DETERMINATION OF ^{226}RA IN FISH

DETERMINATION OF ^{226}RA IN FISH USING LIQUID SCINTILLATION ANALYSIS

By

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A Thesis

Submitted to the School of Graduate Studies

in Partial Fulfillment of the Requirements

for the Degree of

Masters of Science

McMaster University

MASTER OF SCIENCE (2012) McMaster University

(Medical Physics) Hamilton, Ontario

TITLE: DETERMINATION OF ^{226}RA IN FISH USING LIQUID SCINTILLATION ANALYSIS

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NUMBER OF PAGES: xi, 85

Abstract

^{226}Ra is a radionuclide of much concern since it poses a high risk of radio-toxicity when ingested and is well known for its invariably long half life of 1600 years. As such ^{226}Ra concentrations were measured in whole body tissue of fathead minnows (Pimephales promelas) in an experimental set up. Fathead minnows obtained were about two months old and fed on a Radium-226 spiked diet until 115 days. A simple and direct method to determine ^{226}Ra ingested by fish using a homogeneous liquid scintillation counting was developed. The study consisted of three groups; a sham, Radium treatment and acid treatment. Fathead minnows were sampled 75 and 115 days after feeding, and the following end points; mass (w/w), length, specific growth rate, condition factor and radionuclide measurements obtained. Mean end point results were (0.24 ± 0.03 g), (2.78 ± 0.1 cm), (1.75 ± 0.13 % day^{-1}), (1.10 ± 0.06 g cm^{-3}) and (577.06 ± 572.13 mBq g^{-1}) respectively. Also mean total ^{226}Ra level was calculated as (1911.43 ± 868.64 mBq g^{-1}) while the activity in sham and acid treatment resulted in levels below the Minimum Detectable Activity of 7.46 mBq g^{-1}. The mean rate of ^{226}Ra accumulation, known as the concentration factor, by the fathead minnows was determined as 0.35 ± 0.19. Assuming that the ^{226}Ra Isotope is evenly distributed in the fish whole body, the derived dose rate was found to be 5.26 µGy h^{-1}.

Acknowledgement

First and foremost, my thanks go to God Almighty for giving me strength and courage and seeing me through my graduate studies. This work is dedicated to my son Adley and mum who have been my motivation and source of inspiration throughout the pursuit of my Masters.

I would like to express my heartfelt gratitude to my Supervisors Dr. Carmel Mothersill and Colin Seymour for their immensurable support and guidance towards the completion of this work. My sincere thanks also go to Dr. William Prestwich and Byun-Soo Hyun for their encouragement, knowledgeable advice and experience given me during the entire project.

I would also like to thank Kenric for his support in providing a better data acquisition system and Richard Smith who was always ready to help whenever called upon. Also a word of thanks goes to Jim Garret who made available a furnace and fume hood in his lab for this project, not forgetting the Health Physics staff that helped me in one way or the other.

To my friends Kiara, Heidi, Jenn, Christine, Chris, colleagues and to all the Medical Physics and Applied Radiation Sciences Administrative staff who have always been keen to help me, accept my warmest gratitude.

A special acknowledgement to Dr. Donald Amuh who has given his support throughout my entire education, to you I say God richly bless you.

Last but not least, I thank all those who lent their expertise to make this thesis complete, your time and contribution is greatly appreciated.

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1 Introduction

Radiation occurs naturally in the environment as such it is important to monitor its level in the environment due to the effects it has when interacted with matter. Nuclear disasters such as that which occurred in Chernobyl and recently in Fukushima have increased the public's concern of radiation effects such as cancer. Such unforeseen disasters have also heightened the interest of environmental radiation scientists and environmental protection agencies to take adequate measures in protecting the non-human species of this environment. In order to implement such measures, there is the need for a substantial amount of data on the effects of radiation on these non-human species. As such, this study seeks to develop a rapid and reliable method for the determination of radionuclide contamination (especially Radium-226) of aquatic biota (fish), measure the amount of radiation energy deposited by this radionuclide in the fish and to study some important effects on the fish caused by the ingestion of the radionuclide which can have adverse impacts on the individual fish and population.

Radiation is the energy (particles or waves) released from a source and travels through space or a medium. There are two well known types of radiation; ionising and non ionising radiation. Non-ionising radiation is the energy emitted by a substance; this energy is not high enough to produce charged ions. Types of this radiation include radio waves, microwaves and visible light. On the other hand ionising radiation has sufficient energy to ionise (removal of an electron) an atom, examples of these are X-rays and gamma rays (Podgorsak, 2010). Both ionising and non-ionising radiation can cause a certain degree of harm to living organisms and the environment. Ionising radiation (henceforth referred to as radiation) is of particular interest since it more harmful when deposited in living organisms. Radiation is produced by unstable atoms (possess

excess mass or energy) and such atoms are said to be radioactive. For a radioactive element or atom to reach stability, it has to emit radiation in the form of electromagnetic radiation (X-rays and gamma rays), particulate radiation (heavy charged particles; Beta and alpha particles) or both (Cember, 1996; Podgorsak, 2010). Focus will be placed on alpha emitted radiation which has a high linear energy transfer (amount of energy deposited per unit length) and causes severe damage when deposited in matter (living organisms and the environment). Since radiation does not appeal to the senses, that is it cannot be seen, smelt or touched special instruments have been produced to determine its presence. These are known as radiation detectors and function according to the type of radiation to be detected. This project aims to study the measurement of an alpha emitting radionuclide deposited in an environmental organism and the possible effects of the emitted radiation on the organism.

1.1 Environmental Issues involving radionuclide pollution

Canada has been the leading producer of uranium since mid 1950's, accounting for 22% of total world uranium production until 2009 with McArthur River mine being the world's largest production site with most of its treatment plants situated in Elliot Lake area, Figure 1.1 (Natural Resources Canada, 2011; World Nuclear Association, 2011a; Wren, Cloutier, Lim, & Dave, 1987) . One of the aftermaths of uranium mining is the mill tailing produced which causes environmental problems such as Radon emanation, leaching of contaminants like radionuclides and heavy metals into surface and ground waters. 'Activity of such tailings is dependent on the grade of the ore mined and varies from less than 1 Bq/g to more than 100 Bq/g', (IAEA, 2004). Also uranium mill tailings retain majority (approximately 85%) of radioactivity of the mined ore

which have a very long half life and is easily leached or eroded into surface water systems (Mirka, Clulow, Davé, & Limb, 1996). Recently growing concerns from the impacts on public health and natural environment from uranium mining activities and other pollutants has led to the increase of research in these areas. Environmental concerns include the risk of environmental degradation, contamination and reduction in the viability of the ecosystem. Many research reports exist on the contamination of environmental media such as soil, surface and ground waters, lake sediments, air etcetera, however only a few reports consider the biological impacts on non-human species caused from the production of uranium mill tailings (F. V. Clulow, Davé, Limb, & Avadhanula, 1998; F.V Clulow, Davé, & Limb, 1991). Hence this study is to investigate the measurement, uptake, behaviour and effects of Radium-226 in fish.

1.2 Thesis Objectives

Recent occurrences in nuclear disasters such as that of Fukushima and increase in radionuclide contamination of the environment has heightened the public's and scientific researchers concern on environmental protection. This project was thus aimed at:

- Studying and monitoring the uptake of Radium-226 in fresh water fish.
- Determining natural radioactivity in fish.
- Measurement of Radium-226 activity in fish and fish pellets spiked with Radium-226.
- Determine alpha particle dose rate to fish caused by ingestion of Radium-226.
- Studying the physical growth (non-invasive measurement of length and mass) and determine the specific growth rate and condition factor of Radium fed fish.
- Measurement of some end points such as: Fertility, mortality, Biochemical growth indices, Proteomics and ApoA1 marker.

1.3 The Need for Non-human species protection

We live on a planet where the emission of radiation is ubiquitous. While high energy and cosmic radiation penetrates the ozone layer to shine its rays on the earth and its inhabitants, the earth emits radiation from the naturally occurring radionuclides in its crust; anthropogenic activities also pollute the earth's environment with more toxic radionuclides. The age of Uranium mining and milling wastes brings to birth potential human radiation hazards and contamination of natural water aquifer not leaving out its inhabitants. Thus there is the need to protect the flora and fauna of the aquatic ecosystem from radiation and its effects due to mans discovery and negligence. Environmental radiation protection was formerly based on the recommendations of ICRP (1977) which believed that '*if man is adequately protected then other living things are also likely to be protected*' (ICRP, 1977). However it has taken the growing demand of many authors, national and international bodies such as, IAEA, NCRP, CNSC (Pentreath, 2002; D.S. Woodhead, 2003) and ICRP itself (ICRP, 1991), to reconsider its stand in explicitly establishing guidelines for the protection of non-human species from ionising radiation and come up with a committee specifically for the protection of non-human species (ICRP, 2003, 2007, 2008). Protection of human beings has been in the form of adequate control of radiation exposure to prevent cancer risk and heritable radiation effects. In the case of radiation protection of non-human biota, protection has to begin from the individual to the population and the ecosystem as a whole. Though some non-human species have the risk of developing cancer, most radiation effects documented have been physical effects such as morbidity, mortality occurrence in early stages of life and the impairment of reproductive capacity (fertility and fecundity) and DNA damage on the microscopic scale. In order to protect these non-human biota effectively and adequately, the dose and dose rate at which these effects occur have to be determined. To fulfil such a task

various methods of radionuclide detection have been established (Antovic & Svrkota, 2009; Biggin, Cook, MacKenzie, & Pates, 2002; Blackburn & Al-Masri, 1992; IAEA, 2010; Zikovsky, 1991). Since alpha radiation has a high linear energy transfer, and causes great ionising radiation effects to tissue, this project seeks to determine the measurement of one of the most important radiotoxic alpha emitting radionuclide, Radium (which is a decay product of Uranium) in an experimental organism.

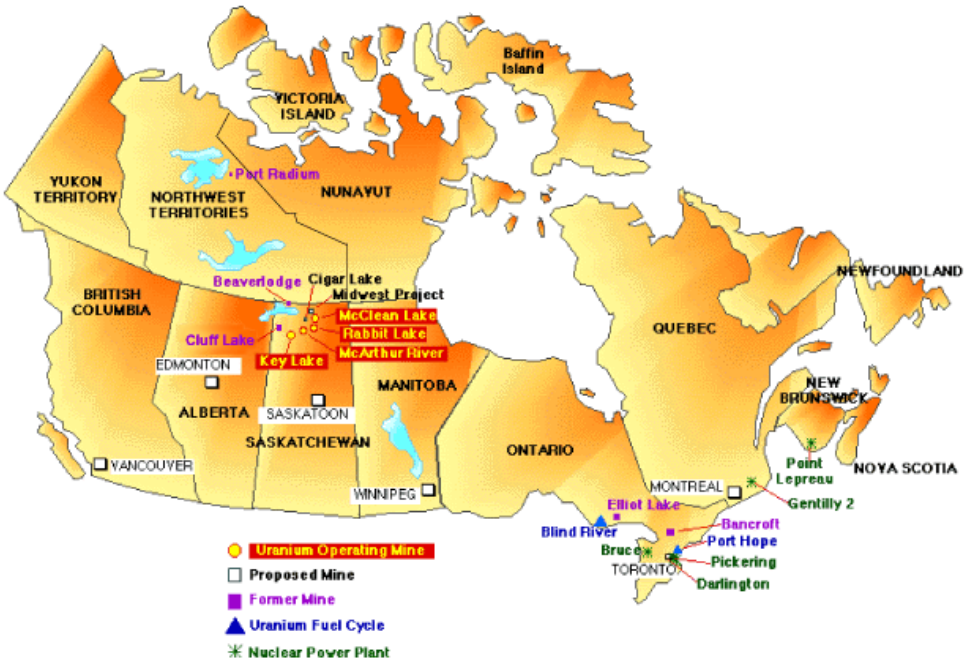

Figure 1.1. Uranium Resources in Canada. Adapted from (World Nuclear Association, 2011b)

1.4 Literature Review

1.4.1 A Radioactive element: Radium

Radium which, is a naturally occurring radioactive element present in Uranium and Thorium elements in the earth's crust was first discovered by Marie and Pierre Curie in 1898 ("Radium," 2005). Radium was used in many applications such as its use as a self illuminating material in

dial clocks and in the application of medical diagnoses and therapy (Radiation Therapy and Brachytherapy), (IAEA, 2010). The most abundant of the Radium isotopes is Radium-226, which is a natural decay product of Uranium-238. Among the radiotoxic radionuclides, Radium-226 is important since it is an alkaline earth metal and has physiological and environmental properties similar to those of Calcium and Barium (Cowart and Burnett, 1994). Thus Radium would compete with Calcium and hence be deposited as a substitute for Calcium in material where the latter is of lesser concentration, especially in the bone. With Radium-226 being an alpha emitter and having a half-life of about 1600 years, it is of utmost interest because when deposited in internal organs of humans, it is known to cause severe radiation damage resulting from the alpha particles and short lived daughter radionuclides of high specific activity emitted in its decay process (Mirka et al., 1996). Such severe radiation damage may cause cancer as in the case of the early Radium dial painters. Also Radium-226 adheres well to soil particles and rocks, sips into ground water due to its ability to form soluble sulphates, chlorides and carbonates and is a major contaminant in mine and milling wastes, like Uranium mill tailings (Whicker and Shultz, 1982; Human Health fact sheet, 2005). Since Ra-226 is easily soluble and deposited in bone tissue when taken up, it is necessary to monitor its uptake in ground and natural waters, by the fauna of these waters. In this thesis the use of Radium, Radium-226, Ra-226 or ^{226}Ra refer to the same radionuclide Radium-226.

1.4.2 Environmental levels of Radium

Analysis of Radium in water and other organic samples is of importance to earth, marine and environment scientists due to public health concerns (Burnett and Tai, 1992) and it being one of the most hazardous elements when it comes to internal exposure (Higuchi et al 1984). Due to

this, US regulations; Title 40 of the U.S Code Regulations (CRF) of July 1, 1992 part 141.25 require that the minimum detection limit for Ra-226 does not exceed 1 pCi L^{-1}. The guideline for Canadian drinking water has established Maximum acceptable Concentration Ra-226 as 0.5 Bq L^{-1}, where as the screening level established by World Health Organisation for drinking water is 0.5 Bq L-1 for gross alpha activity(Health Canada,2009 and WHO 2008). A 14 year radionuclide (Ra-226) monitoring at Lake Elliot from 1983 to 1996 ranged from (0.015 Bq L^{-1} to 0.007 Bq L^{-1}) (Health Canada, 2009).

1.4.3 Fundamentals of Radiation and Radioactivity

Radioactivity was first discovered in 1896 by Henri Becquerel a French scientist, who discovered that radiation from uranium mineral was able to penetrate a photographic plate even though placed in the dark(Bimbot, R.; Bonnin, A ; Deloche, R ; Lapeyre, 1999). Further experiments by Rutherford led to the discovery of two types of rays: alpha and beta in 1899. As described by Rutherford and Soddy in 1902, 'Radioactivity is a spontaneous disintegration of the radioactive element by the emission of particles with the aim that new elements would be formed'(Cember, 1996; Pauwels, 2005). Since radioactivity is a random process, the number of atoms, N decaying in time *dt* for a system where there a N (0) atoms initially present is given by

$$-dN = \lambda N\, dt \qquad \text{Equation 1.1}$$

And λ is the decay constant. For a very small time interval

$$\frac{dN}{dt} = -\lambda N \qquad \text{Equation 1.2}$$

The number of atoms at any time, N (t) is given by integrating with respect to time

$$N(t) = N(0)e^{-\lambda t} \qquad \text{Equation 1.3}$$

Where e is the exponential term. The amount of time it takes for half of the given nuclide to decay is the half-life, τ. Thus

If $N(t) = \frac{N(0)}{2}$ and substituted into the equation above

Then the half-life, τ is expressed as

$$\frac{1}{2} = e^{-\lambda\tau}$$

Thus the half life and decay constant, λ are related by

$$\boldsymbol{\tau = \frac{In(2)}{\lambda} \approx \frac{0.693}{\lambda}} \qquad \text{Equation 1.4}$$

1.4.3.1 How to determine the Activity of a radionuclide

Activity of a radionuclide is expressed as the average number of decays per unit time. However, since radioactivity is a stochastic event the activity, A for a general case of decay and growth of a radionuclide, is given by

$$\boldsymbol{A = \lambda N} \qquad \text{Equation 1.5}$$

The Bq has been adopted as the System International (SI) unit of activity which is equal to one radioactive decay per second. Formerly the Ci was commonly used, where $1Ci = 3.7 \times 10^{10}\ Bq$ (NCRP, 1985). The activity determined in samples have normally been stated as the activity concentration, which is the activity per unit mass or volume of the sample (Bq Kg^{-1} or Bq L^{-1}) or the activity per unit mass of the radioisotope element (specific activity, Bq g^{-1}).

1.4.4 The Radium decay chain series

There are three natural radioactive isotopes associated with Uranium: Uranium-238, Uranium-235 and Uranium-234. The nuclei of radioactive elements are highly unstable and have to undergo radioactive decay by the emission of alpha or beta particles from the nucleus to form other stable elements. The most abundant isotope in a Uranium ore is Uranium-238 which has a half life of 4.5 billion years. It undergoes several decay series one of which is Radium-226 and finally ends with the stable isotope Lead-206, see Figure 1.2 . Most often the progeny (daughter product) of a nuclear decay is also radioactive; a radioactive decay chain is formed until a stable product is produced. For example a parent nuclide N1 decays to a daughter N2, where the daughter is still radioactive and decay to N3 resulting in a successive decay chain as $N_1 \rightarrow N_2 \rightarrow N_3 \rightarrow N_4 \rightarrow \cdots$

The successive radioactive decay was first investigated systematically by Bateman (Bateman, 1910).

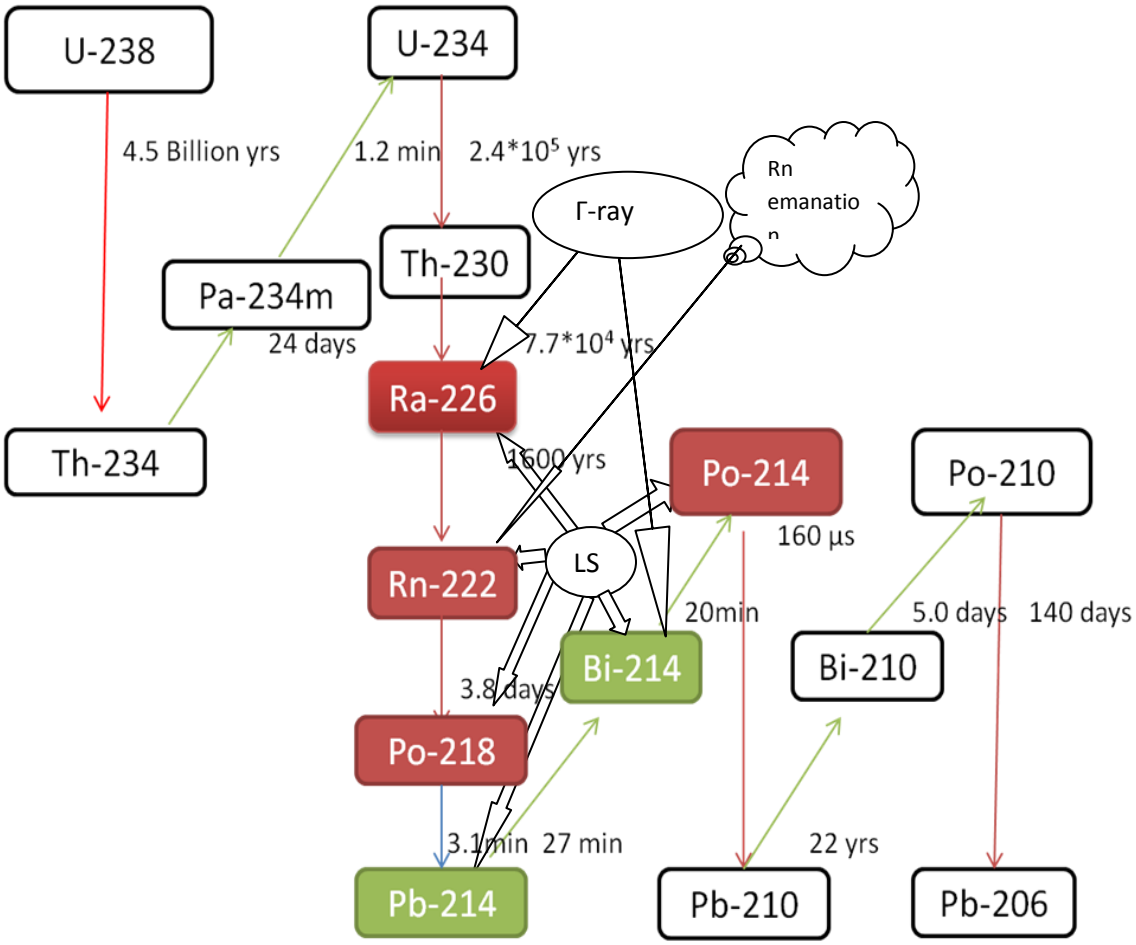

Figure 1.2. Schematic decay of Uranium-238, the coloured arrows used indicate the type of decay (Red: alpha emission, Green: Beta emission) and LSC (Liquid Scintillation Counting).

The Bateman equation for a decay chain assumes that only a single parent nuclide is present at time t=0 while all other decay products (nuclei) are not present yet, that is

$N_1(0) \neq 0$; $N_2(0) = N_3(0) = N_i(0) = 0$

The Radium decay is an example of a successive decay chain and its decay rate equations are given as:

$$Ra^{226} \xrightarrow{\lambda_{Ra}} Rn^{222} \xrightarrow{\lambda_{Rn}} Po^{218} \xrightarrow{\lambda_{Po}} Pb^{214} \xrightarrow{\lambda_{Pb}} Bi^{214} \xrightarrow{\lambda_{Bi}} Po^{214} \xrightarrow{\lambda_{Po}} Pb^{210} \xrightarrow{\lambda_{Pb}} Bi^{210} \xrightarrow{\lambda_{Bi}} Po^{210}$$

$$\xrightarrow{\lambda_{Po}} Pb^{206}(stable)$$

$$\frac{dN_{Ra}}{dt} = -\lambda_{Ra}N_{Ra}$$

$$\frac{dN_{Rn}}{dt} = \lambda_{Ra}N_{Ra} - \lambda_{Rn}N_{Rn}$$

$$\frac{dN_{Po}}{dt} = \lambda_{Rn}N_{Rn} - \lambda_{Po}N_{Po}$$

$$\frac{dN_{Pb}}{dt} = \lambda_{Po}N_{Po} - \lambda_{Pb}N_{Pb}$$

$$\frac{dN_{Bi}}{dt} = \lambda_{Pb}N_{Pb} - \lambda_{Bi}N_{Bi}$$

Where dN_{Ra}/dt represents the decay rate, λ_{Ra} the decay constant, N_{Ra} the number of atoms of Radium-226 and the subsequent equations are for its daughters in the decay chain.

From the equations above, the Bateman solution for the Radium decay chain equation can be derived as

$$N_i(t) = C_1 e^{-\lambda_1 t} + C_2 e^{-\lambda_2 t} + C_3 e^{-\lambda_3 t} + C_i e^{-\lambda_i t}$$

Where,

$N_i(t) =$ The number of atoms of each nuclide of a radioactive decay chain (Ra) produced after a given time t, given that at time t = 0 (initial condition) only a given number of Radium-226 nuclei were present.

$$C_1 = \frac{N_1(0)\lambda_1\lambda_2 \ldots \lambda_{i-1}}{(\lambda_2-\lambda_1)(\lambda_3-\lambda_i)\ldots..(\lambda_i-\lambda_1)}$$

$$C_2 = \frac{N_1(0)\lambda_1\lambda_2 \ldots \lambda_{i-1}}{(\lambda_1-\lambda_2)(\lambda_3-\lambda_2)\ldots\ldots(\lambda_i-\lambda_2)}$$

$$C_i = \frac{N_i(0)\lambda_1\lambda_2 \ldots \lambda_{i-1}}{(\lambda_1-\lambda_i)(\lambda_3-\lambda_i)\ldots\ldots(\lambda_{i-1}-\lambda_i)}$$

$N_i(0) =$ The number of atoms of type i nuclide present at time t = 0 and decaying with decay constant λ_i.

$C_i =$ The detection coefficient of the $i\ th$ nuclide decaying with decay constant λ_i

Constructing the first few terms for Radium-226 gives

$$N_{Ra} = N_{Ra}^0 e^{-\lambda_{Ra}t}$$

$$N_{Rn^{222}} = \frac{\lambda_{Ra}}{\lambda_{Rn}-\lambda_{Ra}} N_{Ra}^0 e^{-\lambda_{Ra}t} + \frac{\lambda_{Ra}}{\lambda_{Ra}-\lambda_{Rn}} N_{Ra}^0 e^{-\lambda_{Rn}t}$$

$$N_{Po^{218}} =$$

$$\frac{\lambda_{Rn}\lambda_{Ra}}{(\lambda_{Po}-\lambda_{Ra})(\lambda_{Rn}-\lambda_{Ra})} N_{Ra}^0 e^{-\lambda_{Ra}t} + \frac{\lambda_{Rn}\lambda_{Ra}}{(\lambda_{Po}-\lambda_{Rn})(\lambda_{Ra}-\lambda_{Rn})} N_{Ra}^0 e^{-\lambda_{Rn}t} + \frac{\lambda_{Rn}\lambda_{Ra}}{(\lambda_{Rn}-\lambda_{Po})(\lambda_{Ra}-\lambda_{Po})} N_{Ra}^0 e^{-\lambda_{Rn}t}$$

...

1.4.5 Radioactive Equilibrium

Suppose a parent nuclide A decays to nuclide B and nuclide B decays to nuclide C

$A \overset{\lambda_A}{\rightarrow} B \overset{\lambda_B}{\rightarrow} C(stable)$. The rate of decay of nuclide B is given by

$$\frac{dN_B}{dt} = \lambda_A N_A - \lambda_B N_B$$

And the number of atoms of nuclide B is

$$N_B(t) = \frac{\lambda_A}{\lambda_B - \lambda_A} . N_A(0)\left(e^{-\lambda_A t} - e^{-\lambda_B t}\right)$$

$$= \frac{\boldsymbol{\lambda_A}}{\boldsymbol{\lambda_B - \lambda_A}} . \boldsymbol{N_A(t)}\left(\mathbf{1} - \boldsymbol{e}^{-(\lambda_A - \lambda_B)t}\right) \qquad \text{Equation 1.6}$$

From the equation above, it can be observed that the rate at which equilibrium is reached is dependent on the half-life of both parent and daughter nuclide. Three distinct cases can be observed.

1.4.5.1 Secular Equilibrium ($\tau_A \gg \tau_B$)

For secular equilibrium to occur the half-life of the parent must be much longer than that of the daughter, and the decay constant $\lambda_A \ll \lambda_B$. When time t has passed many half lives of the daughter nuclide($t \gg \tau_A$), the daughter nuclide begins to decay at the same rate as the parent. Equation 1.7 reduces to

$$N_B(t) = \frac{\lambda_A}{\lambda_B} . N_A\left(1 - e^{-\lambda_B t}\right)$$

Secular equilibrium is reached when the parent and daughter activities are equal, $A_A = A_B, and\ \frac{N_B}{N_A} = \frac{\lambda_A}{\lambda_B} = \frac{\tau_B}{\tau_A}$ where A is the activity (equal to λN) as shown in Figure 1.3.

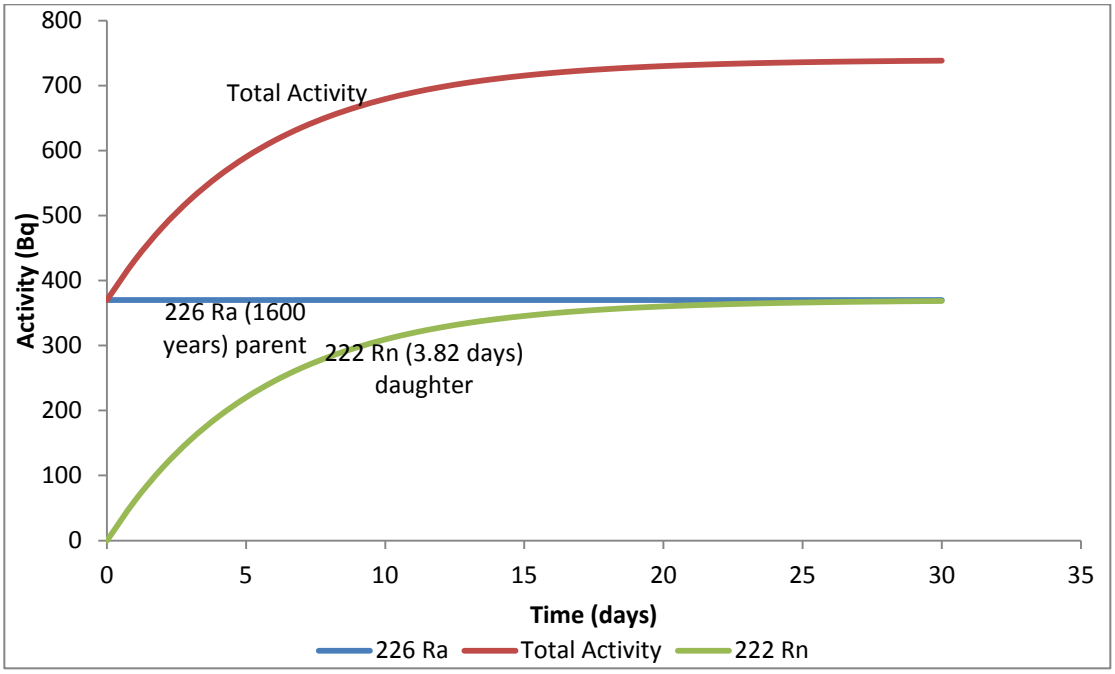

Figure 1.3. Diagram of secular equilibrium between a parent ^{226}Ra and its daughter ^{222}Rn.

1.4.5.2 Transient Equilibrium $(\tau_A \geq \tau_B)$

In transient equilibrium, radionuclide equilibrium is reached between a parent-daughter pair where the half-life of the daughter is shorter than or equal to that of the parent, i.e. $(\tau_A > \tau_B, \lambda_A < \lambda_B)$. The general solution of nuclide B, is from Equation 1.7

$$N_B(t) = \frac{\lambda_A}{\lambda_B - \lambda_A}.N_A(0)\left(e^{-\lambda_A t} - e^{-\lambda_B t}\right)$$

An example can be seen in the case of ^{214}Pb decay shown in Figure 1.4, where the number of atoms for ^{214}Bi increases when time is far smaller than the half-life of ^{214}Pb (27 min). As time t becomes far greater than the half-life of ^{214}Bi (19.9 min) the number of atoms of ^{214}Bi decreases according to $e^{-\lambda At}$.

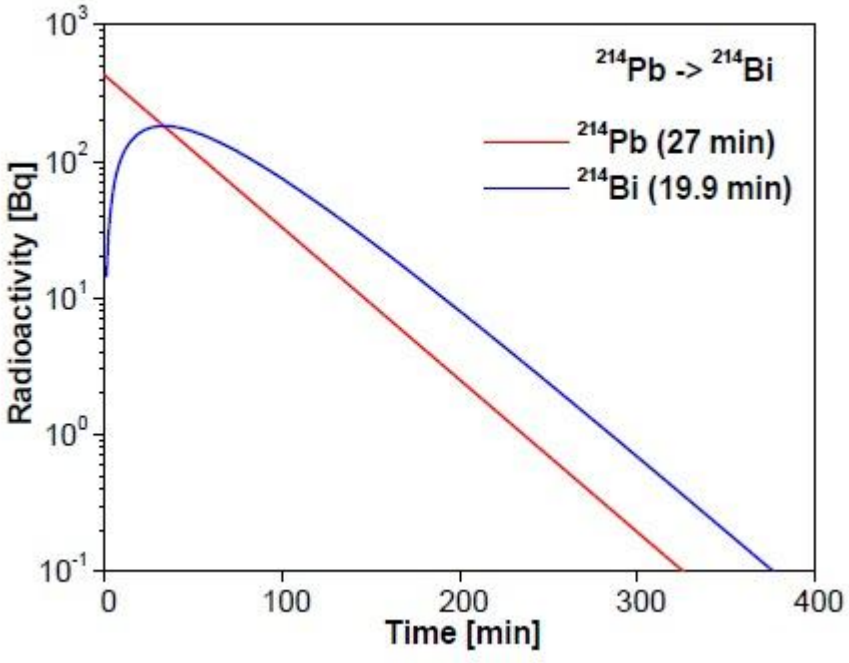

Figure 1.4 Schematic diagram of transient equilibrium between a parent ^{214}Pb and its daughter ^{214}Bi. Adapted from (Byun, 2009)

1.4.5.3 No Equilibrium ($\tau_A < \tau_B$)

When the half-life of the parent is shorter than the daughter half-life, no equilibrium exists. In such a case the daughter nuclide grows to a maximum and begins to decay with its own half-life while the parent nuclide decays to a negligible amount. The decay scheme of ^{146}Ce as shown in is an example.

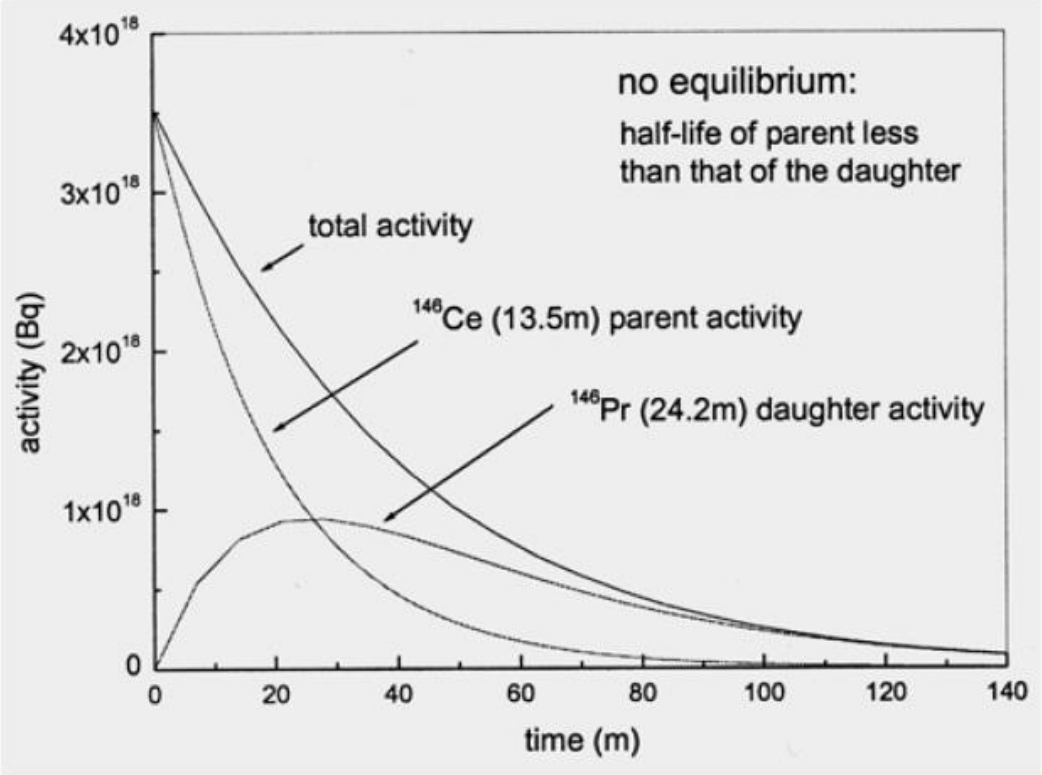

Figure 1.5. Schematic diagram of no equilibrium between a parent ^{146}Ce and its daughter ^{146}Pr. Adapted from (Pauwels, 2005)

1.5 Radium Detection Methods

Many methods exist for the determination of Ra-226 especially in water. Certain methods include the direct determination of Ra-226, whereas most of them are indirect methods from its progeny. The most common methods are measuring by Sorption emanation (Radon emanation) which is based on determining Ra-226 through Rn-222 ingrowth, γ-ray spectrometry, and α spectrometry, co-precipitation with barium sulphate and with liquid scintillation counting having an upper hand in all of these methods.

1.5.1 Radon emanation

This is a technique used to measure Ra-226 via Rn-222 ingrowth. The sample is sealed in a bubbler and stored for Rn-222 ingrowth; the Radon gas is then purged and counted in a scintillation counter after Radon equilibrium with its daughters has been reached. This process is very slow and time consuming, more over large sample volumes are required to analyse low level activity samples (Scheibel, Porstendorfer, & Wicke, 1979).

1.5.2 Gamma-ray spectroscopy

Gamma spectrometry is a process which measures radionuclides from its gamma-radiation emissions. This method allows the simultaneous determination of many radionuclides in a sample in a non-destructive way. Also there is no need for complicated and time consuming radiochemical separation in this process as in alpha spectrometry(Rihs & Condomines, 2002; Semkow, 2002). A rapid method for measurement of Ra-226 by gamma-ray spectroscopy is the direct use of the 186 KeV line(Canet & Jacquemin, 1990), however the γ-emission probability is relatively low and could be subjected to interference from U-235. Measurement via the Bi-214 line, which involves the establishment of equilibrium between Ra-226 and its Bi-214 daughter, is preferable but it is time consuming. The radionuclide measured in this case is shown in

Figure 1.2. Also this method is faced with difficulty in precisely calibrating the efficiency of the detector and acquiring the same geometry of sample and calibration source. Another limitation is the relatively poor efficiency of the high purity Germanium detectors (HPGe) over a wide range of energy and self absorption effects (Jodlowski, 2006).

1.5.3 α - Spectrometry

It is mostly used to determine low level concentrations of Ra-226 by Radium isolation from the sample. In this technique preparation of a robust counting source and electrodeposition of the sample onto a stainless steel tray is required to prevent backscattering and self absorption. This method is direct and the most sensitive due to high resolution of surface barrier detectors(Hancock & Martin, 1991) and low background but is disadvantaged from the chemical isolation of Radium from other group II elements(Crespo, 2000) and low counting efficiency of approximately 25% (Floeckher, 2011; Tinker, Smith, & Cooper, 1995).

1.5.4 Co-precipitation with barium sulphate

Due to the similar chemical properties of barium and Radium, barium co-precipitation method is used in the determination of Radium isotopes. Radium is co-precipitated with barium as Ba (Ra) SO_4 and separated, followed by alpha counting on a gas flow proportional counter, alpha spectrometer or gamma spectrometer; however this is a tedious process (Chui and Dean, 1986).

1.5.5 Liquid Scintillation Analysis

Liquid scintillation counting is one of the most effective methods for radionuclide determination. It is mostly used to determine Radium isotopes in environmental samples particularly for liquid samples (Vrskova, 2006; Wallnner, 2009). This dates back to the late forties and early fifties (G.T. Reynolds, 1950) when it was mainly developed for beta counting, nevertheless it's being used now in determining alpha emitting nuclides due to its high counting efficiency (approximately 100%), simplicity in sample preparation, automization for counting large number of samples (D. L. Horrocks & Studier, 1964, McKlveen, J.; McDowell, 1984)and acceptable detection limits for several Radium isotopes (Repinc & Benedik, 2002; Chalupnik S

1993; Schoenhofer F et. Al 2009). The disadvantage of this counting system is the poor energy resolution of approximately 0.30 MeV for low alpha spectrometry equipment(McDowell, 1992), relatively high background and quenching compared to alpha spectrometry (Burnett & Tai, 1992; Köhler et al., 2002). Moreover simultaneous measurements of both α- and β- emitting radionuclides can be achieved using pulse shape analysis (PSA)(Dazhu, Yongjun, & Möbius, 1991; J. M Pates, 1996).

Previous studies on the low dose contamination ranges of Ra-226 in water and food samples were done with the counting of the decay of the daughter product Radon 222 using the Radon emanation method. Radiochemical separation methods such as co-precipitation (Barium) methods and cation-exchange resins have also been used to isolate Radium from the sample. Other methods involve the determination of Ra-226 without separating it from the sample. These methods use special cocktails and instruments to gain good energy resolution of the alpha peaks produced by Radon-222 and its daughters, to obtain low background and good recovery of Radon-222 (Asikainen *et al,* 1981*;* Higuchi *et al,* 1984*).* However, these methods take a lot of time (waiting for the re-growth of Radium daughters and for secular equilibrium to be reached) before counting and are extremely tedious and complex. Hence, the method proposed for this research is based on the direct measurement of Ra-226 using Liquid Scintillation Counting, where Ra-226 would not be separated from the sample. This method is less time consuming and does not involve complex and tedious radiochemical processes, also measurements can be done without the establishment of equilibrium, and at equilibrium for accuracy. A simple radiochemical method has been derived and is to be used in this experiment to obtain the

samples (whole fish body and food) in the aqueous form for analysis by Liquid Scintillation Counting.

2 Materials and Method

2.1 Experimental Set-up

Initially four different dose range groups of Radium-226 spiked fish flakes were fed to the fish, starting with reported environmental dose ranges to ten and hundred fold increments (natural, 10, 100, 1000, 10000 mBq g^{-1}). A protocol (Appendix A) was developed for the digestion of fish samples and spiked fish food. To test the efficacy of the protocol, preliminary experiments were conducted with Radium-226 injected fat head minnows. Preliminary results obtained for the above dose ranges were below the minimum detectable activity, thus the highest dose range was increased by a factor of ten to obtain a 100 Bq g^{-1} group. The experiment was continued using 100 mBq g^{-1} Radium-226 doses.

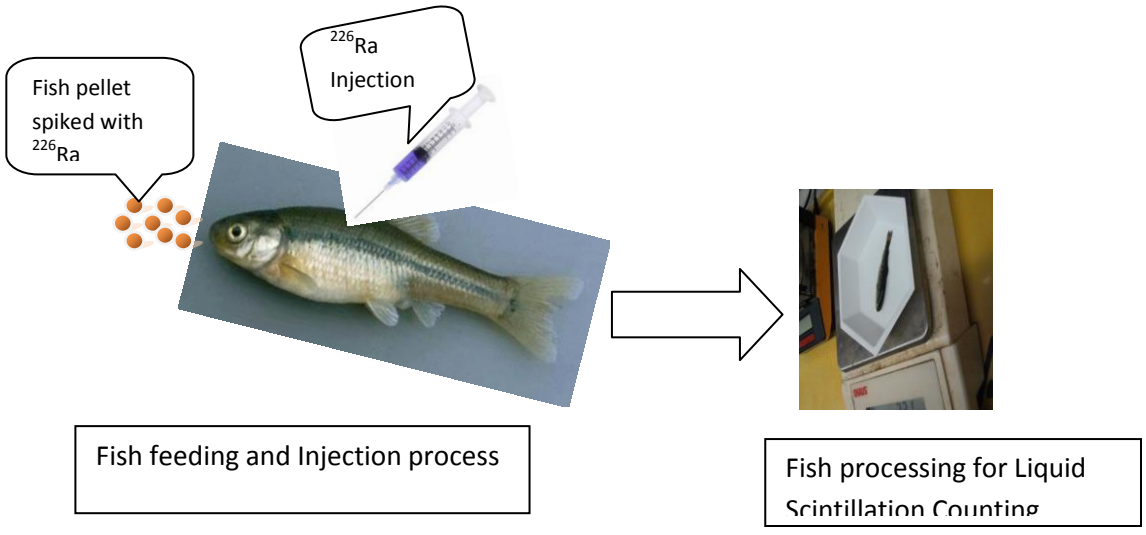

Figure 2.1 Schematic illustration of the Experimental Process.

2.2 Experimental fish

Fat Head Minnow, FHM (Pimephales promelas), a fresh water fish was chosen as the experimental fish model due to its wide distribution across North America, including the Maritime Provinces and Great Slave Lake drainage of Canada (Scott & Crossman, 1973) and its influence on the trophic structure of aquatic ecosystems (Brooks & Dodson 1965). It is also commonly used as a fish model for toxicology studies around the world, thus there is a lot of data on its husbandry. The unique size of the FHM (adult 43-103mm in length) makes them easy to work with and they are continuous spawners, with the females laying about 200 to 700 eggs (Speirs, 2000). This makes them a good species for further studies on their F2 and F3 generation. Also since fat head minnows are bottom feeders, they are more likely to be affected by radionuclide contamination in the environment.

2.3 Adjusted Setup

Each group (15 fish) under study; control, 100 Bq g^{-1} Ra-226 and acid control treatment were fed and maintained in 26 litre plastic tanks. An acid control treatment was added to the experiment to evaluate the effect of the nitric acid, since the Radium standard solution was Radium nitrate. The tanks had a hood with an opening to make feeding easier as well as prevent the fish from jumping out. To prevent leftover food (which could give external dose to the fish) and bacterial growth in the tanks, a flow through system and mechanical filtration was adapted as shown in Figure 2.2. The filters were checked and cleaned once every week and the water temperature was maintained at 20 – 25 ^{o}C.

Figure 2.2. Experimental Fish set up

2.4 Choosing a LSC cocktail

The type of scintillation cocktail used can interfere with several factors in the counting process such as background count rate and counting efficiency. Counting of ^{226}Ra can be done using the homogeneous or two phase method. The choice of which method to use relies greatly on the type of cocktail used. The two phase method is commonly used in the LSC process. In this method the sample is immiscible with the cocktail forming a layer of the aqueous sample beneath the organic cocktail. By using this method to analyse ^{226}Ra, ^{222}Rn diffuses from the aqueous phase to the organic phase. Since Radon is readily soluble in the organic phase, it remains and grows and is detected together with its daughters in this phase. However, problems arise such as Radon leakage from the vial (L. Salonen, 1992), loss of counting efficiency due to the hydrophilic nature of the Radon daughters (Po, Pb and Bi) which prefer the aqueous phase. This results in the loss of about 30% of ^{218}Po and ^{214}Po to the aqueous phase (L. Salonen, 1990). The Ultima Gold (UG) cocktail was chosen since it's an aromatic solvent based cocktail, (Radon

has a high solubility in aromatic solvents) and biodegradable. An aromatic solvent based cocktail is desired because it has a high density of π electrons, which give efficient transfer of radionuclide decay energy (Thompson, 1983). The Radium standard solution prepared formed a homogenous mixture with the cocktail; however the high acidity content in the aqueous prepared fish sample formed a two phase layer when mixed with the cocktail. Counting was proceeded using this method; however repeat counts produced a vast number of irreproducible data. A new set of low ^{40}K glass vials were obtained to repeat the counting process this time counting only ^{226}Ra standards. The inconsistency in the data still pertained until a check for loss of Radon was evaluated. The acidity content of the aqueous fish sample was reduced and a cocktail with a high acid load capacity (UGAB) and good resolution was purchased and used for further experiments. The homogeneous method, where ^{226}Ra and its daughters are uniformly mixed in the cocktail mixture was used thenceforth.

2.5 Spiked food process

A commercial fish diet which contained a balanced proportion of nutrients was used in preparing the ^{226}Ra spiked meal. This food was ground up into fine powder using a mortar and pestle obtained solely for spiked meal and another for the untreated meal. Preliminary trials were made to determine the right amount of water for the mixture of the powder to obtain a fine paste. After measuring the right amount of water needed, the desired activity of Radium aliquots were calculated from the manufactures nominal activity (100Bq/g) and mixed uniformly with the water. A fine paste was obtained by stirring the desired amount of food and Radium solution. To get the paste to dry out evenly, it is put into a syringe and gently squirted out. The

dried ^{226}Ra spiked diet was ground into fine powder and refrigerated in a stored container. The untreated diet for control fish and acid control were prepared in the same way as described above except only water and equivalent amount of nitric acid present in ^{226}Ra stock solution were used respectively.

2.5.1 Feeding process

Fathead minnows feed about one third of their body mass daily and are bottom feeders. For this experiment, fifteen fish were in each group. An adlibitum feeding was done on an alternative daily basis.

2.5.2 Sampling Method & Processing

The first sampling was done after the fish had been fed 34 times within 75 days. Five fish were randomly netted from each group. After measuring their weight and length, the carcases were taken for further processing. Calcination and acid digestion methods were chosen in order to obtain the sample in a liquid form for liquid scintillation counting

2.5.2.1 Calcination

Sampled fish whole bodies were calcined in a muffle furnace with temperature of about 700 ^{o}C, Figure 2.3. At this temperature, the entire organic component is decomposed and this is well below the boiling point or vaporization point of ^{226}Ra which is retained in the skeletal tissue. However any ^{222}Rn formed in the fish tissue due to decay is lost in this process.

Figure 2.3. Calcination of fish whole body

2.5.2.2 Acid Digestion

The residual was digested in aqua regia which consists of one part of HCL to three parts of HNO3. Usually the aqua regia is prepared one hour prior to digestion; 3ml of this is added to the sample and evaporated to dryness. In order to bring the final solution to chloride form, 1.78ml of dilute HCL is added to obtain a RaCl solution. This volume was chosen due to the acidity tolerance of the scintillation cocktail used.

Figure 2.4. Wet oxidation process, showing aqueous fish sample in beaker

2.5.3 Counting process

The Beckman Liquid Scintillation Spectrometer was used to determine Ra-226 in aqueous fish samples. It has a pulse shape analysis which separates the alpha and beta spectrums. Normally liquid scintillators are composed of one or more fluorescent solutes in an organic solvent; this mixture is referred to as scintillation cocktail. The radioactive sample solution was pipette into a 20 ml glass scintillation vial, the scintillation cocktail (Ultima Gold AB) was introduced into the sample and thoroughly mixed. Radiation energy of the radionuclide in the sample is exhausted in the excitation and ionisation process of the solvent. De-excitation of the solvent molecule occurs by the transfer of energy to the Fluor (solute), this energy is subsequently re-emitted as ultraviolet photons whose average wavelength is characteristic of the solute (Thompson, 1983). These are then detected at the photocathode of one or more photomultiplier tubes, amplified

into signals and recorded with a Multichannel Analyser. The magnitude of each pulse produced is proportional to the energy of the radiation particle emitted and deposited in the scintillator. The pulse height information is transferred through an RS-232 port to a computer, where data is collected and analysed. Due to low activity samples being used, the total counting window was used to obtain the total ^{226}Ra activity. The Ra spectrum was observed in channel 400-600.

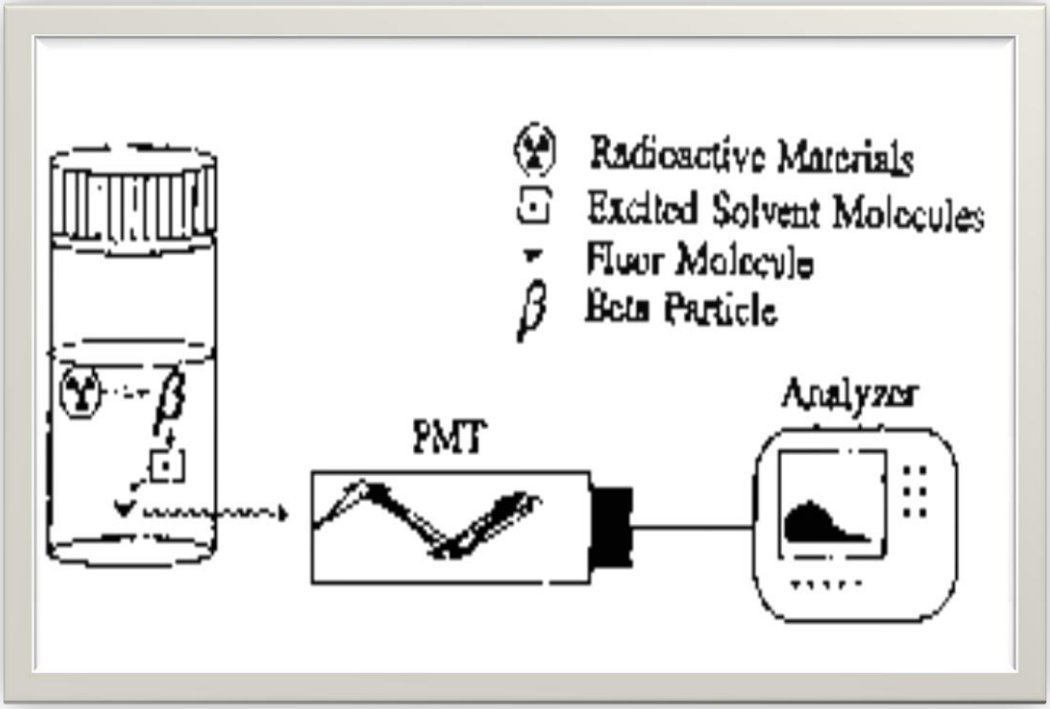

Figure 2.5. Energy transfer process in LSC. Adapted from (Grau Malonda, 1985)**.**

2.5.3.1 Background (Noise)

Background is defined as natural activity counts generated from sources not in the sample, such as instrument noise, Photomultiplier tube cross talk and cosmic or external radiation. When counting a sample the background activity must be determined and subtracted from the sample

count especially when background activity is very high and low level environmental samples are being counted.

2.5.3.2 Sources of Background in Liquid Scintillation Counting

There are many sources that contribute to background (natural radioactivity). These can be split into background produced in the sample and liquid scintillation cocktail, mostly known as quenchable background and that generated outside the cocktail (unquenchable background)(D. Horrocks, 1974). Quenchable events produce about 32% of a total background spectrum while approximately 68% is due to unquenchable events.

2.5.3.2.1 Quenchable Background

Quenchable background activity is due to external high energy radiation interacting with the scintillator and natural radioactivity in the scintillator constituents. Thus quenchable background events increases with increasing cocktail volume and produces light pulses produced similar to true beta events (D. Horrocks, 1974; L'Annunziata, 2003). As can be seen in the results of this work, there was a two fold increase in background counts when the scintillator cocktail was increased from 10 mL to 20 mL.

2.5.3.2.2 Unquenchable Background

The primary source of unquenchable background is from the interaction of high energy cosmic rays or natural radioactivity in the vial wall with the photomultiplier tube (PMT) glass surface. This result in a burst of low photon after pulses depicted in the pulse pattern, due to Cerenkov events produced from the interaction of cosmic rays with the vial or PMT glass envelope, which is distinguishable from true scintillation events (L'Annunziata, 2003). A clear indication of such pulses can be observed in Figure 3.1.

2.5.3.3 Background reduction methods

Background reduction has considerably improved from the age where reduction methods were attempted by instrument users to commercially manufacturing low level scintillation counters, which incorporates background reduction features with microprocessor technology such as Multichannel analyzers and Pulse Shape Analyzers. Also other methods include: Temperature control was essential in the operation of older liquid scintillation counters by refrigerating the PMT's to reduce noise. However, PMT noise has been significantly reduced by the use of coincidence circuits and improved bialkali manufactured tubes. Today temperature control is used for reasons other than PMT cooling such as special sample preparation, reduction of static build-up and chemiluminescence (Cook, Passo Jr., & Carter, 2004). The amount of cosmic rays or environmental radiation present in the counting room can affect the background observed. Specially shielded counting rooms (rooms below ground level and shielding against cosmic rays and environmental radiation) can reduce background levels. An example is a specially designed counting laboratory in the University of Arizona (Kalin, R. M; Long, 1989). The type of cocktails used can also affect background; it is important to use cocktails which are prepared with low natural radioactivity liquid scintillator. For environmental analysis it is necessary to use cocktails with low background and high sample load capacity. Load capacity affects the limit of detection for aqueous samples as shown in Figure 2.6. It is also desirable to use vials produced from relatively low natural radioactivity since this can increase background count rate. Lower background can be achieved with Plastic vials than Glass vials; however care must be taken in the choice of cocktail when using plastic vials. Glass vials produced with very little ^{40}K is advantageous (L. Kaihola, 1991; Lauri Kaihola, 1993).

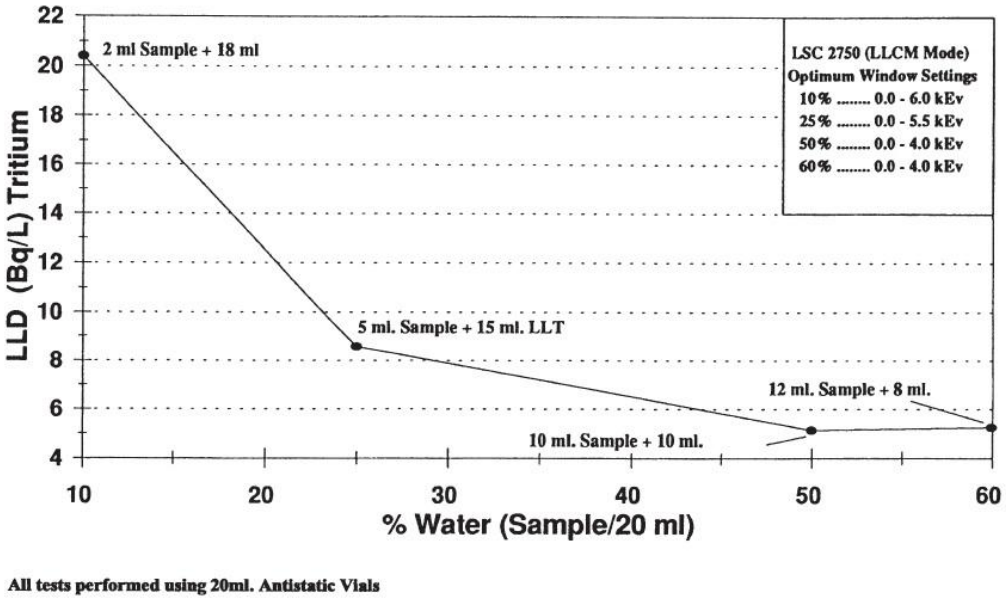

Figure 2.6. Example of Cocktail performance evaluation of aqueous samples. Adapted from (Cook et al., 2004)

2.5.3.4 Sample Quenching

Quenching in Liquid scintillation Counting is anything which reduces the output of Ultra Violet, UV photons (light-transfer process) in the liquid mixture. The light transfer process occurs when the emitted radiation from the sample is transformed by a Fluor in the cocktail and given off as UV photons in the blue energy range (Thomson, 2001). Quenching may be classified in three major forms: Photon, Chemical and Colour quench.

2.5.3.4.1 Photon Quench

The incomplete transfer of Beta or alpha particles to the solvent is known as photon quenching. This occurs when the sample consists of substances which reduce the probability of absorption of ionising radiation by the solvent and subsequent energy transfer processes. Example of such photon quenchers are alcohol and aliphatic hydrocarbons (Cook et al., 2004; Thompson, 1983).

2.5.3.4.2 Chemical Quench

Chemical quench causes a shift in the pulse height to lower energy (loss of energy), see Figure 3.7, by de-excitation of the solvent. Some chemical quenchers have stronger interference than others. Chemical quenchers such as water, chloroform, acetone and carbon tetrachloride, interfere mildly where as Nitric acid and Nitromethane interfere strongly with the scintillation process (Cook et al., 2004).

2.5.3.4.3 Colour Quench

Colour quench is caused by the attenuation of light transmission in the solute. As shown in Figure 2.7, colour quenchers reduce the probability of the transmission of UV photons to the cathode of the photomultiplier tube.

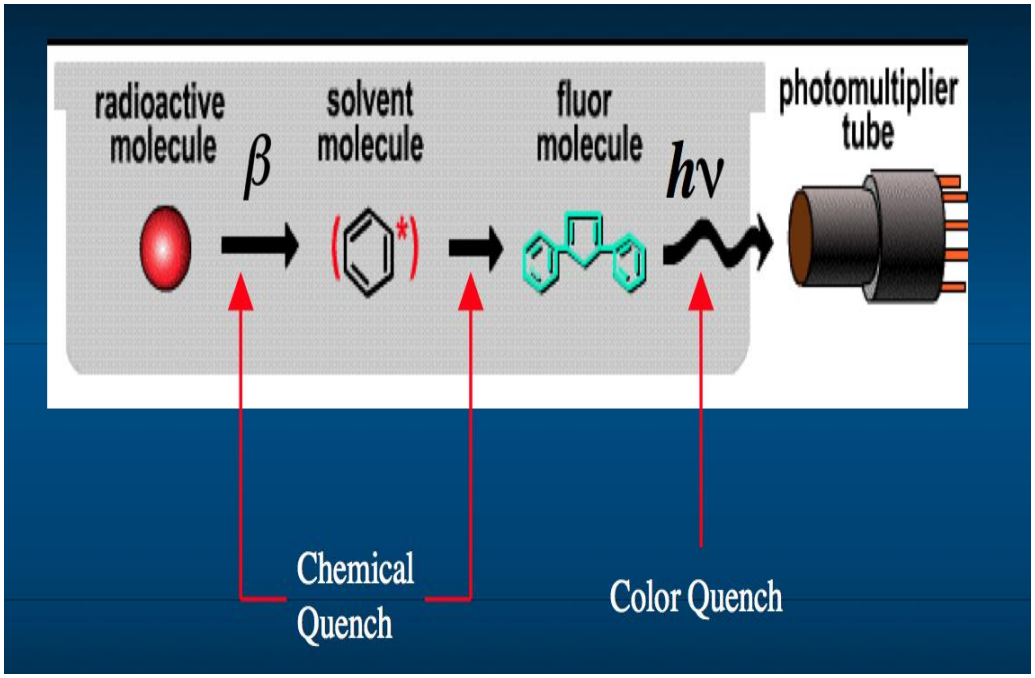

Figure 2.7. LSC Quench classification. Adapted from (Thompson, 1983)

2.5.3.5 Detection Limit

To evaluate the performance of the method for radionuclide analysis, the limit of detection must be determined. Limit of detection comes to mind when one wants to know whether or not a radionuclide is definitely present in a sample. This must take into account the detector background and counting efficiency, sample volume or mass, the count time of both background and sample and the chemical recovery. From (Currie, 1968) two principal aspects of detection can be defined: the critical level L_c (the net counts above which a signal can be detected) and the detection limit L_D (the signal level where a signal at or above this level would be detected). There are two kinds of error associated with this level, since it is a qualitative decision.

i. The Type I error or α, which concludes that there is a true signal when in fact there is not and
ii. Type II error or β, which fails to conclude that there is a true signal when there is

In nuclear counting, the risk (α) is acceptable at 5%, that is 95% (1-α) of the time the conclusion made is true. The critical level, L_c can be set at

$$L_c = K_\alpha \sigma_0 \ = K_\alpha \sqrt{2B}$$

As shown in Figure 2.8. Where $K_{\alpha,}$ represents the set degree of confidence, σ_0 the standard deviation and B the total Background count. When the critical level is established, the detection limit L_D can be defined as the true net signal that can be reliably detected as shown in Figure 2.9.

$$L_D = L_c + K_\beta \sigma_D = K_\alpha \sigma_0 + K_\beta \sigma_D$$

For convenience K_α and K_β can be assigned the same risk level, usually 5% (NCRP, 1985)(the value of k at this risk level is 1.645) and σ_D is the standard deviation for radiation events detected at the detection limit of a detector. The L_D solution is finally simplified to

$$L_D = 2K\sqrt{2B} = 4.65\sqrt{B}\ [counts]$$

For radioactivity calculations, the Lower Limits of Detection (LLD) or Minimum Detectable Activity (MDA) is used to report the Limit of detection and accounts for other factors that influence the counting process. MDA defined by (Currie, 1968) is

$$MDA(Bq/L) = \frac{L_D}{V.T.\varepsilon.60}$$

$$L_D = 2.71 + 4.65\sqrt{C_B T}\ [counts]$$

Where V= volume (L) of sample, T= Time of Background measurement in minutes, ε= counting efficiency, C_B= Background count rate and 60 is the Bq conversion factor.

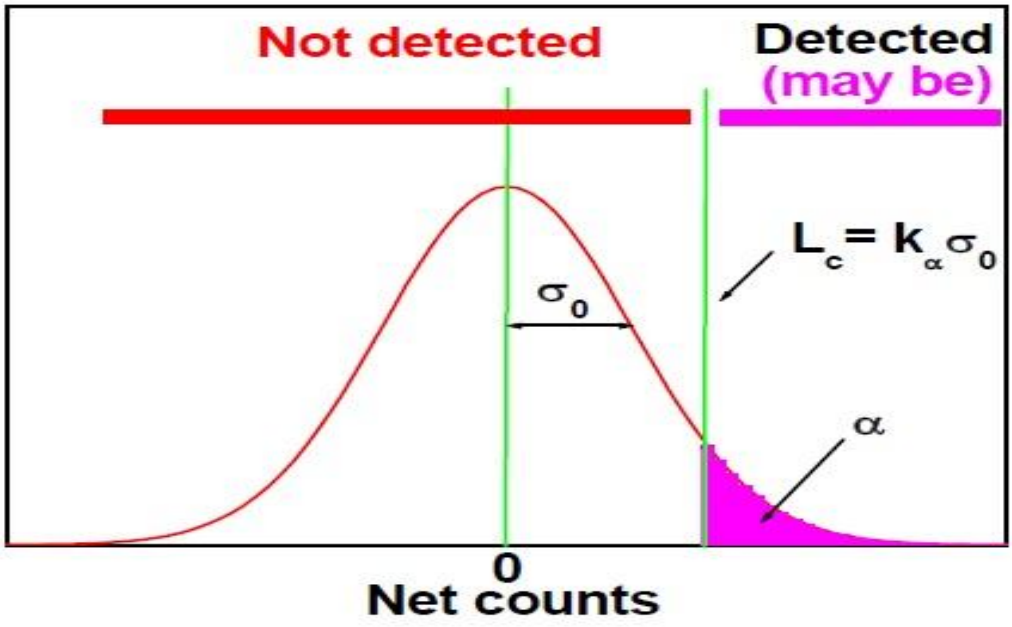

Figure 2.8. Critical Level, Lc detection. Adapted from (Byun, 2009)

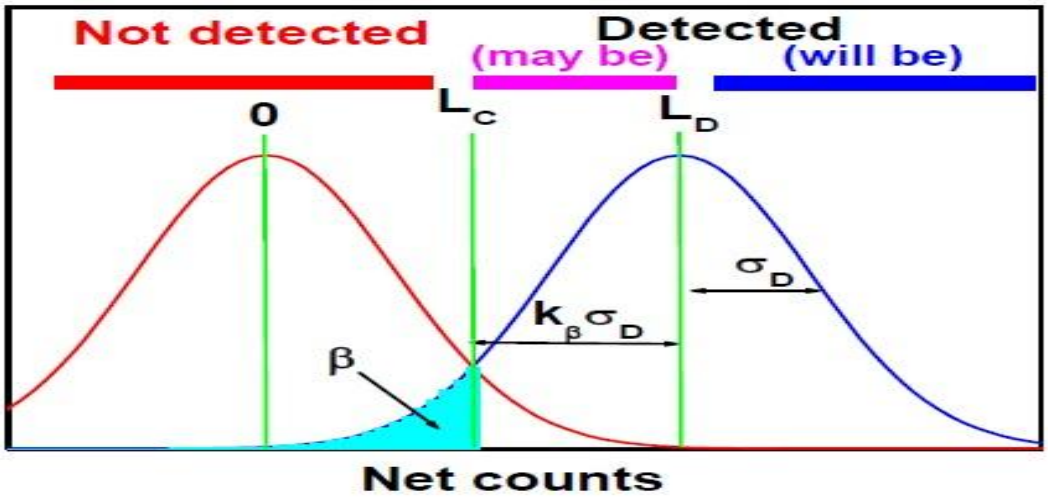

Figure 2.9. Detection Limit, L_D. Adapted from (Byun, 2009)

3 Results

3.1 Background Counts

To determine the detection limit of the Beckman Liquid Scintillation counter used in this experiment, the Blank sample, defined by Currie is a sample which is identical to the sample of interest except that it contains no sample of interest, in this case 10 mL of the Ultima Gold AB (UGAB) scintillation cocktail was put in a low potassium glass vial and counted for 200 mins. The count rate obtained was 70.66 ± 8.41 counts per minute (cpm), two blanks were further counted for the same amount of time and the average count rate was observed as 69.78 ± 8.35 cpm. This count rate value was used in all activity calculations until, changes in the protocol were made and the blank volume had to be adjusted from 10 mL to 20 mL; the average count rate corresponding to the 20 mL blank samples was 106.17 ± 10.31 cpm. Since a low level of Radium-226 concentration was to be measured, particular attention was given to reduce the amount of background counts. It is known that counting in phases rather than one very long count time reduces the background count rate; this phenomenon was taken into consideration and the blank sample was counted for 30 minutes with 11 repeats. Our results obtained were 66.31 ± 2.71 cpm for the replicate count and 68.65 ± 1.18 cpm for a 200 minute; there was no significant difference in the results obtained thus the 200 count time was maintained. Relatively high background values obtained in this experiment could be due to most of the reasons mentioned in sections 2.5.3.2 and 2.5.3.2.1. A typical Background spectrum of UGAB obtained using a LS β spectrometer is shown in Figure 3.1. From the spectrum a peak can be seen between channel 600 and 810 in the channel window. This peak could be a result of the interaction of high energy

and cosmic ray with ^{40}K in the glass vial walls and scintillation cocktail which produce Compton electrons.

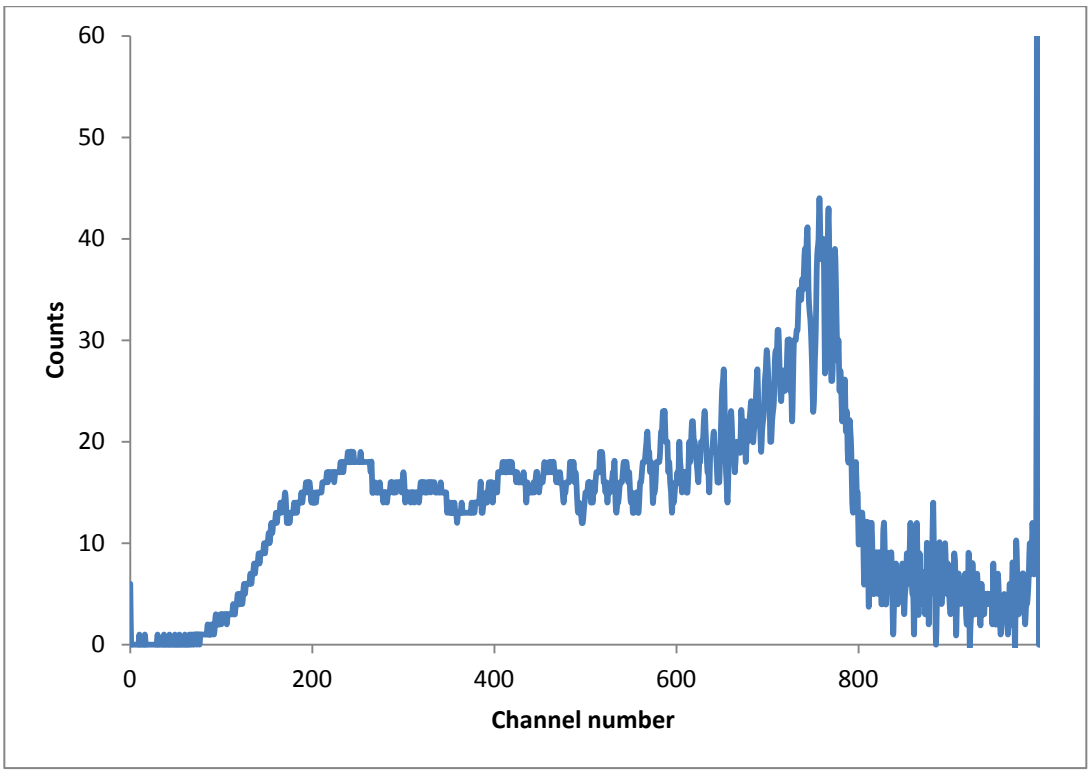

Figure 3.1. A Background spectrum obtained for UGAB using a Beckman LS β Spectrometer.

3.1.1 LSC Detection Sensitivity

The detection limit calculated in this experiment was based on Currie's method, given in section 2.5.3.5. After obtaining a limit of detection, L_D of 680.30 counts the measurable Minimum Detectable Activity (MDA) for ^{226}Ra at 95% confidence level was 7.46 $Bq.L^{-1}$ (7.46 $mBq.g^{-1}$).

3.2 ^{226}Ra standard source Activity

A standard ^{226}Ra source was purchased from Eckert and Ziegler on 1st January 2010 with an activity of 10 µCi in 5 mL which is equal to 370 KBq per 5 mL and purity greater than 99%. The activity of Radium used in preparing the spiked fish food and standard solution (for LSC calibration) was calculated from the manufacturer's said activity. Radium-226 decays with a half-life of 1600 years; however from its decay eight other radioactive daughters are formed. As a result of this the total activity of Radium (Radium plus daughters) must be considered. Since the activity of aliquots of Radium was calculated from the manufacturer's activity, the Bateman equation in section 1.4.4 was employed in calculating the true activity of aliquots. In order to obtain the activity of measured samples, a standard source solution was prepared and counted in the Beckman Liquid Beta Scintillation Counter. The count rate of the standard was used in calculating the activity of all fish samples in this work. An aliquot of Radium-226 with activity of 370 Bq was introduced into UGAB scintillation cocktail in a glass vial and counted for 200 minutes. The count rate obtained immediately and after equilibrium is presented in Table 3.1 together with calculated results using the Bateman equation. To determine, the equilibrium condition at which greater than 99% of Radon would have been formed, a Radium-226 source was counted over time. Equilibrium condition occurs at time greater than 21 days as seen in Figure 3.4 and a typical spectrum of Radium in equilibrium is shown in Figure 3.2. The calculated activity from the Bateman equation shown in Figure 3.3, indicates that 99.99 % (which is approximately 100%) of the Radium activity can be detected when counted immediately (less than 24 hrs) after preparation and only about 11% of Radon and its progeny would have been formed. The counting efficiency from LSC measurement is derived from:

$$Counting\ efficiency\ (\%) = \frac{Counts\ Per\ Minute\ (CPM)}{Disintegrations\ Per\ Minute\ (DPM)} \times 100\%$$

A measured value of a total efficiency of 114.49% was evident when the Radium standard was counted immediately after preparation. Thus from the deductions of the Bateman equation, if it is assumed that 100% of the Radium is detected then the remaining 14% can be attributed to the presence of Radon and its progeny. In equilibrium condition for Radium and its daughter Radon, that is when Radon has passed about 10 of its half-life's (in this case 28 days), the activity of Radon and its progeny is equal to the activity of the parent radionuclide Radium. The total activity can simply be divided by the number of radionuclides present. In this case 6 radionuclides are present; Radium, Radon and four of its progeny (see coloured portion of Figure 1.2). However when the measured total activity in equilibrium as stated in Table 3.1 is divided by 6, the Radium activity is 267.90 Bq which is clearly an underestimation of the calculated value. From Figure 3.4, a counting efficiency of approximately 600% and summate activity which is 6 times the initial Radium activity can be achieved if all the emissions from Radium and its progeny are counted. On the other hand, the measured total activity gave rise to only 4 times the initial activity and not 6 as expected. Such a huge difference could primarily result as a loss of Radon from the counting vial. Hence a ratio of the detectable Radium percentage (99.99%) was used to calculate the activity of Radium in both measured and calculated data. This led to the test of Radon loss, which is explained in a subsequent section.

Measured					
Time	CPM	DPM	Total Activity (Bq)	^{226}Ra Activity (Bq)	Counting Efficiency (%)
Immediate	25415.84	22200	423.6	369.99	114.49
Equilibrium	96445.44	22200	1607.42	369.96	434.44
Calculated					
Immediate	26894.28	24433.8	407.23	369.99	110.07
Equilibrium	658231.84	132489	2208.15	369.94	596.82

Table 3.1 The Activity of a ^{226}Ra standard source Measured (calculated from LSC measurement) and Calculated (calculated from Bateman equation) for two different times.

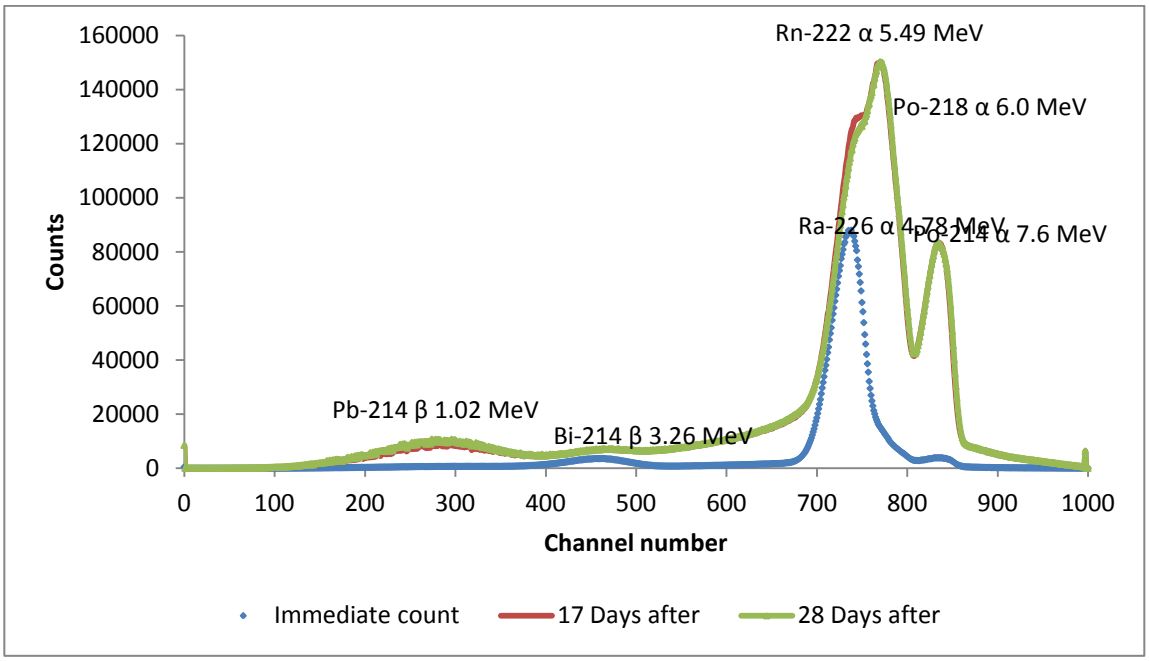

Figure 3.2 Spectrum of ^{226}Ra Standard counted at different time intervals.

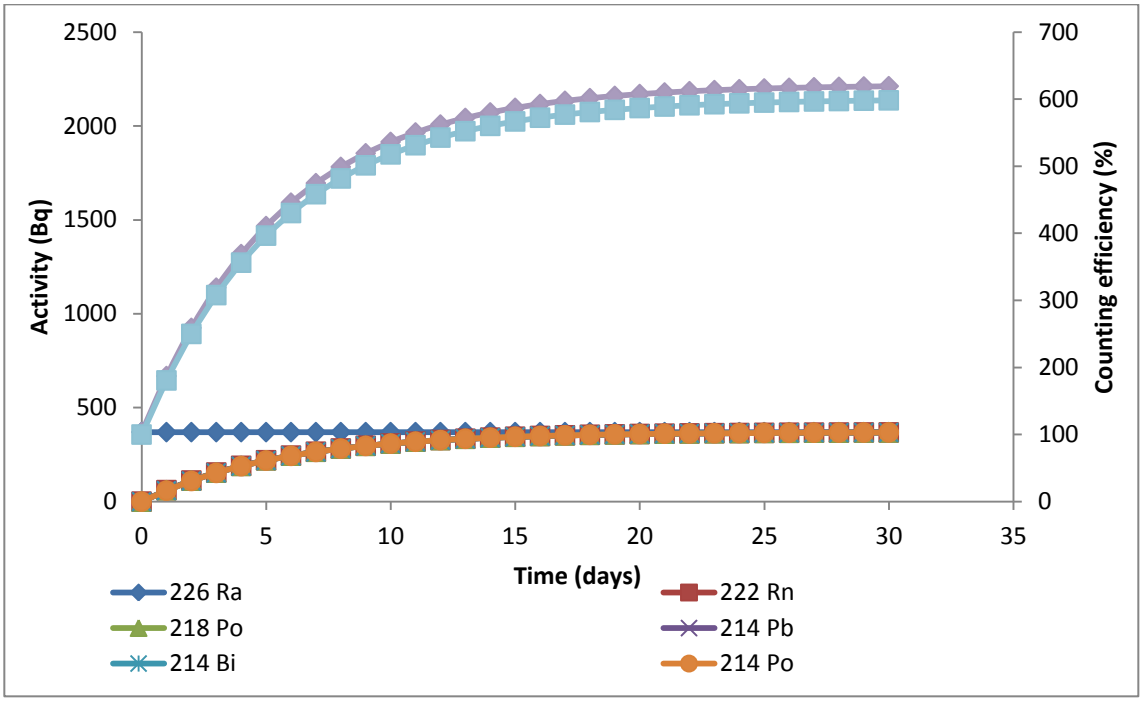

Figure 3.3. Curve showing the number of days it takes to establish equilibrium between Radium with its progeny, Total Activity and Counting efficiency.

3.3 Sample Counting Efficiency

The counting efficiency for α- particles is approximately 100% and 90-100% for β-particles using Liquid Scintillation counting (LSC). In counting ^{226}Ra by LSC method, 3α-particles and 2β-particles emitted from ^{222}Rn and its progeny are counted. This results in a gross counting efficiency at radiological equilibrium of approximately 600% (4α + 2β). On the contrary as seen in Figure 3.4 a counting efficiency of 600% is not realized for measured samples. The mean counting efficiency achieved during this experiment was approximately 447%.

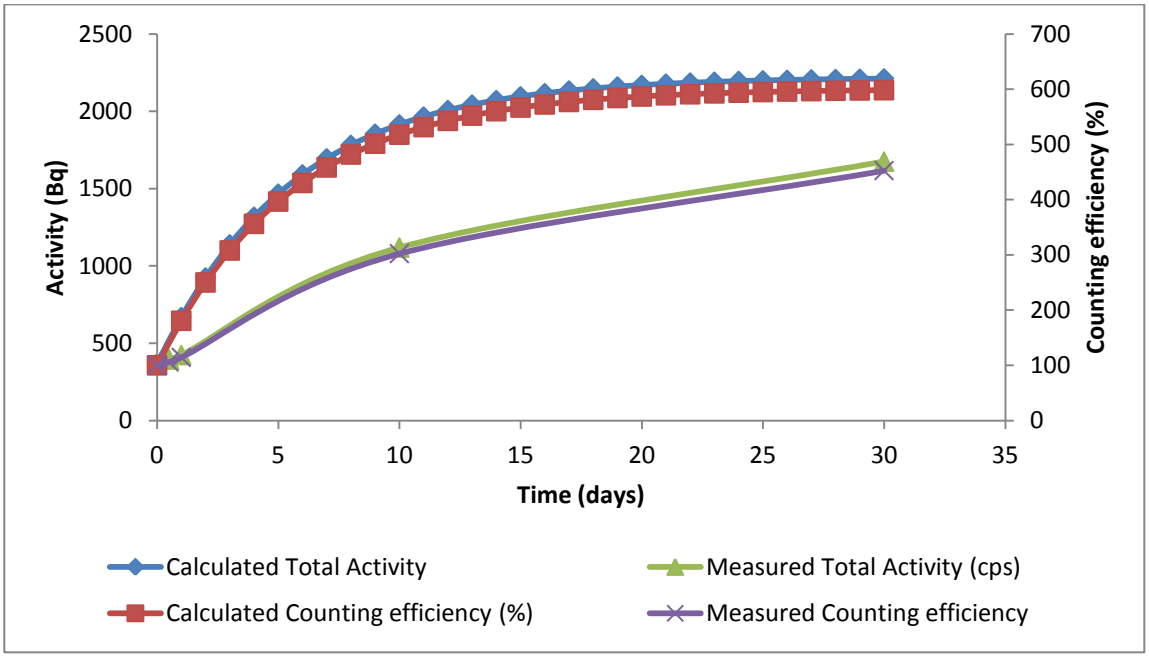

Figure 3.4 Curve showing the, Total Activity and Counting efficiency as a function of Time.

3.4 ^{222}Rn escape

Radium-226 decays by emitting an alpha particle to form Radon-222 which has a half life of 3.15 days. To accurately measure the Radium-226 activity present in a sample by LSC, all of the Radon must be trapped or allowed to escape. Counting of Radium-226 standard solutions for reciprocity resulted in inconsistent data. Several factors had to be considered to determine the cause of such discrepancies. New glass vials with lower background which had aluminum lined covers were used to try and solve the inconsistency problem. However since Radon is an inert gas, it permeates through most things. The use of Teflon to wrap around the glass vial before capping was exploited. This was done by counting the same sample with and without Teflon sealing. The samples were counted for 200 minutes without Teflon sealing at equilibrium (30 days); these were then left open for about 4 hours for most of the in-built Radon and its short

lived daughters to decay. Teflon sealing was applied to the vials and recounted at different time intervals. Results of the pulse height spectrum, Figure 3.5 for one of the standard samples prepared indicate that there was loss of Radon through the vial. Since sealing of the scintillation counting vial with Teflon tape before capping reduced the loss of Radon from the vial, counting vials for all other samples were treated this way. Another Radon loss reduction method employed was to minimize the amount of free air volume above the cocktail by filling the vial (20 mL) to the brim, instead of having 10 mL of scintillation cocktail mixed with the sample. This technique prevents Radon and some of its daughters emanating to the air space above the scintillation cocktail, also inverting the scintillation vial aids in the minimization of Radon loss.

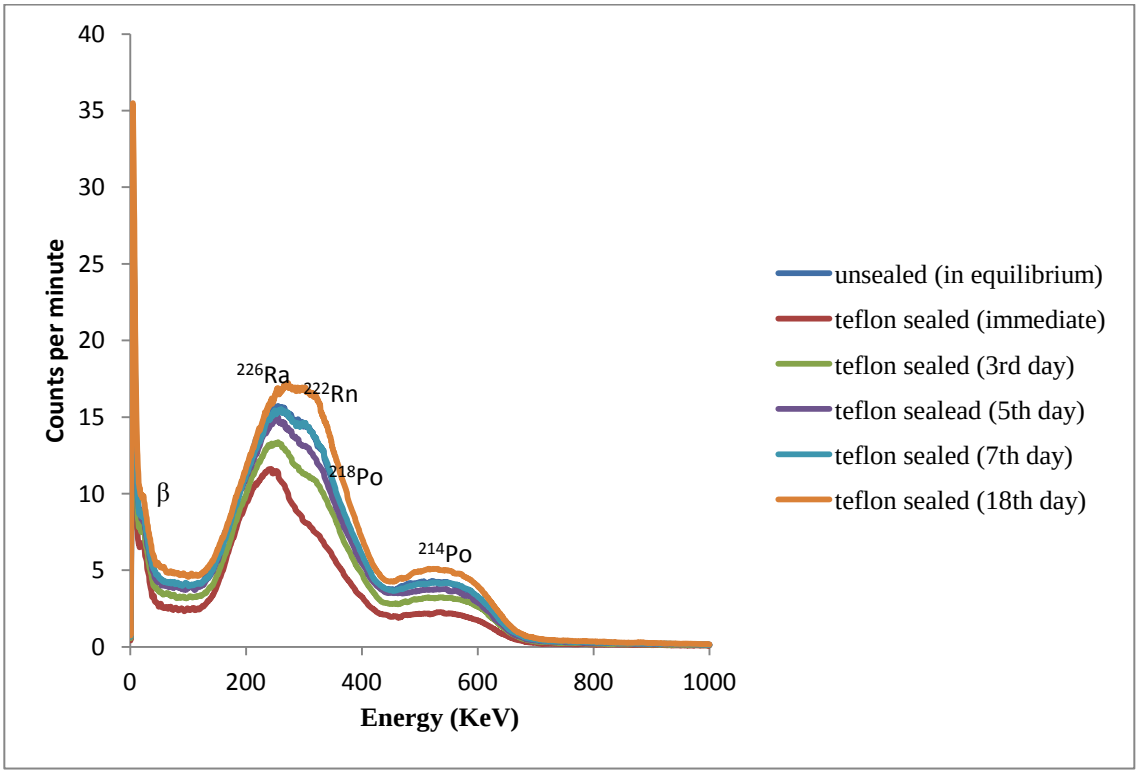

Figure 3.5. Pulse height spectrum of a Radium-226 standard solution in a Teflon sealed (aluminum foil cap plus Teflon tape) and unsealed (aluminum foil cap) vial counted at different time intervals.

3.5 Quenching Effect

As discussed in a previous section, there are three forms of quenching which may occur in Liquid Scintillation Counting. Since the fish sample preparation involves the use of concentrated acids, the effect of chemical quench was analysed. Ten vials containing approximately the same amount of Radium -226 activities (370 Bq) in scintillation cocktail were counted for 5 minutes (corresponds to obtaining 0.5% of a 2s (2 sigma standard deviation)). The Beckman LS Spectrometer used is modified to indicate which standard solution falls within the 0.5% 2s value, thus based on this mechanism nine vials were selected to produce a quench curve (calibration curve of counting efficiency versus a quench indicating parameter, H#), which is used to convert the count rate (CPM) to disintegration rate (DPM). The H # is a quench indicating parameter used in Beckman Liquid Scintillation Spectrometers to indicate the shift of the inflection point of the pulse height spectrum of a quenched sample from an unquenched sample. Thus higher H #s indicate higher quenching in the sample. Increasing amounts, (0 - 75 µL) of a quench agent, nitromethane was added to the standard solutions which were subsequently counted for 200 minutes to acquire a count rate used in the preparation of a quench curve, Figure 3.6. The Quench curve was used to calculate the disintegration rate of Radium-226 in fish whole body samples counted immediately (less than 24 hours) after preparation.

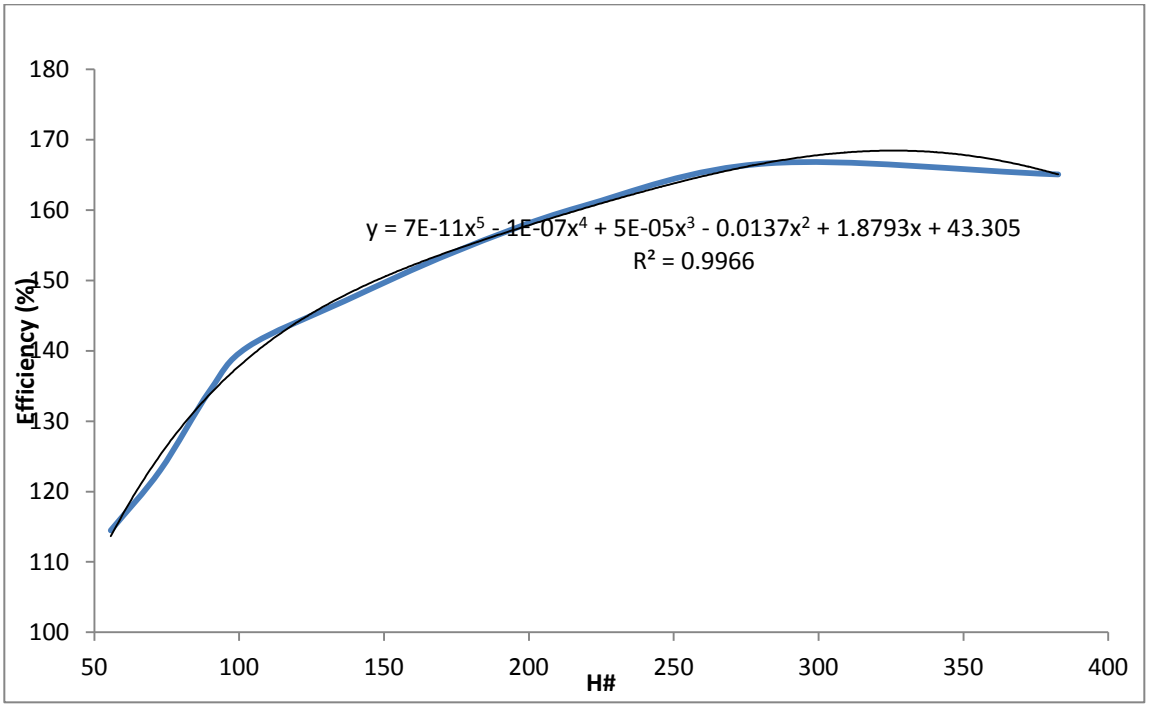

Figure 3.6. A Quench curve of Radium-226 standards counted for 200 mins immediately after preparation showing the line equation and regression coefficient value.

The count rate and corresponding H# data used in calculating the counting efficiency is found in Table 3.2. The results from this table show varying amount of counting efficiency which is due to the growth of Radon and its progeny as every sample is counted for 200 minutes. Samples were counted with a 95 percent confidence level.

^{226}Ra Standard solution sample	H#	CPM	DPM	Efficiency %
1	55.7	$25.42*10^3$±22.87	$22.2*10^3$±0.5	114.49±0.12
2	72.8	$27.32*10^3$±24.58	$22.2*10^3$±0.5	123.04±0.14
3	89.8	$29.81*10^3$±23.85	$22.2*10^3$±0.5	134.29±0.15
4	103	$31.21*10^3$±24.97	$22.2*10^3$±0.5	140.57±0.16
5	136.4	$32.65*10^3$±26.12	$22.2*10^3$±0.5	147.09±0.17
6	174.1	$34.20*10^3$±27.35	$22.2*10^3$±0.5	154.02±0.19
7	218	$35.63*10^3$±24.94	$22.2*10^3$±0.5	160.50±0.20
8	282.6	$37.00*10^3$±25.90	$22.2*10^3$±0.5	166.66±0.20
9	382.7	$36.65*10^3$±25.65	$22.2*10^3$±0.5	165.07±0

Table 3.2 Quench Indicating Parameter, H#, count rate and counting efficiency for standard Radium-226 samples. Samples were counted immediately after preparation for 200 mins.

Results of Radium-226 count rate at equilibrium can be seen in Table 3.3 and Figure 3.7 shows the spectrum of the quenched Radium-226 standards. From the table and spectrum it can be seen that the total area under the pulse height spectrum is not significantly affected by the chemical quenching agent, however the pulse height shifts to the lower energy (lower pulse height) and alpha peak broadening (reduced resolution) occurs which is proportional to the level of quench. A quench curve was prepared from the data obtained at equilibrium, shown in Figure 3.8. Here it can be realised that increase in the quench indicating parameter (H#) does not follow a polynomial increase as in the case of Figure 3.6. As observed, quenching at equilibrium is greatly affected by low energy resolution that is; a shift in pulse height to lower energy. Due to this effect and the fact that the quench indicating parameter (H#) in the fish samples ranged from 100.00-130, the quench curve at equilibrium was not used to calculate the disintegration rate of count rates evaluated for fish samples at equilibrium. The count rate from

the counted fish samples were converted to activity (disintegrations per second) by a comparison with the count rate of the standard Radium solution.

sample	H#	CPM	DPM	Efficiency %
1	54.7	$96.45*10^3$±22.87	$22.2*10^3$±0.5	434.44±0.20
2	72.9	$98.44*10^3$±22.87	$22.2*10^3$±0.5	443.42±0.20
3	91.1	$10.08*10^4$±22.87	$22.2*10^3$±0.5	454.05±0.20
4	101.3	$98.10*10^3$±22.87	$22.2*10^3$±0.5	441.85±0.20
5	137.8	$97.18*10^3$±22.87	$22.2*10^3$±0.5	437.73±0.20
6	175.7	$96.75*10^3$±22.87	$22.2*10^3$±0.5	435.80±0.20
7	220.3	$94.55*10^3$±22.87	$22.2*10^3$±0.5	425.88±0.20
8	287	$94.63*10^3$±22.87	$22.2*10^3$±0.5	426.26±0.20
9	390	$89.83*10^3$±22.87	$22.2*10^3$±0.5	404.65±0.20

Table 3.3. Quench Indicating Parameter, H#, count rate and counting efficiency for standard Radium-226 samples. Samples were counted at equilibrium (28 days) for 200 mins.

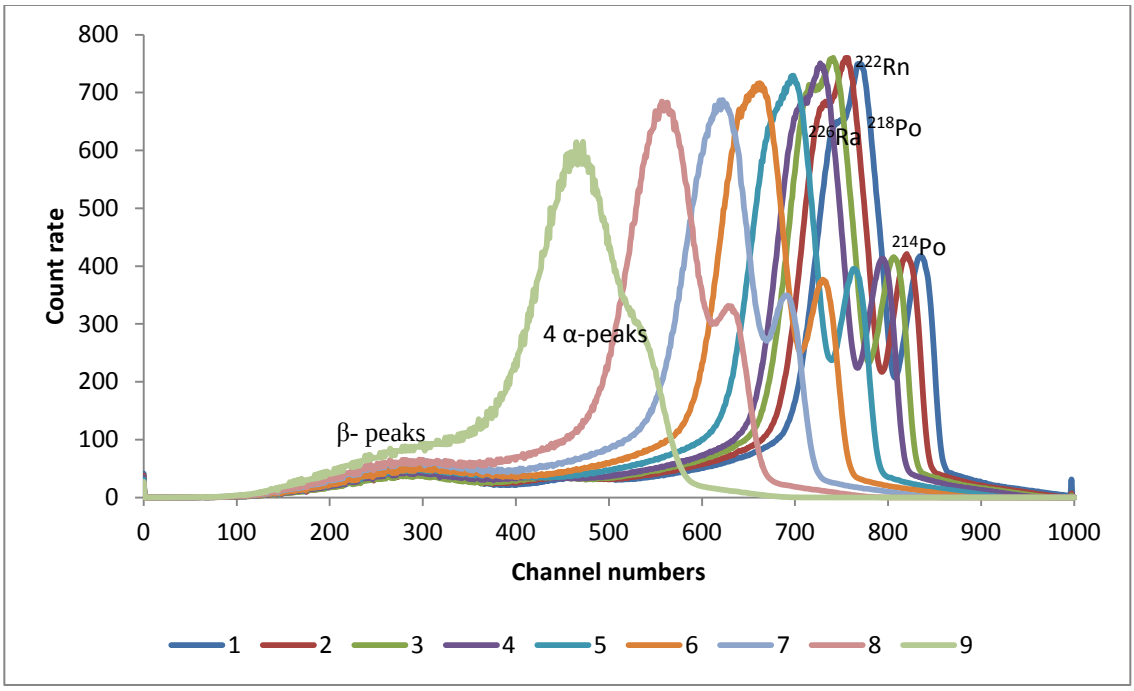

Figure 3.7 Spectrum of ^{226}Ra standard (equilibrium) quenched with nitromethane. The numbers indicate the intensity of quenching with 1 having no quench agent to 9 having the highest level of quenching agent.

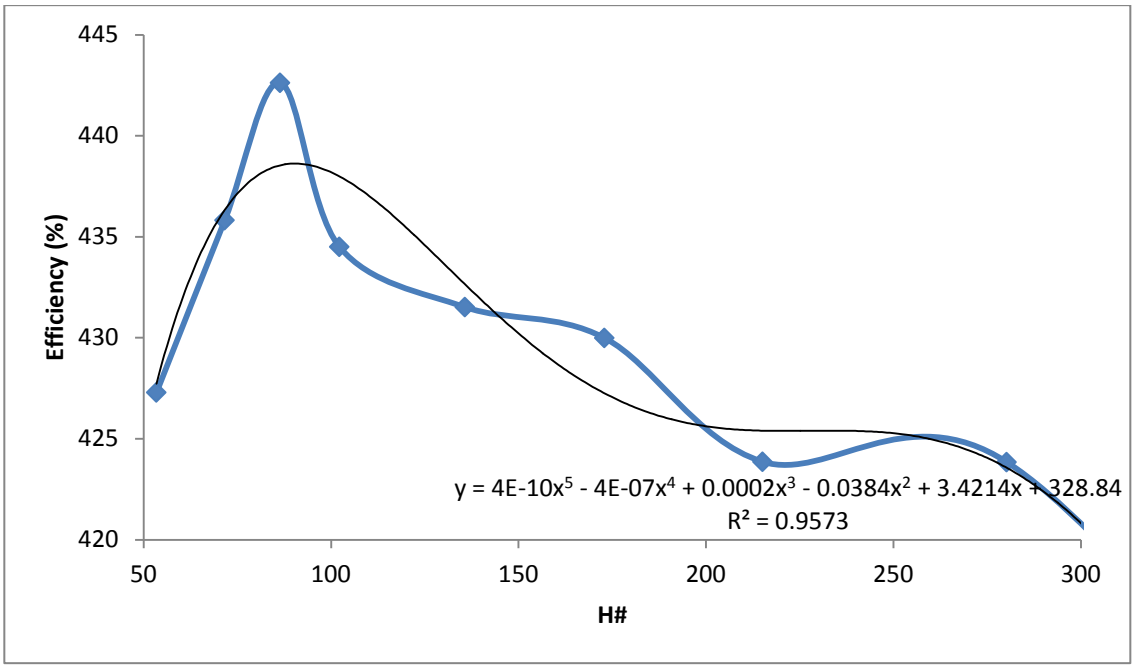

Figure 3.8. . A Quench curve of Radium-226 standards counted for 200 mins at equilibrium (28 days after preparation) showing the line equation and regression coefficient value.

3.6 Injected ^{226}Ra FHM

The protocol developed initially for sample preparation of fish for LSC activity analysis enabled only 10% of the fish sample to be counted at a time. This was due to the high acidity of the final form of fish solution which had to be diluted with distilled water before mixing with scintillation cocktail prior to counting. In view of this, only 10% of the total volume could be counted as a result of the low acidic tolerance of the liquid scintillation cocktail. Since it was more appropriate to count the whole fish sample due to low activity levels, a scintillation cocktail (Ultima Gold AB) with higher acidity tolerance was obtained. The protocol was revised and experimented with the new cocktail. Initial neutralising tests without fish bodies showed good results with 1.78ml sample volume. Further tests with fish bodies proved successful;

approximately 6 µL of Radium stock solution was injected into each fish n=4, these were processed using the protocol (Appendix A) to validate the neutralising method and to determine Radium loss during sample processing (calcination and acid digestion). Two of the fish (control, 1 & 2 and Radium-226 injected fish, Fish 1 & 2) were neutralised with Sodium hydroxide to a volume of 1.78 ml, the other two were taken up to the same volume in 1M of Hydrochloric acid. Figure 3.9 shows the Radium spectrum of the injected fish. Activities calculated for these samples are shown in Figure 3.11 with the results presented in Table 3.4.

FHM sample	^{226}Ra Injected Activity (Bq)
Control 1	0.41 ± 0.03
Control 2	0.94 ± 0.03
Control 3	0.97 ± 0.03
Control 4	1.08 ± 0.03
Fish 1	247.59 ± 0.31
Fish 2	457.41 ± 0.42
Fish 3	444.13 ± 0.41
Fish 4	514.25 ± 0.44

Table 3.4 Measured Natural Background (control) and ^{226}Ra injected fish activity. Neutralised fish are; Control 1 & 2 and Fish 1&2

As shown in Table 3.5, the calculated injected Radium-226 volume falls within range of the estimated value with the exception of the first sample. The spectrum, Figure 3.9 showed no significant difference between the neutralised and acidic fish samples. Hence the fish samples fed with a Radium-226 spiked diet were taken up in dilute Hydrochloric acid for analysis.

FHM sample	^{226}Ra Injected Activity (Bq)	Calculated injected volume of ^{226}Ra (µL)	Estimated injected volume of ^{226}Ra (µL)
Fish 1	247.59 ± 0.31	3.35 ±0.5	5
Fish 2	457.41 ± 0.42	6.16±0.5	5
Fish 3	444.13 ± 0.41	6±0.5	5
Fish 4	514.25 ± 0.44	6.95±0.5	5

Table 3.5 Estimated and Calculated injected volume of ^{226}Ra from individual Fish 1 to Fish 4.

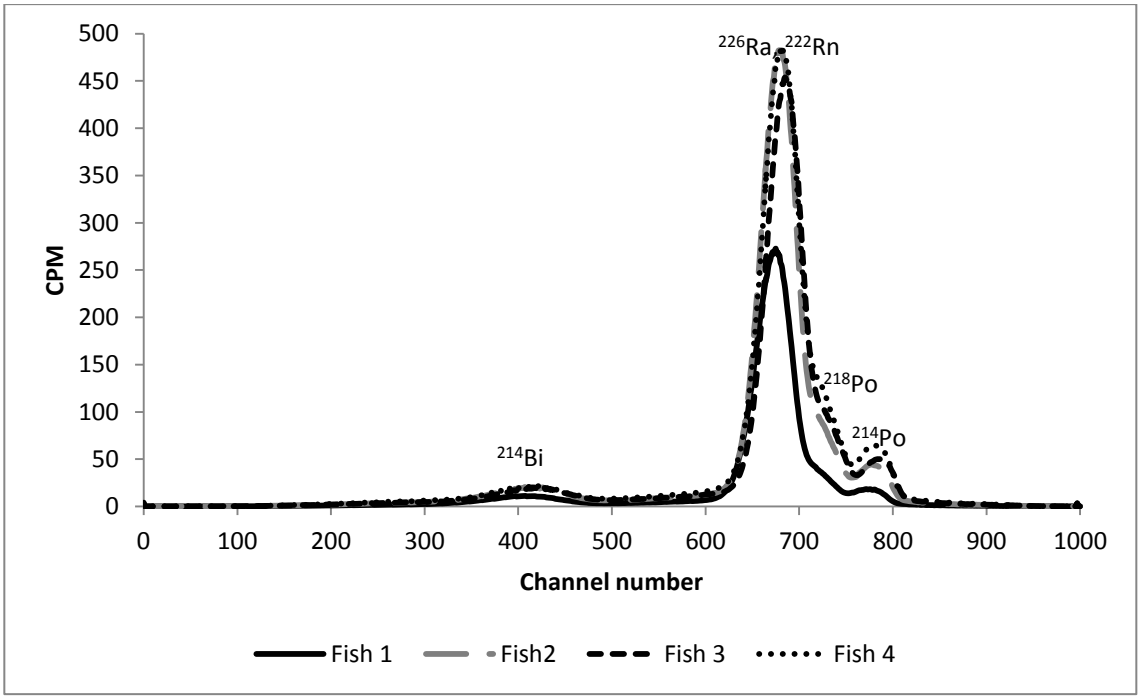

Figure 3.9. ^{226}Ra Injected Fish Spectrum. Fish 1 to Fish 4 are individual fish injected with ^{226}Ra solution. Counted immediately after preparation

A comparison of the injected fish spectrum with quenched and unquenched Radium standards, shown in Figure 3.10 confirms the presence of chemical quench and the area under the pulse height spectrum (CPM) is not significantly affected from the acid digestion process.

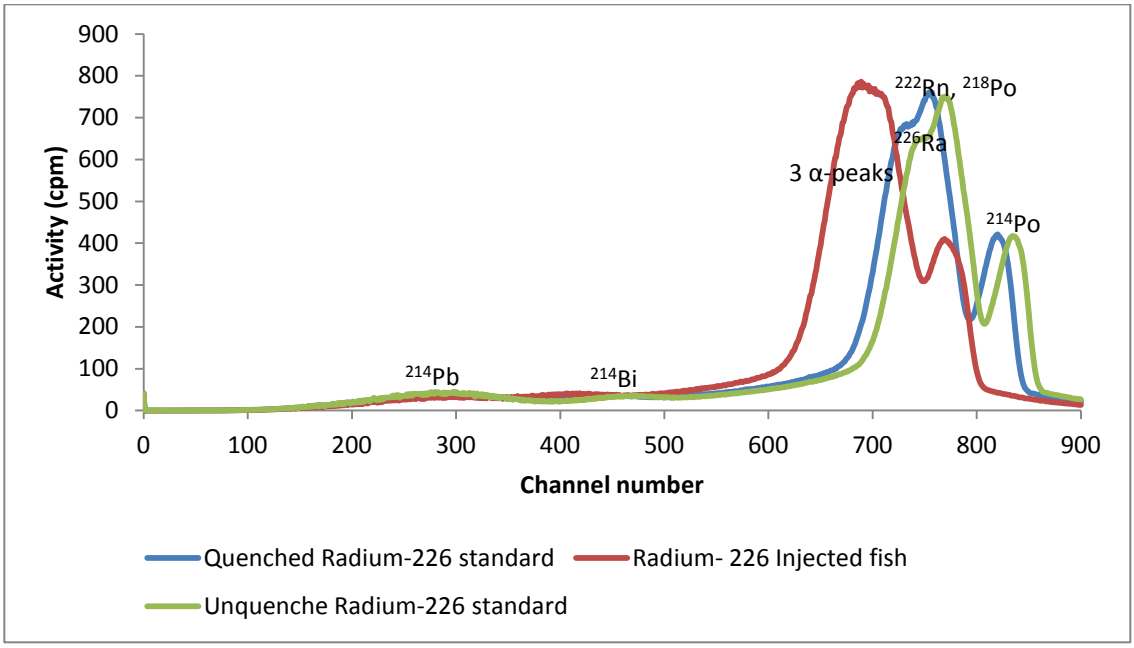

Figure 3.10 Comparison of quenched and unquenched Radium- 226 standard spectrum with injected fish spectrum. Counted at equilibrium.

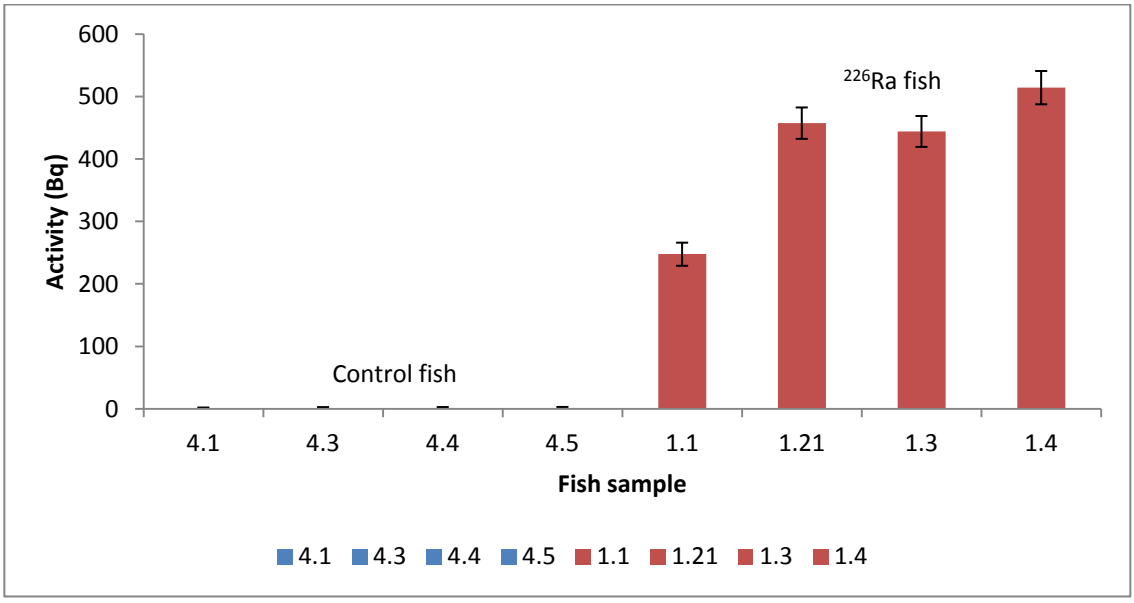

Figure 3.11. Activity of ^{226}Ra Injected Fish. Where 4.1….4.5 are control fish and 1.1….1.4 are fish injected with ^{226}Ra.

3.7 Tissue concentrations of ^{226}Ra and dose rate

Feeding commenced with Fifteen Fathead Minnows (FHM) with an average weight of 0.093 g. These fish were fed adlibitum with a commercial fish food spiked with 100Bq g^{-1} of Radium-226 on an alternate daily cycle. On the 75th day, at which time the fish had been fed 34 times, five fish were randomly netted and euthanized. The fork length of the fish and weight were measured and recorded. This was followed by the processing of the fish by calcination and acid digestion to obtain the sample in an aqueous form for Radium-226 measurement by Liquid Scintillation Analysis. The total food that had been given to the fish at the time of sampling was 1858 mg. Assuming that each fish consumed equal amounts of food, on average each fish should have eaten approximately 123.86 mg of food. Also if all the Radium-226 in the fish food was retained in the whole body of the fish, the corresponding activity should be 12.38 Bq. Based on this analogy, the estimated Radium-226 activity to be detected in each fish should be approximately 12.38 Bq and the total activity 61.9 Bq. Table 3.6 shows the average measured Radium-226 activity retained in the fish as 281.26 mBq and the activity concentration, 1579.25 mBq/g of fresh fish weight (wet wt). Most of the fish in the control and acid treatment groups resulted in count rates less than that of background, thus the activities for these groups have been set to zero.

Column1	weight, w/w (g)	length (cm)	SGR (%/day)	Condition factor (g/cm3)	Measured ^{226}Ra Activity Concentration w/w (mBq/g)	Measured ^{226}Ra Activity (mBq)
Control	0.19 ± 0.02	2.44 ± 0.12	1.95 ± 0.25	1.26 ± 0.05	0 ± 47.03	0 ± 4.80
226Ra Treatment	0.18 ± 0.01	2.62 ± 0.06	2.1 ± 0.06	1.01 ± 0.02	1579.25±294.94	281.26±49.65
Acid Treatment	0.2 ± 0.01	2.68 ± 0.02	2.19 ± 0.03	1.01 ± 0.03	0 ± 34.7	0 ± 9.94

Table 3.6 Average activity concentration and physical growth measurement for the three groups (control=no Radium in food, 226Ra Treatment= food spiked with 226Ra, Acid Treatment= food spiked with nitric acid) studied sampled at 75 days after feeding. Sample size n=5, error (SEM).

The individual results used in calculating mean values after 75 days of Radium exposure have been presented in Table 3.7.

fish sample	wet weight (g)	length (cm)	SGR (%/day)	K factor	Measured ^{226}Ra Activity Concentration w/w (mBq/g)	Measured ^{226}Ra Activity (mBq)
3.1	0.16 ± 0.03	2.6 ± 0.1	2.00 ± 0.12	0.93± 0.05	2267.21±659.50	371.82±111.01
3.2	0.17 ± 0.03	2.5 ± 0.1	2.02 ± 0.12	1.07± 0.05	1808.58±659.50	302.032±111.01
3.3	0.16 ± 0.03	2.5 ± 0.1	2 ± 0.12	1.05± 0.05	1096.77±659.50	179.871±111.01
3.4	0.20 ± 0.03	2.7 ± 0.1	2.2 ± 0.12	1± 0.05	2027.63±659.50	399.44±111.01
3.5	0.22 ± 0.03	2.8 ± 0.1	2.26 ± 0.12	1± 0.12	696.06± 659.50	153.13±111.01
4.1	0.23 ± 0.05	2.6 ± 0.3	2.28 ± 0.56	1.3± 0.12	ND	ND
4.2	0.18 ± 0.05	2.4 ± 0.3	2.09 ± 0.56	1.27± 0.12	ND	ND
4.3	0.22 ± 0.05	2.6 ± 0.3	2.26 ± 0.56	1.25± 0.12	ND	ND
4.4	0.19 ± 0.05	2.6 ± 0.3	2.18 ± 0.56	1.08± 0.12	ND	ND
4.5	0.11 ± 0.05	2.0 ± 0.3	0.96 ± 0.56	1.4± 0.12	ND	ND
7.1	0.19 ± 0.02	2.7 ± 0.1	2.17 ± 0.06	0.96± 0.08	24.49±77.58	4.6±22.2
7.2	0.22 ± 0.02	2.7 ± 0.1	2.27 ± 0.06	1.13± 0.08	ND	ND
7.3	0.21 ± 0.02	2.7 ± 0.1	2.23 ± 0.06	1.04± 0.08	ND	ND
7.4	0.18 ± 0.02	2.7 ± 0.1	2.15 ± 0.06	0.93± 0.08	ND	ND
7.5	0.18 ± 0.02	2.6 ± 0.1	2.11 ± 0.06	1.01± 0.08	ND	ND

Table 3.7. Individual activity concentration and physical growth measurement for the three groups (4.1...4.5=no Radium in food, 3.1...3.5= food spiked with ^{226}Ra, 7.1...7.5= food spiked with nitric acid) studied sampled at 75 days after feeding. Sample size n=15, error (Standard Deviation) and ND are values below detection.

Comparison of the spectrum of the sampled fish from the Radium-226 treatment group, Figure 3.12 with the standard Radium-226 spectrum indicates the presence of Radium. It is rather interesting to note the near absence of a peak in the channel range 700-800 which corresponds to the ^{214}Po peak region even though there is evidence of the presence of its parent ^{214}Bi.

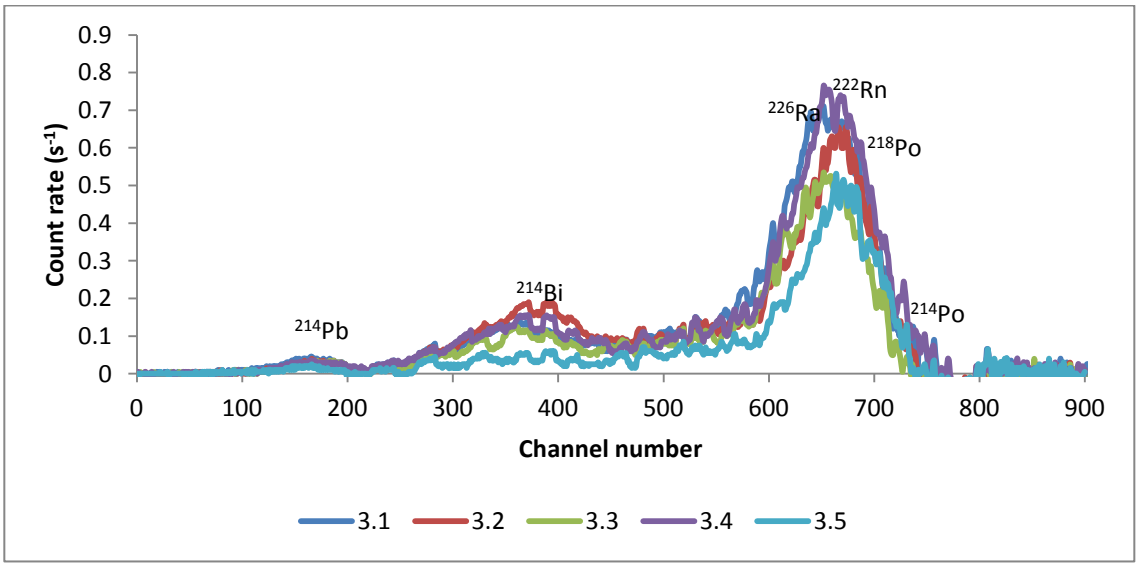

Figure 3.12. Pulse height spectrum of Radium-226 (equilibrium) retained in the sampled fish whole body. The Legend represents individual sampled fish.

The sampled fish were counted in different time intervals to show the growth of Radon-222 and its progeny. As shown in Figure 3.13, the Radon growth curve follows that of the Radium-226 standard sample.

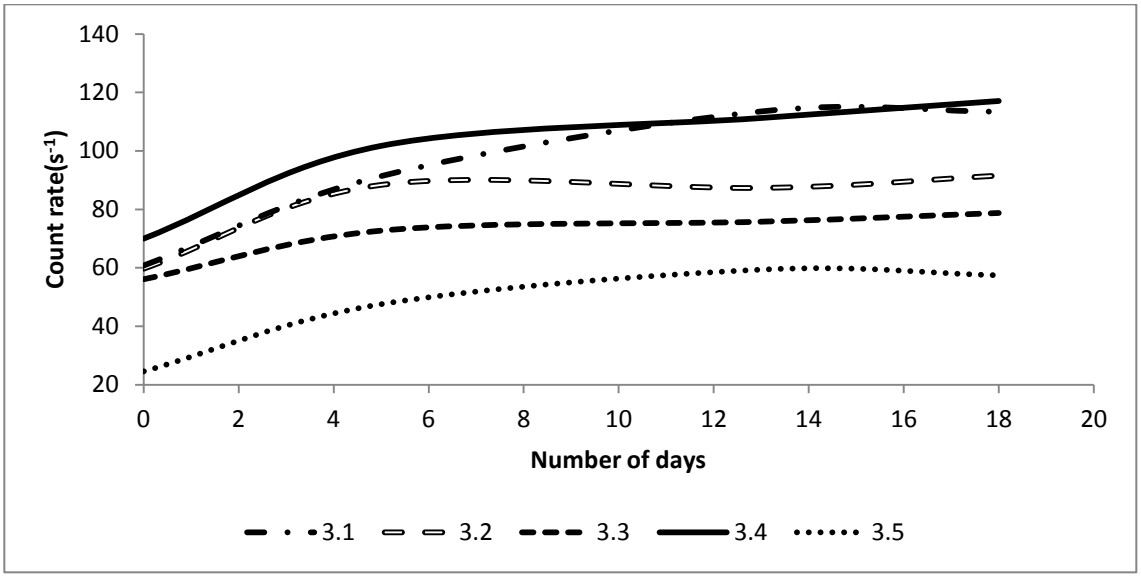

Figure 3.13 ^{226}Ra progeny growth in sampled FHM. 3.1 to 3.5 represent individual fish.

The figure below shows the measured activity in the Radium-226 treatment group, control and acid control group. The activity concentration of the control and acid control group are significantly below the MDA of 7.6 mBq g^{-1}, hence Radium-226 was not detected in these groups of fish. Activity concentrations of fish evaluated from the first sampling have relatively high Radium-226 level of approximately 1579 mBq g^{-1}, however this result must be compared to the second sampling (data found in Table 3.8 and Table 3.9) done 3 days shy of 6 weeks after the first sampling date before any definite conclusion can be made on the Radium uptake. This experiment was terminated upon the second sampling, thus all ten fish remaining were netted and euthanized. By the end of the experiment about 2558 mg (255.8 Bq/g of Ra-226) of food had been fed to the fish; thus assuming that the fish ate equal amount of food each fish as at the second sampling should have 19.38 Bq of Radium-226 if all the Radium in the food was ingested. Length and mass measurements were taken and five of them were processed for

Liquid Scintillation Counting. The other five were sent to Laval University in Quebec for accurate measurement.

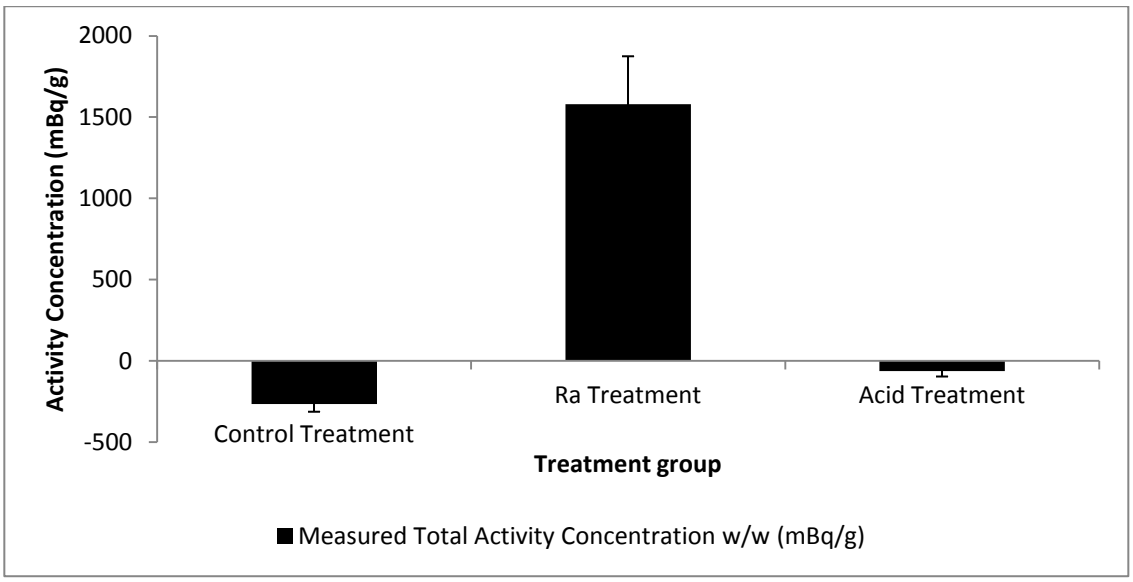

Figure 3.14. Measurement of ^{226}Ra Activity for fish sampled after 75 days of exposure. For each group n=5 and error (SEM).

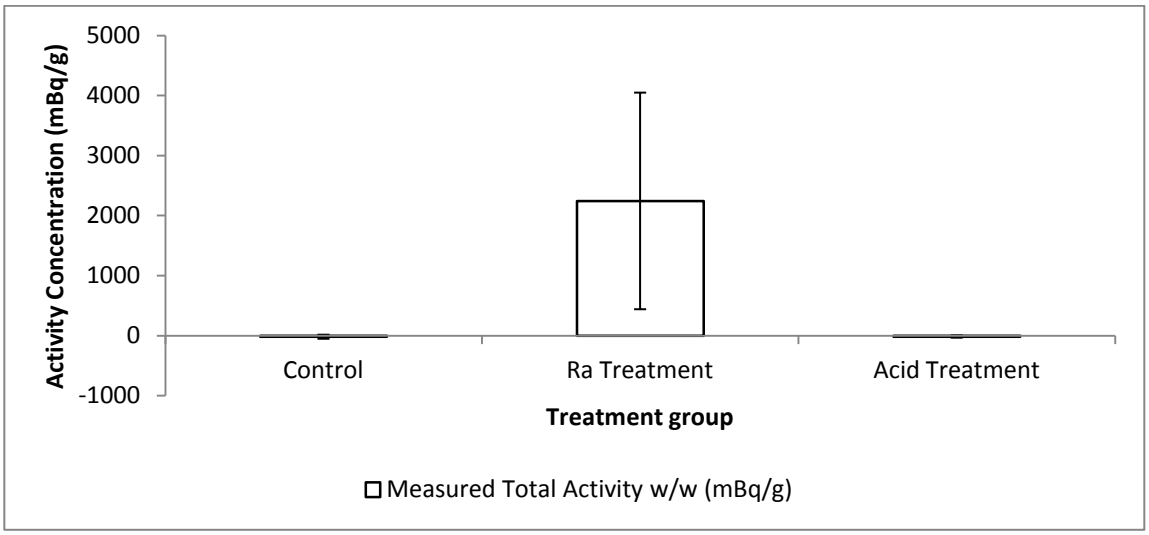

Figure 3.15. Measured Total ^{226}Ra Activity for second fish sampling. For each group n=5 and error (SEM)

Results from the tables below show an increase in weight and length of fish as expected, however one can also observe that the fish in the Radium and acid treatment were heavier than that of the control fish. Even though the fish increased in length and mass they had a relatively low growth rate compared to those sampled earlier and a slightly higher condition factor.

Treatment	weight, w/w (g)	length (cm)	SGR (%/day)	Condition factor (g/cm3)	Measured ^{226}Ra Activity w/w (mBq/g)	Measured ^{226}Ra Activity (mBq)
Control	0.21 ± 0.02	2.82 ± 0.06	1.37 ± 0.12	0.92 ± 0.09	0 ± 33.66	0 ± 6.07
Ra Treatment	0.31 ± 0.02	2.84 ± 0.04	1.47 ± 0.02	1.34 ± 0.08	2243.58 ± 1803.67	722.42 ± 597.03
Acid Treatment	0.38 ± 0.03	3.28 ± 0.10	1.40 ± 0.03	1.07 ± 0.05	0 ± 18.58	0 ± 6.43

Table 3.8. Average activity concentration and physical growth measurement for the three groups (control=no Radium in food, ^{226}Ra Treatment= food spiked with ^{226}Ra, Acid Treatment= food spiked with nitric acid) studied sampled at 115 days after feeding. Sample size n=5, error (SEM).

fish sample	wet weight (g)	length (cm)	SGR (%/day)	K factor	Measured ^{226}Ra Activity Concentration w/w (mBq/g)	Measured ^{226}Ra Activity (mBq)
3.11	0.329	2.9	1.46	1.35	9451.37	3109.50
3.22	0.381	2.9	1.41	1.56	177.21	67.52
3.33	0.286	2.8	1.5	1.3	411.83	117.21
3.44	0.275	2.7	1.5	1.4	517.11	142.20
3.55	0.266	2.9	1.5	1.09	660.40	175.66
4.11	0.125	2.8	0.9	0.57	ND	ND
4.22	0.269	3	1.5	1	7.08	1.90
4.33	0.2	2.7	1.46	1.02	90.75	18.15
4.44	0.247	2.9	1.51	1.01	ND	ND
4.55	0.201	2.7	1.46	1.02	ND	ND
7.11	0.42	3.4	1.37	1.07	ND	ND
7.22	0.321	3.1	1.47	1.08	ND	ND
7.33	0.471	3.6	1.31	1.01	ND	ND
7.44	0.287	3.1	1.49	0.96	57.25	16.43
7.55	0.409	3.2	1.38	1.25	ND	ND

Table 3.9. . Individual activity concentration and physical growth measurement for the three groups (4.11...4.55=no Radium in food, 3.11...3.55= food spiked with ^{226}Ra, 7.11...7.55= food spiked with nitric acid) studied sampled at 115 days after feeding. Sample size n=15, error (SEM) and ND = no detectable values.

The mean concentration of Radium-226 over the entire experimental phase is given in Table 3.10. The retained Radium-226 activity was measured as approximately 501.84 ± 291.83 mBq and the activity concentration was 1911.42 ± 868.64 mBq g^{-1} (wet wt), that in the control and acid control group were below the achieved MDA hence Radium-226 was not detected in these groups.

Treatment	weight, w/w (g)	length (cm)	SGR (%/day)	Condition factor (g/cm3)	Measured ^{226}Ra Activity w/w (mBq/g)	Measured ^{226}Ra Activity (mBq)
Control	0.20 ± 0.02	2.63 ± 0.09	1.66 ± 0.16	1.09 ± 0.07	0 ± 49.52	0 ± 8.08
Ra Treatment	0.24 ± 0.02	2.73 ± 0.05	1.79 ± 0.11	1.18 ± 0.07	1911.42 ± 868.64	501.84 ± 291.83
Acid Treatment	0.29 ± 0.04	2.98 ± 0.11	1.80 ± 0.13	1.04 ± 0.03	0 ± 20.13	0 ± 5.68

Table 3.10. Average activity concentration and physical growth measurement for the three groups (control=no Radium in food, Ra Treatment= food spiked with ^{226}Ra, Acid Treatment= food spiked with nitric acid) studied sampled over the entire experimental period. Sample size n=10, error (SEM).

3.7.1 Concentration Factor and dose rate

To determine the radionuclide transfer of one compartment to the other in this case the transfer of Radium-226 from fish food to fish tissue, the concentration factor (CF) which is a transfer parameter is employed. This transfer parameter is used in assessing radionuclide uptake in an organism. The ^{226}Ra concentration factor (CF) is calculated using the following equation given in (ICRP, 1978) as

$$CF = \frac{radionuclide\ concentration\ in\ tissue\ (mBq\ g^{-1} wet\ weight)}{radionuclide\ concentration\ in\ diet\ (mBq\ g^{-1} wet\ weight)}$$

The ^{226}Ra concentration factor ranged from 0.03 to 2.0 with a mean value of (0.35 ± 0.19).

The amount of radiation energy deposited per unit mass in an organism is known as the absorbed dose. Its unit is Joules Kg^{-1} which is known as gray (Gy). As biological effects occur as a result of the deposition of ionising radiation in matter, and the amount of radiation or energy deposited over time determines the severity of the effect it is important that the amount of radiation deposited is known. To determine the dose rate of Radium-226, which is an alpha emitter, in the fathead minnow it is assumed that the alpha emitting radionuclide is evenly distributed in the whole body. The dose rate can then be calculated from the following equation (D.S Woodhead, 2000)

$$D_{\alpha}^{\infty} = 5.76 \times 10^{-7} \times \bar{E}_{\alpha} \times C_{226_{Ra}}\ \mu Gy h^{-1} (Bq\ g^{-1})^{-1}$$

Where D_{α}^{∞} is α-radiation dose rate to the whole body. $\bar{E}_{\alpha}$ is the average ^{226}Ra energy = 4.78 MeV, $C_{226_{Ra}}$ is the whole body concentration of ^{226}Ra in Bq g^{-1} (wet wt) and 5.76×10^{-7} is a conversion factor. The dose rate from the mean activity concentration was calculated as 5.26 µGy h^{-1}. Individual dose rates to the fathead minnows in this experiment fall within the range of 0.1-30 µGy h^{-1} which fall within the scale where no negative effects on fish have been observed from the 'dose rates-effects' scale (Kryshev & Sazykina, 2011).

3.8 FHM Physical Growth

The fish were weighed with an accuracy of 0.01 (g) and length of 0.01 (cm) with a metric rule. The physiological state of fathead minnows in this experiment was determined by growth indicators such as, specific growth rate and Fulton's condition factor.

The growth rate of fish is dependent on factors such as; age, water temperature, quality and quantity of food. The specific growth rate (SGR), which is a measure of the percentage of increase in body weight per day, was used to ascertain the rate of growth of fish. It is calculated as

$$SGR\left(\%/_{day}\right) = \frac{\ln final\ weight\ (g) - \ln initial\ weight\ (g)}{time\ interval\ between\ weighing\ (days)} \times 100\%$$

Where In is the natural Log.

Data for the specific growth rate for the different treatment groups and sampling times are presented in the tables above.

Condition factor (K) which is used in assessing the overall fish health, robustness and an indication of sexual maturity is the ratio of weight to length of fish (Williams, 2000). It can be calculated using the formula:

$$K = \frac{Weight(g)}{Length\ (cm)^3} \times 100$$

For fish sampled at 75 days, the condition factor ranged from 0.93 in the acid control and Radium treatment to 1.27 in the control, Figure 3.16 reflects the mean of the physical indicators measured within this exposure time. Figure 3.17 can be referred to, to assess the effects of Radium on the physical indicators of fathead minnows after 115 days of exposure in this study, with individual condition factors sampled at 115 days ranging from 0.57 in the control to 1.56 in the Radium treatment.

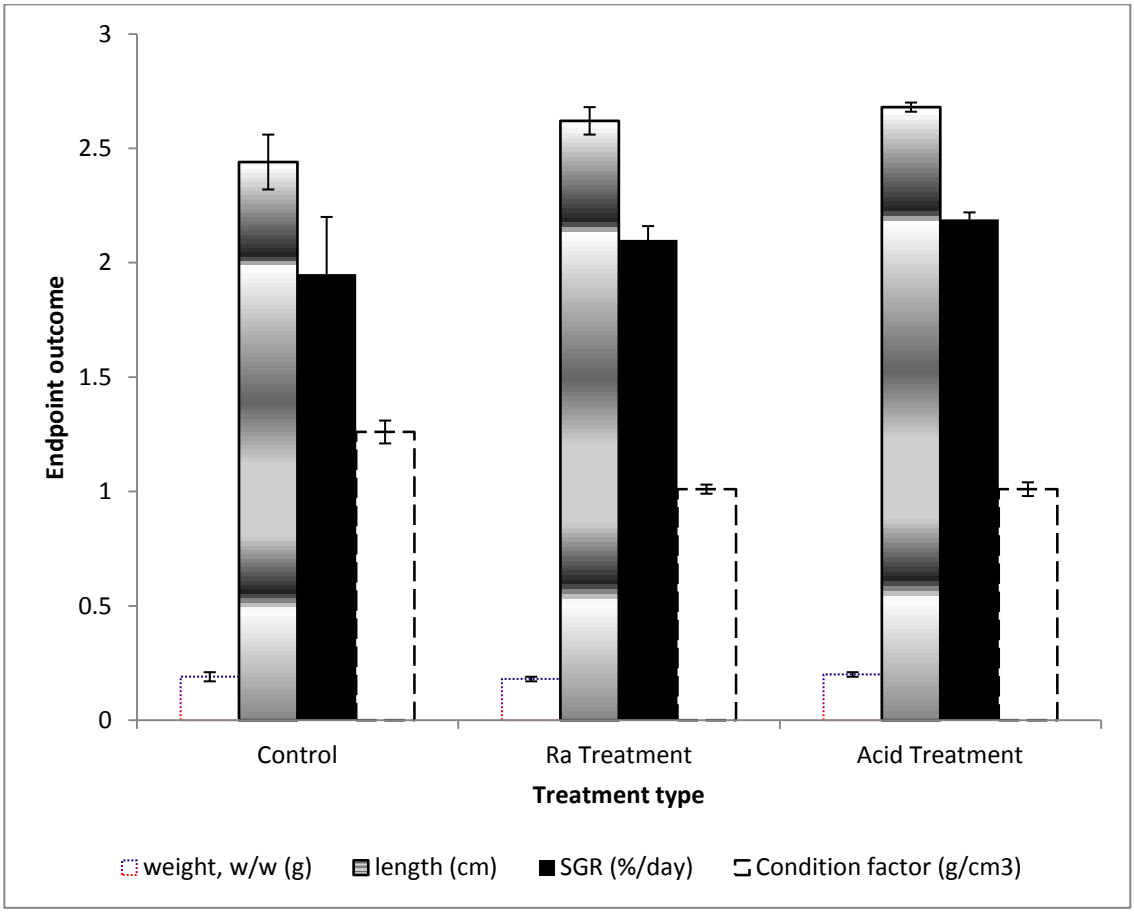

Figure 3.16 . Effects of treatment on various endpoints for first fish sampling. For each group n=5 and error (SEM).

Results for the physical indicators on the total fish during the entire experiment have been identified in Figure 3.18. This gives a visual comparison of the various endpoints determined in the three treatment groups at both sampling times.

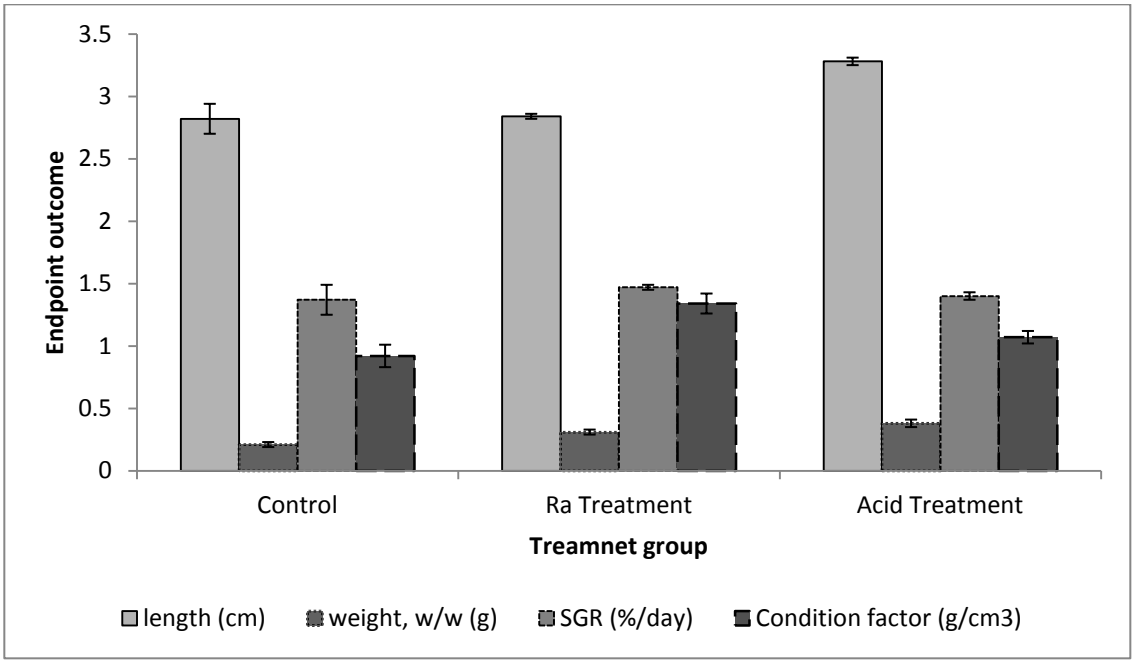

Figure 3.17. Effects of treatment on various endpoints for second fish sampling. For each group n=5 and error (SEM).

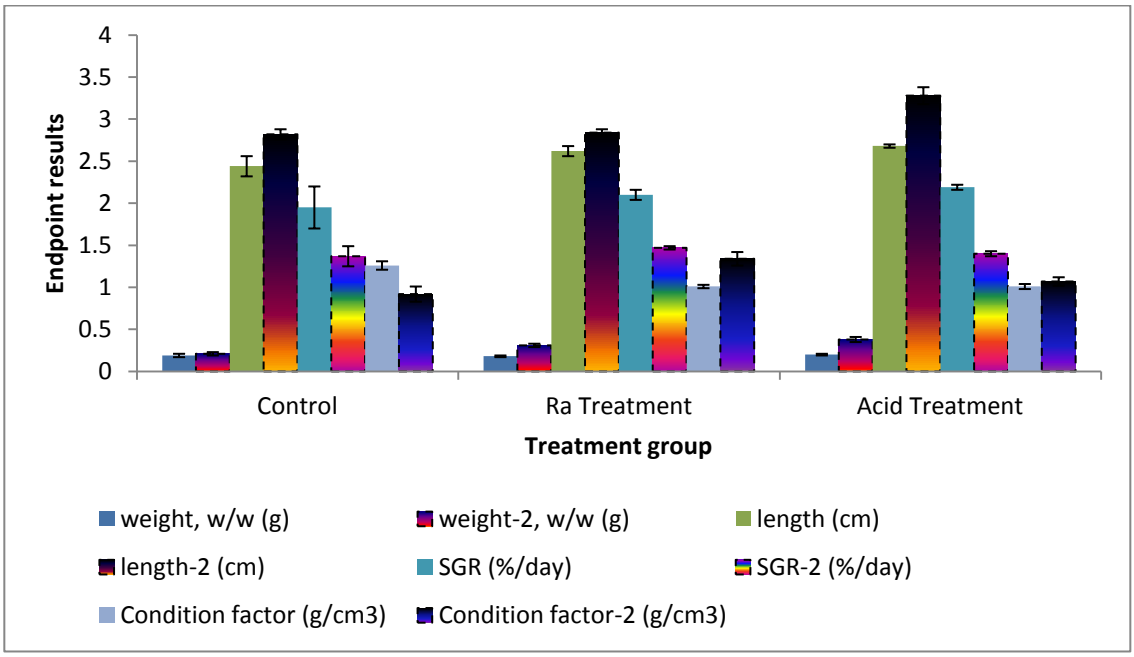

Figure 3.18 Comparison of the Effects of treatment on various endpoints for first and second fish sampling. For each group n=10 and error (SEM). (Legend with -2 represents data for second sampling).

4 Discussion

4.1 Fathead Minnow Detection and Spectral Analysis

This study has focused on determining a simple and rapid method of analysing Radium-226 in fish samples by liquid scintillation counting and observing some critical effects caused by the bioaccumulation of Radium-226 in fathead minnows. The detection limit attained from using the Beckman LS β spectrometer was (7.6 mBq g^{-1}). This limit is relatively high compared to other detection limits reported for ^{226}Ra analysis (Radon ingrowth method) which range from 0.004 Bq mL^{-1} for biological samples to 0.02 Bq L^{-1} for environmental samples. Detectable limits for ^{226}Ra in soil samples have been reported as 2.75 mBq g^{-1} (Sato et al., 2000). However much lower detection limits have also been observed for ^{226}Ra using pulse shape analysis methods; Sato et al reports limits of 0.75 $mBqg^{-1}$ and 0.25 BqL^{-1} (V. Gomez Escobar, 1996). Due to our high detection limit, the activity of a relatively large number of control and acid treatment groups of fish could not be determined. Values for these were thus set as zero, resulting in the conclusion that no Radium-226 was present in these fish.

Ground water and soil samples evaluated for ^{226}Ra using the total alpha peaks (^{226}Ra, ^{222}Rn,^{218}Po and ^{214}Po) resulted in a counting efficiency of 375% in the alpha spectrum region as reported by (Yong-Jae Ki, Chang-Kyu Kim, 2001). Evaluations from (J Aupiais, 2005; Jean Aupiais & Dacheux, 2000) show that ^{226}Ra as every other alpha emitting radionuclide emit several additional rays due to internal conversion with the alpha intensity of the main peak and additional rays being 97.74% and 2.26% respectively. Therefore indicating that the counting efficiency of the alpha particles emitted will not amount to the expected 100% if all of these emissions are not counted. Also (Laina Salonen, 1993) reports lower efficiency of 84% for ^{214}Po, which may be due

to ionisation (photon) quench and the elimination of some ^{214}Po counts. Thus the efficiency of 447% acquired in this experiment is within acceptable range and not just due to Radon loss. As Radium-226 and its daughters attain radioactive equilibrium after 21 days (with little loss of Radon gas) as shown in the results, one would be attempted to assume that once equilibrium is achieved the total disintegrations per second can easily be divided by the number of radionuclides counted in the process to obtain the Radium-226 activity. Our results have shown that by doing so the Radium activity is gravely underestimated by more than 150 percent of the actual activity. Rather the percentage of Radium-226 still present in the sample at the time of counting can be derived from the Bateman equation, which can be further used in calculating the true activity present. Since our initial experiments resulted in variation, due to the loss of Radon and its progeny; standards were counted again at equilibrium for accuracy. (Villa, Moreno, & Manjon, 2004) used both methods in the determination of Radium-226 activities in environmental sediments.

The data obtained from counting the samples was transported through an RS 232 port to a computer which enabled analysis of the data and the derivation of sample spectra. One of the setbacks in Liquid Scintillation Counting is the poor energy resolution, which arises due to the high energy and densely ionising particles emitted. This effect resulted in the poorly resolved alpha peaks of Radium and its progeny which have energies in close range except for the ^{214}Po peak of energy about 7.6 MeV seen in most of the derived spectra.

4.2 Bioaccumulation of ^{226}Ra

The percentage of Radium retention in relation to the total amount of Radium food fed to the fish after 75 days of exposure is 2.27%. From the Radium retention percentage, one can deduce

that once the radionuclide is ingested by the fish, its body undergoes a form of radionuclide decontamination. There are two mechanisms of radionuclide removal from an organism or organ; these are by normal radionuclide decay and biological elimination. If the biological half-life of Radium-226 is known, the effective half-life (which is the time it takes for a radionuclide deposited in a living organism to reduce to half its original value) of Radium-226 in the fish can be evaluated. Johnson et al (1987) estimates the biological half-life of Radium ($^{226}Ra/^{228}Ra$) in fresh water mussel as approximately 9 years as cited by (Bollhöfer, Brazier, Humphrey, Bruce, & Esparon, 2011), however there is no known biological half-life of Radium-226 in fish. Therefore it is necessary to investigate the biological half-life of Radium-226 ingested by the fish. An evaluation of Radium-226 in fish from some lakes in Ontario, Canada showed significant variation in Radium levels among different fish species with higher levels of Radium occurring in bone (6.5-75.6 mBq g^{-1} dry weight) than muscle tissue (1.4-6.4 mBq g^{-1} dry weight) and bottom feeding fish (38-76 mBq g^{-1} dry weight); Radium levels in bone tissue from control lakes was less than 20 mBq g^{-1} dry weight (F. V. Clulow et al., 1998). Though this experiment did not study different species of fish, from Table 3.7 it can be observed that Radium-226 uptake varies by individual fish in the population (group). Also fish in the control and acid control population have Radium activities lower than the detectable limit (ND).

The results for the second sampled fish evaluated by LSC can be found in Figure 3.15, Table 3.8 and Table 3.9 below. The average activity concentration retained in the fish was approximately 2243.58 mBq g^{-1}. A one-way analysis of variance (ANOVA) at 95% Confidence level was used to evaluate the difference in activity concentration for the two sampling times. ANOVA indicated no significant difference between the samplings ($F_{1,2}$ =0.13; p >0.05). As the control and acid

control groups had activity concentration levels below the detection limit, no statistical analysis was performed for these. The individual fish activity concentration is given in Table 3.9, here four of the fish have an activity range of 60-200 mBq and concentration of 170 -700 mBq g^{-1} which is about half the activity observed in the initial evaluated fish. This could be as a result of an adaptive mechanism whereby most of the Radium ingested is quickly removed from the containing organ. The individual fish which weighed the most(0.381 g) and longest (2.9 cm) in the radium treatment group had the lowest activity concentration (177.21 mBq/g), while the second heaviest individual (0.329 g) with the same length (2.9 cm) as that just described rather had the highest activity concentration (9451.37 mBq/g). There was no correlation found between the condition factor that is physical growth and Radium accumulation for the fish at both sampling times. The correlation coefficient, r, values obtained were (-0.39 and -0.006) for the 75 and 115 sampling time respectively. Individual comparison of physical and activity factors only goes to prove that Radium-226 uptake and retention could be related to physico chemical composition of the individual fish. A third sampling would have been appropriate to visualize the feeding behaviour and pattern of Radium-226 in fish exposed over time.

At the time of the second sampling period the activity concentration of Radium-226 in the fish fed with a Radium diet was 0.75% of the total amount of Radium in the diet fed (255.8 Bq in 2.6 g) to the fish. The accumulation of Radium from the fish food can be assumed to be directly proportional to the concentration of Radium in the food ingested. It is however necessary to compare Radium and Calcium concentrations in the fed diet to accurately determine Radium accumulation in these fathead minnows. A study of bioaccumulation of mussels in Australia (Bollhöfer et al., 2011) indicates that there is a strong correlation between the activity

concentration ratio of Radium-226 and Calcium evaluated both in mussel flesh and water. It is known that Radium and Calcium have similar biogeochemical properties, as such Radium can be substituted as Calcium in bone of living organisms (fish) (F. V. Clulow et al., 1998; Cowart & Burnett, 1994; Porntepkasemsan & Nevissi, 1990; S. Swanson, 1982; S. M. Swanson, 1983), and will both be taken up from the food. Even though Calcium uptake was not evaluated in this experiment, it is possible that the body burden of Radium in the fathead head minnow will depend on the Radium to Calcium ratio in the water and food and the uptake rate of Calcium (Bollhöfer et al., 2011; Hesslein & Slavicek, 1984).

Our values of concentration factors obtained are in the same range as concentration ratios calculated for gut to bone of lake trout in two lakes (0.08 and 0.1) (F. V. Clulow et al., 1998). Other studies have however, observed benthic fish to have high ^{226}Ra concentration factors for water to bone varying from 81 to 548 (F. V. Clulow et al., 1998) and fathead minnow had a bioaccumulation factor of 743 in (Hesslein & Slavicek, 1984). Concentration factor for Radium in Benthic fish is also stated as 80 in the ERICA database (Hosseinia, Thørringa, Browna, Saxénb, & Ilus, 2008). Though some studies have documented high levels of Radium for bottom feeding fish, from the perspective of this study it can be deduced that fathead minnows have a low bioaccumulation of Radium-226.

4.3 Dose rate of ^{226}Ra to Fathead Minnows

A large number of data on the dose, dose rate and effects of acute radiation delivered at high dose rate on fish exists. However, only a few of such experiments have been focused on exposure to chronic low dose radiation (Real, Sundell-Bergman, Knowles, Woodhead, & Zinger, 2004). The values achieved in this study, 0.1 to 30 µGy h^{-1} for individual fish and the mean dose

rate of 5.26 µGy h^{-1} are somewhat higher than the dose rate observed for ^{226}Ra in fresh water benthic fish (0.11 µGy h^{-1}) (Hosseinia et al., 2008). Although there have been a couple of effects associated with such low dose rates, the threshold for statistically significant effects is approximately 10^2 µGy h^{-1} (Real et al., 2004) and 1 mGy day^{-1} (41.6 µGy h^{-1}) for the early effects of morbidity to occur (Kryshev & Sazykina, 2011). Our results correspond with that of other data since no mortality was recorded during the experimental period and no significant morbidity effects were observed.

4.4 Effects of Physical growth parameters on Fathead Minnow

There is no known effect or values reported for condition factor of ^{226}Ra chronic exposure to fish, however a research conducted on the effects of nutrient enrichment for fathead minnows resulted in mean condition factors of 1.24 to 1.27 for juvenile and male and female in their treatment group and 1.14 to 1.21 for reference groups (Grant & Tonn, 2002). The mean condition factors obtained at the end of the experiment were 1.09 ± 0.07 for control, 1.18 ± 0.07 in Radium treatment and 1.04 ± 0.03 in the acid group. Differences between the condition factor in our first and second samplings were significant (ANOVA: $F_{c1,c2}$ =10.72; p=0.01). This was followed by a student's t-Test: Two Sample (Unequal Variances) which failed to indicate any significant difference between the treatment groups in the first sampling, control and acid control groups in the second sampling, however there was a significant difference between the control and Radium treatment from the second sampling. Our results for the two control groups relate well with condition factors of fathead minnows which are fairly nourished. From the condition factor for the Radium group compared to the other groups, it can be said that Radium seems to act as a beneficial element to the fathead minnows rather than a detrimental one.

Other physical growth indicators which I considered in this experiment were the weight and length of the fish. The weight of fathead minnows in the Radium treatment for both samplings showed a high significant variation (ANOVA: $F_{Raw1,Raw2} = 26.83$; p= 0.00084). The treatments did not seem to have an effect on the weight of the fish in the first 75 days of the experiment, however after this period fish weights in the acid control group doubled and that of the Radium treatment almost doubled compared to the control. One would expect that the weights of fish in the Radium treatment would decrease, since Radium is known to cause detrimental effects however this is not the case. Rather the mass of fish in the Radium group was higher than the other treatment groups. Fathead minnow fork length (that is the distance between the tip of the snout to the tip of the median caudal fin) was measured for each fish at both sampling times. Results for the fish length and other growth indicators in comparison with the treatment groups can be found in Figure 3.16, Figure 3.17 and Figure 3.18. Analysis for variation in length among the treatment groups was evaluated using student's t-Test; two sample for unequal variances. There was no correlation found between length of fish for control treatment and Radium treatment at 75 days and 115 days after treatment (P> 0.05), neither was there any significant variation between fish length in the control and acid treatment at 75 days post treatment.

An Analysis of Variances (ANOVA: F = 0.83; p = 0.451) failed to indicate any significant difference in the specific growth rate between the treatment groups and sampling times. Nevertheless at 115 days post treatment, a variation in fish length was observed among control and acid treatment groups (p=0.004). Though no mortality of the fathead minnows was recorded in this experiment and the treatments did not adversely affect the growth rate of the fish, their health condition in entirety was significantly affected by the various treatments. This could in turn

affect the sexual maturity and reproduction of individual fish and the population as a whole, which is evident in Grant's research, where the size and growth of fathead minnows influence egg production and survival of juvenile fish and the relation of fecundity to the body size of female fish. Since the whole body of the sacrificed fish had to be analysed for radionuclide concentration, other important end points such as fertility, fecundity and DNA damage which affect the population could not be investigated.

5 Conclusion and future work

In this thesis an experimental approach was taken to investigate the effects of radionuclide contamination in fresh water aquatic biota. The radionuclide under investigation, Radium-226 was chosen due to its relatively long half-life and among the radiotoxic radionuclides it's the most important due to its biogeochemical similarities as Calcium, which is an essential element in the development of living organisms. On the other hand benthic fresh water fish was studied, since they have been known to bioaccumulate Radium-226 because they come into direct contact with sediments (which have high Radium-226 levels). Fathead minnow, a benthic fish which can be easily found in Canadian lakes were fed with a Radium-226 fish diet over time. The activity concentration of Radium-226 accumulated in the fish during the experimental time was measured by liquid scintillation counting and converted to absorbed dose rate which is more applicable in assessing the biological effects caused by the radiation emitted from the radionuclide.

A simple and fast method has been developed to determine the activity of Radium-226 in fathead minnow by liquid scintillation counting. The Radium activity can be evaluated within 24 hours after transforming the solid sample into an aqueous one by counting the aqueous sample using the whole counting window of the liquid scintillation counter. By using this method the Radium-226 activity would be 99% of the total count rate which results in a 114% counting efficiency. To determine the accuracy of this method, the samples were left sealed for about 30 days to enable the Radium progeny attain radiological equilibrium with the parent radionuclide Radium-226. At this equilibrium state the samples were counted with a counting efficiency of approximately 447%. By using the Bateman equation to calculate the activity and counting

efficiency, a much higher counting efficiency of about 597% is obtainable at equilibrium. However from this experiment it was observed that such a counting efficiency is not obtainable by our LSC system used due to factors such as Radon loss which results in disequilibrium of Radon-222 and its daughters. Also lower counting efficiency of 84% for ^{214}Po has been reported (Laina Salonen, 1993). In spite of this the activities obtained at both times were in good agreement, however only the activity calculated at equilibrium was reported in this thesis for accuracy.

Much lower limits could have been observed in this work if the pulse shape analysis method had been used, since our detector has the capability of discriminating alpha and beta pulses. However due to insufficient funds to purchase a pure alpha and beta source, which are extremely expensive, to calibrate the detector this method was not adapted.

The fathead minnow activity concentration of Radium-226 bioaccumulated over the 115 days of the experiment was determined as 1911.42 ± 868.64 mBq g^{-1} fish wet weight. Concentration factors (CF) were calculated for individual fish studied in this experiment to evaluate the accumulation of Radium-226 in the fish food to the fish. The mean CF was found to be 0.35 ± 0.19, this is slightly higher compared to values (0.08 to 0.1) obtained from gut to fish bone; however CF from water to bone has been reported as (81 to 548) for benthic fish (F. V. Clulow et al., 1998). In contrast to our results a bioaccumulation of Radium -226 for fathead minnows has been reported in (Hesslein & Slavicek, 1984) as 743. Therefore on the basis of this experiment, it can be said that fathead minnows used in this study did not bioaccumulate Radium-226 over a chronic exposure.

An important but simple way of indicating physical growth in fish, condition factor, was determined for fathead minnows in this experiment. The condition factor tells whether the fish are healthy or not and serves as an indication for sexual maturity. The condition factor for fish sampled after 75 days from the start of the experiment showed no significant difference, however the fish sacrificed at 115 days after the first feeding varied greatly with the Radium treatment fish having the highest condition factor of 1.56 compared with the control fish which was 1.0. The mean condition factor of the fish in this experiment was calculated as 1.08 ± 0.07, 1.18 ± 0.07, and 1.04 ± 0.03, for fish in the control, Radium treatment and acid control groups respectively. It is somewhat difficult to make a comparison with other published condition factors of fish since most of these are for salmonids which have a different morphological structure compared to fathead minnows. In spite of this our results are much lower than condition factors obtained for fathead minnows fed with a high nutrient diet. Also limited data has been established on the condition factor of fathead minnows from chronic exposure to radionuclides, thus the condition factors presented in this thesis serves as a first source of reference for the evaluation of the general condition of fish exposed to Radium. Since no mortality was recorded during the experiment it can be said that the fish were generally in good health.

Another physical growth indicator which was studied was the rate of growth, specific growth rate (SGR), of the fat head minnow under experimentation. Growth rate percentile for fathead minnows in all the three treatment groups ranged from 1.3 to 2.0 percent per day. The different treatment did not have a great impact on the growth of the fish.

To determine the amount of radiation energy deposited per unit over the course of this experiment, the dose rate to the fish was established. This was calculated as 5.26 μGy h^{-1}, which falls within the dose rate group where no severe radiation effects have been evident. Though no severe effects such as mortality and morbidity were observed in the fish during this study which could pose an impact on their population; other effects such as fertility, fecundity, second and third generational hereditary effects and on the microscopic level DNA damage (which can lead to increased risk of cancer) should be studied.

The effects on fertility and fecundity were to be studied in this experiment; however a lot of difficulties were encounted in getting an appropriate lab for processing the fish and food samples for dosimetry. In processing the food samples for Liquid Scintillation Counting, it was realised that silica was present in the residue. This made the radiochemistry process extremely difficult since the silica could not dissolve and formed precipitates which affected the scintillation process by causing colouration of the cocktail. Hydrogen Fluoride was used to dissolve the sample; however this did not produce any good results either. The food processing took up most of the time not to talk of finding suitable means in obtaining lower background counts since lower count rates were expected for the fish samples. All of these did not leave much time to focus on the above mentioned relevant end points.

Notwithstanding all of the above experimental constraints, this study has been able to develop a fast and simple method to determine the Radium activity concentration exposed to fathead minnows, establish condition factors which can be used in the evaluation of the health of fish, and show that at dose rates below 0.1 Gy per hour effects such as mortality and morbidity are

not likely to occur. Also on the basis of this experiment, one can argue that fathead minnow is a fish which does not bioaccumulate Radium at low levels.

6 Appendix

6.1 PROTOCOL APPENDIX A: LAB PROTOCOL

PREPARATION OF DIGESTION OF FISH SAMPLE (WHOLE BODY) AND FISH FOOD FOR LIQUID SCINTILLATION COUNTING

A. Place a clean and labelled crucible in a muffle furnace at 700 °C for 30 minutes. Turn off the furnace and transfer crucible (partially cooled) with metal tongs into a desiccator and cool to room temperature.

B. Weigh crucible quickly (to prevent possible absorption of moisture).

C. Weigh whole fish and 1-2 grams of fish food into the pre-weighed crucible.

D. Transfer crucible into the furnace set at 700°C for 12-18 hours. Burn off the organic material until the sample is rid of carbon and appears as light grey or white ash.

E. Turn off oven and allow for cooling. Do not open oven while hot, as ash could be blown out of the crucible. Open cooled oven and cover crucible with lid or a watch glass.

F. Transfer partially cooled crucible with metal tongs into a desiccator. Keep cooling to room temperature, and then when cooled, weigh crucible quickly (to prevent moisture absorption).

G. Weigh ash sample (e.g. food, whole body) in a tarred 100ml beaker. Add 3ml of freshly made aqua regia.

H. Place beaker with watch glass on a hot plate, allow mixture to simmer and evaporate acid mixture to dryness until a white-grey precipitate is formed.

I. Remove beaker from hot plate. Rinse watch glass with 3ml of 1M HCL into beaker. Heat gently to evaporate acid mixture to 2ml.

J. Add 18ml Ultima Gold scintillant and 1.78ml diluted sample to a scintillation vial. Seal vial with Teflon tape, cap vial and shake well to mix. Allow to cool in the dark for 1 hour and count.

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