RE-EVALUATION OF LETHAL RADIATION DOSE IN DROSOPHILA MELANOGASTER USING 350 KEV ELECTRONS

by

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Abstract

Radiation dosimetry from a 350 KeV electron beam and its impact on Drosophila melanogaster response serve as the focal points of this study. While previous research has established an LD_{50} (lethal radiation dose for 50 % of the population) exceeding 452 Gy across mainly all developmental stages, this investigation extends the understanding by examining dose-dependent differences throughout development, from the pupae stage into adulthood.

Fruit flies, irradiated at their most sensitive stage, the egg phase, displayed a notable reduction in effective LD_{50}, especially when accounting for long-term effects. Specifically, the LD_{50} values decreased to approximately 59 Gy for the maximum pupae population observed at 187 hours after eggs were laid down, and further reduced to about 15 Gy for the entire adult population at 310 hours. These findings underscore the importance of prolonged observation, revealing a delayed pattern wherein fruit fly eggs progress through various developmental stages after irradiation.

This study prompts a critical re-evaluation of the LD_{50} concept, advocating for its modification based on observed developmental stages and time. Additionally, it identifies optimal radiation conditions for fruit fly development, extending implications to other biological systems. The potential for engineering a radiation-resistant biological system offers promising applications in various living organisms, contributing significantly to advancing radiation biology and enhancing resilience to radiation exposure.

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CHAPTER ONE: INTRODUCTION

1.1 Background of the study

Radiation is the emission and propagation of waves transmitting energy through space or through some medium [1]. A great definition of radiation was published in McGraw-Hill far back in 1989 where radiation was described as a stream of various particles, such as electrons, protons, neutrons, alpha particles, high-energy photons, or a mixture of these.

There are two principal categories of radiation: ionizing and non-ionizing. Ionizing radiation possesses a significant amount of energy, sufficient enough to move out electrons of atoms. Meanwhile, non-ionizing radiation possesses enough energy to cause atoms to vibrate and move but within a molecule. So, the main difference between them lies in the fact that ionizing radiation has enough energy to remove electrons from atoms, while non-ionizing radiation does not [2].

Radiation occurs naturally; it was not invented but discovered [1]. Non-ionizing radiation is omnipresent in our daily lives [2], and people are continuously exposed to it [1]. In particular, visible light, ultraviolet, uranium, thorium, and radium in the earth's crust, radiofrequency energy emitted by cell phones, radio waves, and numerous others are all sources of radiation [1]. Non-ionizing radiation presents no peril to living organisms [1, 3, 4].

Ionizing radiation, pervasively encountered within our quotidian existence, encompasses electromagnetic emissions such as X-rays, alpha (α) and beta (β) particles, gamma rays (γ), and ultraviolet radiation. The perilous nature of ionizing radiation arises from its capacity to induce molecular perturbations within biological tissues, precipitating deleterious

consequences that have the potential to afflict both human and animal organisms in due course [4, 5].

Advancements in society, technology, and science have engendered substantial apprehensions regarding radiation dosimetry, manifesting in escalated radiation exposures experienced by individuals compared to previous periods. This trend has been particularly discernible among those exposed to ionizing radiation from diverse origins. High-risk populations encompass individuals undergoing medical treatments, healthcare practitioners, doctors, researchers, and those encountering environmental radiation.

In accordance with the imperative tenets of radiological safety and health, the International Commission on Radiological Protection (ICRP) has promulgated stringent benchmarks for individual dose limits and normative criteria to forestall deleterious ramifications stemming from radiation exposure to the human population.

In relation to ICRP, any radiation exposure exceeding the natural background radiation levels should be kept as low as reasonably archivable (ALARA) but below the specified individual dose limits. The average individual dose limit for radiation workers over a five-year period is 100 mSv, and for the general public, it is 1 mSv per year [6, 7, 8].

Ionizing radiation can initiate direct or indirect, irreversible processes within human tissues, DNA, and cells. This can result in DNA damage or mutation [5]. The effects of ionizing radiation can be avoided with precautionary and curative measures. The precautionary measures include distance, time and shielding which has limitations. However, ionizing radiation can be harnessed for beneficial inquiries in society. Applying

proper knowledge and expertise scientists are treating patients with cancer employing ionizing radiation.

Individuals exposed to ionizing radiation are among those who face potential health risks from low-dose radiation (LDR) exposure. It is particularly challenging to predict biological effects of LDR, as each organism represents a unique set of settings, characterized by its fundamental and initially inborn, deep-rooted system with biological responses to ionizing radiation [9]. The use of LDR in case of patient treatment adds further complexity due to the cells adaption into different stressors or different intensities of stress [10] as well as low-dose hyper-radiosensitivity (HRS), an effect in which cells die from excessive sensitivity to small single doses of ionizing radiation but become more resistant (per unit dose) to larger single doses [11].

LDR has no immediately noticeable effects on humans; nevertheless, there is great interest in its long-term biological effects, which may include genetic defects in progeny. LDR mainly causes indirect DNA damage by triggering the production of reactive oxygen species. Research into the biological effects of LDR exposure is hindered by a lack of assays sensitive enough to measure the relevant cellular alterations [9, 12].

Exposure to high doses of radiation (HDR) poses a significant risk to human health [4]. The relationship is directly proportional: the higher the radiation dose, the more rapidly the effects will manifest, and the greater the likelihood of mortality. Moreover, high levels of radiation can cause cellular death, and in severe instances, it can result in tissue and organ damage, causing an acute whole-body reaction referred to as "acute radiation syndrome”.

The time interval between radiation exposure and the appearance of cancer is called the "latent period." This phenomenon was observed in atomic bomb survivors in 1945 and emergency workers who responded to the Chernobyl nuclear power plant accident in 1986. In the Chernobyl disaster, around 134 plant workers and firefighters were exposed to high radiation doses ranging from 70,000 to 1,340,000 mrem (700 to 13400 mSv), resulting in acute radiation sickness, and 28 of them died from their injuries [13].

While the effects of radiation vary from person to person, exposure to 500 rem of radiation all at once can be lethal without medical intervention. Similarly, a single dose of 100 rem can cause nausea or skin reddening (with eventual recovery), and around 25 rem may cause temporary sterility in men. However, if these doses are spread out over time, rather than delivered all at once, their effects are typically less severe, but also can lead to mutations in DNA and changes in genetic structure [13].

1.2 Objectives

The initial goal of this thesis was to measure radiation dosages with biological systems such as Drosophila melanogaster and HeLa cells. The findings of the research highlighted the importance of re-evaluation of lethal radiation dose in Drosophila melanogaster using 350 KeV electrons.

What the research is attempting to address are questions seeking solutions for:

1. Is it possible to measure radiation dosages using biological systems as model organisms?

2. Can we rely on the response of biological systems to radiation as a convenient means of ensuring radiation dose?
3. Can we use LD_{50} as a sufficient parameter of finding radiation dose?
4. Explore the level of accuracy of the results.
5. Is it possible to identify particular underlined genetics that effectively assist living organisms in resisting radiation?

1.3 Significance of the study

Considering occupational differences, certain individuals encounter radiation exposure more frequently than others. In accordance with the guidelines set forth by ICRP, which have established appropriate protocols, commensurate protective measures must be afforded to each individual, and specific dose limits and standards must be rigorously upheld. Despite this, recent worker studies have reported statistically discernible dose-related increased risks of cancer; however, results must be interpreted with care, and occupational radiation doses need to be treated with particular attention [14]. Research examining the effects of high dose radiation is crucial in comprehending the possible dangers and health hazards linked with exposure to such levels of radiation for human population. Such studies are a key-factor of development of safety protocols, safeguarding measures for workers and the public, and evaluating the potential long-term impacts on human health and the surroundings. By conducting such studies, we can prepare for potential hazards, predict the radiation dose, anticipating threats to health and life or a response of human body into received radiation dose [14]. Having researched underlying

genetics and mechanism of radiation resistance in Drosophila melanogaster exposed to varying radiation dosages we can implement acquired knowledge into diverse biological systems. The prospect of integrating a well-researched and predictable genetic system of radiation resistance of human population is one of the most immense questions for this day.

1.4 Justification

Drosophila melanogaster, commonly referred to as the fruit fly, has been reported to have high radiation tolerance as that of other insects [15, 16], and varying levels of radiation tolerance at different ages [15, 17]. Due to its higher radiation tolerance and postmitotic tissue organization, Drosophila melanogaster has emerged as a suitable model to study radiation and its effect [16]. Drosophila melanogaster and humans share lots of similarities in biological processes and mechanisms which is extremely beneficial in research purposes.

HeLa Cells are one of the most used biological materials in radiation research. This is simply explained by the ability to use human cells in biological research and testing which has obvious advantage over the use of laboratory animals, as it eliminates the necessity for species extrapolation. Hela Cells are the oldest and most studied immortal cell line. They are the first human cells to be grown and multiplied endlessly in the lab. HeLa Cells served as a key tool that significantly expanded our understanding of the fundamental mechanisms of cells, microbiology, and research related to numerous diseases and their treatment.

CHAPTER TWO: LITRETURE RIVIEW

2.1 Ionizing radiation and dosages

Radiation with wavelengths shorter than 10^{-10} m is considered ionizing radiation, because it can ionize the molecules it encounters. Examples of ionizing radiation are alpha, beta, gamma, X-rays, e-beam [18]. Ionizing radiation can induce changes in molecules within our body cells and genetic material. When ionizing radiation pierces a human body or an object it deposits energy. The energy which is absorbed by the body from exposure to radiation is called a dose [18].

Radiation dose tells us the effect radiation has on tissue. It can be measured in multiple ways: absorbed dose, equivalent dose, and effective dose.

2.1.1 Absorbed dose

Absorbed dose is used to assess the potential for biochemical changes in specific tissues [19]. Absorbed dose is the concentration of energy deposited in tissue due to exposure to ionizing radiation. Ionizing radiation can impale deep into the body and deposit energy in internal organs.

Absorbed dose describes the intensity of the energy deposited in any small amount of tissue located anywhere in the body.

A dose of 1 Gy (Gy, international unit) is equivalent to 1 joule of energy deposited in a kilogram of the substance [18,19].

Ionizing radiation has the capacity to traverse the entire human body system, releasing energy within organs and tissues while concurrently continuing into the environment, with the capability to ionize additional objects.

2.1.2 Equivalent dose

Equivalent dose is used to assess how much biological damage is expected from the absorbed dose.

When radiation is absorbed in living matter, a biological effect may be observed. Different types of radiation have different effects even if absorbed dose is the same [20]. The reason for that is a type of radiation, for example alpha, beta, gamma, or neutrons. 1 Gy of alpha radiation is more harmful to a given tissue than 1 Gy of beta radiation. To get the equivalent dose, the absorbed dose is multiplied by a specified radiation weighting factor (wR) to reflect the relative biological effects of the different types of radiation. The equivalent dose is expressed in a measure called the sievert (Sv). This means that 1 Sv of alpha radiation will have the same biological effect as 1 Sv of beta radiation. In other words, the equivalent dose is expressed as a single unit that accounts for the degree of harm that different types of radiation would cause to the same tissue [18].

2.1.3 Effective dose

Effective dose is used to assess the potential for long-term effects that might occur in the future from exposure (cancer or hereditary effects and many others). Measuring effective dose helps to compare predictable health effects from different exposure situations.

Because designated value is an approximation, not a physical quantity, it cannot be used to predict an individual effect for different people at each unique situation [21].

Different body parts have different sensitivities to radiation. Effective dose takes the absorbed dose and adjusts it for radiation type and relative organ sensitivity [21].

There are 3 crucial factors that are taken into consideration while calculating an effective dose:

- the absorbed dose to all organs of the body,
- the relative harm level of the radiation, and
- the sensitivities of each organ to radiation [19].

2.1.4 LD_{50}

The way of expressing the lethality of a dose is LD_{50}. This means the dose that will result in a specified endpoint, in this case, death, to 50% of the population. For humans, the LD_{50} is thought to be about 3 to 3.5 Gy (300 to 350 rads) for healthy adults irradiated but with no mechanical intervention. The lethal dose varies depending on the individual's health and whether medical intervention is available.

Figure 1 illustrates the relationship between the percentage of deceased subjects and the administered dose, with the slope representing the dose-response curve [44].

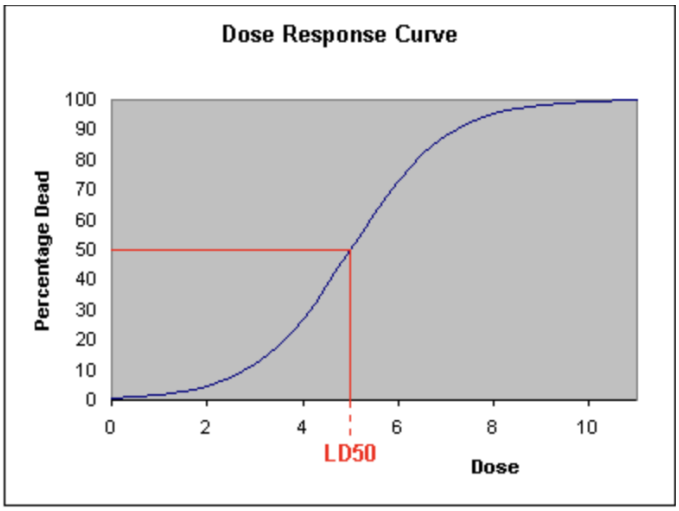

Figure 1: Percentage dead graphed versus dose representing dose response curve for LD_{50} [44].

The LD_{50} is a parameter utilized for determining the optimal radiation dose to induce effective mutations in genetic improvement programs. This occurs due to the observation that at low doses, the influence on the genome is minimal and challenging to discern, whereas at high doses, the genome may undergo multiple substantial impacts, consistently resulting in abnormalities or adverse alterations [24, 25]. Furthermore, low radiation doses may induce an increase in the cell division or enzyme activity [24].

In contrast, high radiation doses may also produce physical or chemical damage in the cells, due to the destruction of enzymes and hormones, among others. However, because these changes might not be produced through genetic modifications, they will not be passed on to the next generations. In this sense, low and high radiation doses may generate phenotypic changes that may be mistaken for genetic changes [25].

Therefore, the optimal radiation dose must be established before starting a mutagenesis-based genetic improvement program; this will increase the possibility of finding effective

mutations. The optimal radiation dose is established through the mean lethal dose or the mean growth reduction dose [22,26].

2.2 Impact of ionizing radiation on the human body

High levels of exposure to ionizing radiation can result in irreversible damage to the skin or tissues [4]. This damage potentially leads to eventual harm, such as disrupting of chemical bonds of DNA by breaking the bonds or direct changes in the molecular structure, such as the bond between base-pairs.

This destruction could be effectively remedied by cellular mechanisms, improperly repaired causing a genetic mutation, or it could result in the death of the cell or the development of cancer [27]. Figure 2 illustrates this procedure and represents various scenarios along with their respective results.

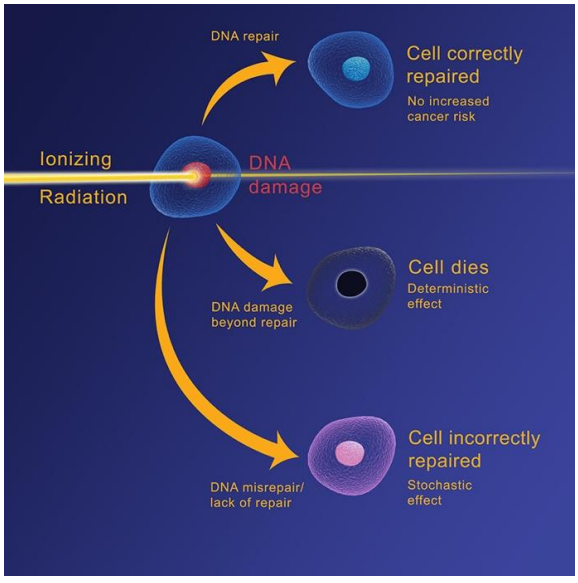

Figure 2: Effect of ionizing radiation on DNA [46].

Heath effects from radiation doses can be grouped into two categories: deterministic and stochastic.

Deterministic effects occur after surpassing a specific threshold dose. Therefore, doses below the threshold are not anticipated to cause particular effects. Comprehensibly, that with the increase of dose the severity of the effect increases linearly. Skin reddening (erythema), cataracts to lens of the eye, sterility (temporary/permanent), epilation (loss of hair) are examples of these effects. Although it may not accurately describe all deterministic health effects, they are sometimes described as "short-term" health effects [28]. Stochastic effects occur by statistical chance. The probability of the effect occurring, rather than its severity, is assumed to be a linear function of dose without threshold [27]. Cancer represents the primary stochastic outcome that may arise from radiation exposure, frequently manifesting many years after the initial exposure. It is generally assumed that stochastic health effects do not exhibit a dose threshold below which they are absent. This is the reason that no level of radiation dose is considered to be completely "safe" and why doses should always be kept ALARA. Although it may not accurately describe all stochastic health effects, they are sometimes described as "long-term" health effects [28].

2.3 Cancer and tumorigenesis

The last 50 years of cancer research and treatment have generated huge and diverse body system of knowledge, revealing cancer to be a disease involving dynamic changes in the genome of living organisms. The foundation of cancer has been set in the discovery of mutations that produce oncogenes with dominant gain of function and tumor suppressor

genes with recessive loss of function [29]. Tumorigenesis is a complicated multistep process that drives the progressive transformation of normal human cells into highly malignant derivatives by specific rules in molecular, biochemical, and cellular traits [30]. Not all types of tumors exhibit malignancy. Some kinds of tumors remain at one site and exhibit restrained proliferation, refraining from infiltrating adjacent tissues or metastasizing throughout the organism. Such types of tumors are classified as benign tumors and are characterized non-cancerous as depicted in Figure 3 [31].

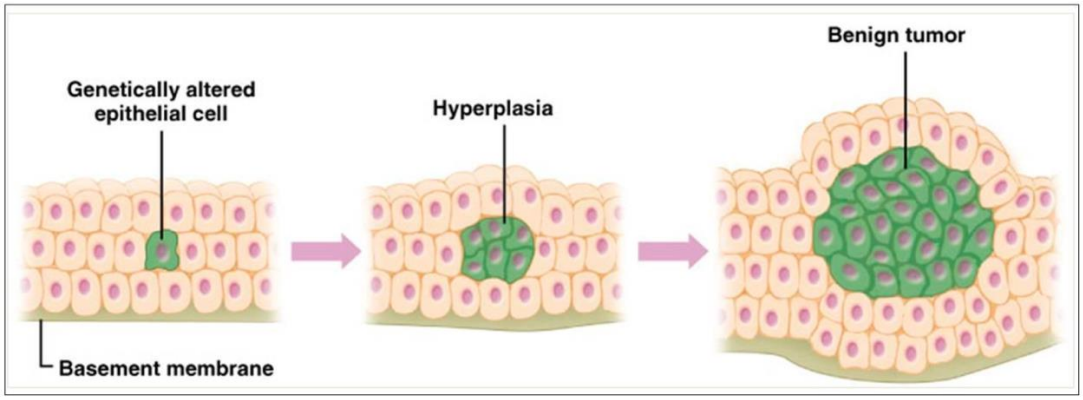

Figure 3: Development of benign tumor [45].

Cancerous tumors are characterized by uncontrolled growth, tumor formation, and invasion of the surrounding body parts. Tumor development takes a couple stages. Figure 4 describes this process. The process of tumorigenesis commences when a cell undergoes genetic alterations in its function, often triggered by various factors, such as ionizing radiation, for instance. As genetically modified cells progress through their life cycle, they manifest unregulated and exceedingly swift proliferation. At this point this process is called hyperplasia. A stage where overgrowing cells change their original form is called

dysplasia. It consists of more immature cells than mature. In situ cancer denotes a neoplastic lesion characterized by arrested cellular differentiation, the loss of tissue-specific identity, and uncontrolled proliferation. Conversely, malignant tumors involve the unbridled growth of cells that infiltrate adjacent regions by breaching the basal membrane. Metastasis is the process by which cancer cells disseminate to remote anatomical locations via the lymphatic and circulatory systems and creating new colonies of tumors at different parts of the body. Metastasis is the case of more than 90% of cancer-related deaths [31].

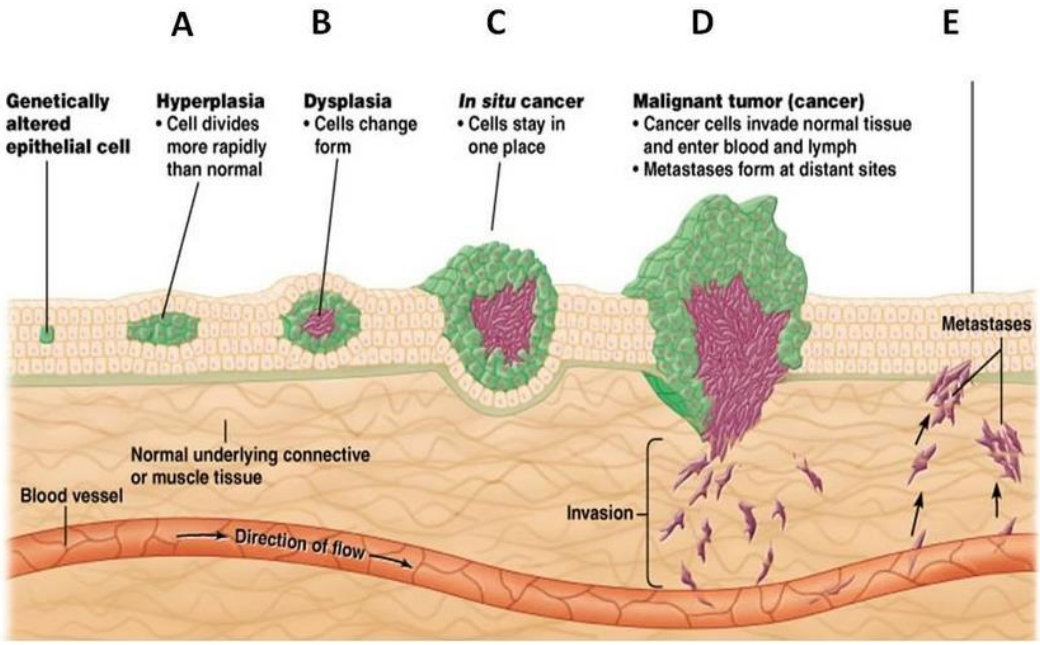

Figure 4: Cancerous tumor development and mechanism of metastasis [45].

It is important to understand that the same type of cancer that occurs at one individual is very different than the cancer that occurs at another individual, just like those individuals are different. The tumor of one person will be very different from a tumor in another person

therefore treatment approach is a unique process and requires personal and extremely specific implementation of sources and knowledge in each of those cases.

2.4 Radiation therapy and diagnostics

Radiation therapy or radiotherapy is a cancer treatment that uses high doses of radiation to kill cancer cells and shrink tumors [32]. Depending on type of cancer, size of tumor, its location in the body as well as relative position to normal tissues and their sensation to radiation, general health and medical history of patient, age, different types of radiation therapy might be applied.

There are 2 types of radiation therapy: external beam radiation (teletherapy) and internal radiation (brachytherapy).

External beam therapy is radiation delivered from a machine that aims radiation from outside the body and directed at the patient's cancer site. This type of radiotherapy is a local treatment, which means it treats a specific part of the body, not a whole body [33].

Internal radiation therapy is a treatment in which a source of radiation is put inside the body. The radiation source can be solid or liquid [32].

At high doses radiation therapy kills cancer or slows down their growth by damaging their DNA. Cancer cells whose DNA is damaged beyond repair stop dividing or dying. When the damaged cells die, they are broken down and removed by body [32].

Radiation therapy does not immediately eliminate cancer cells; instead, it requires several days or even weeks of treatment to sufficiently damage the DNA within cancer cells,

causing them to eventually perish. Furthermore, even after radiation therapy concludes, cancer cells continue to die off over several weeks or months.

There are a lot of different types of exams in Diagnostic Imaging which use ionizing radiation. Such medical exams include:

- X-rays
- PET
- CT
- Nuclear medicine procedures

At the Diagnostic Nuclear Medicine, a radioactive marker (tracer, isotope) is injected, ingested, or inhaled with a vector molecule into human body. The vector molecule is absorbed by a certain specified structure of substances (organ, tissue, liquid). The radioactive label serves as a "transmitter": it emits gamma rays, which are registered by a gamma camera (scintigraphy). The amount of injected radiopharmaceutical is such that the radiation emitted by it is easily captured, but at the same time it does not have a toxic effect on the body. Figure 5 illustrates this process. Technetium-99m (Figure 5a) and I-123 (Figure 5b) were administered intravenously at doses of 30.0 mCi and 4.95 mCi, respectively, for Myocardial scintigraphy stress images and Brain DaTscan rest images at Mankato Clinic under the supervision of Katie Grissman. The gamma scan (scintigraphy) is depicted in Figure 6.

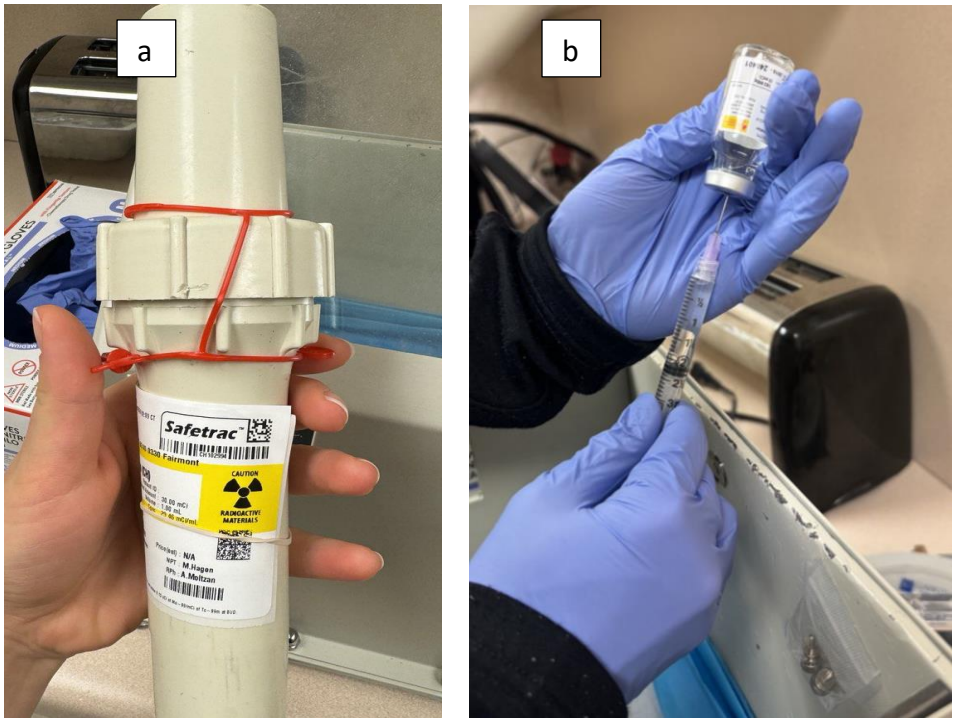

Figure 5: Technetium-99m (a) and I-123 (b) are shown in the preparation phase before intravenous injection for Myocardial scintigraphy stress images and Brain DaTscan rest images.

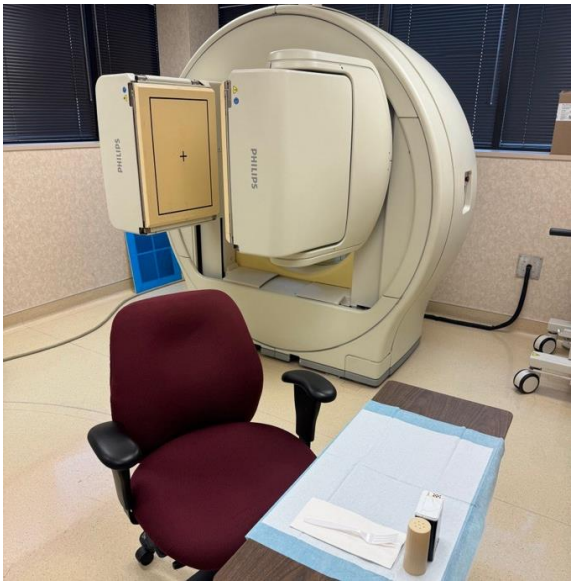

Figure 6: A gamma scan (scintigraphy) machine located at the Mankato Clinic is ready and prepared for GES (gastric emptying scan).

2.4.1 Electron beam radiation therapy

Electrons tend to release their energy close to the skin's surface and are often used to treat tumors close to the surface of the skin. Apparently, the dose of radiation from electron beam to deeper tissues is minimal [34]. Electron beams are typically employed to treat skin tumors, certain lymph nodes, keloids, and the scars resulting from lumpectomy procedures following breast surgery.

Electron beam radiation therapy can be given as spot treatment (used to treat tumors on the surface of the skin) or as total skin electron beam therapy (TSEBT) when entire surface of the skin is treated.

2.5 CSDA range – NIST/ESTAR

While 99 % of the energy of the electron beam is depositing into the surface, around 1 % of it is being redirected elsewhere. The National Institute of Standards and Technology (NIST) provides the ESTAR program which calculates stopping power, density effect parameters, CSDA range, and radiation yield tables for electrons in various materials. Having an option of managing material and desired energy it is easy to find continuous slowing down approximation range (CSDA range) using this tool.

The CSDA range is a very close approximation to the average distance traveled by a charged particle as it slows down to rest. It can be calculated by integrating the reciprocal of the total stopping power with respect to energy regarding the formula (unit is g/cm^2 in our case):

$$R_{CSDA} = \int_0^{T_0} \left(\frac{dT}{\rho dx}\right)^{-1} dT \quad \left({}^{g}/_{cm^2}\right)$$

where,

T = kinetic energy, T_0 = initial energy, ρ = density of medium, x = path length.

While working with biological materials, water stands out as the most appropriate choice due to its prevalence in living organisms. Approximately 60 % of the human body and all life forms consist of water.

Figure 7 represents the CSDA range in a water (liquid) as material [47].

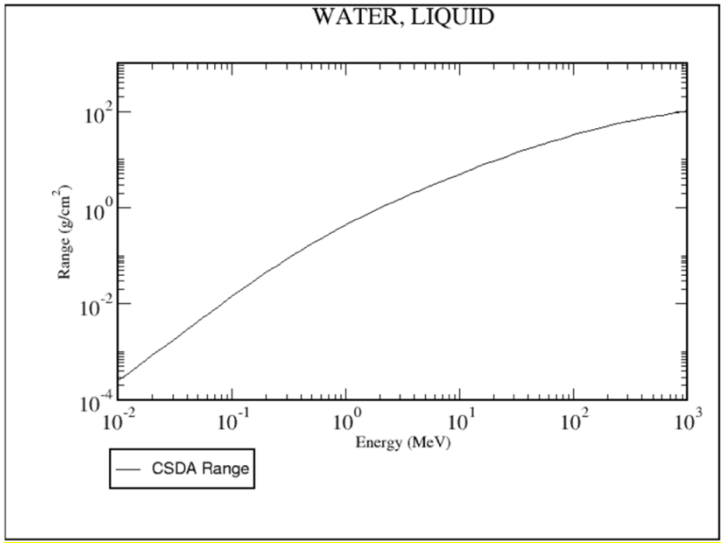

Figure 7: ESTAR graph for CSDA range of electron beam dumping into the water [47].

(required) Kinetic Energy (MeV)	Stopping Power (MeV cm²/g)			CSDA Range (g/cm²)	Radiation Yield
	Collision	Radiative	Total		
3.000E-01	2.355E+00	5.514E-03	2.360E+00	8.421E-02	1.331E-03
3.500E-01	2.235E+00	5.914E-03	2.241E+00	1.060E-01	1.496E-03
4.000E-01	2.148E+00	6.339E-03	2.154E+00	1.288E-01	1.658E-03
4.500E-01	2.083E+00	6.787E-03	2.090E+00	1.523E-01	1.818E-03

Table 1: ESTAR stopping power and range table for electrons in water (liquid) [47].

Regarding to ESTAR data table (see Table 1), for 350 KeV (3.5×10^{-1} MeV)[47] (highlighted with yellow frame in Table 1) around 99.73 % of all stopping power is going into the collision, about 0.26 % is going into the radiative stopping power which is the average rate of energy loss per unit path length due to collisions with atoms and atomic electrons in which bremsstrahlung quanta are emitted, and around 0.065 % of energy is called lost energy which is a radiation yield.

CSDA range is equal to 1.060×10^{-1} (g/cm^2) for 3.5×10^{-1} MeV. The depth to which a certain mass per unit area penetrates depends on the material's density, which, in this case, is water.

The formula to calculate the depth (d) is:

$$d = \frac{\sigma}{\rho}$$

where,

σ - is the surface density (1.060×10^{-1} g/cm^2)

ρ - is the density of the water, which is approximately 1 g/cm^3 (1000 kg/m^3).

$$d = \frac{0.1060\ \text{g/cm}^2}{1\ \text{g/cm}^3} = 0.1060\ \text{cm} = 1.060\ \text{mm}.$$

So, based on this, we can draw the conclusion that for 3.5×10^{-1} MeV CSDA range is equal to 1.060×10^{-1} (g/cm^2) which means that electron beam will go depth into the water approximately for 1.060 mm.

2.6 High Dose Radiation calculation

The following calculation is performed based on fruit flies vial dimensions. Water is chosen as the material that most closely resembles the biological system. A cylinder of water with diameter 10 mm and length 0.8mm (from NIST for 300 KeV electrons).

1. Let`s find out how many electrons per second in 1 nA.

$$1\ \text{nA} = 1\ \times\ 10^{-9}\ \text{A}$$

$$1\ \text{A} = {}^{\text{C}}/_{\text{s}}$$

Electron charge is equal to:

$$1\ \text{e} = 1.6 \times 10^{-19}\ \text{C}$$

$$1\ \text{A} = \frac{1\ \text{e}}{1.6\ \text{x}\ 10^{-19}\text{C}} = 6.25 \times 10^{18}\ {}^{\text{e}}/_{\text{s}}$$

In beam of 1 nA are:

$$1\ \text{nA} = (6.25 \times 10^{18}\ {}^{\text{e}}/_{\text{s}}) \times (1\ \times\ 10^{-9}\ \text{C}) = 6.25 \times 10^{9}\ {}^{\text{e}}/_{\text{s}}$$

2. Energy in terms of J/seconds:

The total bream power can be found by multiplication:

$$(300 \times 10^{3}\ \text{eV}) \times (6.25 \times 10^{9}\ {}^{\text{e}}/_{\text{s}}) = 187.5 \times 10^{13}\ {}^{\text{eV}}/_{\text{s}} = 3 \times 10^{-4}\ {}^{\text{J}}/_{\text{s}}$$

3. Volume of cylinder:

Volume of cylinder can be calculated by formula: $\pi r^2 L$

$$\text{Volume} = \pi \times (0.5\ \text{cm})^2 \times (0.08\ \text{cm}) = 6.3 \times 10^{-2}\ \text{cm}^3$$

4. Mass of sample (water):

Density of water = 10^{-3} (g/cm^3)

Mass of sample can be found by:

$$10^{-3}\ \text{g/cm}^3 \times (6.3 \times 10^{-2}\ \text{cm}^3) = 6.3 \times 10^{-3}\ \text{kg}$$

5. Dose:

$$1\ \text{Gy} = 1\ \text{J/kg}$$

$$\text{Dose rate} = \text{Power/Mass} = (3{\times}10^{-4}\ \text{J/s})\ /\ (6.3 \times 10^{-5}\ \text{kg}) = 4.6\ \frac{\frac{\text{J}}{\text{kg}}}{\text{s}} = 4.6\ \text{Gy/s}$$

$$\text{D} = 4.6\ \text{Gy/s} = 460\ \text{Rad/s}$$

A large amount of radiation is distributed over a small volume.

2.7 Drosophila melanogaster and its appliance in research

Drosophila melanogaster is a widely used model organism in a variety of research as it has a unique combination of characteristics which sets Drosophila melanogaster apart from the competition. Its small size, ease and low-cost of maintenance, low breeding expenses, short generation time, high fecundity, simple genome, and availability of numerous mutants, such as gene mutations and chromosomal recombination, make it an ideal model organism for developmental and genetic studies [35]. Fruit flies can be easily anesthetized and manipulated individually with unsophisticated equipment.

Using Drosophila melanogaster as testing biological system has led to significant advancements in understanding of the fundamental principles of genetics, including cell

growth, differentiation, aging, cancer, and identification of genes involved in those processes. Its application in research has been particularly beneficial in unraveling the genetic processes and molecular mechanisms underlying various biological phenomena such as development as well as late development, mutations, behavior, and disease.

Deep studies of mechanisms of radiation tolerance in Drosophila melanogaster will open wide and more advanced understanding in approach for radioprotection. The faith of successful applying radiation resistant system into humas comes directly from the teachings of cell biology that virtually all mammalian cells carry a similar molecular machinery regulating their proliferation, differentiation, and death.

2.8 HeLa Cells as biological material in research

HeLa Cells was the first human cell line established in culture [36] and has since become the most widely used human cell line in biological research including cancer biology, infectious disease, fundamental microbiology, and many others.

For the successful process of endless multiplying in the lab cells may be grown in a culture medium of biological origin such as blood serum or tissue extract, in a chemically defined synthetic medium, or in a mixture of the two. For cells to be studied effectively, it is essential that the medium has the correct nutrient proportions and is adjusted to the suitable pH level. Cultures are typically cultivated in one of two ways: either as monolayers on glass or plastic surfaces or as suspensions in liquid or semi-solid mediums.

To initiate a culture, a tiny sample of the tissue is dispersed on or in the medium, and the flask, tube, or plate containing the culture is then incubated, usually at a temperature close

to that of the tissue's normal environment. Sterile conditions are maintained to prevent contamination with microorganisms [37].

Researchers achieved success in utilizing HeLa Cells for polio research, contributing significantly to global efforts to eradicate this virus from most countries. HeLa Cells have been instrumental in uncovering the link between the presence of Human Papillomavirus (HPV) and the development of specific types of cervical cancer. This groundbreaking discovery regarding the connection between HPV and cervical cancer laid the foundation for the development of one of the earliest anti-cancer vaccines.

2.9 Tolerance in different life stages of Drosophila melanogaster to radiation

To grasp the mechanisms involved and assess existing hypotheses regarding fruit fly radiation tolerance, it is essential to acquire knowledge about sensitivity at specific developmental stages.

As published early twenty-four hours post-irradiation with gamma rays, 100 % mortality was recorded for eggs at 1000 Gy. One hour post irradiation 100 % mortality was recorded at 1300 Gy for first instar larvae, 1700 Gy for second instar larvae, 1900 Gy for feeding third instar larvae and 2200 Gy for non-feeding third instar larvae. Early pupae were found to be prone to radiation, whereas the non-feeding third instar larvae were most resistant among all stages. Post-irradiation complete failure of emergence (100 % mortality) was observed at 130 Gy for early pupae and 1500 Gy for late pupae; 100 % mortality was observed at 1500 Gy for adults [38]. The values of LD_{50} were recorded as 452 Gy for eggs,

1049 Gy for first instar larvae, 1350 Gy for second instar larvae, 1265 Gy for feeding third instar larvae, 1590 Gy for non-feeding third instar larvae, 50 Gy for early pupae, 969 Gy for late pupae, 1228 Gy for adult males and 1250 Gy for adult females. Eggs were observed for survival 24 h post-irradiation. There was a continuous decrease in the percentage of hatching with increased dose, and an inability to hatch was considered as the death of egg. The untreated group showed 100 % hatching. The threshold dose for inhibition of hatching of eggs was found to be (50 ± 3) Gy, whereas (1000 ± 30) Gy dose was required to stop complete hatching. LD_{50} was recorded at 452 Gy which is represented by Figure 9 [38]. There was a significant negative correlation found between the survival percentage of eggs and the dose of gamma rays. Post-irradiation observation showed a high correlation between gamma irradiation and mortality [38]. The correlation between doses of gamma radiation and survival in different life stages of Drosophila melanogaster differed from one stage to another. Figure 8 represents the mean percent survival of all stages plotted against irradiation dose [38]. LD_{50} is increasing with the stage of the development.

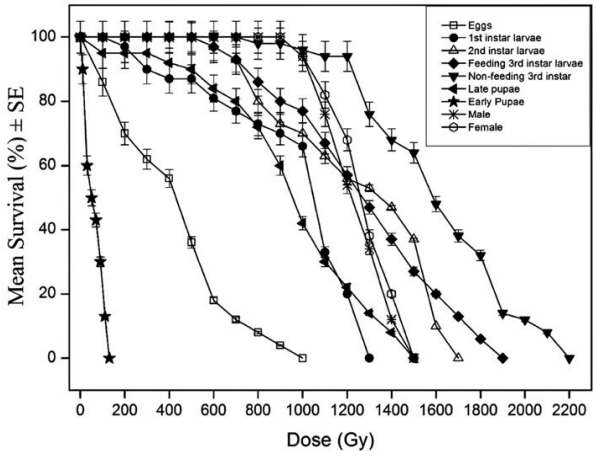

Figure 8: Gamma radiation tolerance measured post-irradiation at different developmental stages of Drosophila melanogaster. Here eggs, larvae (1st instar, 2nd instar and 3rd instar), pupae (late and early) and adult flies (male and female) were gamma irradiated and their survival rate measured at different doses ranging from (0 – 2200) Gy. Y-axis represents mean survival % (± SE) and X-axis represents gamma radiation treatment at different dose level [38].

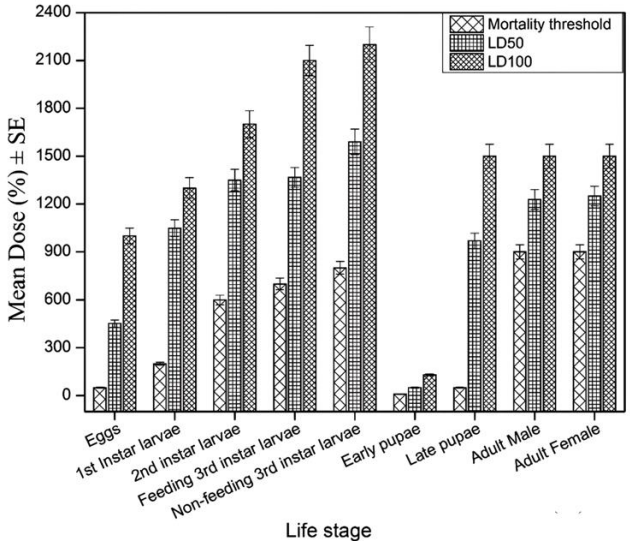

Figure 9: Threshold of mortality, LD_{50} and LD_{100} values during gamma irradiation at different developmental stages of Drosophila melanogaster. Y-axis represents mean dose of gamma radiation (Gy) ± SE ranging from (0 – 2200) Gy. X-axis represents different developmental stages

of Drosophila melanogaster including eggs, 1st instar, 2nd instar, 3rd instar (feeding and non-feeding), pupae (early and late) and adults (male and female) used for gamma irradiation treatment [38].

Significant age-dependent decline in the radiation resistance of both males and females was observed [39], [38].

The larvae of field populations had a higher survival rate at 51 % as compared to 43 % in the case of cultured flies and thus more resistant. The III instar larval stage (lab culture) had a LD_{50} of 26 Gy as compared to LD_{50} of 928 Gy in case of adult flies have ~160 times higher tolerance compared to humans. Prolonged rearing comparable to 'domestication' might have induced reduction in tolerance. Larval stages have a lower tolerance than adults possibly due to higher metabolic rate. Adults are post-mitotic with a low cell division rate. This may contribute to higher tolerance [40].

CHAPTER THREE: MATERIALS AND METHODS

3.1 Van de Graff accelerator

The Applied Nuclear Physics lab operates a re-built 350 KeV Van de Graff accelerator (see Figure 10) which is shooting an electron beam through the thin window into the room. Electrons are suited perfectly for this task compared to protons which are too slow to extract at low energy. Using electrons, we can deposit high dose rates over small depths that are relevant to our biological system.

Increasing the KVp generally results in a higher beam intensity, but it may not necessarily affect the number of electrons in the beam itself. Beam current relies more on factors such as the emission of electrons from the cathode. Consequently, conditions with high energy but low beam current are very likely to occur. Once electrons are dispersed into the room, the energy becomes too high for sensitive detectors to accurately determine radiation dose, while the beam current remains too low. In such circumstances, conventional physics techniques for measuring radiation dose, such as the parallel plate chamber, prove to be less effective, necessitating the exploration of alternative methods.

The Applied Nuclear Physics Lab conducted numerous experiments to ascertain radiation dosages utilizing electron beam. Energy was deposited into personal radiation badges, Drosophila melanogaster eggs, and HeLa Cells.

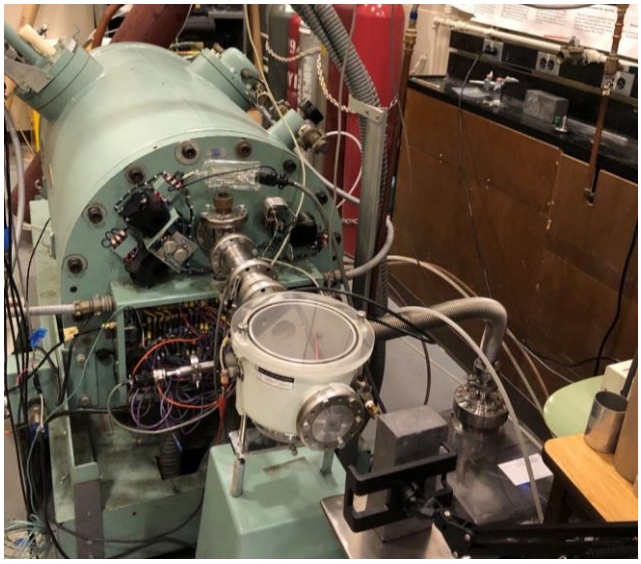

Figure 10: Van de Graff accelerator located at the Applied Nuclear Physics lab in the Department of Physics and Astronomy at Minnesota State University, Mankato.

3.2 Personal radiation badge experiments

The first set of experiments was made using 2 personal radiation badges. They were placed 8 cm away at 0 ° from the source and irradiated for 5 and 15 min, respectively (see Figure 11). Both exceeded their limits which is 10 Gy, suggesting a minimum dose rate of 120 Gy/h.

For the second set the badge was placed 12 cm away at 0 ° from the source for 1:45 min. It exceeded its limit in 10 Gy, suggesting the same dose rate.

The third badge experiment was done using 2 badges. The first badge was placed 8 cm away at 0 ° from the source using vial tube, cut at the bottom, to imitate a Drosophila melanogaster set up. The badge was irradiated for 5.5 seconds. Beta dose was equal to 66070 mR = 0.66 Gy, which gives dose approximately equal to 432 Gy/h.

For the second badge surface-to-source distance (SSD) was equal to 12 cm at 0 °. The badge was irradiated for 5 sec showing 19070 mR = 0.19 Gy, which is giving a dose approximately equal to 137.3 Gy/h.

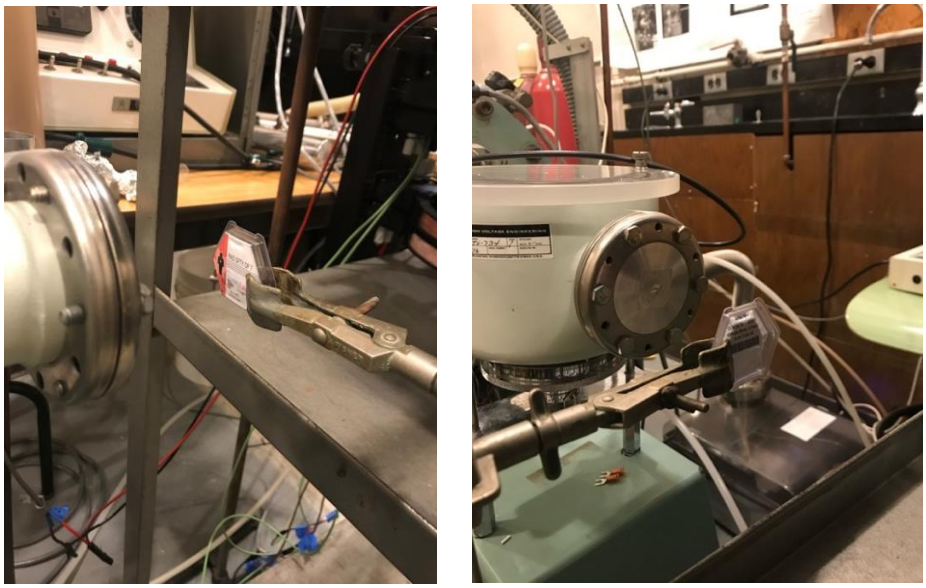

Figure 11: Set up for the first Badge experiment. Badges were placed in front of the accelerator window at 0 ° through which electrons were emitted.

3.3 Drosophila melanogaster experiment set up

In collaboration with Dr. Daniel Toma from the Biology Department at Minnesota State University, Mankato we started our work with Drosophila melanogaster. The initial goal of the experiment was to calibrate the Van de Graaff accelerator based on the response to radiation of the biological system. Before initiating an experiment, it is advisable to confirm whether Drosophila melanogaster is a suitable model organism for our research and whether it is compatible with the conditions of our laboratory. The size of Drosophila

melanogaster eggs measures 1 mm in length and 0.2 mm in width. The eggs were laid down on top of the food. Food was placed at the bottom of the vials utilized for irradiating the samples. The food mixture consisted of sugar, corn syrup, and yeast. Food is an essential component for these purposes, serving as a supportive element crucial for the development of Drosophila melanogaster through all stages of development. Throughout the entire research process, all samples were consistently prepared using the same food ingredients. At 350 KeV, the electron beam penetrates the sample to a depth of 1.060 mm. Given the size of the eggs, this indicates that in our lab conditions, the 350 KeV beam will irradiate all eggs as soon as it reaches the sample. Consequently, the set-up shown in Figure 12 is advantageous, as 99.73% of the deposited energy is directed towards irradiating the eggs.

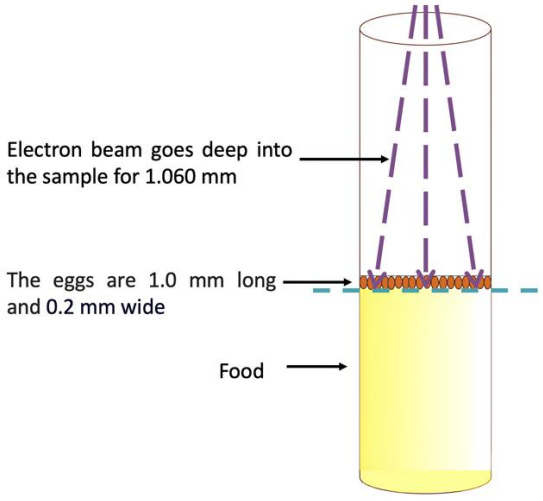

Figure 12: The illustration demonstrates the advantageous utilization of Drosophila melanogaster eggs for experiments, highlighting how the size of the eggs aligns with the depth at which the electron beam penetrates the sample, making them good system for irradiation purposes.

As published earlier, LD_{50} is greater than 452 Gy for almost all development stages [41]. Samples were placed in front of the accelerator window at 0 ° with the vial opening towards the window which is shown in Figure 13. After the irradiation, all samples (experimental and controls) were placed into the incubator at the temperature of 26 °C and were kept there till the end of each experiment throughout all development stages.

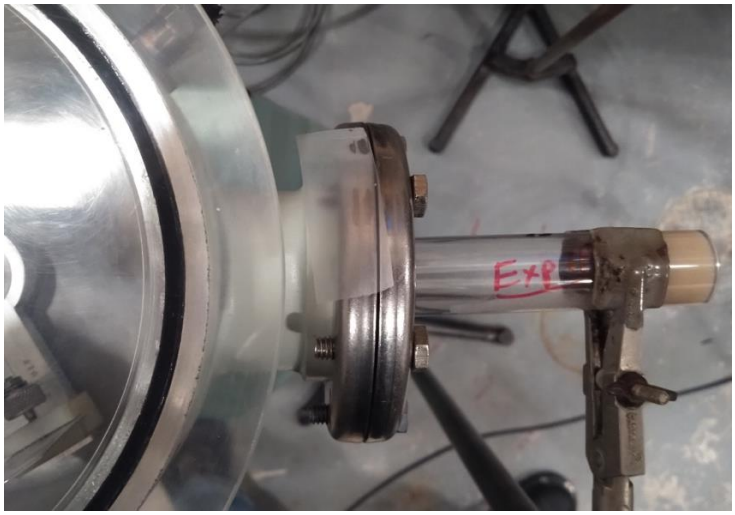

Figure 13: Drosophila melanogaster set-up prior irradiation with an electron beam.

3.3.1 Fruit flies Experiment 1

Experiment 1 was done with 4 vials: 2 experimental and 2 controls. Samples were laid down (24 ± 3) hours before irradiation. First vial was placed 6 cm away from the source at 0 ° and irradiated for 40 minutes, rotated for 90 ° and irradiated for additional 20 min. Second vial was placed at the same position and irradiated for 5 hours with rotation of 90 ° each hour. Control-1A was shielded using led bricks (shown in Figure 14 a), Control-1B

was shielded with led bricks inside of incubator at temperature of 26 ℃ (shown in Figure 14 b). After the procedure, all vials were placed and kept at the incubator at the temperature of 26 ℃.

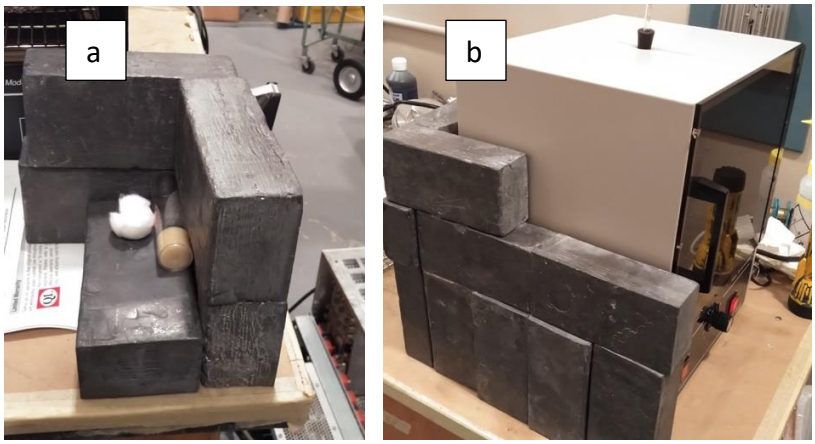

Figure 14: Control vials during Experiment 1 procedure. Control-1A (a) was shielded using led bricks during the experiment, while Control-1B (b) was shielded with led bricks inside of incubator.

The findings of Experiment 1 are depicted in Figure 15. In the Control vials, the presence of a black band at the top indicated ongoing life processes, while in the Experimental vials, no such black bands were observed atop the egg masses, signifying population demise. These results indicate that no fruit fly eggs survived in either experimental vial throughout the duration of the experiment.

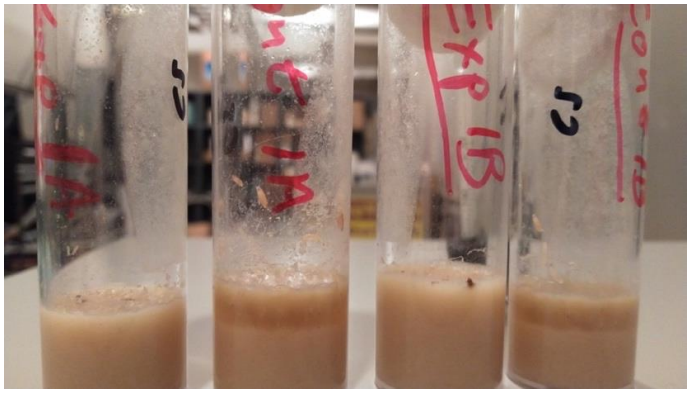

Figure 15: The findings from Experiment 1 are as follows: In the two control vials, distinct signs of life processes are apparent, characterized by the presence of black bands. However, in the experimental vials, there is a notable absence of any such indicators of life.

3.3.2 Fruit flies Experiment 2

Experiment 2 was made using 4 experimental (A, B, C, and D) and 2 control vials. Samples were laid down (24±3) hours before irradiation. They were exposed to radiation 6 cm away from the source at 0 ° for 45 sec, 5, 15, 30 min accordingly. Control vials were shielded at room temperature during the experiment to maintain consistent conditions for all samples. After the procedure, all vials were placed into the incubator at the temperature of 26 ℃. Compared to the control group late development was noticed at all experimental vials. First adults were noticed in a vial A, 196 h after irradiation. At other vials pupae never developed to that stage. In vials B, C, and D all fruit flies pupae died before returning into adults. After 432 h adults started to die at the control vials. The subsequent step involved scattering irradiation into smaller time intervals to observe changes in the development rate and identify noticeable stages.

3.3.3 Fruit flies Experiment 3

Experiment 3 had 2 parts: part 3A and 3B.

Samples were laid down (24±3) hours before irradiation. Part 3A had 5 vials: 2 controls and 3 experimental (A, B and C). Vial A was irradiated for 20 min with rotation of 180 ° every 10 min. Vial B was irradiated for 2 min with rotation of 180 ° after a minute. Vial C was irradiated for 60 min with rotation of 90 ° every 30 min, then it was left to chill for 50 min and irradiated for an additional hour with rotation of 90 ° every 30 min.

Samples were placed 8 cm away from the window at 0 °. All samples were kept out of the incubator until the experiment was finished. Control vials were shielded at room temperature during the experiment to maintain consistent conditions for all samples. After the procedure, all vials were placed into the incubator at the temperature of 26 °C.

Part 3B had 7 vials: 1 control and 6 experimental (A, B, C, D, E and F), that were irradiated for 1, 5, 15, 30, 45, and 90 min. Samples were laid down (24±3) hours before irradiation. During the experiment, all samples were located outside of the incubator at room temperature.

Sample A had a rotation of 180 ° after 30 sec. Vial B was rotated for 120 ° after 90 sec and then was additionally irradiated for 110 sec and 100 sec with rotation of 120 ° in between. Vial C had 2 rotations of 120 ° every 5 minutes. Sample D was rotated 2 times for 120 ° every 10 min. Vial E had 2 rotations of 120 ° every 15 min. Sample F was rotated 2 times for 120 ° every 30 min. After all samples were irradiated, they were placed into the incubator with the temperature of 26 °C.

Experiment 3A showed late development compared to the control group which is represented by Figure 16. None of the experimental sample showed development to the adult stage.

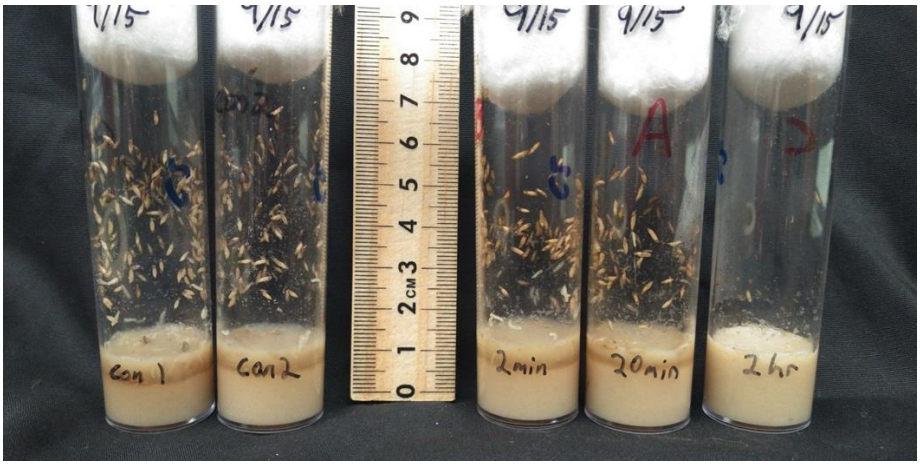

Figure 16: Experiment 3A at 235 hours after the eggs were laid down. Visually noticeable late development was observed in the experimental vials (on the right) compared to the control ones (on the left). No adults were observed in the experimental vials.

Experiment 3B was the first experiment where we were able to monitor the development of the population till the stage of adult. The first adult appeared in 264 h after eggs were laid down at vial A, which was irradiated for 1 min. This was a milestone moment that gave an understanding of the direction for future work. Figure 17 represents Experiment 3B, highlighting visual late development and the presence of an adult population for the first time throughout the experiments.

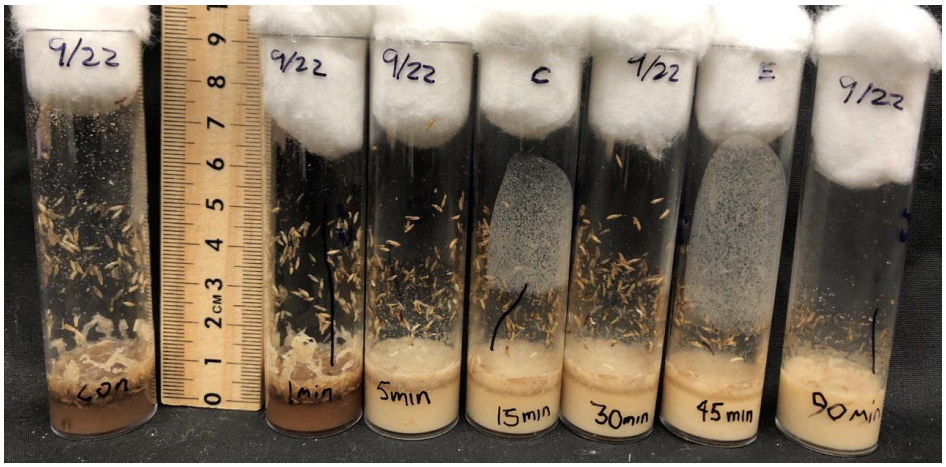

Figure 17: At 357 hours after the eggs were laid down, Experiment 3B illustrates late development as well as the emergence of the first adult population in this series of experiments.

3.3.4 Fruit flies Experiment 4

Experiment 4 had 2 parts as well: part 4A and 4B.

The eggs were laid down (24±3) hours before irradiation.

Experiment 4A had 5 vials: 1 control and 4 experimental (A, B, C, and D) which all were irradiated for 15 min with 2 rotations of 120 ° every 5 min. The difference in this set up compared to previous experiments was in a position of the vials and irradiation time. Having a guess that radiation dose might be too high for the potential survival of population due to its location we decided to experiment with the distance from the window of the source to the samples. Vials were irradiated at 8, 10, 12, and 14 cm accordingly. All of them were positioned at 0 ° away from the source.

Part 4B had 5 vials as well: 1 control and 4 experimental (A, B, C, and D). All of them were irradiated at 8.8 cm away from the source at 0 °. Vial A was irradiated for 30 sec with

one rotation of 180 ° in 15 sec. Vial B for a minute with one rotation of 180 ° in 30 sec. Sample C and D were irradiated for 2 and 3 min accordingly with one rotation of 180 ° in between. All control samples were kept at room temperature during the experiment and placed at the incubator with the temperature of 26 °C after irradiation was completed.

Experiment 4 showed us that we need to change the position of our samples in the set up. The dose rate was too high at 8 cm which was not beneficial for survival rate. Therefore, our work was concentrated on placing samples farther away from the window in the next experiments. In this way we were trying to find a perfect position in lab conditions where generations would get irradiated but still able to develop and reproduce. Figure 18 supports this idea. A comparison of the experimental vials reveals a noticeable increase in survival rate attributed to the greater distance between the radiation source and the samples. Sample D from part 4A was the only one that showed the stage of adult population. The time of irradiation was kept between 1-3 min for future experiments as this window allowed us to see the development rate.

Experiment 4B, as depicted in Figure 19, presented inconclusive data regarding the development rate across all irradiated samples. Late development was observed compared to the control sample. Furthermore, pupae in the experimental samples gradually caught up in number with those in the control vial over time.

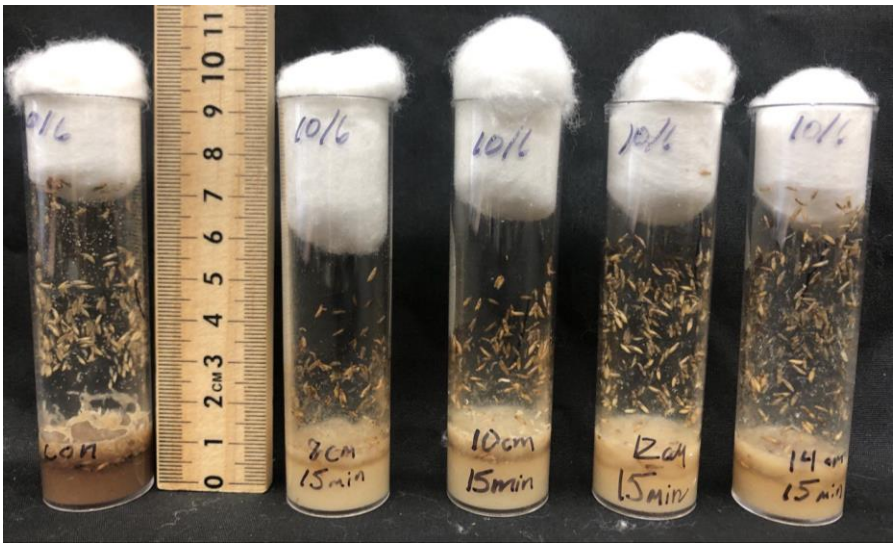

Figure 18: Experiment 4A at 336 hours after the eggs were laid down demonstrated a crucial effect on development with an increase in the distance from the source to the sample. The survival rate was higher in the samples that were irradiated farther from the source.

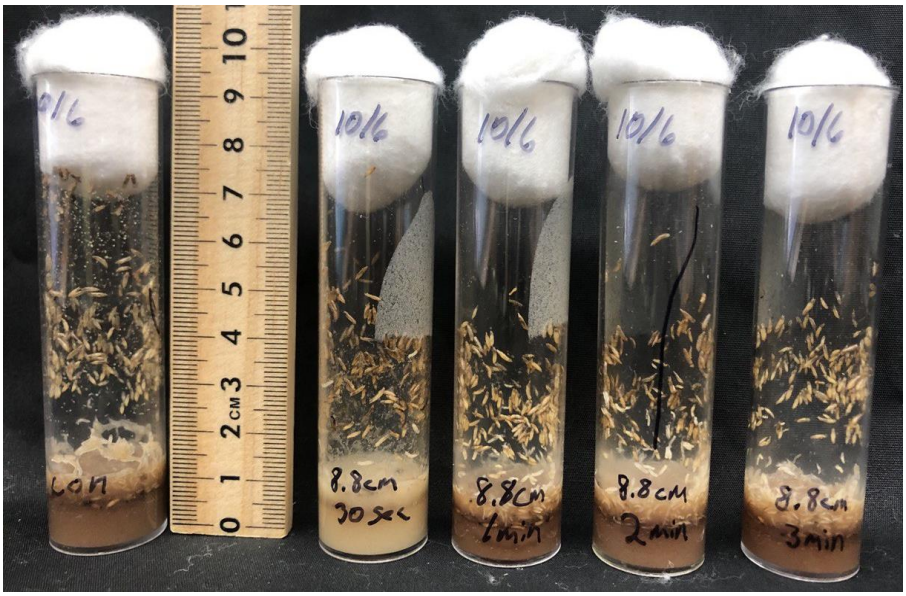

Figure 19: Experiment 4B at 336 hours after the eggs were laid showed inconclusive difference in development rate among the 4 different samples.

3.3.5 Fruit flies Experiment 5

Experiment 5 had two parts: 5A and 5B. They both had 4 vials each (1 control and 3 experimental) and were irradiated at 10, 12, and 14 cm away from the window at 0 °. The eggs were laid down (24±3) hours before irradiation.

At Experiment 5A all 3 samples (A, B, and C) were irradiated for 1 minute with rotation of 180 ° in between.

At Experiment 5B all 3 samples (A, B, and C) were irradiated for 3 minutes with rotation of 180 ° in between.

After irradiation, all samples were placed into the incubator at the temperature of 26 °C.

Experiment 5 did not yield a clear indication of the optimal position for the samples, which was expected to be a perfect placement for LD_{50} measurement. Both parts of Experiment 5 (5A and 5B) demonstrated an unpredictable increase in population for samples placed 14 cm away from the irradiated window, following exposures of 1 and 3 minutes as shown in Figures 20 and 21. This observation led to the assumption of a beneficial effect from irradiation on the population.

Notably, samples located 12 cm away exhibited a significant radiation effect, resulting in the emergence of pupa populations and adults. Consequently, the predicted optimal location of 12 cm was selected for further experimentation.

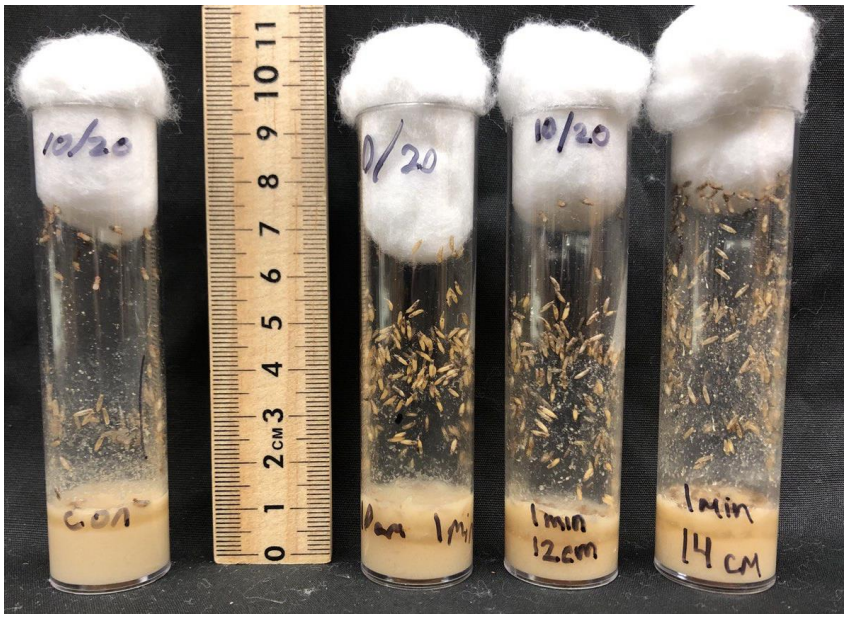

Figure 20: Experiment 5A at 318 h after eggs were laid down showed a different development rate compared to the control group. Increased population was obvious and unexpected.

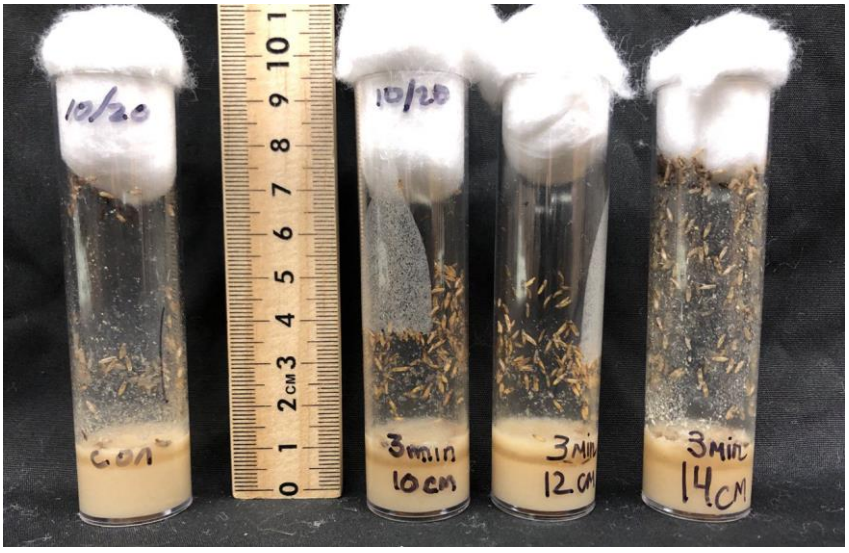

Figure 21: Experiment 5B at 318 h after eggs were laid down. Population increased 3 times compared to the control group in the vial C which was irradiated for 3 min at 14 cm away from the source.

3.3.6 Fruit flies Experiment 6

Experiment 6 separated into 2 parts in order of time when eggs were laid down.

Part 6A had 3 samples (F, D, and B). The eggs were laid down (24±3) hours before irradiation.

Part 6B had 4 samples (E, G, C, and A). The eggs were laid down (48±3) hours before irradiation.

All samples were placed 12 cm away from the window at 0 ° and were irradiated for 2 min with rotation of 180 ° in between.

After the experiment was finished, all samples were moved to the incubator at the temperature of 26 °C.

Experiment 6, depicted in Figure 22, showed that 2 days old laid eggs (part 6B) all produced adults which led to the idea of using older eggs as they might be more radiation resistant. The result showed an importance of scattering 12 cm point into wider selection of irradiation times aiming to find a time which can show 50 % lethal dose of population.

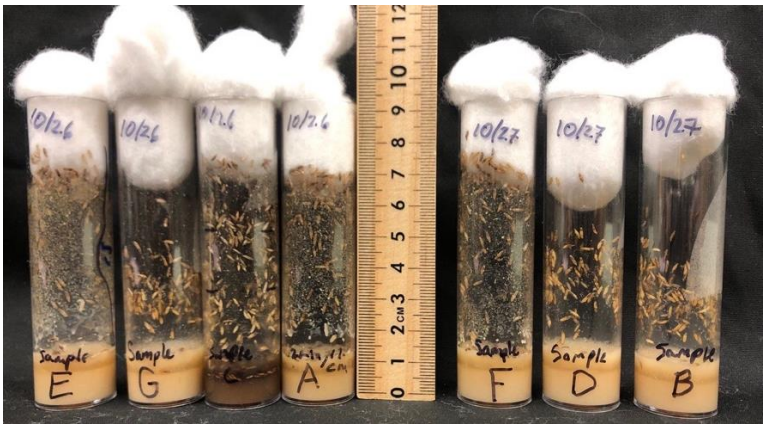

Figure 22: Experiment 6 (part 6A and 6B together) at 358 and 382 h after eggs were laid down showing higher radio resistance in older eggs.

3.3.7 Fruit flies Experiment 7

For the Experiment 7 eggs were laid down (48±3) hours before irradiation in all samples. Vials were placed 12 cm away from the source at 0 °.

We had 12 samples: 2 controls and 10 experimental that were irradiated for 20, 3, 30, 30 sec, 1, 1.5, 5, 10, 15 and 45 min, in this particular order, with 1 rotation of 180 ° in between. During the experiment, all samples were outside the incubator at room temperature. After irradiation was completed, all vials were moved to the incubator at the temperature of 26 °C.

The result of this run showed that shots of 20, 3, and 30 min had the lowest number of adults compared to the rest, while runs 5, 10, and 15 min had the highest. Figure 23 depicts this phenomenon, particularly evident in the vial irradiated for 3 minutes, which exhibited more pronounced effects of radiation compared to vials irradiated for longer durations. This led to possible systematic problems and a potential drop in the dose rate. The runs from 0.5 sec to 15 min were made sequentially which resulted in data that should be re-recorded. It was decided to repeat this trial while minimizing the possibility of dose rate dropping in a way to mix up irradiation times. Also, the suggested warming up time of the machine was added.

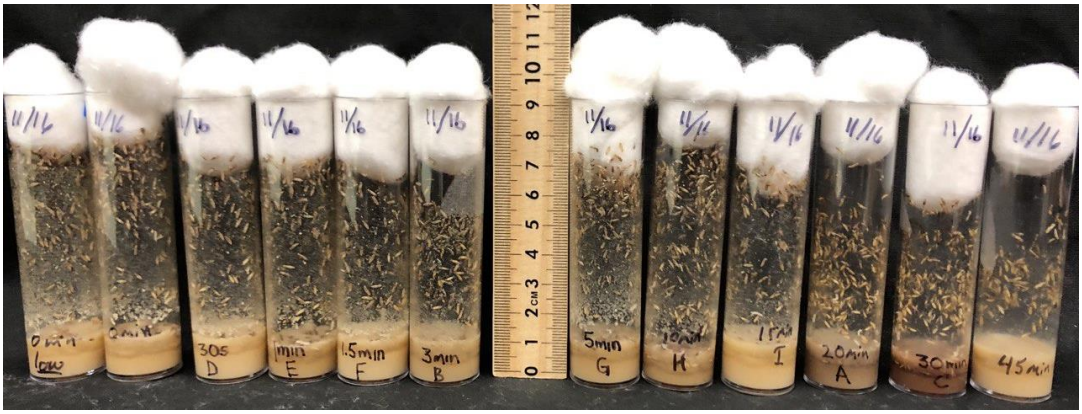

Figure 23: Experiment 7 at 341 h after eggs were laid down. Inconsistent adult rate is noticed at the samples due to the sequence of irradiation.

3.3.8 Fruit flies Experiment 8

In Experiment 8 we used eggs that were laid down (48±3) hours before irradiation in all samples as well.

Experiment 8 had 10 samples: 1 control and 9 experimental and were irradiated for 0.5 sec, 1, 1.5, 3, 5, 10, 15, 20, and 30 min respectively with 1 rotation of 180 ° in between.

All samples were irradiated at 8 cm away from the source at 0 °.

During the experiment, all samples were located outside of the incubator at room temperature. After irradiation was completed, all vials were moved to the incubator at the temperature of 26 ℃.

Experiment 8 exhibited a pupae population, highlighting the phenomenon of late development rate, as depicted in Figure 24.

Experiment 8 did not produce explicit adult population in all samples. Adults were shown in vials that were irradiated for 0.5, 1, 1.5, 3, and 10 min, but not in those that were

irradiated for 5, 15, 20, and 30 min. Population exceeded maximum of 1-8 adults depending on a sample. The lack of data stemming from the mortality of fruit flies at various stages prevented us from determining a dose rate.

The next step was taken towards moving samples back to 12 cm point away from the window, as it allowed to see population in each sample developing from pupa to adult stage. In this case, we could observe the maximum peak of each stage and determine LD_{50}.

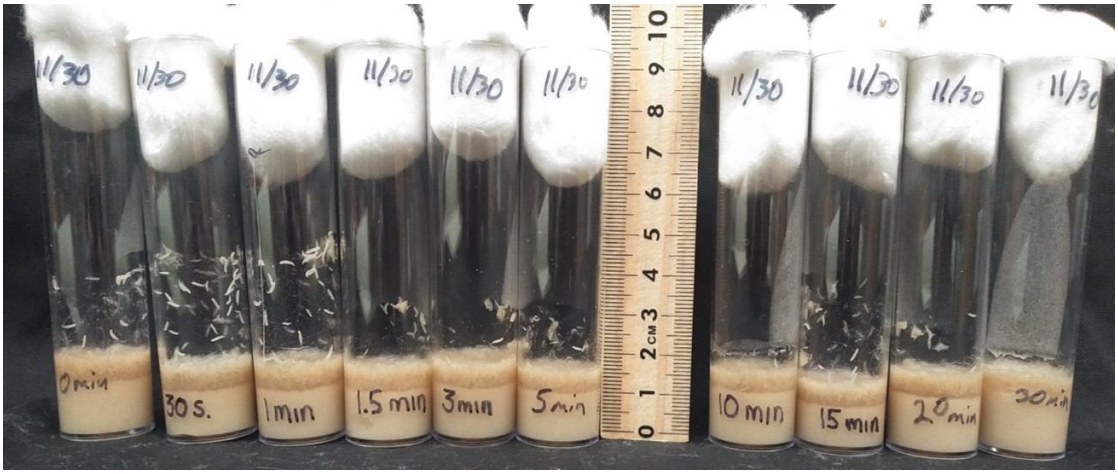

Figure 24: Experiment 8 at 101 h after eggs were laid down, highlights the phenomenon of late development.

3.3.9 Fruit flies Experiment 9

Experiment 9 was based on Experiment 7. Eggs were laid down (48±3) hours before irradiation in all samples.

Samples were placed 12 cm away from the window at 0 °.

We had 11 vials: 1 control and 10 experimental that were irradiated for 15, 3, 10, 20, 5, 30, 1, 15, 3, and 10 min in this specific order. They were marked 15A, 3A, 10A, 20, 5, 30, 1, 15B, 3B, and 10B accordingly. All samples had 1 rotation of 180 ° in between. During the experiment, all samples were outside the incubator at room temperature. After irradiation was completed, all vials were moved to the incubator at the temperature of 26 °C.

Experiment 9 generated a dataset covering all stages of fruit fly development. We were able to monitor evolution together with such processes as late development, mutation, and death. This experiment produced a data-set that was used along with Experiment 7 to determine lethal radiation dose of populations. Figure 25 (a and b) illustrates how the pupae population gradually caught up in development rate with the control group. Although there is still a noticeable decrease in development rate, even the condition of the walls of the vials indicates that the number of pupae in the control group was higher than in the experimental ones. Furthermore, the number of adults decreased with an increase in irradiation time.

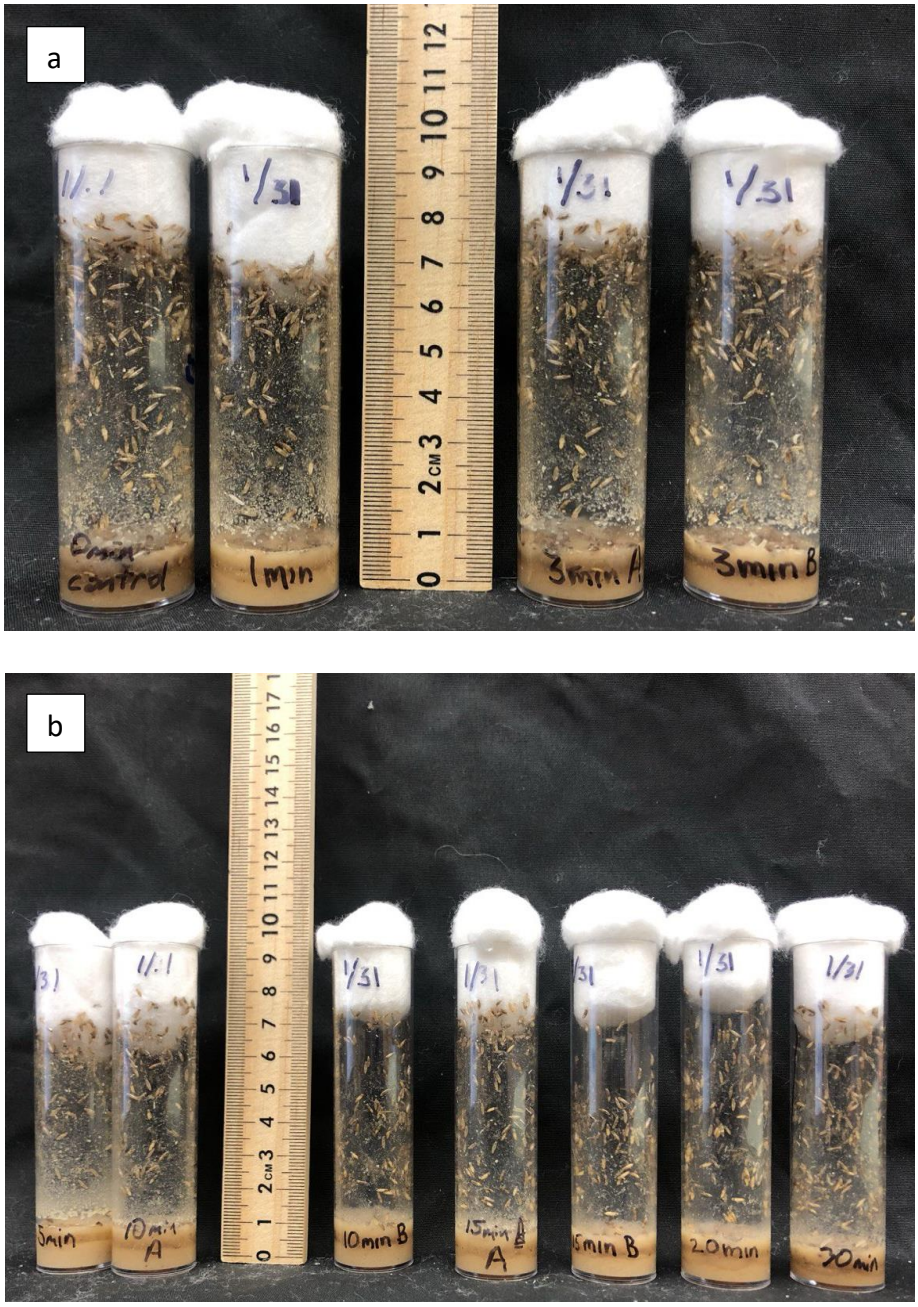

Figure 25: Experiment 9 at 342 h after eggs were laid down. The dataset produced by this experiment covered all development stages.

3.4 HeLa Cells Experiments

The Applied Nuclear Physics Laboratory, in collaboration with Dr. Allison Land from the Biology Department at Minnesota State University, Mankato, conducted a series of experiments using HeLa Cells.

The primary objective of the experiment was to calibrate the Van de Graaff accelerator by assessing the response of HeLa Cells to radiation. Initially, our goal was to determine the LD_{50} parameter. We sought to identify the position, location, and duration of irradiation at the assay site that would result in a lethal dose of 50 % for the cells.

Figure 26 shows the Experimental set up for the HeLa Cells Experiments. The assay plate was positioned in front of the accelerator window at 0 °. Each assay plate had a control group, which was shielded by lead brick.

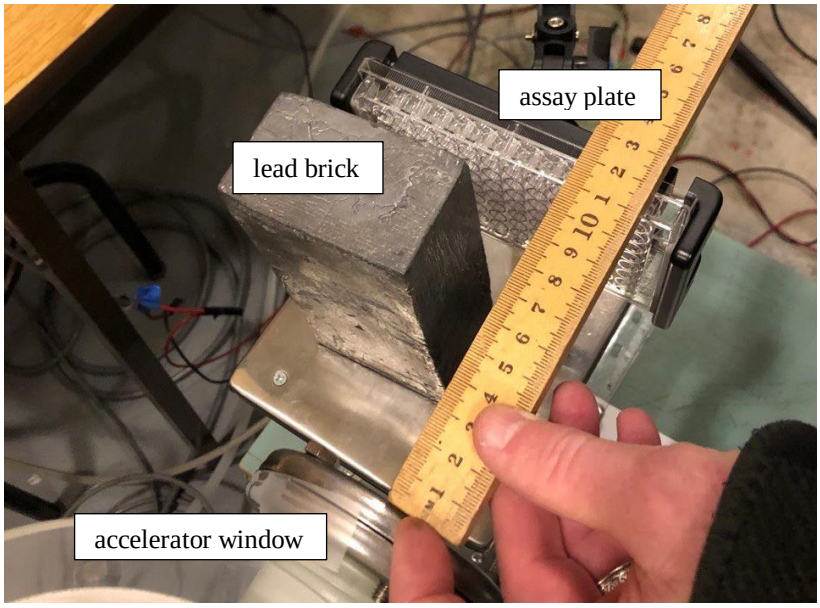

Figure 26: Experimental set-up before HeLa Cells irradiation.

3.4.1 First HeLa Cells Experiment

For the first set of experiments, we had 2 assay plates.

The first plate was located 8 cm away from the source at 0 ° and was irradiated for 3 min. SSD for plate 2 was 40 cm and was irradiated for 3 min.

HeLa Cells were grown in the lab and placed in assay plates with media before irradiation using a hemocytometer. Each assay plate well contained 5000 cells. The wells were in the shape of "X" for more effective positioning of sample in front of the window. The beam was aimed at the center of "X" of the assay plate. Right before irradiation the media was tapped out from the plate. Hands were sanitized with alcohol in advance.

The control group had 4 wells (D1, D2, E1, E2) for both cases and was covered with a copper disk on assay plates. Immediately following irradiation, the assay plate was replenished with fresh media. This step was undertaken to sustain cell viability, as cells require nutrient material to proliferate effectively outside the human body. Subsequently, the cells underwent a viability assessment using an MTS assay (a colorimetric method for determining cell viability).

3.4.2 HeLa Cells Experiment 2

In the second setup, we also utilized two plates. The first plate was positioned 12 cm away from the source's window at 0 ° and was irradiated for 15 min. Second plate had the same SSD but was irradiated for 30 min. The control group had 5 rows of wells: C, D, E, F, G – columns 1-3 which were shielded with the lead brick. For both experimental plates the

number of cells at wells had the same configuration shown in Table 2. The beam was aimed right at the center of each experimental set of assay plates.

Right after irradiation was completed the media was refilled back into the assay plate.

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C	5000	5000	5000				2500	2500	2500	2500	2500	2500
D	2500	2500	2500				5000	5000	5000	5000	5000	5000
E	1250	1250	1250				2500	2500	2500	2500	2500	2500
F	625	625	625				5000	5000	5000	5000	5000	5000
G	0	0	0									
H												
	controls						experimental					

Table 2: Configuration of wells for both assay plates for the second set up of HeLa Cells Experiment.

3.4.3 HeLa Cells Experiment 3

In the third setup we used 3 assay plates. SSD for all of them was 12 cm. All 3 assay plates were irradiated for 20 min at 0 °. The control group had 5 rows of wells: C, D, E, F, G – columns 1-3 which were shielded with the lead brick. The configuration of cells for Experiment 3 was the exact same as for Experiment 2 (see Table 2). For all plates, the number of cells on wells were the same. The beam was aimed right at the center of each experimental set of assay plates.

Right after irradiation was completed the media was refilled back into the assay plate.

CHAPTER FOUR: RESULTS AND DISCUSSION

4.1 Determining LD_{50}

Through all the experiments the best position for the samples which allowed us to observe all development stages was recorded at 12 cm away from the source. Experiments 7 and 9 gave the best data and were taken as analysis base to determine LD_{50} for pupae and adult population.

All counting was done manually for all development stages every time during data collection. The data was collected 2-3 times a day throughout all development stages for each experiment. The effectiveness of data analysis using Python was hindered by the shape of the vials and time constraints. Machine counting posed challenges, as it necessitated capturing a 360-degree picture of each sample repeatedly. Additionally, throughout the lifespan of the fruit flies, their excrement accumulated on the vial walls, which the machine mistakenly included as objects (see Figure 27).

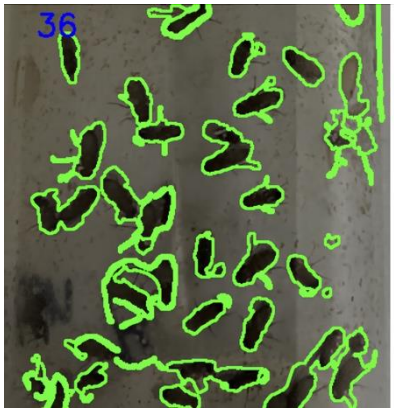

Figure 27: An example of data analysis using Python, showcasing inaccuracies as the machine erroneously included other objects as part of the adult population.

During the counting, fruit fly adults tend to move to the top of the vial if one was placed horizontally at the surface. It was a crucial step at counting the population of adults. Figure 28 illustrates one of the experimental data collections.

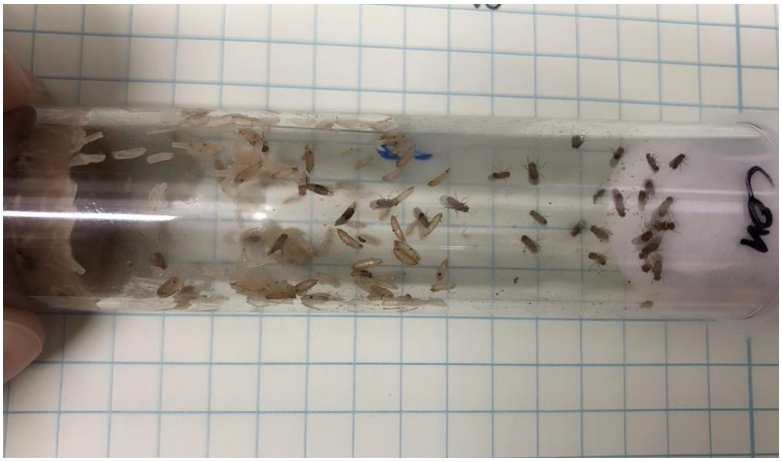

Figure 28: Example one of the vials for adult population count.

Pupae populations for Experiment 7 and 9 are represented in Tables 3 and 4, respectively. The population peak was recorded at 188 h and 187 h respectively after eggs were laid down. At this point, none of the vials contained any adults. Dose rates were determined and subscribed for each sample based on badge readings for SSD 12 cm.

Experiment 7	**pupaes**		laid 11/16/22		12 cm							
dose(Gy)	**0**	**0**	1.1442	2.2884	3.4326	6.8652	11.442	22.884	34.326	45.768	68.652	102.978
hours	**0**	**0**	**30 sec**	**1**	**1.5**	**3**	**5**	**10**	**15**	**20**	**30**	**45**
148.15	29	2	12	3	0	3	3	2	0	0	0	0
161.5	77	40	52	62	44	27	15	9	7	3	10	2
173.5	91	65	68	83	64	56	67	40	52	9	20	8
188	103	119	76	112	70	89	110	95	112	19	45	28

Table 3: Experimental data for pupae population from Experiment 7.

Experiment 9		pupas	laid 01/31/23		12 cm	2 days old eggs					
hours	0	1	3A	3B	5	10A	10B	15A	15B	20	30
144	4	3	5	0	24	4	5	3	11	6	0
149.4	21	8	15	4	50	8	7	7	16	10	1
162	80	65	33	60	92	58	12	24	43	14	34
168	94	76	65	87	97	75	27	58	51	20	57
172.2	151	148	107	106	117	109	49	111	79	45	81
187.2	211	188	212	198	160	143	104	173	94	123	122
193	178	152	200	146	111	129	95	132	91	112	128
199	240	168	229	194	116	169	120	219	126	145	139
210	180	191	217	182	112	142	136	164	110	155	152
215.2	232	202	240	224	107	159	167	185	129	158	148
221.5	243	195	231	221	118	189	183	192	130	160	156
262.4	256	273	280	247	91	260	190	197	143	147	145
269.6	232	253	282	242	88	251	193	200	130	146	143
286.2	241	262	273	240	82	241	189	195	120	143	140
294.4	238	252	266	230	76	235	188	195	116	140	141

Table 4: Experimental data for pupae population from Experiment 9.

Based on the data recorded from the badge Experiment 3, at 12 cm away from the source, the dose rate is equal to 137.304 Gy/h which is letting to determine radiation dose for irradiated samples. Table 5 represents compressed data from Experiments 7 and 9 and their average values of peak pupae population at each irradiation time and uncertainties.

	h laid down								
Dose (Gy)		0	2.2884	6.8652	11.442	22.884	34.326	45.768	68.652
min		0	1	3	5	10	15	20	30
Exp 7	188	103	112	89	110	95	112	19	45
Exp 9	187.2	211	188	212	160	143	173	123	122
exp 7		119		198		104	94		
Average	187.6	144.333333	150	166.333333	135	114	126.333333	71	83.5
n	2	3	2	3	2	3	3	2	2
σ_{n-1}	0.56568542	47.5803414	37.9868398	54.9717099	24.979992	20.808652	33.7918465	51.9903837	38.4870108
uncerrtainty	0.4	27.4705229	26.860752	31.7379315	17.6635217	12.0138809	19.5097317	36.7627529	27.2144263

Table 5: Average values of pupae at their peak population with uncertainties for experiment 7 and 9 combined.

The uncertainties were found through the formulas:

$\sigma_{n-1} = \sqrt{\frac{1}{n-1}} \sum_{i=1}^{n} (x_i - \bar{x})^2$, standard sample deviation and

$\delta A = \frac{\sigma_{n-1}}{\sqrt{n}}$, uncertainty of the mean value.

The adult population data are shown in Tables 6, 7 and 8 with the peak population for each Experiment.

Experiment 9	adults		laid 01/31/23		12 cm	2 days old eggs					
hours	0	1	3A	3B	5	10A	10B	15A	15B	20	30
242	0	0	0	0	2	0	0	0	2	1	0
258.3	4	5	8	4	20	3	3	7	6	4	4
262.2	17	7	7	6	32	8	3	7	9	3	7
269.4	12	22	9	15	51	13	7	10	7	4	11
284	30	40	20	20	50	16	10	20	15	8	12
286	49	51	37	32	53	26	15	28	9	7	15
294.15	64	67	71	43	51	23	10	34	8	8	19
310.15	76	68	79	61	46	31	14	33	7	9	7
319.5	88	77	63	52	61	32	16	44	6	9	19
329.3	100	80	70	50	60	20	15	30	5	6	11
333.5	93	85	80	54	61	34	16	36	7	6	14
341	98	76	86	62	46	32	12	39	6	5	12

Table 6: *Experimental data for adult population from Experiment 7.*

Experiment 7	adults		laid 11/16/22								
dose(Gy)	0	0	1.1442	2.2884	3.4326	6.8652	11.442	22.884	34.326	45.768	68.652	102.978
hours	0	0	0.5	1	1.5	3	5	10	15	20	30	45
238.5	0	0	0	0	0	1	0	0	0	0	0	0
243.5	4	1	0	1	0	1	1	0	0	0	0	0
256	30	3	10	7	5	4	0	0	0	0	0	0
267	50	15	23	18	12	10	7	4	2	0	0	0
283.45	70	50	30	25	30	15	25	20	20	0	0	1
296.05	92	79	40	57	37	16	65	39	30	0	0	1
309.4	96	89	34	59	34	12	90	53	30	0	0	0
318.01	98	87	42	56	39	13	87	60	37	0	0	0
333.04	96	86	39	50	36	13	83	67	34	0	0	0
341.45	78	58	45	48	30	11	97	71	29	0	0	0

Table 7: *Experimental data for adult population from Experiment 9.*

Table 8 represents compressed data from Experiments 7 and 9 and their average values of peak adult population at each irradiation time and uncertainties.

	h laid down								
Dose (Gy)		**0**	2.2884	6.8652	11.442	22.884	34.326	45.768	68.652
min		**0**	**1**	**3**	**5**	**10**	**15**	**20**	**30**
Exp 7	309.4	96	59	12	90	53	30	0	0
Exp 9	310.15	76	68	79	46	31	33	9	7
exp 7		89		61		14	7		
Average	309.775	87	63.5	50.6666667	68	32.6666667	23.3333333	4.5	3.5
n	2	3	2	3	2	3	3	2	2
σ_{n-1}	0.53033009	8.22597512	4.38748219	28.2940905	21.977261	15.9338912	11.5710366	4.38748219	3.35410197
uncerrtainty	0.375	4.74926895	3.10241841	16.3356007	15.5402703	9.19943638	6.68054112	3.10241841	2.37170825

Table 8: Average values of adults at their peak population with uncertainties for experiment 7 and 9 combined.

Plotting the population count of the peak of pupae and adult populations against the dose rate reveals two exponential series shown in Figure 29.

Blue series represents peak of the pupae population at 188 h after eggs were laid down, while orange represents peak of the adult population at 310 h.

By analyzing this graph, we can ascertain the LD_{50} values for both pupae and adult populations. LD_{50}, representing the lethal dose for 50 % of the population, is determined as follows: for the peak of the pupae population, 50 % corresponds to 83 (counts), equivalent to 59 Gy; for the peak of the adult population, 50 % corresponds to 44 (counts), equivalent to 15 Gy. LD_{50} values are marked with stars in Figure 29.

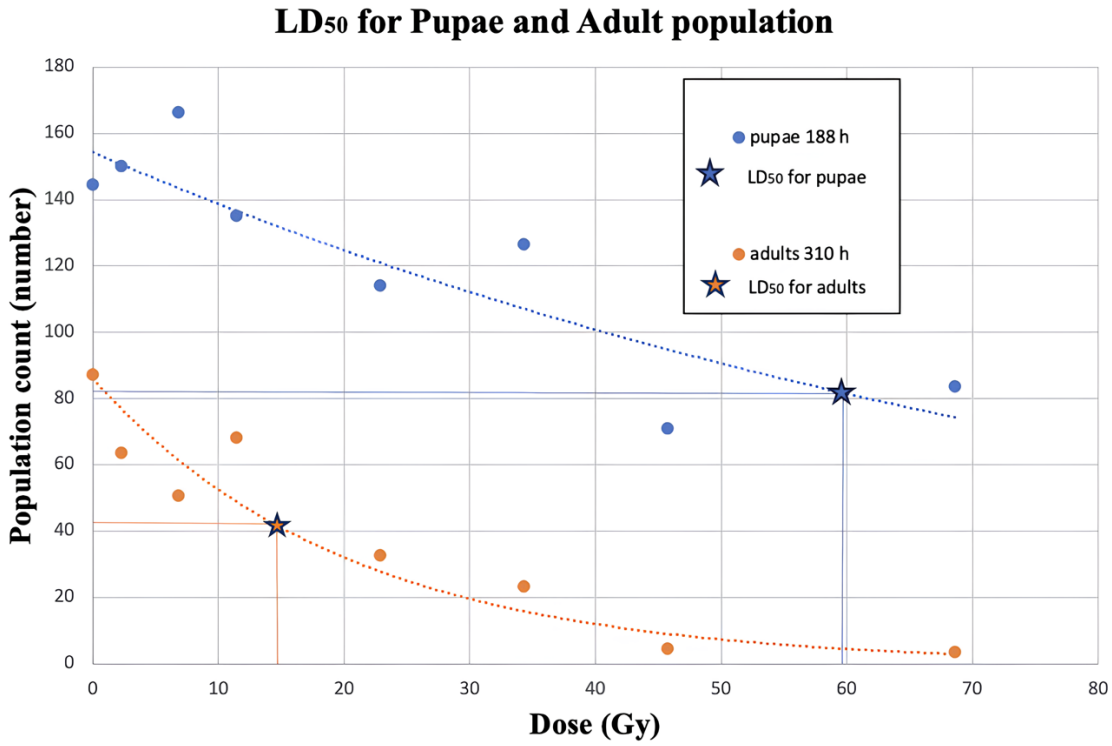

Figure 29: LD_{50} for pupae and adult population.

LD_{50} for the peak of pupae population, recorded at 187 h after eggs were laid down (140 h after irradiation), is about 15 Gy, while for the peak of adult population at 310 h (262 h after irradiation) it is about 59 Gy.

Previous published data claimed LD_{50} for Drosophila melanogaster egg stage (2-6 h old) as 452 Gy and was recorded 24 h post-irradiation[38].

Our researched data shows drastic difference compared to the published ones [38]. LD_{50} for egg stage is significantly reduced. If observed long enough LD_{50} for the maximum

population of pupae, which was recorded at 188 h after laid down (140 h after irradiation) is close to 59 Gy.

Published data of LD_{50} for two-day-old adults is 1228 Gy for adult males and 1250 Gy for adult females. Data was recorded at 1h post-irradiation and at 24 h and 48 h post-irradiation times.

Our outcome of LD_{50} in adult population is showing dose rate around 15 Gy. This value was recorded for the maximum of the adult population at 310 h after eggs were laid down (262 h after irradiation).

The significant importance of this research lies down in the difference between the observed development stage and dose rate compared to the published one [38].

If Drosophila melanogaster eggs are observed long enough drastic change in dose rate will be noticed. Fruit fly eggs will not die right away after radiation, they will develop to the pupa, late pupa or adult formation phase and die at various stages. LD_{50} parameters cannot be observed and assigned to one of the stages at one specific time only. However, it should be taken more precisely and accurately. LD_{50} value is very sensitive to the time, and it will change and vary based on the stage of observation. The concept of LD_{50} must be modified based on the observed stage of development.

4.2 Influence of radiation into adult population

Along all the experiments we noticed such processes as late development, mutations, fast dying of adult generation, necrosis.

Experiment 9 produced flies with glutted wings which were dying fast, as well as flies with no wings and smaller in size compared to the rest of the population. These phenomena are visible in Figures 30 and 31.

Some of the flies were able to develop to the 3rd instar larvae and die inside of cocoon before turning into adults.

Figure 30: Experiment 9 at 270 h after eggs were laid down. Irradiated sample that developed till adult stage exhibit a mutation characterized by the absence of wings and visual difference in size compared to the rest of the same generation.

Figure 31: Experiment 9 captured 270 h after egg deposition. An irradiated sample, having developed into the adult stage, exhibits mutations characterized by enlarged wings.

4.3 Future research with Drosophila melanogaster

The global aim of this study is to identify optimal radiation effect conditions on fruit flies for the development of the next generation which may lead to the creation of a radiation-resistant biological system that can be applied to living organisms as well as be transferable to HeLa Cells and other biological systems (see Figure 33 [48,49,50]). The Applied Nuclear Physics lab will continue its work towards the goals in trial to produce generation by generation. After the initiation, the selection of the best population should be made. Once adults lay eggs, the eggs will be irradiated. As the fruit flies develop into the adult population, they will be evaluated. If they prove to be the fittest solution, this process will undergo several cycles. However, if not, they will be replaced, and the process will be repeated. Figure 32 represents this dynamic process.

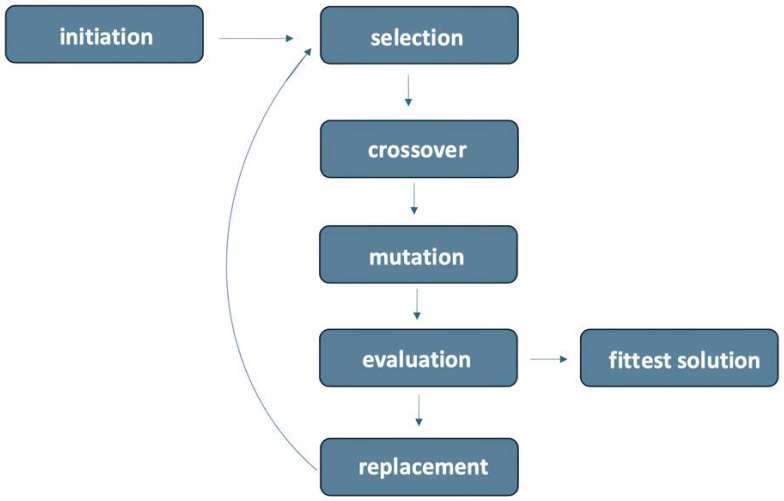

Figure 32: The process of selecting the fittest solution for one irradiation cycle.

After several cycles of irradiation, the comparison of the underlying genetics between the initial population and the last population should be conducted as shown in Figure 33 [48,49,50]. This aims to identify changes in protein coding regions or alterations in regulatory regions, and potential transferable mechanisms to other living organisms and biological systems.

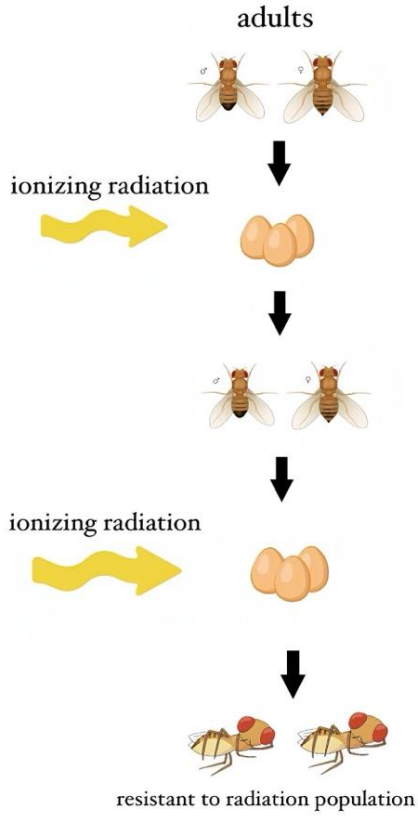

Figure 33: Illustration of the next steps in the research which take place in trials to find changes in underlined genetics in radiation resistant population that might be transferable to living organisms as well as other biological systems [48,49,50].

4.4 HeLa Cells Experiment Results

4.4.1 First HeLa Cells Experiment

In spectrophotometry, absorbance is typically directly proportional to the concentration of a solution and the path length of the light passing through it (does not have units). If the cells are alive, they will convert the substrate to a product that absorbs at 490 nm. If a reagent is dark, it means that it absorbs a significant amount of light. High absorbance indicates that more light is being absorbed by the solution, which suggests higher concentration or a longer path length. The absorbance rate was plotted against the number of cells for both assay plates in this experiment, as depicted in Figure 34.

Rates were color-coded using the MTS assay method, with higher rates shown in green and the gradient changing into the yellow spectrum as the rate decreased in the wells. The theoretically expected lower rate at the middle of the assay plate and higher rates at its edges were not observed in the experimental data of the first set (refer to Table 9). Negative control wells, indicated by red filling, contained no cells.

The data generated from this trial indicated slight saturation at the 5000-cell titration level. It was suggested to examine various cell counts and their responses to irradiation. As the next experimental trial, the mix of 5000 and 2500 cells per well were selected, anticipating a potentially clearer contrast.

	1	2	3	4	5	6	7	8	9	10	11	12
A						1.273	1.375					
B						1.201	1.39					
C						1.296	1.354					
D	1.404	1.443	1.326	1.354	0.939	1.305	1.332	1.385	1.241	1.308	1.33	1.343
E	1.41	1.464	1.385	1.385	1.358	1.417	1.356	1.303	1.392	1.44	1.459	1.459
F						1.418	1.406					
G						1.43	1.377					
H						1.437	1.408				0.269	0.268

plate 1

	1	2	3	4	5	6	7	8	9	10	11	12
A						1.268	1.294					
B						1.124	1.106					
C						1.09	1.296					
D	1.316	1.365	1.207	1.381	1.264	1.326	1.002	1.326	1.422	1.314	0.972	1.411
E	1.347	1.317	1.364	1.318	1.278	1.275	1.145	1.39	1.407	1.382	1.282	1.294
F						1.162	1.281					
G						1.032	1.089					
H						1.208	1.379				0.264	0.254

plate 2

Table 9: The result of the first set of experiments with HeLa Cells for both plates. Numbers were colored-scattered for better visualization. With the green color were marked vials where number of cells was higher, with orange/red – lower.

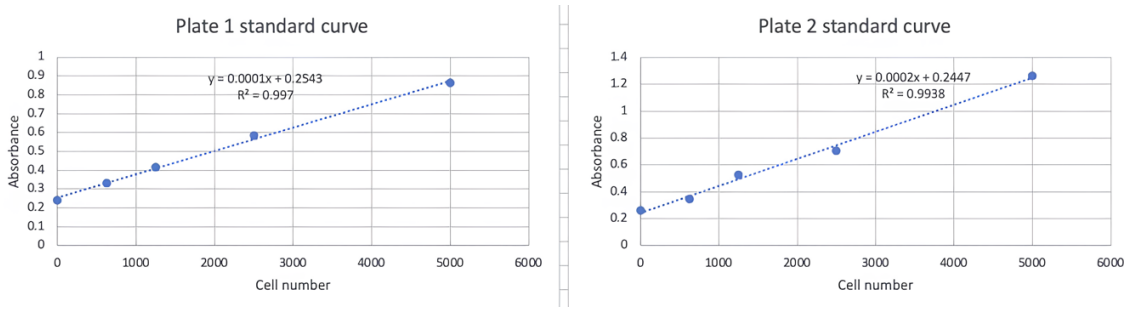

Figure 34: Absorbance rate for assay plates for the first set of experiment.

4.4.2 HeLa Cells Experiment 2

The result for Experiment 2 revealed cell death, although not to the extent anticipated. Table 10 displays the results of the Experimental group for both assay plates. Column 7 appears to be the most affected in both cases. A plausible explanation for the high percentage of remaining cells could be that cell death was not immediate. It is possible that the cells were damaged or undergoing necrosis. Therefore, suggesting a recovery period in the media could aid in clarifying the data.

7	8	9	10	11	12
125.48	130.28	107.08	127.48	129.08	122.68
90.94	113.14	135.54	116.94	123.54	121.54
133.48	135.08	133.48	142.68	121.48	137.88
135.94	111.14	110.74	124.94	115.74	117.14

% of cells remaining

63.86	89.06	64.46	64.86	95.06	99.26
59.93	99.73	101.13	97.43	101.33	86.83
86.86	88.86	92.86	97.66	90.26	81.86
70.63	71.13	92.03	101.83	105.03	106.33

% of cells remaining

Table 10: Results of Experiment 2 for the remaining cell % for plate 1 (first table from the top) and plate 2 (bottom data table).

4.4.3 HeLa Cells Experiment 3

The outcome of Experiment 3 revealed that the top of all assay plates produced a lower % of cells. This is shown in Table 11. The percentage of remaining cells increased at different parts of assay plates, rendering the data less effective for research purposes. This was attributed to numerous factors such as cells not being hit accurately by the beam, rapid growth of cells between counts or before irradiation. The examination of methodologies, distribution of the beam, and dosage will offer significant advantages for this aspect of the research.

7	8	9	10	11	12
34.900015	117.73212	110.15122	61.491337	42.618986	106.26494
136.87016	153.593	137.9844	82.992449	97.545152	134.24025
71.268336	80.004622	105.25184	53.341889	107.84259	123.65649
120.95254	134.1591	130.0069	130.12463	139.22093	79.250998
% of cells remaining					
66.798696	87.054383	92.528596	93.089608	125.62263	135.35441
78.395576	84.71725	111.80991	126.33802	116.20409	127.82264
96.551525	108.10277	79.598786	117.04211	96.267027	112.33576
105.85596	125.9266	92.073913	95.121621	37.91198	78.559138
% of cells remaining					
103.9291	67.834117	88.639523	121.27931	107.98778	94.664752
146.47293	63.868071	81.79051	74.973195	68.519203	101.26338
142.40179	160.97717	162.64091	124.58389	149.05779	174.1342
152.12915	166.78589	154.72694	80.036029	122.86775	164.27536
% of cells remaining					

Table 11: Results of Experiment 3 for the remaining cell % for plate 1 (first table from the top), plate 2 (middle table), and plate 3 (bottom table).

CHAPTER FIVE: CONCLUSION

This research was aiming to re-evaluate LD_{50} for Drosophila melanogaster using a beam of 350 KeV electrons. Drosophila melanogaster was examined across all development rates, from pupae into adulthood stage with dose-dependent differences in radio-resistance. Results of this research showed dependence between different life stages and radiation tolerance. A drastic drop-in dose rate related to the stage of observation was noticed.

Prior to irradiation, samples were laid down for a period of (48±3) hours, as it was determined that they exhibited greater resistance to radiation compared to those prepared (24±3) hours before irradiation.

The previous published data LD_{50} for Drosophila melanogaster eggs was 452 Gy [38], while our recording showed LD_{50} parameter closer to 59 Gy. Published data of LD_{50} for two-day old adults is 1228 Gy for adult males and 1250 Gy for adult females [38], while our results are showing it being close to 15 Gy. The dose-dependent effects obtained in this work are below the published LD_{50} dosages [38].

The outcome of this work showed that the concept of the LD_{50} cannot be generalized based on the measurement at one particular time for a specific development state. The LD_{50} parameter should be taken more dynamically and precisely. Compared to the established and published data [38], our records show that based on the time after irradiation and development rate LD_{50} will differ at each case. Those differences are crucial.

The next phase of this research project will involve conducting validation trials to ensure that the biological system exhibits consistent behavior and responses to radiation, as observed in this study. Administering ether as an anesthetic to adults could be a beneficial approach for enhancing accuracy in counting and minimizing uncertainties during data collection. Future work will be built in a way of breeding new generations of fruit flies that survived through the first radiation run. After that it will be exposed to radiation again. In addition, our research lab will be working towards applying the established methods obtained for the Drosophila melanogaster into HeLa Cells as well as for other biological systems.

The personal badge experiment revealed a radiation dose of 137.3 Gy/h at SSD of 12 cm, irradiated for 5 seconds at 0 °. At an SSD of 8 cm at 0 °, with an irradiation time of 5.5 seconds, the radiation dose was measured at 432 Gy/h.

The HeLa Cells set of experiment showed inconclusive data. Variability in cell growth was noted across the assay plates, with certain wells exhibiting more pronounced effects than others. Therefore, repeating the experiment with other assay plates with new HeLa Cells as a function of the parameters involved (time, distance) is suggested.

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