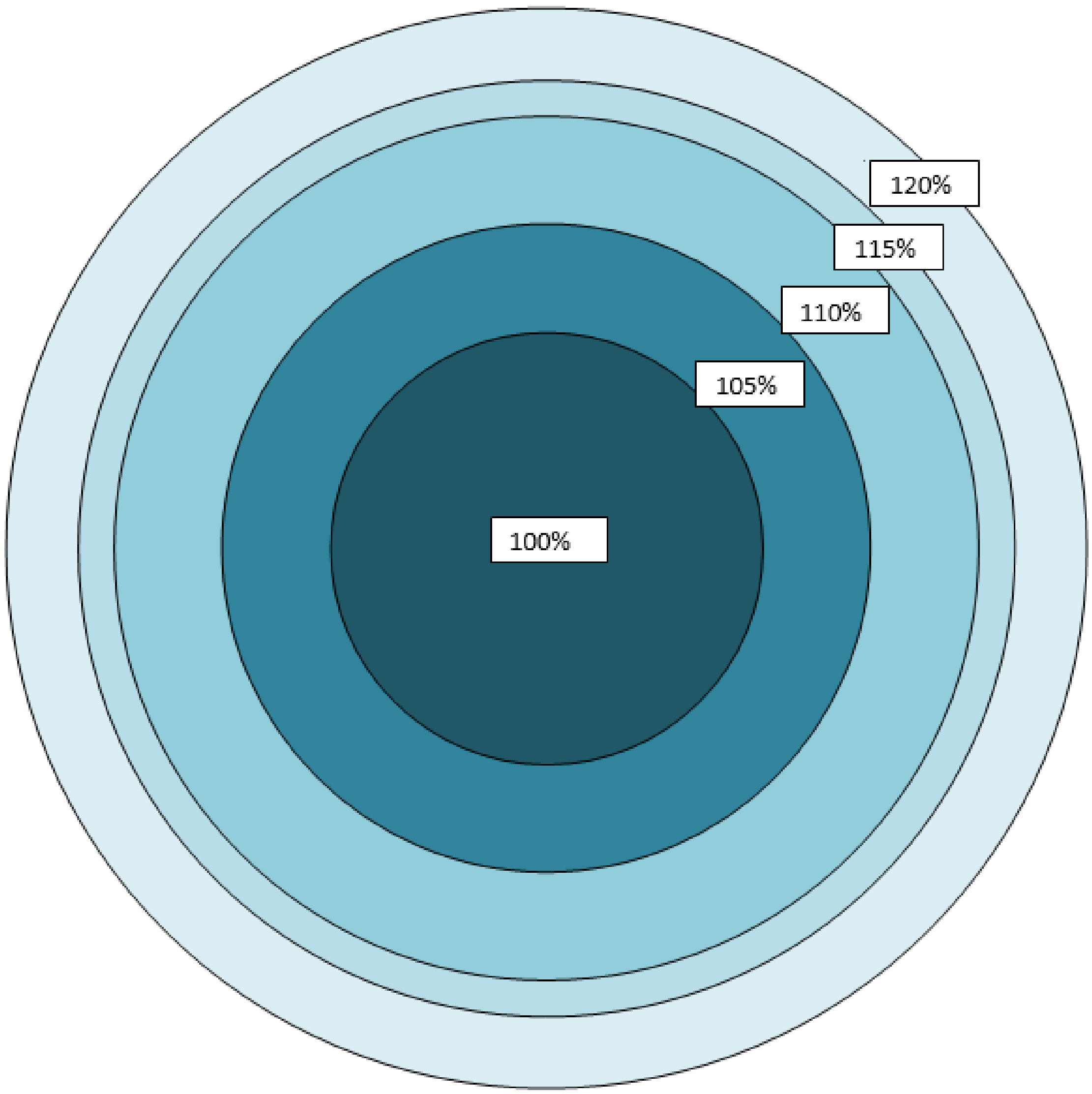


Figure 2.11 Estimated Gammacell 220 chamber horizontal cross-section isodose curve between the height 3-5" (7.6-12.7 cm) range with dose increasing outwards

Iodine-128

Stage two of the study involves adding beta exposure in addition to the gamma exposure from stage one. Iodine-128 was selected as the nuclide for several reasons. It has a relatively short half-life which in turn will make containment and safety protocols easier to design. However, it is a health risk to the thyroid of the radiation worker due to the nature of iodine absorption into the thyroid (Hall & Giaccia, 2006).

Iodine-128 is a short lived nuclide with a half-life of 24.99 minutes that is formed by bombarding natural iodine with thermal neutrons: $^{127}I\ (n, \gamma)\ ^{128}I$ (Kahn & Kleinberg, 1977). The nuclide can decay by either beta-particle emission to Xenon-128 (93.1%) or electron capture to Tellurium-128 (6.9%). The beta-particle decay pathway releases beta-particles with a 2.119 MeV endpoint energy and gamma-ray of 442.9 keV. Electron capture has a decay energy of 1.252 MeV and a gamma of 743.5 keV (KAERI, 2013). Figures 2.12 and 2.13 show the decay schemes of both decay methods.

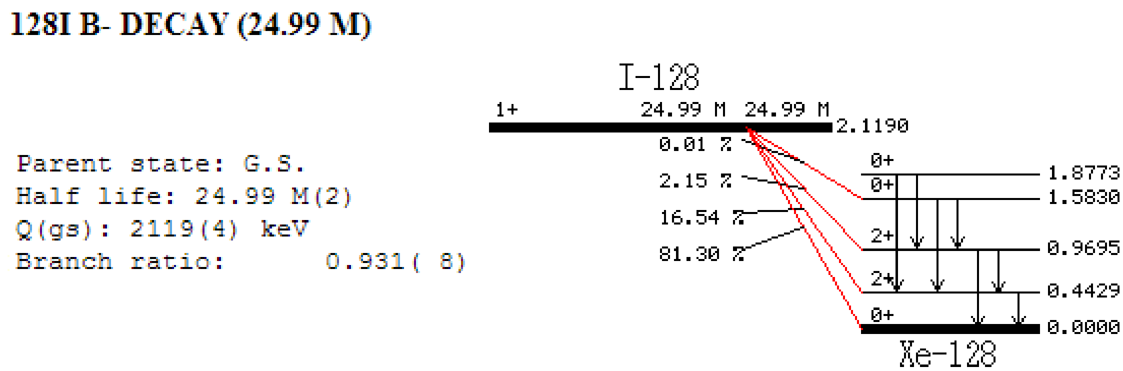


Figure 2.12 I-128 beta decay scheme (KAERI, 2013)

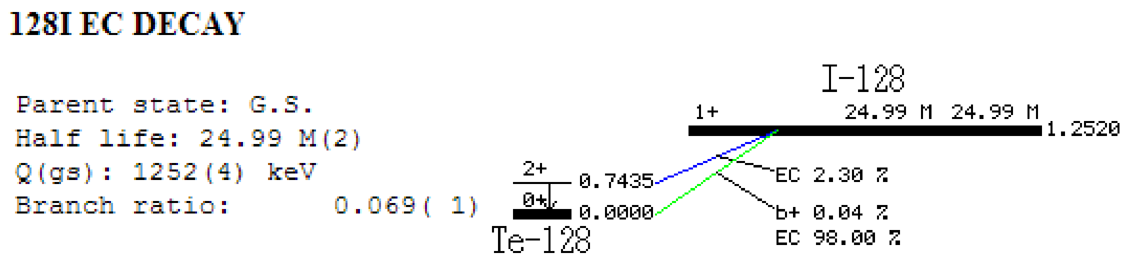


Figure 2.13 I-128 electron capture decay scheme (KAERI, 2013)

Relativistic Heavy Ion Collider (RHIC)

For the final stage of this study the zebrafish will be exposed to HCP at the RHIC in order to mimic the space radiation environment.

The RHIC is located at BNL and is the industry standard for radiation space biology experiments (Figure 2.14). It is a particle accelerator that collides two beams of gold ions at nearly the speed of light. The beams travel along a 2.4-mile long, two-lane "racetrack" and then collide at one of the six intersections (BNL, 2013).

One of the intersections, labeled 1 on Figure 2.14, leads to the Electron Beam Ion Source (EBIS) (Figure 2.15). The EBIS is heart of the NASA Space Radiation Laboratory and where most space radiation biology experiments are conducted. It provides all stable ion species from deuterons to uranium (BNL, 2013).

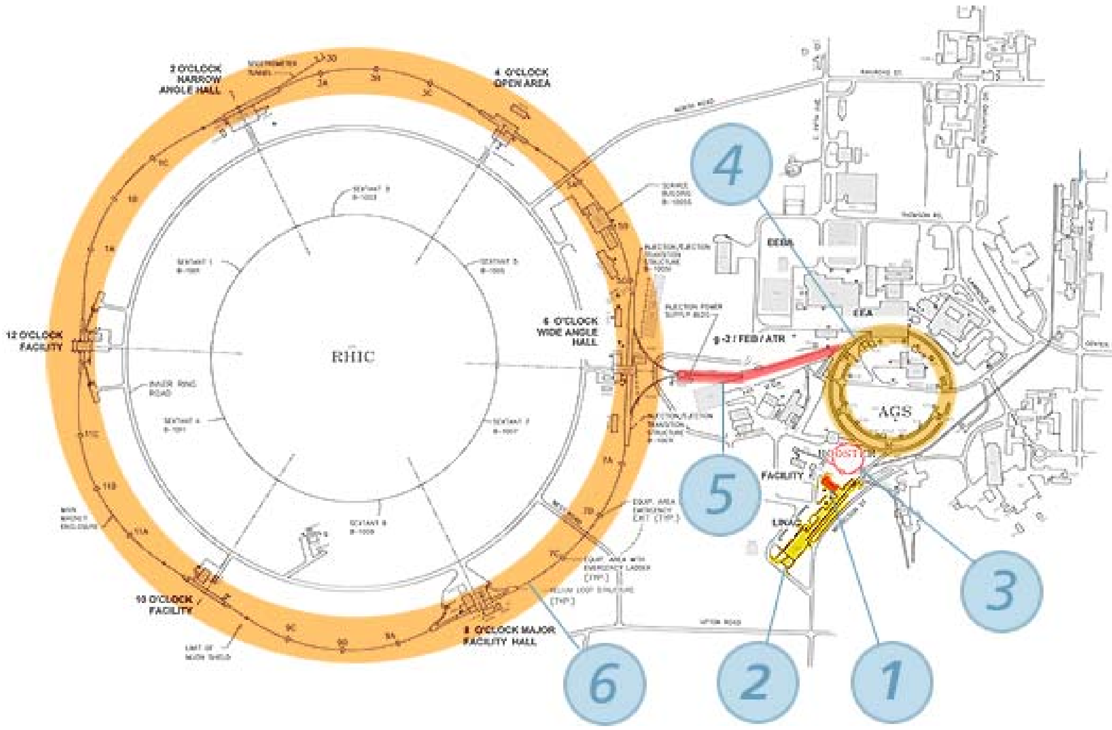


Figure 2.14 RHIC ion accelerator complex (BNL, 2013)

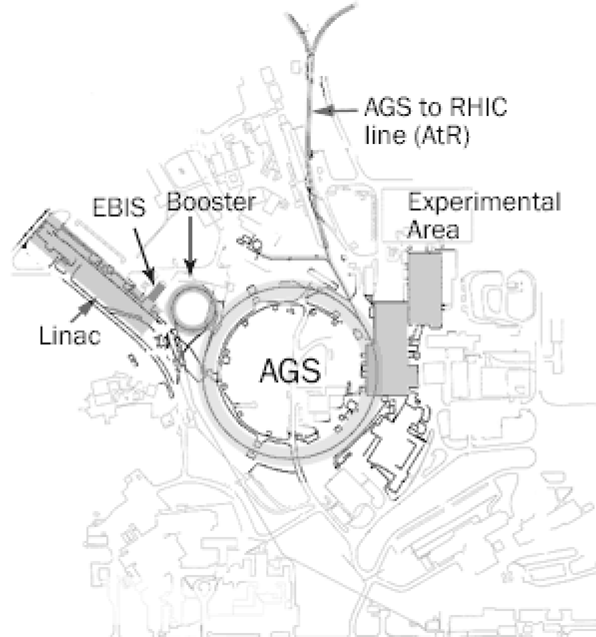


Figure 2.15 Electron Beam Ion Source (BNL, 2013)

Chapter 3 Methods and Materials

Dose Response

The first phase of the project was to determine dose response and survival of embryonic wild-type zebrafish when exposed to gamma radiation. In order to achieve this, 24-hour-old zebrafish embryos were collected and exposed to varying gamma doses ranging from 5-50 Gy in increments of 5 Gy. Graphical analysis and descriptive statistics were performed to explore the data. All analyses were completed using the statistical package R 3.0.1 (R Core Team, 2013).

Materials

a. Gammacell 220
b. One (1) zebrafish embryo per cell (24 hpf)
c. 96 cells per tray
d. 3 trays per dose increment
e. 1 tray for control group per dose increment
f. Trays wrapped in paraffin and aluminum foil
g. Thermoluminescent dosimeter (TLD) worn by radiation workers

Methods

At 4 hours post fertilization (hpf), embryo chorions, an acellular envelope, was removed enzymatically, and transferred to individual wells of a 96-well plate with 100 µl of prepared solution at 6 hpf. Exposure plates were sealed with paraffin to prevent evaporation and wrapped with aluminum foil in case the samples were light sensitive. Embryos were collected from Sinnhuber Aquatic Research Laboratory (SARL) and transported to the OSU radiation Center (RC) for exposure at 24 hpf.

Exposure time was determined by using a dose calculation Excel spreadsheet developed by the Radiation Center staff. Table 3.1 shows the irradiation times and dates for each set of samples.

The aluminum foil was removed and three trays were placed into the gamma cell on a 3” (7.6 cm) elevation block. This was repeated for each increment of 5 Gy. Controls trays were with the exposed trays at all times with the exception of being placed in the gamma cell. Trays were then rewrapped in aluminum foil and returned to SARL. In order to keep embryos in the 24 hpf range, gamma exposures were performed August 8-15, 2012 from 8:30-9:30 AM.

Assessments of the zebrafish were performed by Lisa Truong of SARL on August 8-15, 2012. At 24 hpf, mortality, and developmental progression was evaluated. The static nanoparticle exposure continued under standard laboratory conditions in aluminum covered, sealed plates until 120 hpf. By 120 hpf, each individual embryo was euthanized and scored for mortality and multiple morphological malformations.

Table 3.1 Gammacell 220 irradiation times for 24 hpf, dose converted from rad to gray (controls = 960, exposed = 2880, n= 3840)

Dose (Gy)	Irradiation Time (Seconds)	Date
5	60	8/8/2012
10	120	8/9/2012
15	180	8/9/2012
20	240	8/10/2012
25	300	8/13/2012
30	361	8/14/2012
35	421	8/14/2012
40	481	8/14/2012
45	541	8/15/2012
50	601	8/15/2012

Four-Day-Old Dose

The next phase of the project was to investigate cataract formation in embryonic wild-type zebrafish when exposed to gamma radiation. In order to achieve this, 4-day-old zebrafish embryos were collected and exposed to varying gamma doses ranging from 5-25 Gy in increments of 5 Gy. Four-day-old embryos were selected since the lens of the eye should be fully developed by that point and assessments could be performed within the five day time frame allowed by the institutional review board (IRB).

Materials

a. Gammacell 220
b. One (1) zebrafish embryo per cell (4 dpf)
c. 96 cells per tray
d. 3 trays per dose increment
e. 1 tray for control group per dose increment
f. Trays wrapped in paraffin and aluminum foil

Methods

At 4 hpf, embryo chorions, an acellular envelope, was removed enzymatically, and transferred to individual wells of a 96-well plate with 100 µl of prepared solution at 6 hpf. Exposure plates were sealed with paraffin to prevent evaporation and wrapped with aluminum foil in case the samples were light sensitive. Embryos were collected from SARL and transported to the RC for exposure at 4 days post fertilization (dpf).

Exposure time was again determined by using a dose calculation Excel spreadsheet. Table 3.2 shows the irradiation times and dates for each set of samples. Aluminum foil was removed and three trays were placed into the gamma cell on a 3" elevation block. This was repeated for each increment of 5 Gy. Controls trays were with the exposed trays at all times with the exception of being placed in the Gammacell. Trays were then rewrapped in aluminum foil and returned to SARL. In order to keep embryos in the 4 dpf range, gamma exposures were performed October 31 –November 5, 2012 from 8:30-9:30 AM.

Assessments of the zebrafish were performed by Chapell Miller of SARL on November 1-6, 2012. At 5 dpf each individual embryo was euthanized and scored for mortality and multiple morphological malformations.

Table 3.2 Gammacell 220 irradiation times for 5 dpf, dose converted from rad to gray (controls = 480, exposed = 1440, n = 1920)

Dose (Gy)	Irradiation Time (Sec)	Date
5	62	10/31/2012
10	124	10/31/2012
15	185	10/31/2012
20	248	11/5/2012
25	310	11/5/2012

Experimental Design of Stage Two

Stage two of the study will be conducted over two to five years in order to investigate the long term effects of radiation exposure on zebrafish. In order to carry this out the zebrafish will need to have a dedicated space and staff with husbandry protocols already in place. The John L. Fryer Salmon Disease Laboratory (SDL) at OSU has the space and qualified staff that will be able to maintain a long term study such as the zebrafish cataract formation experiment.

Materials

1. Iodine-128
2. Iodine Gas Detector
3. NaI(Tl) scintillation detector
4. Geiger-Müller counter
5. Fume hood
6. Plexiglas tank for dosing zebrafish
7. Deionized water
8. Lead brick shielding
9. Dosimetry
    a. For radiation worker(s)
        i. TLD 100
        ii. Ring dosimetry
    b. For laboratory
        i. TLD 100
    c. For fish
        i. TLD 100 (chip form)
10. Personal Protective Equipment (PPE)
    a. Eye goggles with sides
    b. Face shield at least 8” in length
    c. Impervious clothing (lab coats, closed toed shoes, pants etc.)
11. One (1) zebrafish juvenile per cell (30 dpf)
12. 24 cells per tray
13. 4 trays per dose type
    a. Control

   b. Beta and gamma
   c. Gamma only

14. Trays wrapped in paraffin and aluminum foil
15. Slit-lamp biomicroscope

Methods

Radiation Safety Protocols

Due to the nature of iodine the radiation safety protocols will have to be increased compared to the use of the Gammacell 220. A radiation work permit (RWP) will need to be written detailing the experiment procedures, monitoring devices, and emergency procedures in the case of a spill.

Personal dosimetry required will include finger dosimetry and a TLD (thermoluminecent dosimeter) which will be required at all times when handling the iodine. Additional dosimetry will be placed inside the laboratory. A Geiger-Müller counter will be used to ensure there is no contamination on the radiation worker or equipment before leaving the laboratory. Swipes will be provided to check contamination on the Plexiglas tank, around the vent hood, and any other surface that may have come into contact with the iodine.

If a radioactive spill occurs the procedures described by The Department of Public Safety & Oregon State Police will be followed:

1. Alert people in the immediate area of the spill
2. Notify Radiation Safety
3. Wear protective equipment, including safety goggles, disposable gloves, shoe covers, and a long-sleeve lab coat
4. Place absorbent paper towels over liquid spill. Place towels dampened with water over spills of solid materials
5. Using forceps, place towels in plastic bag. Dispose in radioactive waste container
6. Monitor area, hands, and shoes for contamination with an appropriate survey meter or method. Repeat clean-up until contamination is no longer detected

In addition to traditional radiation monitoring an OSHA (Occupational Safety & Health Administration) approved iodine particulate detector will be required (Table 3.3). If the permissible exposure limit (PEL) of 0.1 ppm is exceeded the radiation worker will leave the laboratory immediately and inform the radiation health physicist on duty.

Table 3.3 OSHA regulations for iodine in workplace atmosphere (OSHA, 2012)

OSHA PEL - Iodine	**0.1 ppm (ceiling)**
Collection Device	An air sample is collected using a calibrated sampling pump and a glass tube containing impregnated activated beaded carbon (IABC). A modified sampling tube (MST) can be used to preclude any iodide-containing particulate, if necessary. **Loss of iodine using IABC has been noted when sampling in relative humidity's (RHs) > 50%. See Special Precautions below.**
Sampling rate	0.5 L/min
Minimum sampling time	5 min
Analytical procedure	The sampling medium is desorbed using an aqueous solution containing 1.5 mMol sodium carbonate (Na_2CO_3) and 1.5 mMol sodium bicarbonate ($NaHCO_3$). An aliquot of this solution is analyzed for iodine (as iodide, I^-) by an ion chromatograph equipped with a pulsed electrochemical detector.
Detection limit	0.0004 ppm as I_2 (2.5-L air sample)
Validation range	0.05 to 0.20 ppm
Overall error	±11.5%

Pathways

The pathways of concern for iodine exposure are inhalation, injection, and ingestion. In order to protect these pathways best engineering practices will be implemented to reduce exposure. This practice is known in the NRC as ALARA (as low as reasonably achievable).

To reduce the chance of inhalation a fume hood will be used whenever handling the iodine. If the iodine air sample exceeds 0.1 ppm the radiation worker will immediately leave the lab and inform the health physicist on duty.

The iodine will not be placed into a syringe or anything else that may penetrate the skin. In order to protect against ingestion consumption of food, liquid, or gum will not be allowed inside the laboratory.

Dose will be minimized in three ways, time, distance, and shielding. The radiation worker will minimize time in the laboratory by preparing the area with all necessary equipment prior to bringing in the sample. Procedures will be well known and practiced before using sample. After iodine is in solution and zebrafish trays are in place the radiation worker will exit the room until required dose is achieved. The shielding will be a combination of Plexiglas and lead shielding to protect against beta and gamma radiation respectively.

Shielding

Shielding is vital to reducing the dose to the radiation worker. The two materials used will be Plexiglas to protect from the beta source and lead bricks to protect from the gamma source. The Plexiglas will also prevent some of the zebrafish from receiving a beta dose which will allow for comparison between a beta-gamma group and a gamma only group.

The general shielding equation is as follows:

$$\dot{D} = \dot{D_0}\, e^{-\mu x} \tag{1}$$

Where:

$\dot{D} = dose\ rate\ with\ shielding$

$\dot{D_0} = dose\ rate\ without\ shielding$

$\mu = linear\ attenuation\ coefficient$

$x = thickness\ of\ the\ shileding$

Rearranged to find thickness of shielding:

$$x = -\mu \cdot ln\left(\frac{\dot{D}}{\dot{D_0}}\right) \tag{2}$$

The dose rate with shielding will be 2 mrem/hour in order to classify the laboratory as an unrestricted area (NRC, 2013). Dose without shielding will be the maximum dose to be used in the experiment which will be 25 Sv ($2.5x10^6$ mrem). Time for exposure will be approximately ten half-lives which is 4.2 hours. Therefore the maximum dose rate will be 6 Sv/hour ($6x10^5$ mrem/hour).

The linear attenuation coefficient can be found using the mass attenuation coefficient for Plexiglas and lead and the multiplying by the densities of each material respectively for the maximum photon energy released (Table 3.4).

Table 3.4 Attenuation coefficients for a .8 MeV photon (Hubbell & Seltzer, 2011)

	Lead	**Plexiglas**
Mass attenuation coefficient for 0.8 MeV photon (cm^2/g)	$8.87x10^{-2}$	1.18
Density (g/cm^3)	11.34	1.18
Linear attenuation coefficient (cm^{-1})	1.01	0.09

<u>Dose Calculations</u>

Several calculations and modeling in MCNP (Monte Carlo N-Particle) will need to be performed to ensure the zebrafish are receiving the appropriate doses. The first calculation will be to determine what activity will be needed for each dose range. The simple definition of dose is energy deposited per unit of mass and dose rate is dose per unit of time. In order to find the absorbed dose rate for a gamma source the following equation is used:

$$\dot{D} = \dot{\Psi}\frac{\mu_{en}}{\rho} = \frac{CE}{4\pi r^2}\frac{\mu_{en}}{\rho} \quad (3)$$

Where:

$\dot{D} = Dose\ Rate$

$\dot{\Psi} = energy\ fluence\ rate\ (MeV/cm^2 sec)$

$C = activity\ (Bq)$

$E = energy\ per\ decay\ (MeV)$

$\mu_{en}/\rho = mass\ energy - absorption\ coefficient\ in\ air\ \left(cm^2/g\right)$

$(\sim same\ for\ photons\ between \sim 60keV\ and\ 2\ MeV$

Since the beta is a charged particle a modified equation is used. The absorbed dose rate for a low energy beta

$$\dot{D} = 1.60x10^{-10}\ A\bar{E}\ (Gy\ /sec) \quad (4)$$

Where:

$A = activity\ concentration\ (Bq/g)$

$\bar{E} = average\ beta\ energy\ (MeV/disintigration)$

By rearranging both of these equations the activities necessary for each dose can be found.

Gamma activity:

$$C = \frac{D}{t} \cdot \frac{4\pi r^2}{E} \cdot \frac{\rho}{\mu_{en}} \tag{5}$$

Beta activity:

$$A = \frac{d/t}{1.60x10^{-10}\,\bar{E}} \tag{6}$$

The next step will be to determine how long the sample will need to be irradiated for in the reactor in order to achieve maximum activation. This can be found using the following equation:

$$t_m = \frac{\log((\lambda+\sigma\Phi)/(\sigma\Phi))}{(\lambda+\sigma\Phi)-(\sigma\Phi)} \tag{7}$$

Where:

$\lambda = decay\ \ constant\ of\ activation\ product\ (min^{-1})$

$\sigma = thermal\ neutron\ capture\ cross - section\ of\ the\ target\ nuclide\ (cm^2)$

$\Phi = themal\ neutron\ fluence\ rate\ (n/\ cm^2\ \ /sec)$

Next, the mass for each dose will need to be determined. Mass can be found by rearranging the neutron activation equation:

$$A = N\sigma\Phi\left(1 - e^{-\lambda t_a}\right)e^{-\lambda t_w} \quad (8)$$

Where:

$N = number\ of\ target\ atoms$

$\sigma = thermal\ neutron\ capture\ cross - section\ of\ the\ target\ nuclide\ (cm^2)$

$\Phi = themal\ neutron\ fluence\ rate\ (n/\ cm^2\ \ /sec)$

$\lambda = decay\ \ constant\ of\ activation\ product\ (min^{-1})$

$t_a = activation\ time$ (min)

$t_w = time\ between\ end\ of\ activation\ and\ count$ (min)

Rearranged activation equation to find mass needed:

$$N = \frac{A}{\sigma\Phi\left(1-e^{-\lambda t_a}\right)e^{-\lambda t_w}} \quad (9)$$

Plexiglas Tank Design

The Plexiglas tank will serve several functions. The first will be to contain the deionized water and I-128 it will need to be watertight. The second function will be to shield the radiation worker from radiation.

The tank itself will be made of Plexiglas and will shield against beta-particles. There will be a lid which will have a window cut out that will fit a tray containing zebrafish. Notches will be cut into the lid equal distance from the side as the window for the second tray of zebrafish. This will maintain good geometry and ensure proper placement of trays for each exposure. This design will allow for the zebrafish to be exposed to beta-particles and gamma-rays through the window and gamma-rays only in the other position.

It will be surrounded by lead bricks in order to shield against gamma-rays. An additional layer of Plexiglas will be placed around the lead bricks to protect against secondary particles produced in the lead. Figure 3.1 presents the basic mockup of the tank design.

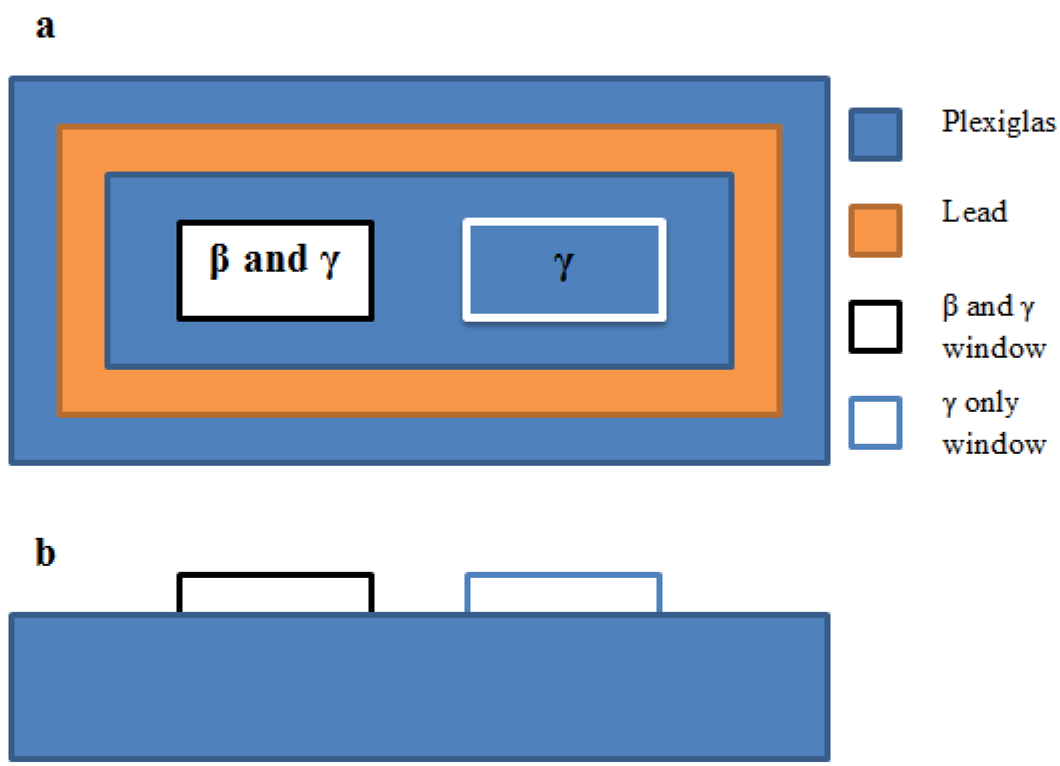


Figure 3.1 Holding tank and basic shielding design (not to scale) (a) overhead view, (b) side view

Zebrafish Irradiation Steps

1. Place specified amount of iodine into reactor to obtain Iodine-128
2. Perform calibration count with a NaI(Tl) scintillation detector to verify activity
3. Prepare I-128
4. Add to Plexiglas tank
    a. Ensure tank has been filled with deionized water
5. Place chip TLD 100 in one of the cells in each tray
6. Place trays in positions shown in Figure 3.1
7. Leave irradiation room until 5 half-lives have passed (2 hours 5 minutes)
8. Remove trays from tank
9. Perform swipe of trays using the Geiger counter before removing them from the room
10. Take fish to the SDL
11. Husbandry and evaluation will be determined by SDL staff

Chapter 4 Results and Analysis

Dose Response

The zebrafish were examined a few hours after irradiation and 96 hours after irradiation. Just after irradiation mortality was fairly consistent between 5-15%. At 45-50 Gy the 30-46% of surviving zebrafish began forming multiple malformations. Tables 4.1 and 4.2 show the percentages and relative risks of mortality and morbidity at 24 hpf. Figure 4.1 Shows the percentages of mortality and morbidity at 24 hpf.

Relative risk (RR) is the ratio of the probability of an event occurring (Pagano & Gauvreau, 2000). It can be found using the following equation:

$$RR = \frac{Probability\ of\ event\ when\ exposed}{Probability\ of\ event\ when\ non\text{-}exposed} = \frac{a/(a+b)}{c/(c+d)} \quad (10)$$

Where:

Risk	**Disease Status**	
	Death (Defects)	Survived (No defects)
Exposed	a	b
Non-exposed	c	d

In the most basic terms, if RR is > 1 then the event is more likely to occur in the exposed group than the unexposed group. If RR is < 1 then the event is less likely to occur in the exposed group than the unexposed group. In general, the relative risks in this study are not statistically significant. This is most likely due to examining the zebrafish so soon after irradiation and therefore not enough events have occurred.

Table 4.1 Mortality at 24 hpf (* statistically significant)

Dose	Cumulative Mortality	Total	% Mortality	Relative Risk	95 % Confidence Interval	
0	58	766	8%			
5	24	288	8%	1.10	0.70	1.73
10	20	288	7%	0.92	0.06	1.50
15	27	288	9%	1.24	0.80	1.91
20	44	288	15%	2.02*	1.40	2.91
25	34	287	12%	1.56*	1.05	2.34
30	15	288	5%	0.69	0.40	1.19
35	19	288	7%	0.87	0.53	1.44
40	18	287	6%	0.83	0.50	1.38
45	18	288	6%	0.83	0.50	1.38
50	44	288	15%	2.02*	1.39	2.90
	n	3644				
	χ^2	> 0.001				
	Degrees freedom	10				

Table 4.2 Morbidity at 24 hpf (* statistically significant)

Dose	Developmental Defects	Surviving Total	% Defects	Relative Risk	95 % Confidence Interval	
0	11	719	2%			
5	0	254	0%	0.12	0.01	2.06
10	6	268	2%	1.44	0.54	3.86
15	3	261	1%	0.74	0.21	2.63
20	3	244	1%	0.79	0.22	2.81
25	4	253	2%	1.02	0.33	3.16
30	2	273	1%	0.47	0.11	2.11
35	6	268	2%	1.44	0.54	3.86
40	7	270	3%	1.67	0.65	4.26
45	87	270	32%	20.74*	11.25	38.22
50	113	244	46%	29.81*	16.33	54.41
	n	3324				
	χ^2	> 0.001				
	Degrees freedom	10				

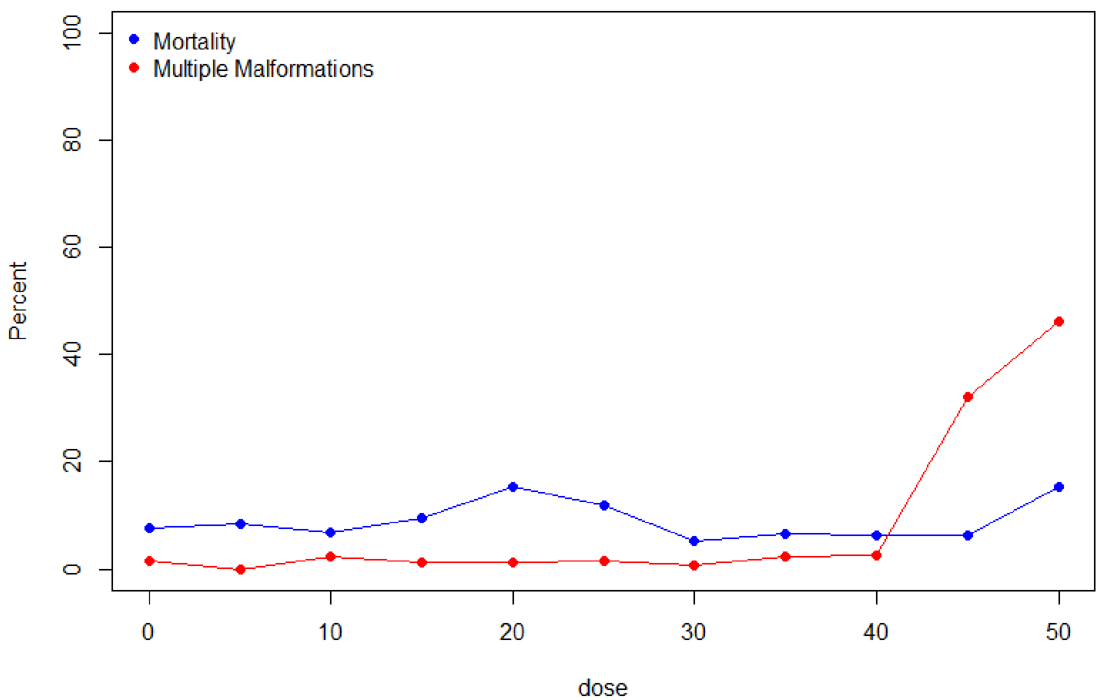


Figure 4.1 Mortality and multiple malformations in zebrafish at various doses at 24 hpf

Next a logit (log-odds) model was calculated in R version 3.0.1 (R Core Team, 2013). The logit function can be found using the following equation.

$$logit(p) = \log\left(\frac{p}{1-p}\right) \quad (11)$$

Where:

p = probability

Tables 4.3 and 4.4 show the logit models for mortality and morbidity respectively. As with the RR the logit models have only a few dose exposures with statistical significance. Mortality at 50 Gy is 2.20 times the odds of mortality at 0. Multiple morphological defects are apparent at ≥ 45 Gy. Morbidity at 45 Gy is 30.14 times the odds of mortality at 0. The confidence intervals for morbidity are fairly large which indicates a low level of precision.

Table 4.3 Log-odds model of mortality for fish at 24hpf (*statistically significant)

Dose	Estimate	Estimate (exp)	95% Confidence Interval (exp)	
0	-2.50	0.08*	0.06	0.11
5	0.10	1.11	0.66	1.18
10	-0.09	0.91	0.53	1.51
15	0.23	1.26	0.77	2.02
20	0.79	2.20*	1.44	3.34
25	0.50	1.64*	1.04	2.55
30	-0.40	0.67	0.36	1.17
35	-0.15	0.86	0.49	1.45
40	-0.20	0.82	0.46	1.38
45	-0.21	0.81	0.46	1.38
50	0.79	2.20*	1.44	3.34

Table 4.4 Log-odds model of morbidity for fish at 24hpf (*statistically significant)

Dose	Estimate	Estimate (exp)	95% Confidence Interval (exp)	
0	-4.15	0.016*	0.01	0.027
5	-24.13	0.00	0.00	0.00
10	0.37	1.45	0.50	3.85
15	-0.31	0.74	0.17	2.38
20	-0.24	0.79	0.17	2.55
25	0.0018	1.02	0.28	3.01
30	-0.76	0.47	0.07	1.76
35	0.37	1.45	0.50	3.85
40	0.52	1.69	0.61	4.32
45	3.41	30.14*	16.44	60.81
50	4.00	54.65*	29.89	110.24

The zebrafish were then examined 96 hours after irradiation (120 hpf). Mortality was fairly consistent below 20% between 0-25 Gy. Mortality increased to over 80% ≥ 30 Gy. Morbidity increases to 58% at 10 Gy and between 70-100% ≥ 15 Gy. Tables 4.5 and 4.6 show the percentages and relative risks of mortality and morbidity at 120 hpf. Figure 4.2 Shows the percentages of mortality and morbidity at 120 hpf.

The mortality rate is lower than the results from Travers 2004 study which found an MLD of 45 Gy. This is to be expected since zebrafish embryos are in rapid development which will make them more radiosensitive than adult zebrafish.

Tables 4.5 and 4.6 show the logit models for mortality and morbidity respectively. The logit models show statistical significance for mortality at over 20 Gy and morbidity over 10 Gy. Again the confidence intervals for morbidity are fairly large which indicates a low level of precision. Mortality at 30 Gy significantly increases to 52.06 times the odds of mortality at 0. Multiple morphological defects are apparent at ≥ 10 Gy. Morbidity at 10 Gy is 33.0 times the odds of mortality at 0.

Another reason why there is variation in the response variable is the nature of the Gammacell 220. Since the dose varies with placement within the Gammacell 220 each dose is more like a range rather than an exact dose. Table 4.9 shows the distribution of dose as exposure increases. At the low end there is only a variation of 5-6 Gy, at the high end there is a variation of 50-60 Gy.

Table 4.5 Cumulative mortality at 120 hpf (*statistically significant)

Dose	Cumulative Mortality	Total	% Mortality	Relative Risk	95 % Confidence Interval	
0	99	766	13%			
5	34	288	12%	0.91	0.63	1.32
10	32	288	11%	0.86	0.59	1.25
15	48	288	17%	1.29	0.93	1.77
20	81	288	28%	2.17*	1.68	2.82
25	54	287	19%	1.46*	1.08	1.97
30	255	288	89%	6.85*	5.67	8.27
35	250	288	87%	6.74*	5.57	8.14
40	239	287	83%	6.38*	5.26	7.72
45	281	288	98%	7.55*	6.27	9.08
50	270	288	94%	7.25*	6.02	8.74
	n	3644				
	χ^2	> 0.001				
	Degrees freedom	10				

Table 4.6 Morbidity at 120 hpf (*statistically significant)

Dose	Multiple Morphological Defects	Surviving Total	%Multiple Morphological Defects	Relative Risk	95 % Confidence Interval	
0	27	667	4%			
5	13	254	5%	1.26	0.66	2.40
10	149	256	58%	14.38*	9.80	21.10
15	239	240	100%	24.60*	17.00	35.60
20	205	207	99%	24.47*	16.90	35.41
25	205	233	88%	21.74*	14.98	31.55
30	24	33	73%	17.97*	11.75	27.47
35	35	37	95%	23.37*	16.02	34.08
40	49	51	96%	23.73*	16.34	34.49
45	7	7	100%	24.70*	17.07	35.75
50	18	18	100%	24.70*	17.07	35.75
	n	3644				
	χ^2	> 0.001				
	Degrees freedom	10				

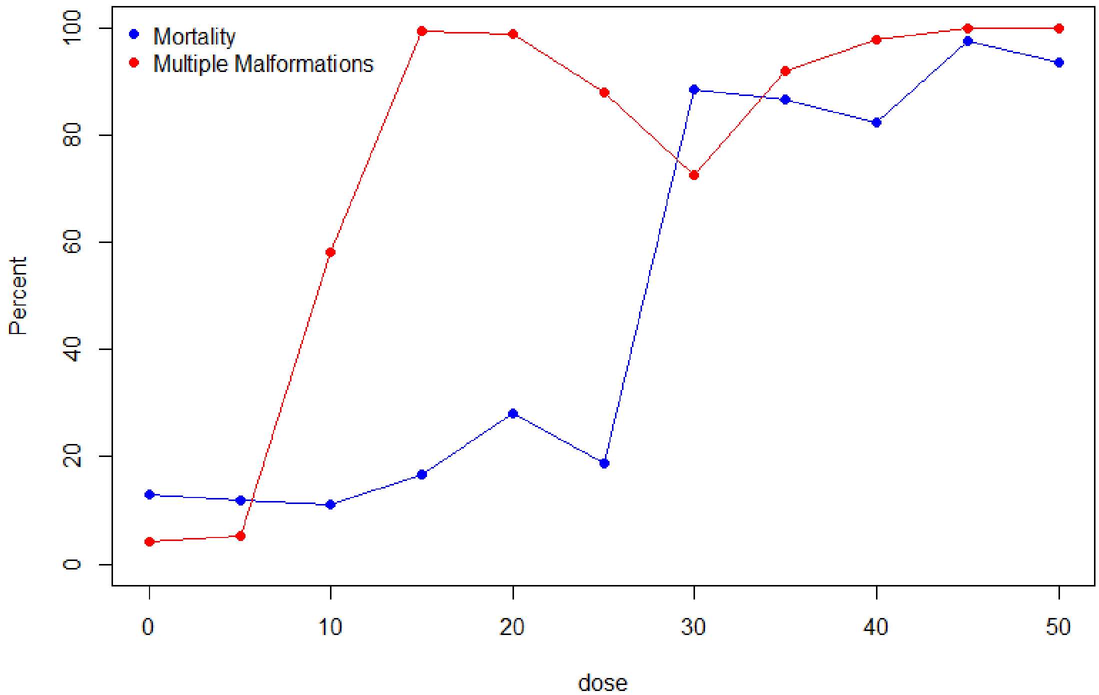


Figure 4.2 Mortality and multiple malformations in zebrafish at various doses at 120 hpf

Table 4.7 Log-odds model of mortality for fish at 120hpf (*statistically significant)

Dose	Estimate	Estimate (exp)	95% Confidence Interval (exp)	
0	-1.91	0.15*	0.12	0.18
5	-0.10	0.90	0.59	1.35
10	-0.17	0.84	0.54	1.27
15	0.30	1.35	0.92	1.95
20	0.97	2.64*	1.89	3.68
25	0.45	1.56*	1.08	2.24
30	3.95	52.06*	34.68	80.40
35	3.79	44.32*	30.01	67.05
40	3.46	31.94*	22.22	46.70
45	5.60	270.46*	133.47	648.72
50	4.62	101.06*	61.57	175.73

Table 4.8 Log-odds model of morbidity for fish at 120 hpf (*statistically significant)

Dose	Estimate	Estimate (exp)	95% Confidence Interval (exp)	
0	-3.17	$4.22x10^{-2}$*	$2.80\ x10^{-2}$	$6.07\ x10^{-2}$
5	0.25	$1.28\ x10^{0}$	$6.30\ x10^{-1}$	$2.47\ x10^{0}$
10	3.50	$3.30x10^{1}$*	$2.12\ x10^{0}$	$5.31\ x10^{1}$
15	8.64	$5.67x10^{3}$*	$1.20\ x10^{3}$	$1.01\ x10^{5}$
20	7.80	$2.43x10^{3}$*	$7.25\ x10^{2}$	$1.52\ x10^{4}$
25	5.16	$1.74x10^{2}$*	$1.02\ x10^{2}$	$3.08\ x10^{2}$
30	4.15	$6.32x10^{1}$*	$2.77\ x10^{1}$	$1.56\ x10^{2}$
35	5.62	$2.77x10^{2}$*	$9.24\ x10^{1}$	$1.20\ x10^{3}$
40	7.06	$1.61x10^{3}$*	$2.40\ x10^{2}$	$2.09\ x10^{4}$
45	27.98	$1.41x10^{12}$*	0.00	NA
50	28.82	$3.28x10^{12}$*	0.00	NA

Table 4.9 Various dose levels within the Gammacell 220

Dose at 100%	105%	110%	115%	120%
5	5.25	5.50	5.75	6.00
10	10.50	11.00	11.50	12.00
15	15.75	16.50	17.25	18.00
20	21.00	22.00	23.00	24.00
25	26.25	27.50	28.75	30.00
30	31.50	33.00	34.50	36.00
35	36.75	38.50	40.25	42.00
40	42.00	44.00	46.00	48.00
45	47.25	49.50	51.75	54.00
50	52.50	55.00	57.50	60.00

Four-Day-Old Dose

The zebrafish were irradiated at four dpf and examined at 5 dpf. Mortality, multiple morphological defects, and eye defects are all below 10% and fairly even across all doses. This is most likely due to examining the zebrafish after a short period of time. Fewer effects are apparent in the fish exposed at 4 dpf than those exposed at 24 hpf since embryo development has slowed down.

Tables 4.10, 4.11, and 4.12 show the percentages and relative risks of mortality, morbidity, and eye defects respectively at 4 dpf. Figure 4.3 depicts the percentages of the aforementioned assessments.

Tables 4.13, 4.14, and 15 show the logit models for mortality, morbidity, and eye defects respectively. As with the RR the logit models have no statistical significance. The various exposures show no discernable difference in mortality, morbidity, or eye defects. This was the goal of this portion of the study since the next phase will be to grow the fish out to 2-5 years-old.

Table 4.10 Mortality at 5 dpf

Dose	Cumulative Mortality	Total	% Mortality	Relative Risk	95 % Confidence Interval	
0	45	435	9.38%			
5	19	269	6.60%	0.70	0.42	1.18
10	26	262	9.03%	0.96	0.61	1.53
15	18	270	6.25%	0.67	0.39	1.13
20	20	268	6.94%	0.74	0.45	1.23
25	22	266	7.64%	0.81	0.50	1.32
	n	1920				
	χ^2	0.54				
	Degrees freedom	5				

Table 4.11 Morbidity at 5 dpf

Dose	**Multiple Morphological Defects**	**Surviving Total**	**% Defects**	**Relative Risk**	**95 % Confidence Interval**	
0	12	423	2.76%			
5	9	260	3.35%	1.21	0.52	2.84
10	8	254	3.07%	1.11	0.46	2.67
15	2	268	0.74%	0.27	0.06	1.19
20	5	263	1.87%	0.68	0.24	1.90
25	7	259	2.63%	0.95	0.38	2.39
	n	1737				
	χ^2	0.39				
	Degrees freedom	5				

Table 4.12 Eye defects at 5 dpf

Dose	Eye Defects	Surviving Total	% Eye defects	Relative Risk	95 % Confidence Interval	
0	11	424	2.53%			
5	5	264	1.86%	0.74	0.26	2.09
10	2	260	0.77%	0.30	0.07	1.35
15	4	266	1.48%	0.59	0.18	1.82
20	5	263	1.87%	0.74	0.25	2.10
25	6	260	2.26%	0.89	0.33	2.38
	n	1737				
	χ^2	0.67				
	Degrees Freedom	5				

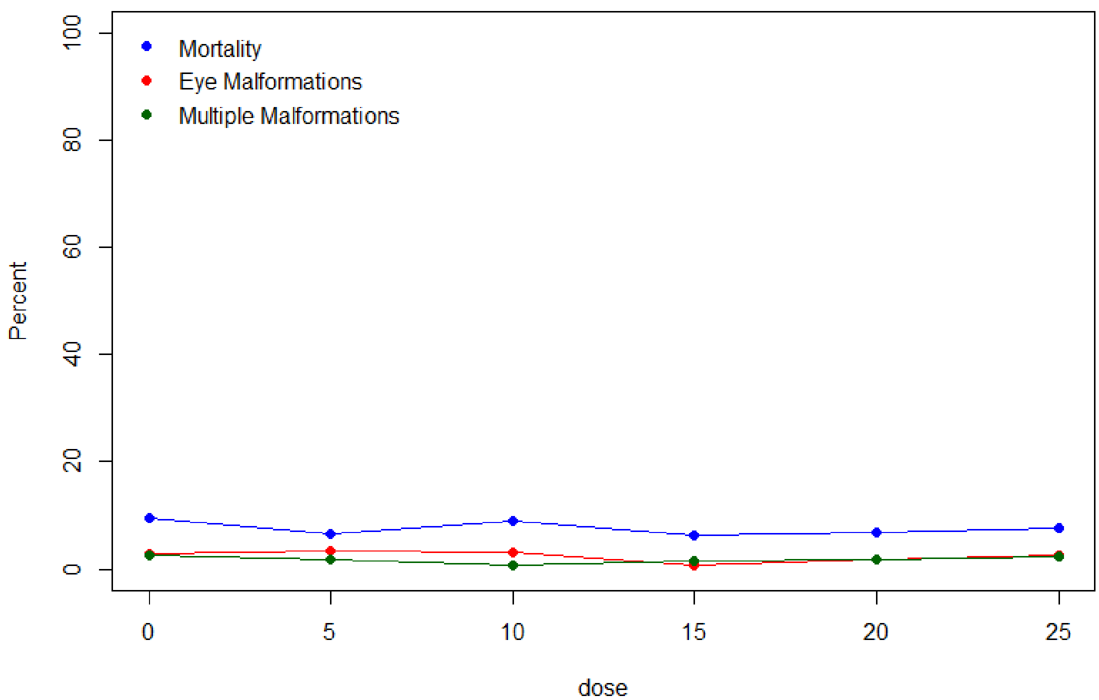


Figure 4.3 Mortality, multiple malformations, and eye defects in zebrafish at various doses at 4 dpf

Table 4.13 Log-odds model of mortality for fish at 4 dpf (*statistically significant)

Dose	Estimate	Estimate (exp)	95% Confidence Interval (exp)	
0	-2.27	0.10*	0.08	0.14
5	-0.38	0.68	0.38	1.17
10	-0.042	0.96	0.57	1.58
15	-0.44	0.64	0.36	1.12
20	-0.33	0.72	0.41	1.23
25	-0.22	0.80	0.46	1.34

Table 4.14 Log-odds model of morbidity for fish at 4 dpf (*statistically significant)

Dose	Estimate	Estimate (exp)	95% Confidence Interval (exp)	
0	-3.56	0.028*	0.015	0.048
5	0.20	1.22	0.49	2.92
10	0.10	1.11	0.43	2.72
15	-1.33	0.26*	0.041	0.97
20	-0.40	0.67	0.21	1.83
25	-0.049	0.95	0.35	2.40

Table 4.15 Log-odds model of eye defects for fish at 4 dpf (*statistically significant)

Dose	Estimate	Estimate (exp)	95% Confidence Interval (exp)	
0	-3.65	0.026*	0.013	0.045
5	-0.31	0.73	0.23	2.03
10	-1.22	0.30	0.046	1.12
15	-0.55	0.58	0.16	1.71
20	-0.31	0.73	0.23	2.03
25	-0.11	0.89	0.30	2.37

Experimental Design of Stage Two

The next stage of this experiment will be carried out by another researcher. Several more steps need to be completed prior to initiation of stage two. The exact dose and shielding configuration will need to be calculated in MCNP. In addition, the researcher will need to construct the tank, acquire all necessary detectors, and meet approval of the Animal Care Use and Proposal.

Chapter 5 Conclusion

The results presented in this work followed a similar pattern to previous studies examining the radiosensitivity of zebrafish. At low doses there tends to be no discernable effect. Mid-ranged doses of ionizing radiation show a marked increase in malformations and high doses show a marked increase in mortality. Age of exposure is also important regarding radiosensitivity. Younger embryos are more radiosensitive than older embryos and much more radiosensitive than adult zebrafish.

Future work will expose adult zebrafish to ionizing radiation and observe mortality and morbidity over a two to five year period with the main objective of establishing a threshold for cataract formation in adult zebrafish. Finally, the zebrafish will then be exposed at the RHIC to determine the difference in damage from HCP, β-particles, and γ-rays.

Space radiation biology is an emerging field of research with many unknowns. Establishing guidelines and protection for astronauts from space radiation is of utmost importance for future crewed deep-space missions. If a threshold for cataract formation in zebrafish can be equated to the threshold for cataract formation in astronauts then zebrafish could potentially be made into a biosensor for ionizing radiation exposure during space missions.

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Appendices

Appendix A: R Code

```
#################
###Relative Risk###
#################

calcRelativeRisk <- function(mymatrix,alpha=0.05,referencerow=2)
{
 numrow <- nrow(mymatrix)
 myrownames <- rownames(mymatrix)
 for (i in 1:numrow)
 {
  rowname <- myrownames[i]
  DiseaseUnexposed <- mymatrix[referencerow,1]
  ControlUnexposed <- mymatrix[referencerow,2]
  if (i != referencerow)
  {
   DiseaseExposed <- mymatrix[i,1]
   ControlExposed <- mymatrix[i,2]
   totExposed <- DiseaseExposed + ControlExposed
   totUnexposed <- DiseaseUnexposed + ControlUnexposed
   probDiseaseGivenExposed <- DiseaseExposed/totExposed
   probDiseaseGivenUnexposed <- DiseaseUnexposed/totUnexposed

   # calculate the relative risk
   relativeRisk <- probDiseaseGivenExposed/probDiseaseGivenUnexposed
   print(paste("category =", rowname, ", relative risk = ",relativeRisk))

   # calculate a confidence interval
   confidenceLevel <- (1 - alpha)*100
   sigma <- sqrt((1/DiseaseExposed) - (1/totExposed) +
            (1/DiseaseUnexposed) - (1/totUnexposed))
   # sigma is the standard error of estimate of log of relative risk
   z <- qnorm(1-(alpha/2))
   lowervalue <- relativeRisk * exp(-z * sigma)
   uppervalue <- relativeRisk * exp( z * sigma)
   print(paste("category =", rowname, ", ", confidenceLevel,
         "% confidence interval = [",lowervalue,",",uppervalue,"]"))
  }
 }
}
```

```
###########
###24hpf###
###########

##Mortality##

mymatrix <-
matrix(c(34,254,20,268,27,261,42,244,34,253,15,273,19,268,18,270,18,270,44,244,58,71
9),nrow=11,byrow=TRUE)

colnames(mymatrix) <- c("Mortality","Survival")
rownames(mymatrix) <-
c("5Gray","10Gray","15Gray","20Gray","25Gray","30Gray","35Gray","40Gray","45Gra
y","50Gray","Unexposed")
print(mymatrix)

calcRelativeRisk(mymatrix, referencerow=11)
chisq.test(mymatrix)

##mobidity##

mymatrix <-
matrix(c(0,254,6,262,3,258,3,241,4,249,2,271,6,262,7,263,87,183,113,131,11,612),nrow
=11,byrow=TRUE)

colnames(mymatrix) <- c("Defects","No Defects")
rownames(mymatrix) <-
c("5Gray","10Gray","15Gray","20Gray","25Gray","30Gray","35Gray","40Gray","45Gra
y","50Gray","Unexposed")
print(mymatrix)

calcRelativeRisk(mymatrix, referencerow=11)
chisq.test(mymatrix)
```

```
############
###120hpf###
############

##Mortality##

mymatrix <-
matrix(c(34,254,32,256,48,240,81,207,54,233,255,33,250,37,239,51,281,7,270,18,99,667
),nrow=11,byrow=TRUE)

colnames(mymatrix) <- c("Mortality","Survival")
rownames(mymatrix) <-
c("5Gray","10Gray","15Gray","20Gray","25Gray","30Gray","35Gray","40Gray","45Gra
y","50Gray","Unexposed")
print(mymatrix)

calcRelativeRisk(mymatrix, referencerow=11)
chisq.test(mymatrix)

##mobidity##

mymatrix <-
matrix(c(13,241,149,107,239,1,205,2,205,28,24,9,35,2,49,2,7,0,18,0,27,640),nrow=11,by
row=TRUE)

colnames(mymatrix) <- c("Defects","No Defects")
rownames(mymatrix) <-
c("5Gray","10Gray","15Gray","20Gray","25Gray","30Gray","35Gray","40Gray","45Gra
y","50Gray","Unexposed")
print(mymatrix)

calcRelativeRisk(mymatrix, referencerow=11)
chisq.test(mymatrix)
```

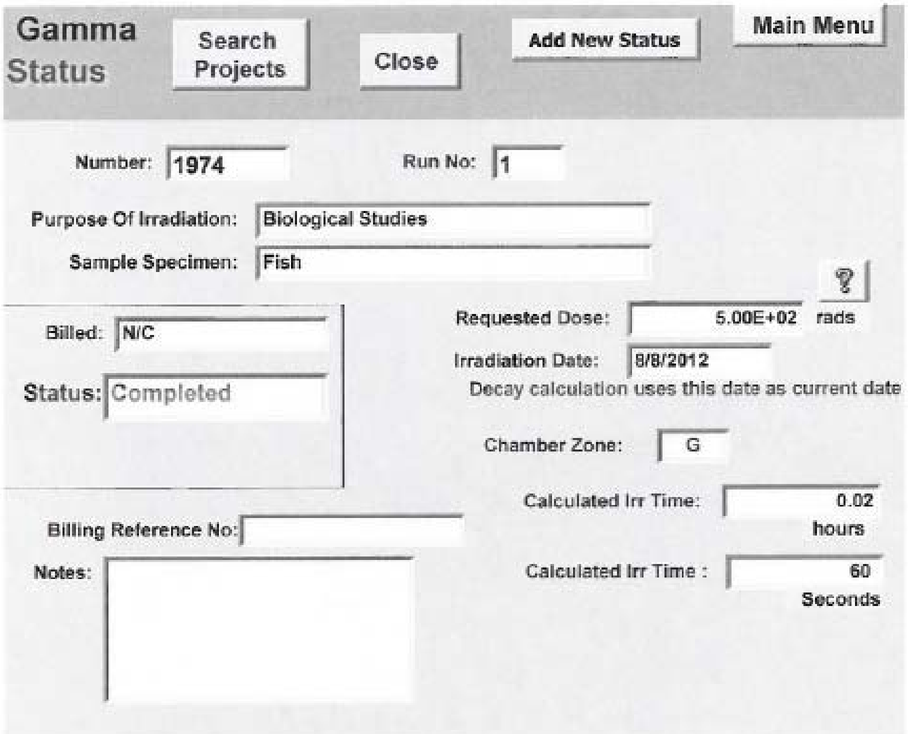
```
##########
###4dpf###
##########

##Mortality##

mymatrix <-
matrix(c(19,269,26,262,18,270,20,268,22,266,45,435),nrow=6,byrow=TRUE)

colnames(mymatrix) <- c("Mortality","Survival")
rownames(mymatrix) <-
c("5Gray","10Gray","15Gray","20Gray","25Gray","Unexposed")
print(mymatrix)

calcRelativeRisk(mymatrix, referencerow=6)
chisq.test(mymatrix)

##defects##

mymatrix <-
matrix(c(9,260,8,254,2,268,5,263,7,259,12,423),nrow=6,byrow=TRUE)

colnames(mymatrix) <- c("Mortality","Survival")
rownames(mymatrix) <-
c("5Gray","10Gray","15Gray","20Gray","25Gray","Unexposed")
print(mymatrix)

calcRelativeRisk(mymatrix, referencerow=6)
chisq.test(mymatrix)

##eye##

mymatrix <-
matrix(c(5,264,2,260,4,266,5,263,6,260,11,424),nrow=6,byrow=TRUE)

colnames(mymatrix) <- c("Mortality","Survival")
rownames(mymatrix) <-
c("5Gray","10Gray","15Gray","20Gray","25Gray","Unexposed")
print(mymatrix)

calcRelativeRisk(mymatrix, referencerow=6)
chisq.test(mymatrix)
```

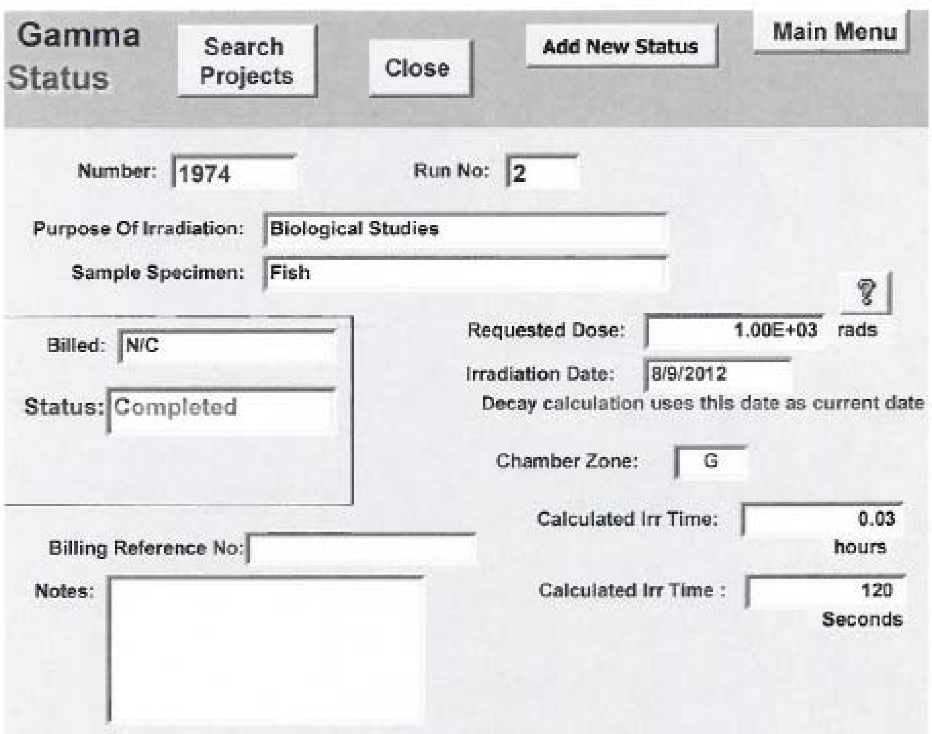
```
###########
###24hpf###
###########
hpf <- read.delim("C:/Users/haskinsh/Dropbox/thesis/Rfiles/hpf.txt")
 View(hpf)
attach(hpf)

mort24hpf= glm(cbind(mort_24,surv_24)~factor(dose),family=binomial)
summary(mort24hpf)
exp(coef(mort24hpf))
exp(confint(mort24hpf))

###
morb24hpf= glm(cbind(dp,no_dp)~factor(dose),family=binomial)
summary(morb24hpf)
exp(coef(morb24hpf))
exp(confint(morb24hpf))

###Plot###

plot(dose,(mort_24*100/(mort_24+surv_24)), col=c("Blue"), pch=16, type="o",
    ylim=c(0,100), ylab="Percent")
points(dose,(dp*100/(dp+no_dp)), col=c("red"), pch=16,type="o")

legend("topleft", c("Mortality", "Multiple Malformations"),
      col=c("blue","red"), pch=16, bty="n")
```

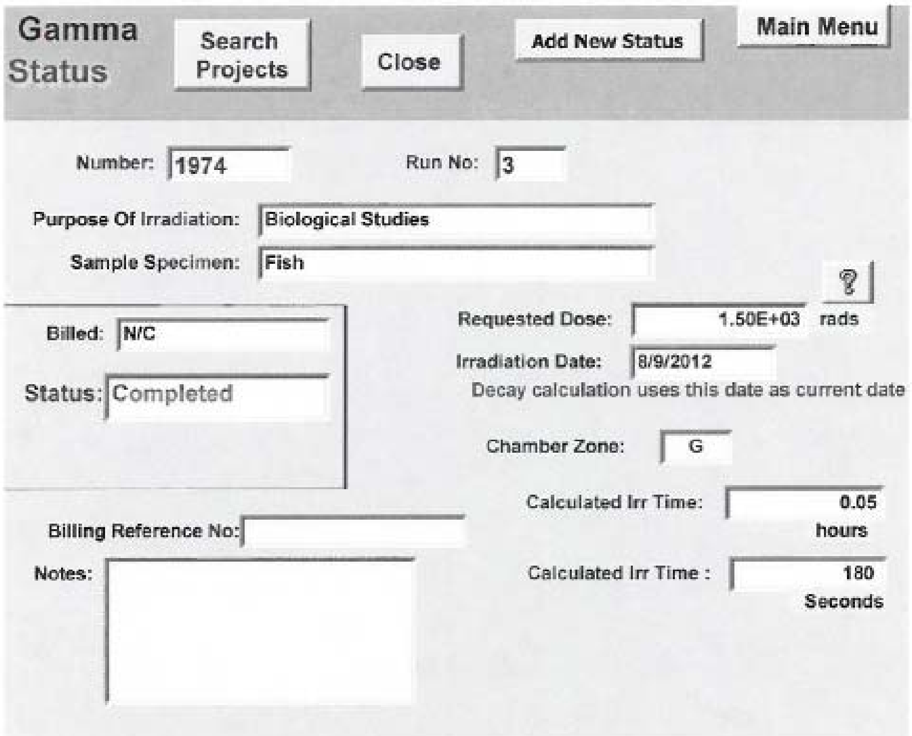
```
############
###120hpf###
############

mort120hpf= glm(cbind(mort_120,surv_120)~factor(dose),family=binomial)
summary(mort120hpf)
exp(coef(mort120hpf))
exp(confint(mort120hpf))

###
morb120hpf= glm(cbind(yse,no_yse)~factor(dose),family=binomial)
summary(morb120hpf)
exp(coef(morb120hpf))
exp(confint(morb120hpf))
###Plot###

plot(dose,(mort_120*100/(mort_120+surv_120)), col=c("Blue"), pch=16,
   type="o", ylim=c(0,100), ylab="Percent")
points(dose,(yse*100/(yse+no_yse)), col=c("red"), pch=16, type="o")

legend("topleft", c("Mortality", "Multiple Malformations"),
      col=c("blue","red"), pch=16, bty="n")

##########
###4dpf###
##########
dpf <- read.delim("C:/Users/haskinsh/Dropbox/thesis/Rfiles/dpf.txt")
View(dpf)
attach(dpf)

###
mortdpf= glm(cbind(mort,surv)~factor(dose),family=binomial)
summary(mortdpf)
exp(coef(mortdpf))
exp(confint(mortdpf))

###
morbdpf= glm(cbind(yse,no_yse)~factor(dose),family=binomial)
summary(morbdpf)
exp(coef(morbdpf))
exp(confint(morbdpf))
```

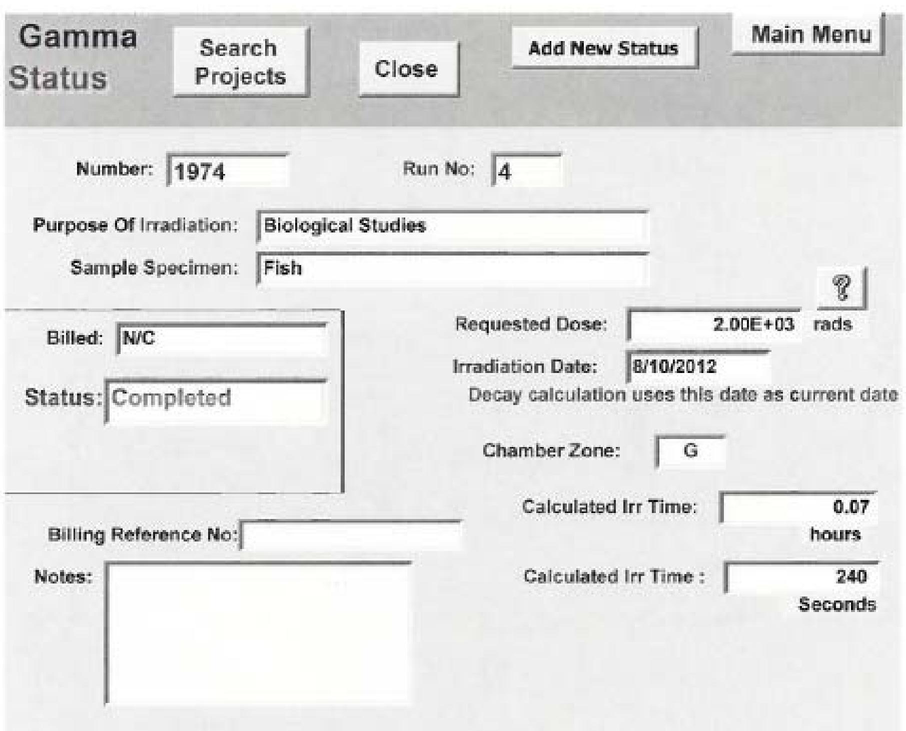
```
###
eyedpf= glm(cbind(eye,no_eye)~factor(dose),family=binomial)
summary(eyedpf)
exp(coef(eyedpf))
exp(confint(eyedpf))

###Plot###

plot(dose,(mort*100/(mort+surv)), col=c("Blue"), pch=16,type="o",
    ylim=c(0,100), ylab="Percent")
points(dose,(yse*100/(yse+no_yse)), col=c("red"), pch=16,type="o")
points(dose,(eye*100/(eye+no_eye)), col=c("darkgreen"), pch=16,type="o")

legend("topleft", c("Mortality", "Eye Malformations", "Multiple Malformations"),
      col=c("blue","red","darkgreen"), pch=16, bty="n")
```

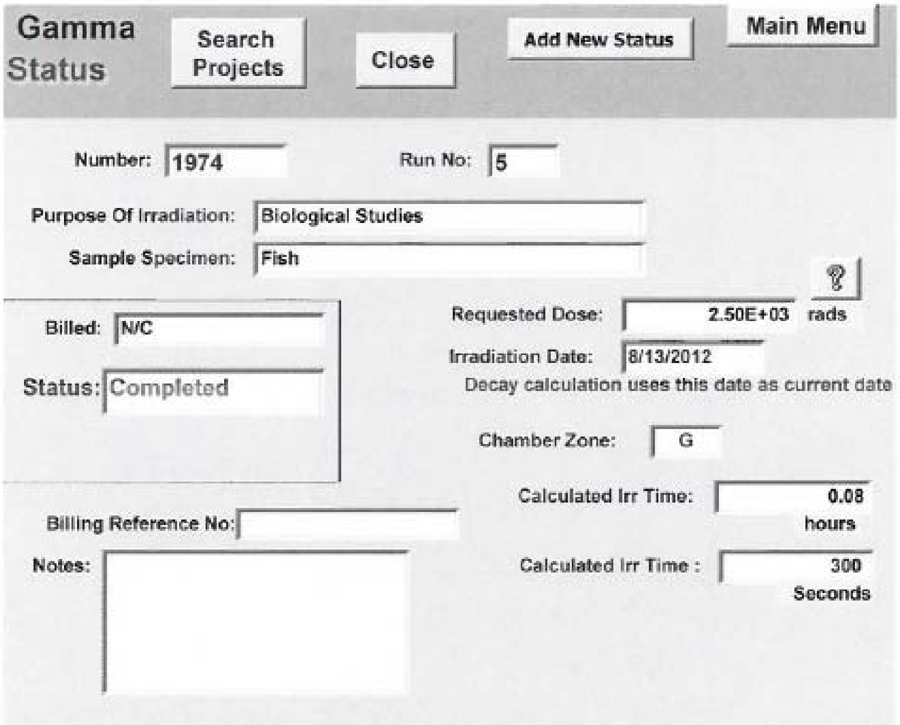
Appendix B: Radiation Center Gamma Cell Exposure Times


Gamma Status
Search Projects
Close
Add New Status
Main Menu
Number: 1974
Run No: 1
Purpose Of Irradiation: Biological Studies
Sample Specimen: Fish
Billed: N/C
Status: Completed
Requested Dose: 5.00E+02 rads
Irradiation Date: 8/8/2012
Decay calculation uses this date as current date
Chamber Zone: G
Calculated Irr Time: 0.02 hours
Billing Reference No:
Notes:
Calculated Irr Time : 60 Seconds


Gamma Status
Search Projects
Close
Add New Status
Main Menu
Number: 1974
Run No: 2
Purpose Of Irradiation: Biological Studies
Sample Specimen: Fish
Billed: N/C
Status: Completed
Requested Dose: 1.00E+03 rads
Irradiation Date: 8/9/2012
Decay calculation uses this date as current date
Chamber Zone: G
Calculated Irr Time: 0.03 hours
Billing Reference No:
Notes:
Calculated Irr Time : 120 Seconds


Gamma Status
Search Projects
Close
Add New Status
Main Menu
Number: 1974
Run No: 3
Purpose Of Irradiation: Biological Studies
Sample Specimen: Fish
Billed: N/C
Status: Completed
Requested Dose: 1.50E+03 rads
Irradiation Date: 8/9/2012
Decay calculation uses this date as current date
Chamber Zone: G
Calculated Irr Time: 0.05 hours
Calculated Irr Time : 180 Seconds
Billing Reference No:
Notes:


Gamma Status
Search Projects
Close
Add New Status
Main Menu
Number: 1974
Run No: 4
Purpose Of Irradiation: Biological Studies
Sample Specimen: Fish
Billed: N/C
Status: Completed
Requested Dose: 2.00E+03 rads
Irradiation Date: 8/10/2012
Decay calculation uses this date as current date
Chamber Zone: G
Calculated Irr Time: 0.07 hours
Calculated Irr Time : 240 Seconds
Billing Reference No:
Notes:



Gamma Status | Search Projects | Close | Add New Status | Main Menu

Number: 1974   Run No: 5

Purpose Of Irradiation: Biological Studies

Sample Specimen: Fish

Billed: N/C

Status: Completed

Requested Dose: 2.50E+03 rads

Irradiation Date: 8/13/2012

Decay calculation uses this date as current date

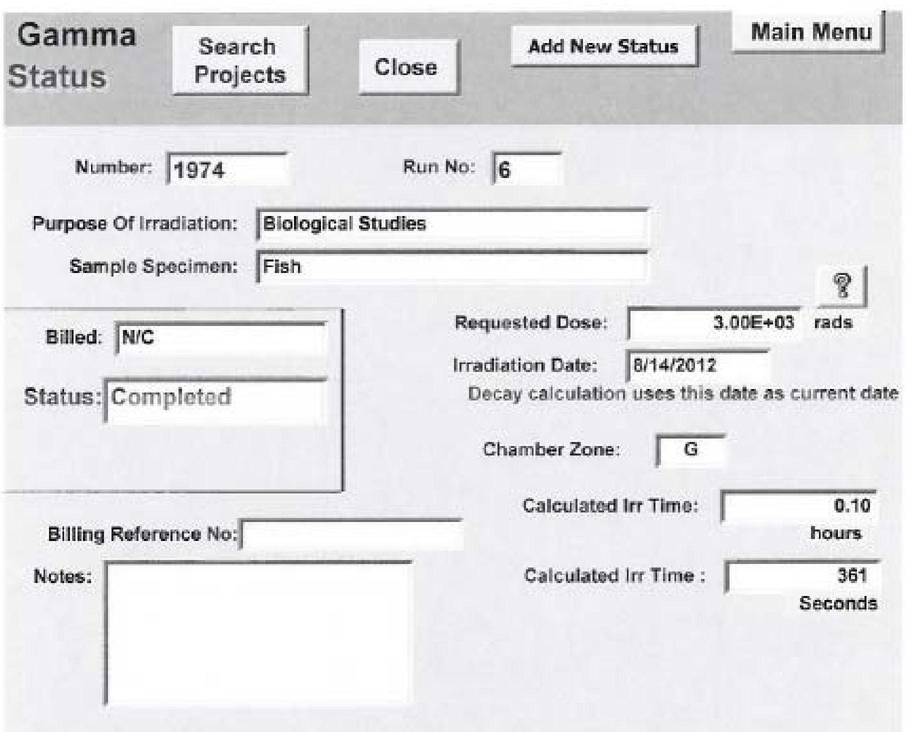
Chamber Zone: G

Calculated Irr Time: 0.08 hours

Calculated Irr Time : 300 Seconds

Billing Reference No:

Notes:



Gamma Status | Search Projects | Close | Add New Status | Main Menu

Number: 1974   Run No: 6

Purpose Of Irradiation: Biological Studies

Sample Specimen: Fish

Billed: N/C

Status: Completed

Requested Dose: 3.00E+03 rads

Irradiation Date: 8/14/2012

Decay calculation uses this date as current date

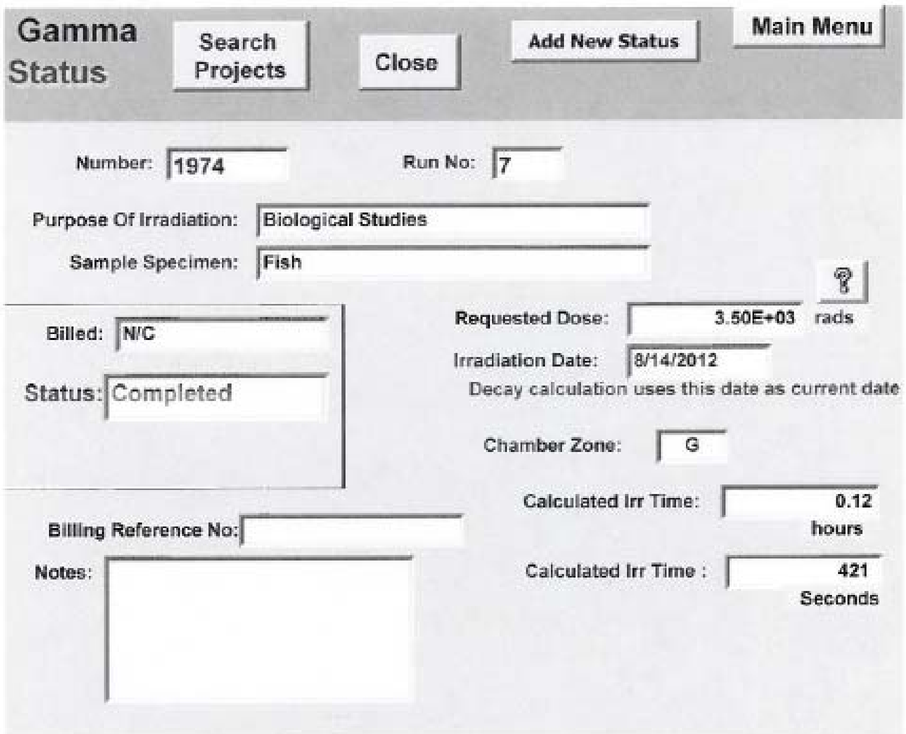
Chamber Zone: G

Calculated Irr Time: 0.10 hours

Calculated Irr Time : 361 Seconds

Billing Reference No:

Notes:



Gamma Status | Search Projects | Close | Add New Status | Main Menu

Number: 1974 | Run No: 7

Purpose Of Irradiation: Biological Studies

Sample Specimen: Fish

Billed: N/C

Status: Completed

Billing Reference No:

Notes:

Requested Dose: 3.50E+03 rads

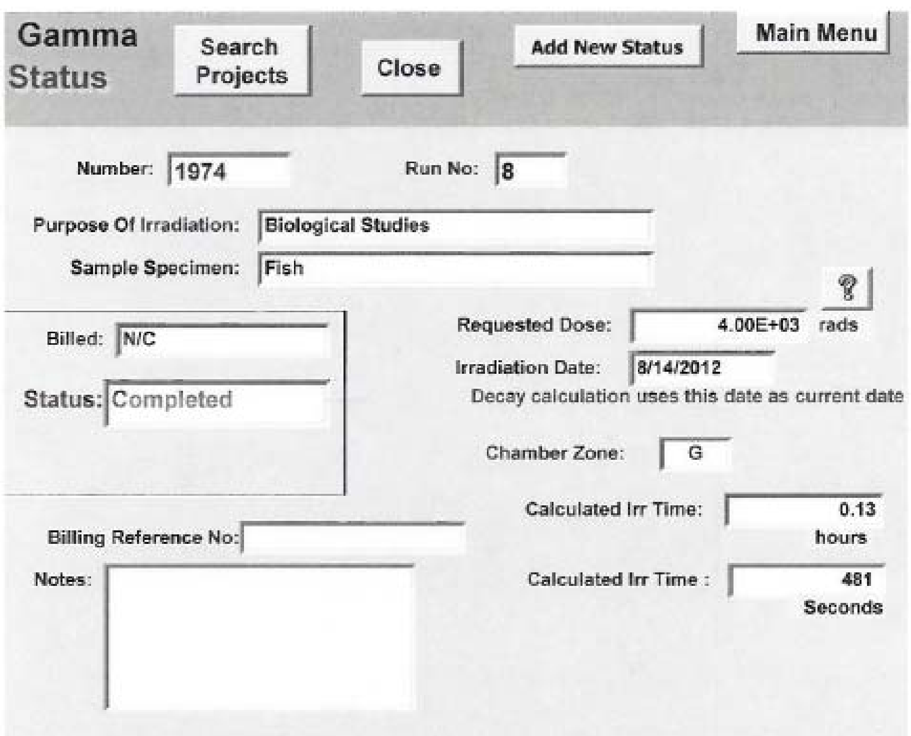
Irradiation Date: 8/14/2012

Decay calculation uses this date as current date

Chamber Zone: G

Calculated Irr Time: 0.12 hours

Calculated Irr Time : 421 Seconds



Gamma Status | Search Projects | Close | Add New Status | Main Menu

Number: 1974 | Run No: 8

Purpose Of Irradiation: Biological Studies

Sample Specimen: Fish

Billed: N/C

Status: Completed

Billing Reference No:

Notes:

Requested Dose: 4.00E+03 rads

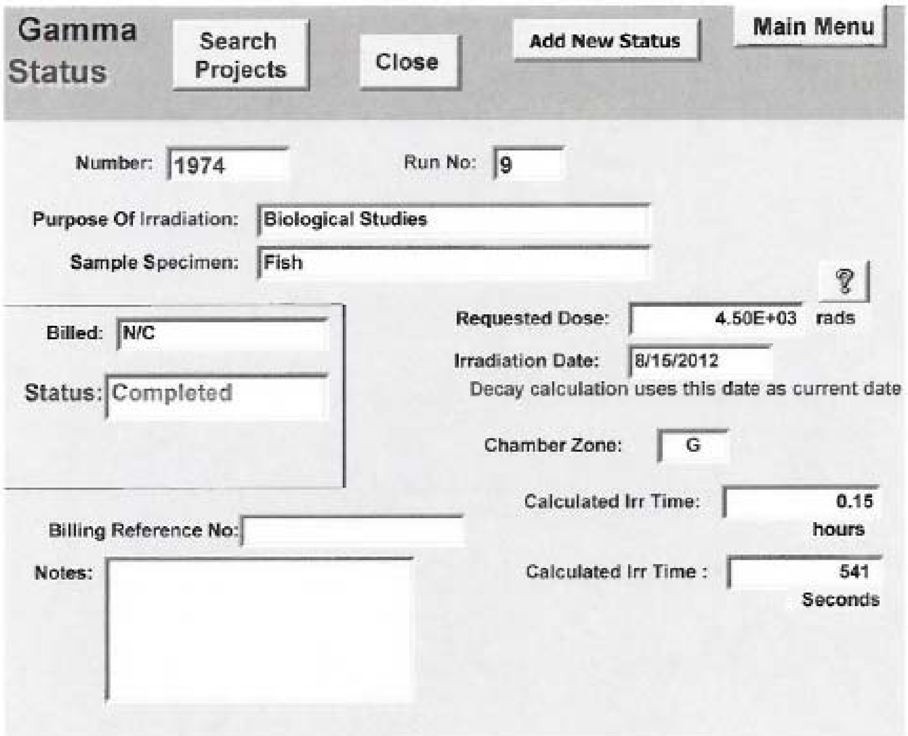
Irradiation Date: 8/14/2012

Decay calculation uses this date as current date

Chamber Zone: G

Calculated Irr Time: 0.13 hours

Calculated Irr Time : 481 Seconds



Gamma Status | Search Projects | Close | Add New Status | Main Menu

Number: 1974 Run No: 9

Purpose Of Irradiation: Biological Studies

Sample Specimen: Fish

Billed: N/C

Status: Completed

Requested Dose: 4.50E+03 rads

Irradiation Date: 8/15/2012

Decay calculation uses this date as current date

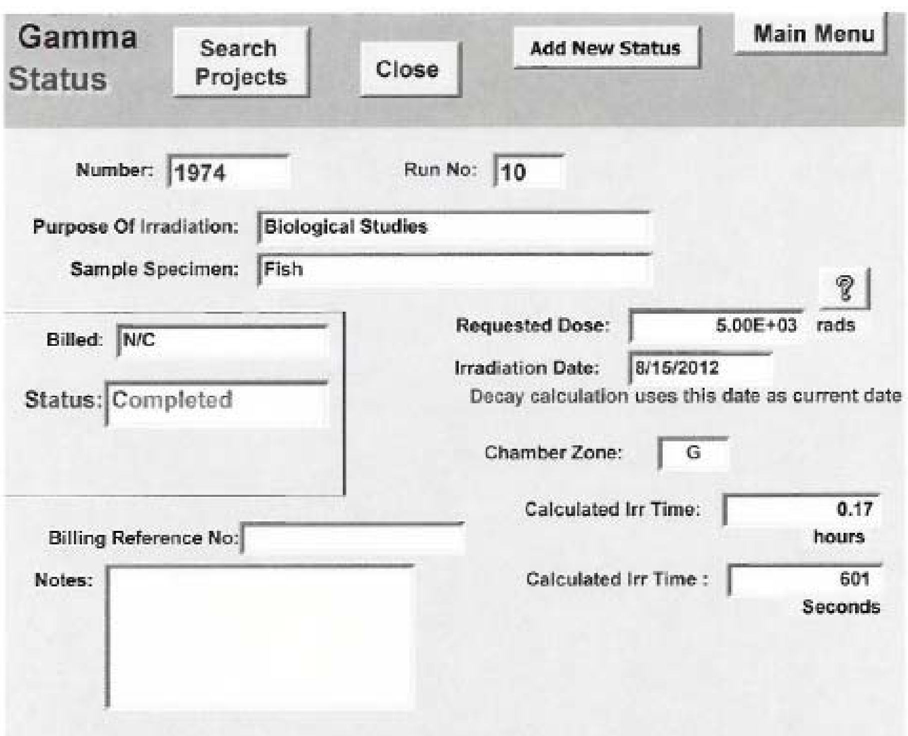
Chamber Zone: G

Calculated Irr Time: 0.15 hours

Calculated Irr Time : 541 Seconds

Billing Reference No:

Notes:



Gamma Status | Search Projects | Close | Add New Status | Main Menu

Number: 1974 Run No: 10

Purpose Of Irradiation: Biological Studies

Sample Specimen: Fish

Billed: N/C

Status: Completed

Requested Dose: 5.00E+03 rads

Irradiation Date: 8/15/2012

Decay calculation uses this date as current date

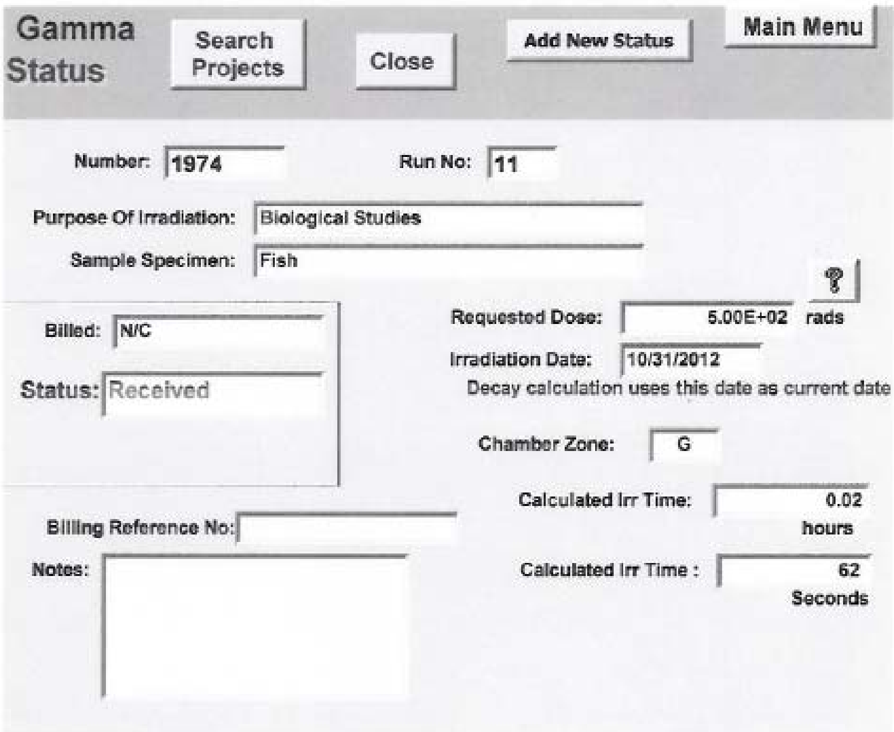
Chamber Zone: G

Calculated Irr Time: 0.17 hours

Calculated Irr Time : 601 Seconds

Billing Reference No:

Notes:



Gamma Status | Search Projects | Close | Add New Status | Main Menu

Number: 1974 Run No: 11

Purpose Of Irradiation: Biological Studies

Sample Specimen: Fish

Billed: N/C

Status: Received

Requested Dose: 5.00E+02 rads

Irradiation Date: 10/31/2012

Decay calculation uses this date as current date

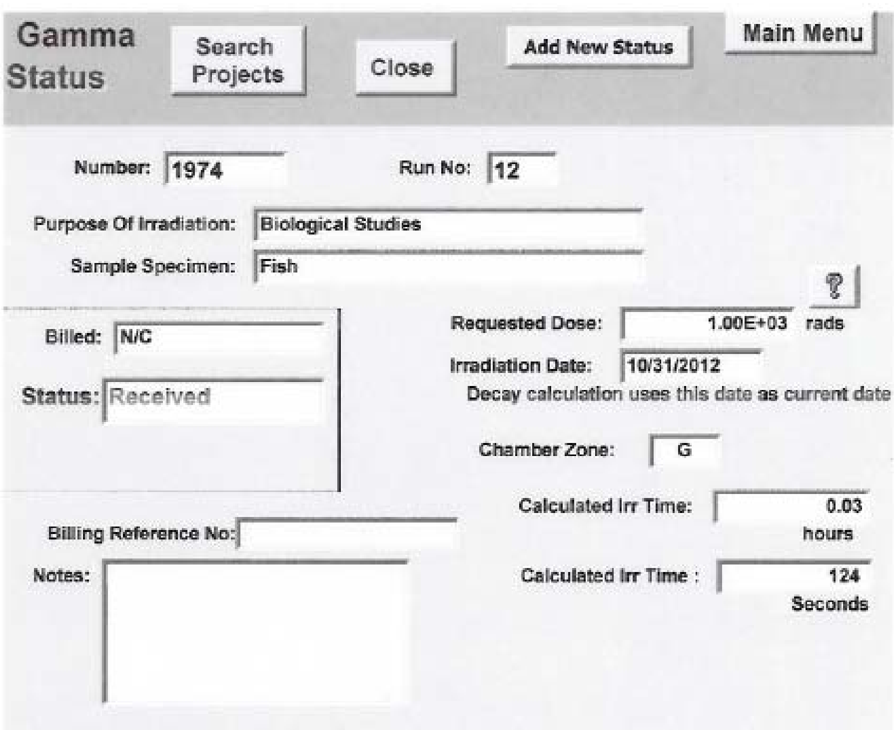
Chamber Zone: G

Calculated Irr Time: 0.02 hours

Calculated Irr Time : 62 Seconds

Billing Reference No:

Notes:



Gamma Status | Search Projects | Close | Add New Status | Main Menu

Number: 1974 Run No: 12

Purpose Of Irradiation: Biological Studies

Sample Specimen: Fish

Billed: N/C

Status: Received

Requested Dose: 1.00E+03 rads

Irradiation Date: 10/31/2012

Decay calculation uses this date as current date

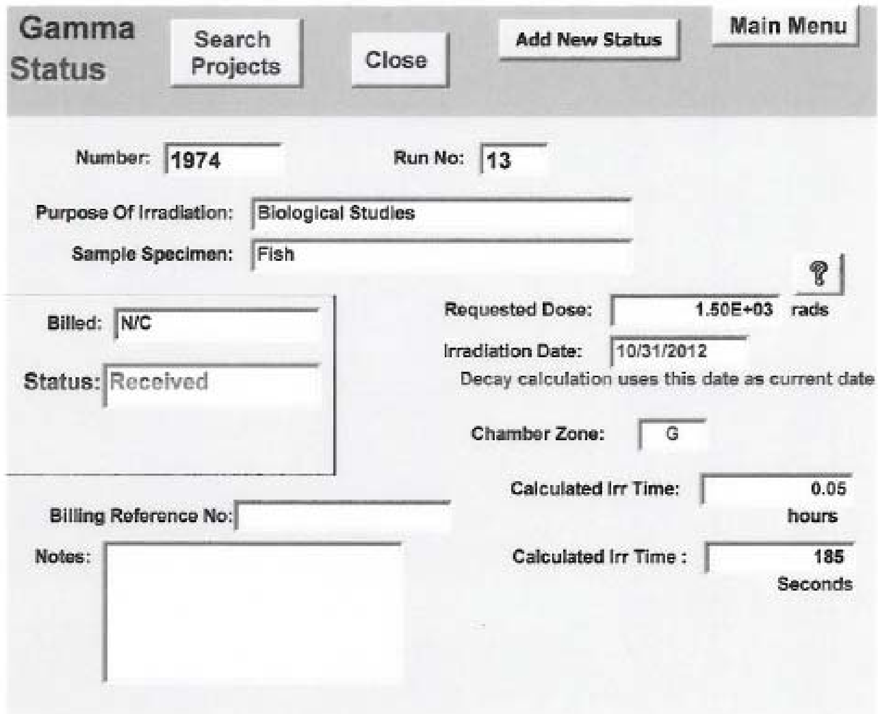
Chamber Zone: G

Calculated Irr Time: 0.03 hours

Calculated Irr Time : 124 Seconds

Billing Reference No:

Notes:



Gamma Status | Search Projects | Close | Add New Status | Main Menu

Number: 1974 Run No: 13

Purpose Of Irradiation: Biological Studies

Sample Specimen: Fish

Billed: N/C

Status: Received

Requested Dose: 1.50E+03 rads

Irradiation Date: 10/31/2012

Decay calculation uses this date as current date

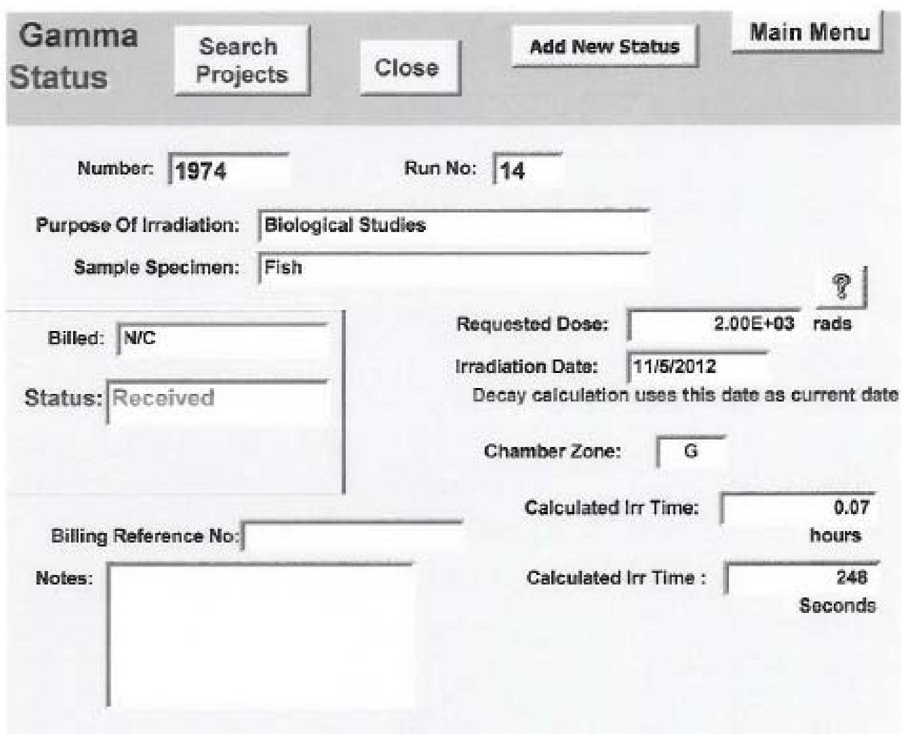
Chamber Zone: G

Calculated Irr Time: 0.05 hours

Calculated Irr Time : 185 Seconds

Billing Reference No:

Notes:



Gamma Status | Search Projects | Close | Add New Status | Main Menu

Number: 1974 Run No: 14

Purpose Of Irradiation: Biological Studies

Sample Specimen: Fish

Billed: N/C

Status: Received

Requested Dose: 2.00E+03 rads

Irradiation Date: 11/5/2012

Decay calculation uses this date as current date

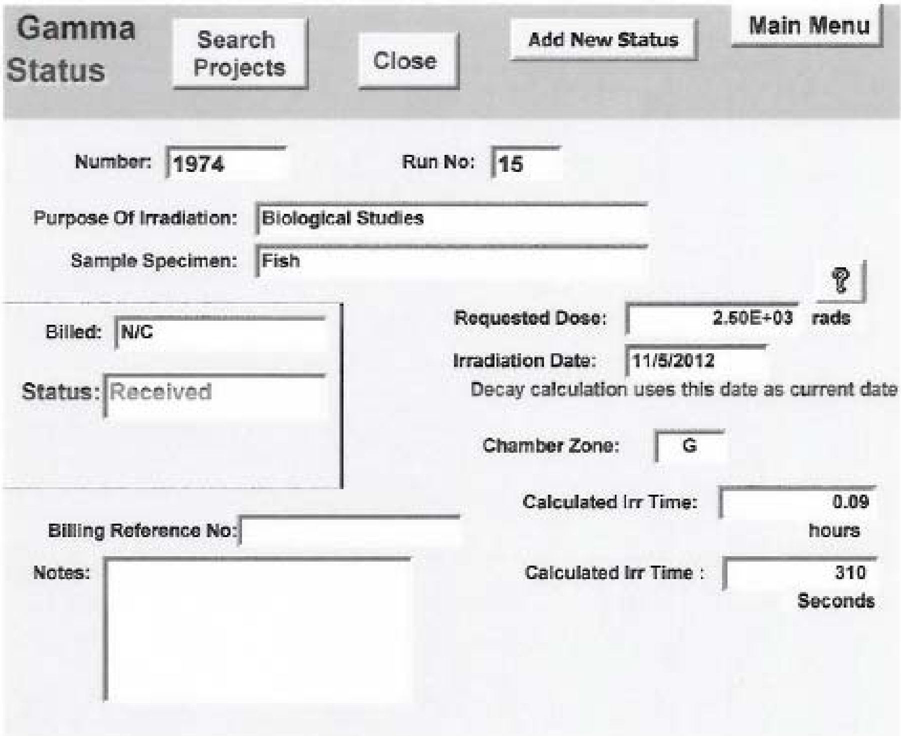
Chamber Zone: G

Calculated Irr Time: 0.07 hours

Calculated Irr Time : 248 Seconds

Billing Reference No:

Notes:


Gamma Status
Search Projects
Close
Add New Status
Main Menu
Number: 1974
Run No: 15
Purpose Of Irradiation: Biological Studies
Sample Specimen: Fish
Billed: N/C
Status: Received
Requested Dose: 2.50E+03 rads
Irradiation Date: 11/5/2012
Decay calculation uses this date as current date
Chamber Zone: G
Calculated Irr Time: 0.09 hours
Calculated Irr Time : 310 Seconds
Billing Reference No:
Notes:

Appendix C: 24 Hours-Old Dose Response Raw Data

ID	CONC	ConcNumber	MO24	DP24	MORT	YSE_
Radiation | 1 | |0 CONT	0 CONT	0	11|96	0	15|96	4|81
Radiation | 11 | |0 CONT	0 CONT	0	10|192	3|182	23|192	7|169
Radiation | 15 | |0 CONT	0 CONT	0	9|95	1|86	11|95	8|84
Radiation | 19 | |0 CONT	0 CONT	0	9|95	1|86	18|95	2|77
Radiation | 29 | |0 CONT	0 CONT	0	4|96	2|92	7|96	3|89
Radiation | 36 | |0 CONT	0 CONT	0	15|192	4|177	25|192	3|167
Radiation | 4 | |5 B	5 B	5	9|96	0	9|96	7|87
Radiation | 3 | |5 M	5 M	5	8|96	0	8|96	4|88
Radiation | 2 | |5 T	5 T	5	7|96	0	17|96	2|79
Radiation | 5 | |10 B	10 B	10	3|96	2|93	4|96	67|92
Radiation | 6 | |10 M	10 M	10	12|96	4|84	15|96	60|81
Radiation | 7 | |10 T	10 T	10	5|96	0	13|96	22|83
Radiation | 8 | |15 B	15 B	15	4|96	2|92	7|96	88|89
Radiation | 9 | |15 M	15 M	15	6|96	1|90	17|96	79|79
Radiation | 10 | |15 T	15 T	15	17|96	0	24|96	72|72
Radiation | 12 | |20 B	20 B	20	33|96	2|63	49|96	46|47
Radiation | 13 | |20 M	20 M	20	5|96	1|91	15|96	80|81
Radiation | 14 | |20 T	20 T	20	6|96	0	17|96	79|79
Radiation | 16 | |25 B	25 B	25	11|95	0	15|95	76|80
Radiation | 17 | |25 M	25 M	25	12|96	2|84	19|96	69|77
Radiation | 18 | |25 T	25 T	25	11|96	2|85	20|96	60|76
Radiation | 23 | |30 B	30 B	30	5|96	0	93|96	3|3
Radiation | 24 | |30 M	30 M	30	6|96	2|90	81|96	15|15
Radiation | 25 | |30 T	30 T	30	4|96	0	81|96	6|15
Radiation | 26 | |35 B	35 B	35	7|95	2|88	83|95	10|12
Radiation | 27 | |35 M	35 M	35	10|96	2|86	85|96	11|11
Radiation | 28 | |35 T	35 T	35	2|96	2|94	82|96	14|14
Radiation | 20 | |40 B	40 B	40	4|96	1|92	74|96	21|22
Radiation | 21 | |40 M	40 M	40	8|96	4|88	92|96	4|4
Radiation | 22 | |40 T	40 T	40	6|96	2|90	71|96	24|25
Radiation | 30 | |45 B	45 B	45	6|96	58|90	94|96	2|2
Radiation | 31 | |45 M	45 M	45	4|96	11|92	93|96	3|3
Radiation | 32 | |45 T	45 T	45	8|96	18|88	94|96	2|2
Radiation | 33 | |50 B	50 B	50	12|96	60|84	79|96	17|17
Radiation | 34 | |50 M	50 M	50	12|96	42|84	95|96	1|1
Radiation | 35 | |50 T	50 T	50	20|96	11|76	96|96	0

Appendix D: Five-Day-Old Dose Response Raw Data

CONC	ID	ConcNumber	MO24	DP24	SM24	NC24
0 gra5	Radiation | 10 | |0 gra5	0	0	0	0	0
0 gra10	Radiation | 6 | |0 gra10	0	0	0	0	0
0 gra15	Radiation | 8 | |0 gra15	0	0	0	0	0
0 gra 20	Radiation | 2 | |0 gra 20	0	0	0	0	0
0 gra 25	Radiation | 4 | |0 gra 25	0	0	0	0	0
5 gra	Radiation | 9 | |5 gra	5	0	0	0	0
10 gra	Radiation | 5 | |10 gra	10	0	0	0	0
15 gra	Radiation | 7 | |15 gra	15	0	0	0	0
20 gra	Radiation | 1 | |20 gra	20	0	0	0	0
25 gra	Radiation | 3 | |25 gra	25	0	0	0	0
0	NAME|0	0	0	0	0	0

ConcNumber	MORT	YSE_	AXIS	EYE_	SNOU	JAW_
0	2|96	4|94	1|94	2|94	3|94	3|94
0	14|96	3|82	2|82	2|82	2|82	2|82
0	9|96	1|87	1|87	2|87	2|87	2|87
0	9|96	1|87	1|87	2|87	2|87	1|87
0	11|96	3|85	3|85	3|85	3|85	3|85
5	19|288	9|269	9|269	5|269	6|269	5|269
10	26|287	8|261	5|261	2|261	2|261	2|261
15	18|288	2|270	3|270	4|270	10|270	16|270
20	20|288	5|268	5|268	5|268	10|268	11|268
25	22|288	7|266	5|266	6|266	6|266	6|266
0	0	0	0	0	0	0

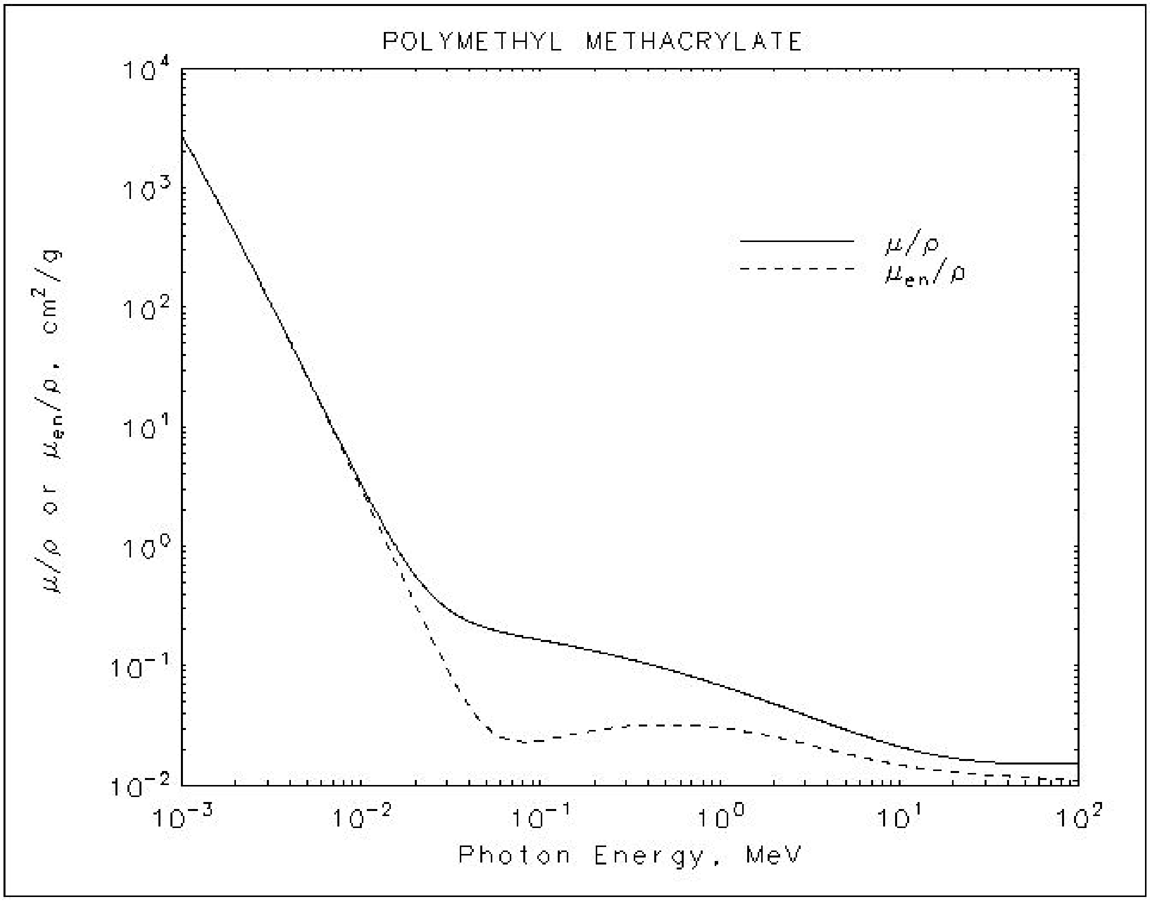
ConcNumber	OTIC	PE__	BRAI	SOMI	PFIN	CFIN
0	0	4|94	1|94	1|94	1|94	1|94
0	2|82	2|82	2|82	2|82	2|82	0
0	1|87	1|87	0	1|87	1|87	3|87
0	0	1|87	0	1|87	1|87	2|87
0	1|85	3|85	3|85	3|85	3|85	1|85
5	5|269	13|269	5|269	5|269	6|269	7|269
10	1|261	4|261	2|261	1|261	2|261	1|261
15	1|270	2|270	2|270	3|270	3|270	5|270
20	0	5|268	2|268	4|268	3|268	5|268
25	2|266	8|266	5|266	5|266	5|266	1|266
0	0	0	0	0	0	0

ConcNumber	PIG_	CIRC	TRUN	SWIM	NC__	TR__
0	4|94	0	4|94	0	0	0
0	2|82	2|82	2|82	2|82	0	2|82
0	0	0	2|87	0	0	0
0	0	0	2|87	0	0	2|87
0	3|85	3|85	3|85	3|85	1|85	3|85
5	6|269	0	6|269	0	1|269	16|269
10	2|261	2|261	2|261	2|261	0	2|261
15	1|270	0	3|270	1|270	0	2|270
20	0	3|268	5|268	0	1|268	3|268
25	5|266	5|266	5|266	5|266	0	4|266
0	0	0	0	0	0	0

Appendix E: X-Ray Mass Attenuation Coefficients

Plexiglas (Polymethyl Methacrylate) (Hubbell & Seltzer, 2011)

ρ (g/cm^3)	**Energy (MeV)**	**Energy (keV)**	**μ/ρ (cm^2/g)**	**μ_{en}/ρ (cm^2/g)**	**μ (cm^{-1})**
1.18	1.00E-03	1.00	2794.00	2.79E+03	3296.920
	1.50E-03	1.50	915.30	9.13E+02	1080.054
	2.00E-03	2.00	403.70	4.02E+02	476.366
	3.00E-03	3.00	123.60	1.23E+02	145.848
	4.00E-03	4.00	52.47	5.18E+01	61.915
	5.00E-03	5.00	26.81	2.63E+01	31.636
	6.00E-03	6.00	15.45	1.50E+01	18.231
	8.00E-03	8.00	6.49	6.11E+00	7.663
	1.00E-02	10.00	3.36	3.03E+00	3.961
	1.50E-02	15.00	1.10	8.32E-01	1.299
	2.00E-02	20.00	0.57	3.33E-01	0.674
	3.00E-02	30.00	0.30	9.65E-02	0.358
	4.00E-02	40.00	0.24	4.60E-02	0.277
	5.00E-02	50.00	0.21	3.07E-02	0.245
	6.00E-02	60.00	0.19	2.53E-02	0.227
	8.00E-02	80.00	0.18	2.30E-02	0.207
	1.00E-01	100.00	0.16	2.37E-02	0.194
	1.50E-01	150.00	0.15	2.66E-02	0.172
	2.00E-01	200.00	0.13	2.87E-02	0.157
	3.00E-01	300.00	0.12	3.10E-02	0.136
	4.00E-01	400.00	0.10	3.19E-02	0.122
	5.00E-01	500.00	0.09	3.21E-02	0.111
	6.00E-01	600.00	0.09	3.19E-02	0.103
	8.00E-01	800.00	0.08	3.12E-02	0.090
	1.00E+00	1000.00	0.07	3.02E-02	0.081
	1.25E+00	1250.00	0.06	2.88E-02	0.072
	1.50E+00	1500.00	0.06	2.76E-02	0.066
	2.00E+00	2000.00	0.05	2.53E-02	0.057
	3.00E+00	3000.00	0.04	2.21E-02	0.045
	4.00E+00	4000.00	0.03	2.00E-02	0.039
	5.00E+00	5000.00	0.03	1.84E-02	0.034
	6.00E+00	6000.00	0.03	1.73E-02	0.031
	8.00E+00	8000.00	0.02	1.58E-02	0.027
	1.00E+01	10000.00	0.02	1.48E-02	0.025
	1.50E+01	15000.00	0.02	1.35E-02	0.021
	2.00E+01	20000.00	0.02	1.28E-02	0.020

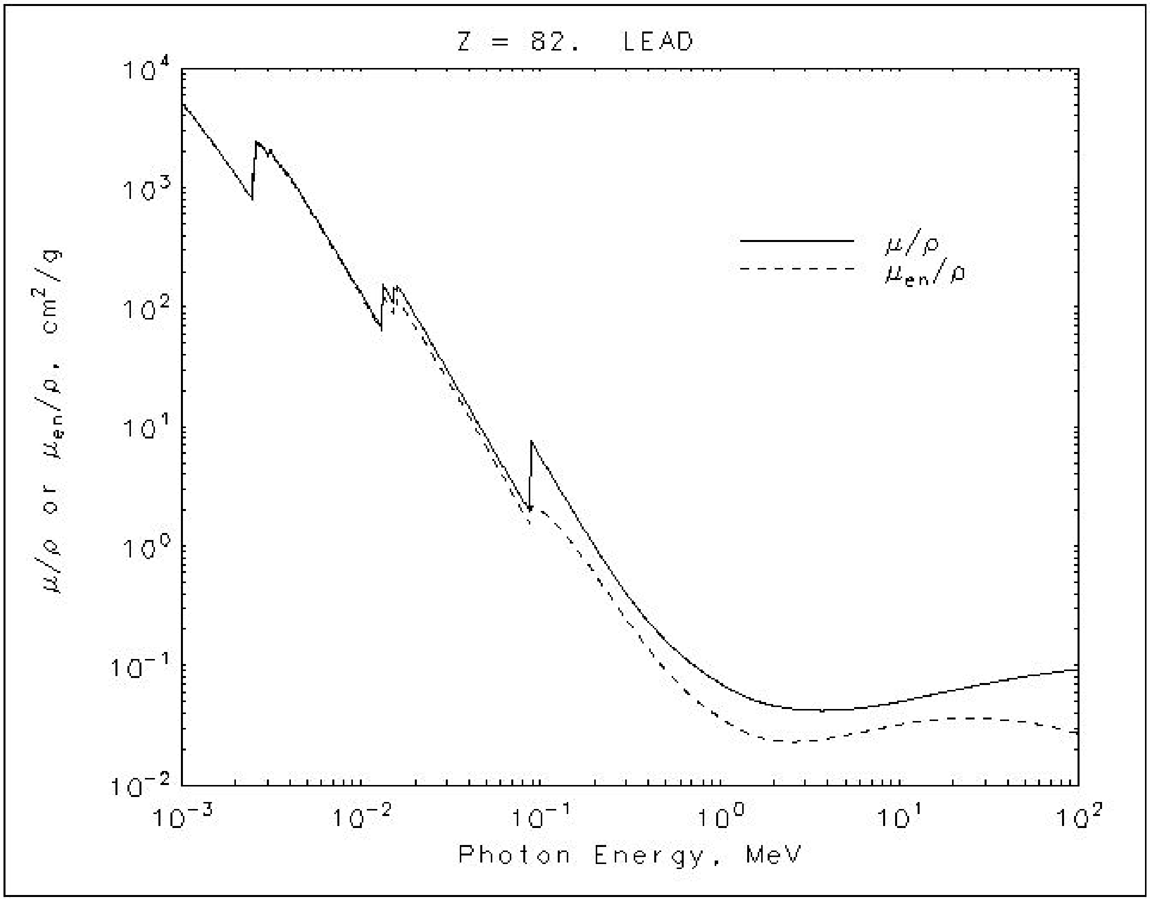


X-Ray Mass Attenuation Coefficient Distribution of Plexiglas (Hubbell & Seltzer, 2011)

Lead (Hubbell & Seltzer, 2011)

ρ (g/cm³)	Energy (MeV)	Energy (keV)	μ/ρ (cm²/g)	μ_{en}/ρ (cm²/g)	μ (cm⁻¹)
11.34	1.00E-03	1.00	5210.00	5197.00	59081.40
	1.50E-03	1.50	2356.00	2344.00	26717.04
	2.00E-03	2.00	1285.00	1274.00	14571.90
	2.48E-03	2.48	800.60	789.50	9078.80
	2.48E-03	2.48	1397.00	1366.00	15841.98
	2.53E-03	2.53	1726.00	1682.00	19572.84
	2.59E-03	2.59	1944.00	1895.00	22044.96
	2.59E-03	2.59	2458.00	2390.00	27873.72
	3.00E-03	3.00	1965.00	1913.00	22283.10
	3.07E-03	3.07	1857.00	1808.00	21058.38
	3.07E-03	3.07	2146.00	2090.00	24335.64
	3.30E-03	3.30	1796.00	1748.00	20366.64
	3.55E-03	3.55	1496.00	1459.00	16964.64
	3.55E-03	3.55	1585.00	1546.00	17973.90
	3.70E-03	3.70	1442.00	1405.00	16352.28
	3.85E-03	3.85	1311.00	1279.00	14866.74
	3.85E-03	3.85	1368.00	1335.00	15513.12
	4.00E-03	4.00	1251.00	1221.00	14186.34
	5.00E-03	5.00	730.40	712.40	8282.74
	6.00E-03	6.00	467.20	454.60	5298.05
	8.00E-03	8.00	228.70	220.70	2593.46
	1.00E-02	10.00	130.60	124.70	1481.00
	1.30E-02	13.04	67.01	62.70	759.89
	1.30E-02	13.04	162.10	129.10	1838.21
	1.50E-02	15.00	111.60	91.00	1265.54
	1.52E-02	15.20	107.80	88.07	1222.45
	1.52E-02	15.20	148.50	113.10	1683.99
	1.55E-02	15.53	141.60	108.30	1605.74
	1.59E-02	15.86	134.40	103.20	1524.10
	1.59E-02	15.86	154.80	118.00	1755.43
	2.00E-02	20.00	86.36	68.99	979.32
	3.00E-02	30.00	30.32	25.36	343.83
	4.00E-02	40.00	14.36	12.11	162.84
	5.00E-02	50.00	8.04	6.74	91.18
	6.00E-02	60.00	5.02	4.15	56.94
	8.00E-02	80.00	2.42	1.92	27.43
11.34	8.80E-02	88.00	1.91	1.48	21.66

8.80E-02	88.00	7.68	2.16	87.13
1.00E-01	100.00	5.55	1.98	62.93
1.50E-01	150.00	2.01	1.06	22.84
2.00E-01	200.00	1.00	0.59	11.32
3.00E-01	300.00	0.40	0.25	4.57
4.00E-01	400.00	0.23	0.14	2.63
5.00E-01	500.00	0.16	0.09	1.83
6.00E-01	600.00	0.12	0.07	1.42
8.00E-01	800.00	0.09	0.05	1.01
1.00E+00	1000.00	0.07	0.04	0.81
1.25E+00	1250.00	0.06	0.03	0.67
1.50E+00	1500.00	0.05	0.03	0.59
2.00E+00	2000.00	0.05	0.02	0.52
3.00E+00	3000.00	0.04	0.02	0.48
4.00E+00	4000.00	0.04	0.02	0.48
5.00E+00	5000.00	0.04	0.03	0.48
6.00E+00	6000.00	0.04	0.03	0.50
8.00E+00	8000.00	0.05	0.03	0.53
1.00E+01	10000.00	0.05	0.03	0.56
1.50E+01	15000.00	0.06	0.03	0.64
2.00E+01	20000.00	0.06	0.04	0.70



X-Ray Mass Attenuation Coefficient Distribution of Lead (Hubbell & Seltzer, 2011)

Appendix F: Acronyms

Acronym	**Meaning**
ALARA	as low as reasonably achievable
BNL	Brookhaven National Laboratory
dpf	days post fertilization
GCR	galactic cosmic rays
HCP	heavy charged particles
hpf	hours post fertilization
HZE	high charge Z and high energy E
ICRP	International Commission on Radiation Protection
ISS	International Space Station
LET	linear energy transfer
LHSA	Longitudinal Study of Astronaut Health
MCNP	Monte Carlo N-Particle
NASA	National Aeronautics and Space Administration
NCRP	National Council on Radiation Protection
NEO	near Earth orbits
NRC	Nuclear Regulatory Commission
OSU	Oregon State University
PEL	permissible exposure limit
RBE	relative biological effectiveness
RBE_{α}	Dose rate of γ-radiation causing an effect / Dose rate of α-radiation causing the same effect
RC	Radiation Center
RHIC	Relativistic Heavy Ion Collider
RWP	radiation work permit
SARL	Sinnhuber Aquatic Research Laboratory
SDL	John L. Fryer Salmon Disease Laboratory
SPE	solar-particle event
TLD	thermoluminecent dosimeter
UO	University of Oregon

Acronym	**Meaning**
Cataract types	
COR	cortical cataract
NUC	nuclear cataract
PSC	posterior subcapsular cataract
Nuclides	
Co-60	Cobalt-60
Cs-137	Cesium-137
I-128	Iodine 128
Ni-60	Nickle-60
Te-128	Tellurium-128
Xe-128	Xenon-128