AN ABSTRACT OF THE THESIS OF

Christopher Glenn Shaw for the degree of Master of Science in Radiation Health Physics presented on February 12, 2009.

Title: Exposure of Ionic Hyper-Regulated Artemia to Chlorine – 36 in a Marine System

Abstract approved:

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Kathryn A. Higley

Chlorine – 36 (^{36}Cl) is produced naturally, through neutron activation of stable chlorine during nuclear weapons detonation, or from neutron capture on residual chlorine in graphite piles. It has a half-life of 301,000 years and decays by the means of beta decay with an average energy of 236.33 keV. Due to ^{36}Cl's long half-life and mobility in the environment, it is of potential concern in the long-term management of certain nuclear waste facilities or when decommissioning plutonium-production reactors.

The existing body of research data on ^{36}Cl is limited and often contradictory—showing extremely high uptake factors into biologic media. This study is part of a larger effort to understand both the environmental and biological mobility of ^{36}Cl, in this case using the common Brine shrimp (*Artemia Salina*). These species are of interest because of their use of osmoregulation, which allows them to hyperregulate and survive in an environment with variable salinity.

Brine shrimp were exposed in two simulated marine ecosystems: Tank #1 at a low water concentration ^{36}Cl of 1.64 +/- 1.28 Bq/ml and tank #2 at a ten times higher concentration of 16.40 +/- 4.04 Bq/ml. Uptake was evaluated for mature Artemia over a period of 20 days. The uptake in both the low and high ^{36}Cl concentration tanks followed similar cycles of uptake and efflux of ^{36}Cl. The Brine shrimp exposed to the low activity concentration of ^{36}Cl had an uptake rate of 35.77 +/- 6.77 (Bq/g)/day with an efflux rate of 35.79 +/- 7.26 (Bq/g)/day. The Brine shrimp exposed to ten times the concentration of ^{36}Cl had an uptake rate of 70.02 +/- 11.81 (Bq/g)/day and an efflux rate of 62.98 +/- 11.81 (Bq/g)/day.

Two conceptual models were developed using the isee software STELLA. The first model followed the hypothesis of a buildup and plateau. The low ^{36}Cl concentration system conceptual model predicted a maximum concentration of

7.14×10^{1} Bq/g. For the high ^{36}Cl concentration system, a maximum concentration of 1.115×10^{2} Bq/g was predicted.

A second conceptual model was developed taking into account a cycling effect that closely mimicked the research data for both the high-concentration and low-concentration systems. The low ^{36}Cl concentration conceptual model predicted a maximum concentration of 7.33×10^{1} Bq/g and minimum concentration of 1.58×10^{1} Bq/g. The high ^{36}Cl concentration conceptual model yielded a maximum concentration of 1.22×10^{2} Bq/g and minimum concentration of 1.79 Bq/g.

Exposure of Ionic Hyper-regulated Artemia to Chlorine – 36 in a Marine System

by

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APPROVED:

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Dean of the Graduate School

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

Christopher Glenn Shaw, Author

ACKNOWLEDGEMENTS

I would like to thank all of my family, my friends, and the faculty of Oregon State University who have put up with me and helped me to get this far. Trust me, it was no small task.

I would also like to make a point to thank my mother individually for all of her help and support throughout my education—through the good and the bad: Thank you.

TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

LIST OF FIGURES

LIST OF FIGURES (Continued)

LIST OF FIGURES (Continued)

Figures Page

LIST OF FIGURES (Continued)

LIST OF FIGURES (Continued)

Figures Page

LIST OF TABLES

LIST OF EQUATIONS

LIST OF APPENDIX A FIGURES

LIST OF APPENDIX B

DEDICATION

This work is in memory of my father, Calvin Glenn Shaw (Aug/27/1951 – Nov/5/2002) and to all of those in and outside of my life who have been touched by cancer, whose names unfortunately infinitely outnumber the number of words printed in this paper. No one ever truly dies as long as they are remembered, continue fighting and never give up!

I love you, Pop.

Exposure of Ionic Hyper-regulated Artemia to Chlorine – 36 in a Marine System

Chapter 1

INTRODUCTION

Chlorine–36 (^{36}Cl) is a long-lived and highly mobile element whose behavior in the biosphere has recently become of interest. ^{36}Cl is produced naturally though the spallation of Argon–36 in the atmosphere with cosmic ray protons, or it can be produced naturally through neutron activation of ^{35}Cl found in rocks and soil. Vast amounts of ^{36}Cl were also created during the nuclear weapons testing from 1952-1958 (Davis et al. 1998). Another source of ^{36}Cl is neutron capture on residual chlorine in graphite piles of operating nuclear power reactors (BIOPROTA 2006).

Chlorine-36 is a radioactive isotope of chlorine with a half-life of 301,000 years and decays by Beta minus (β^-) emission with an average energy of 236.33 keV and a maximum energy of 709 keV (Nuclides and Isotopes 2002).

Due to the long half-life of ^{36}Cl and its environmental mobility, it is of potential concern in the long-term management of certain nuclear waste facilities or when decommissioning plutonium-production reactors (BIOPROTA 2006).

The existing body of research on ^{36}Cl is limited and often contradictory, showing extremely high uptake factors into biologic media. This is likely due to the fact that chlorine is a biological essential element (Selinus et al. 2005). These factors, combined with a general lack of experimental data on ^{36}Cl, motivate new research on all aspects and behaviors of ^{36}Cl in the biosphere. This study is part of a larger effort to understand both the environmental and biological mobility of ^{36}Cl; in this case measuring uptake by Artemia.

Artemia are of interest because of their use of osmoregulation, which allows them to hyper-regulate key ions and survive in environments with moderate to high salinity. Hyper-regulation is the process by which ions are actively pumped in and out of a cell to maintain a desired concentration of biologically key ions. Key ions are any ion that is necessary to maintain life functions. One such ion is chlorine, which is maintained at a low concentration in contrast to the concentration in the environment. One of the reasons that Artemia were chosen for this study is for their ability to hyper-regulate ionic levels of biologically important ions such as chlorine in environments where the levels of such ions are in much greater concentrations than is possible to maintain life (Croghan 1958 B).

As stated previously, ^{36}Cl is highly environmentally mobile and is key in the creation of the potential difference in the nervous system, which allows the

nervous system to function as well as maintain osmotic pH balance in the cell (Selinus et al. 2005).

^{36}Cl is especially mobile in aquatic systems where dispersion can happen rapidly and can be considered ubiquitously dispersed in the aqueous environment after introduction. The rate of dispersion can depend on the rate at which the solution is moving and its turbidity.

Chapter 2

Literature Review & Background

2.1 Literature Review

There have only been a few studies that have looked at the rates of uptake and efflux of ^{36}Cl in Artemia. These will be discussed in the following section, along with several other studies and articles related to the research presented here.

Very few studies have been conducted on the uptake and efflux rates of a beta emitting radionuclide that chemically resemble biologically key ions like calcium, magnesium and potassium in Artemia. There have been only two studies that have looked at either efflux or uptake pathways of ^{36}Cl (Smith 1969 & Croghan 1958 A).

The study that has the closest relationship to the research done in this paper was written by P.G. Smith in 1969 and was entitled, "The Ionic Relations of Artemia Salina (L.)." Smith found that approximately 70% of the chloride efflux is due to exchange diffusion, which is the carrier transport of ions across the cell membrane in manner faster then would occur in diffusion. Most of the remainder is due to active transport of chloride across the cell membrane or epithelium.

Smith also found that the chloride flux across the epithelium was approximately 7,000 pmole cm^{-2} sec^{-1}.

Smith did not look at the rate of uptake of chloride in his research, instead he loaded Artemia with ^{36}Cl by placing them in a vial containing 0.925 MBq/ml overnight, then removed them after the loading period was complete. According to Smith, long-term efflux studies were not possible because the 700 keV Beta emitted by ^{36}Cl was not detectable with the liquid scintillation counter that he also used in his study to count his sodium samples.

Another ionic efflux study conducted using Artemia Salina was done in 1958 by P.C. Croghan (A) and was entitled, "Ionic fluxes in Artemia Salina." In this study, the author felt that the best way to study chloride efflux was by replacing it with labeled Bromide–82 (^{82}Br). The author had shown in a previous study that bromide (Br^-) moved in a manner very similar to that of chloride (Cl^-) and thus could be substituted for chlorine. Croghan, like Smith, did not look at rates of uptake in the environment; he loaded Artemia overnight in a 100 ml vial with ^{24}Na and ^{82}Br in the amount of 30 mg each in the form of $^{24}Na^{82}Br$ and $^{24}NaHCO_8$.

After being removed from the spiked vials, Artemia were transferred to a nonradioactive solution of 500 mMole/L NaCl solution. This concentration closely resembles that of seawater. Croghan found that the rate of efflux for ^{24}Na was slightly higher than that of ^{82}Br. He concluded that it would be unwise to

assign too much significance to this difference because there was a possibility that the rate of Br^- exchange is slightly different than that of Cl^-. As a point of interest the author pointed out that, according to his study, unlike most of the previous work, he found that most of the rapid flux of NaCl into Artemia occurs not through the gut epithelium but across the outer surface of the body via the process of exchange diffusion.

2.2 Artemia

Hyper-regulation is the process of pumping ions in and out of the cell in opposition to that of the concentration of the surrounding environment and can be a mix of both active and passive transport mechanisms (Kirschner 2004). In fresh water systems the majority of organisms exist in ionic equilibrium with their environment and thus lack a mechanism for hyper-regulation. It is only when looking at saltwater organisms that all organisms exhibit at least some ability to hyper-regulate in their environment to maintain life. Artemia were chosen because of their ability to hyper-regulate ionic concentrations in moderate to very high salinity environments without affecting the overall health of the Artemia.

The life cycle of an Artemia starts with either a live birth or from cysts. The cysts are inactive eggs, which are produced certain times of the year and remain

inactive until the introduced into the right environment. Once the egg is hatched, both the live born and the freshly hatched Artemia begin to follow the same life cycle (Figure 2.1). The free-swimming freshly-hatched Artemia or live born Artemia are defined as nauplius and are in the first larval stage. They are still reliant on yolk reserves to survive, as the digestive system is not functional yet. After approximately 8 hours the Artemia molts into the second larval stage where the digestive track is now functional. The Artemia continues to grow through about 15 moltings. It is in the later adult pre-egg producing stage when sexual differentiation occurs between the male and females of the species. After the second larval stage they enter the first stage of being adult Artemia, which is adult non-egg producing, followed by adult egg producing. The entire process from nauplius to adult takes approximately 8 days. Under good conditions an Artemia can live for several months and in turn can reproduce at a rate of up to 300 nauplii or cysts every 4 days (Stappen 1996).

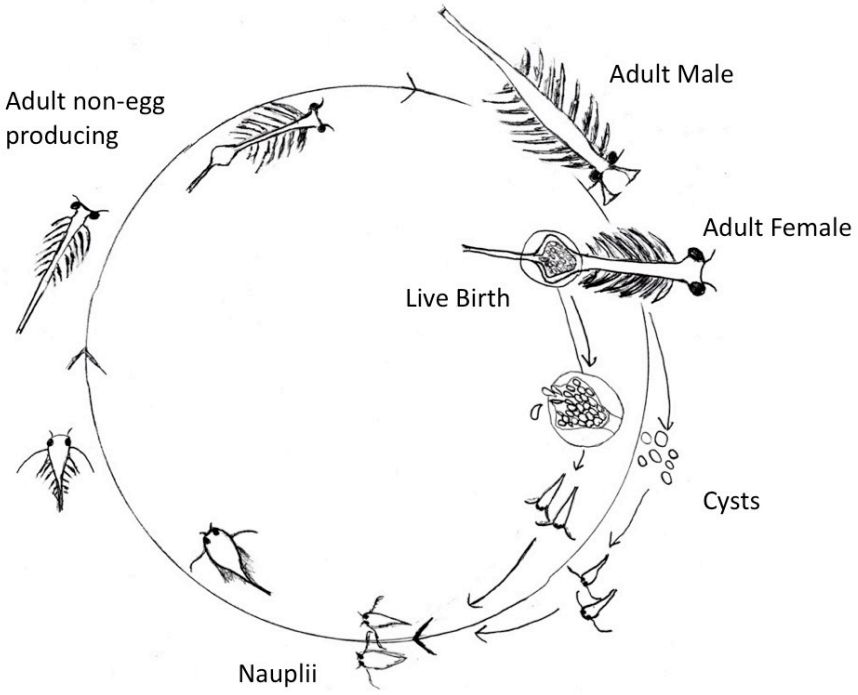

Figure 2.1: Recreated Artemia Life cycle[1].

For this experiment, Artemia were received from the vender in the life cycle stage of adult pre-egg producing. The vendor[2] sells the Artemia as a food for various aquarium systems and at this life-stage they are at the highest nutritional value. It was beneficial to use mature Artemia, as they had finished with their molting phases, they had stopped the majority of their growth, and would presumably have a more constant uptake and feeding rate.

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[1] Recreated from: http://ut.water.usgs.gov/shrimp/images/lifecycle2.jpg
[2] The vendor can be found at: http://livebrineshrimp.com/

The mature Artemia seen in Figure 2.2 have an elongated body with two stalked complex eyes and a linear digestive track with eleven pairs of functional thoracopods or appendages (Stappen 1996). In the wild they can reach a length of up to 10mm, although when in the right environment some have grown as long as 20 mm. Their color can vary from a greenish to a bright red depending on the type of food and oxygen concentrations within their environment. [3]

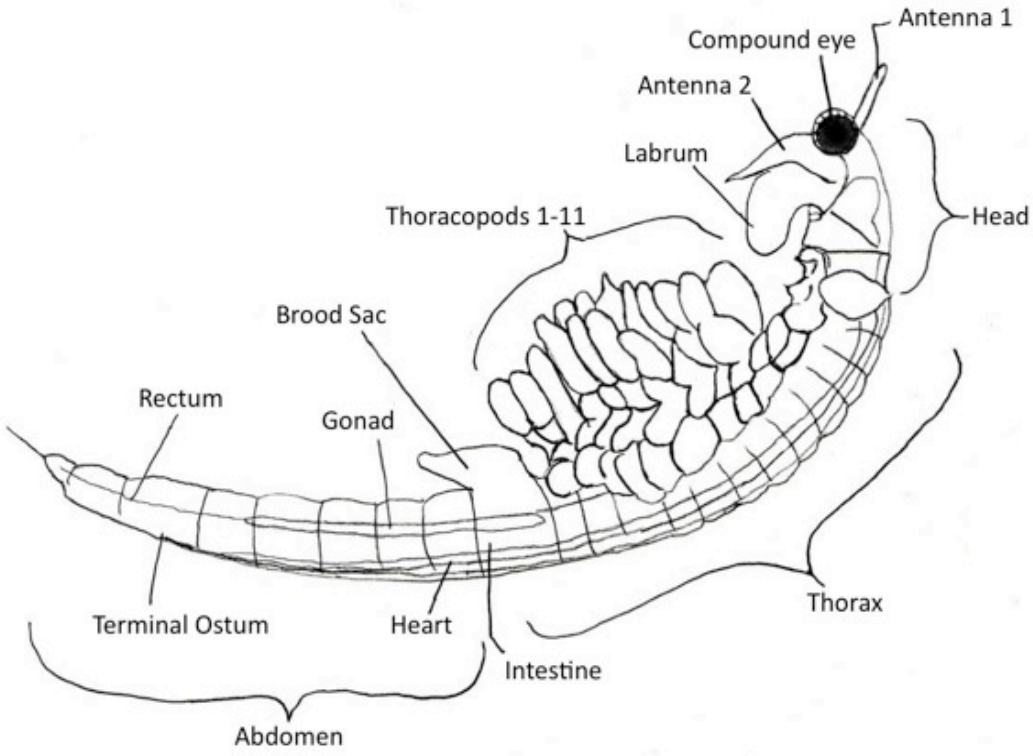


Figure 2.2: Recreated Artemia depiction[4].

[3] http://www.artemiaworld.com/home
[4] Recreated from http://webs.lander.edu/rsfox/invertebrates/artemia.html

Artemia are also non-selective filter feeders, meaning that everything in the their environment passes through their digestive system (Stappen 1996). This allows for maximum potential for uptake as the solution from the surrounding environment passes through the digestive track. There is also evidence that the digestive track can uptake solution from the environment from either end of their digestive track (Croghan 1958 C).

2.3 Liquid Scintillation Counter

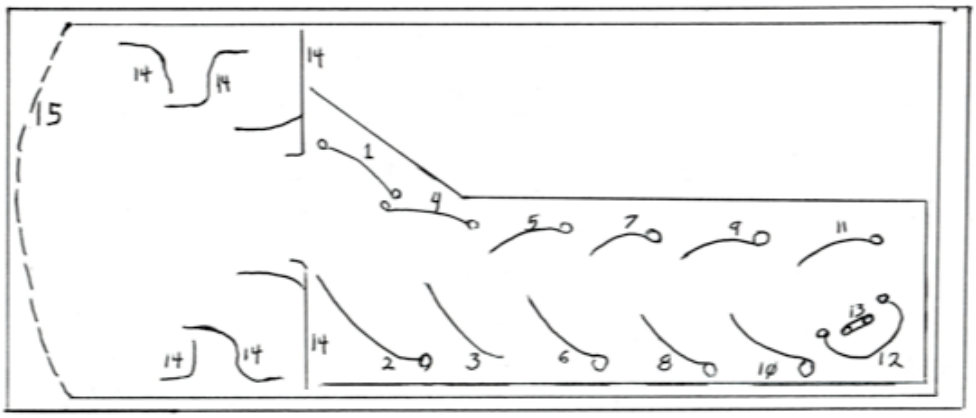
Analysis of ^{36}Cl in Artemia was preformed using a Liquid Scintillation Counter (LSC). The detection efficiency for betas in a LSC is very high, approximately 100%, for beta energies higher then 300 keV. The high efficiency for beta detection is due to the fact that the source is surrounded by a media, which emits light when it interacts with a β^-. The β^- particle emitted by the sample is done so in a 4π geometry so that it must pass through a portion of the scintillator medium the sample is within, depositing the particle's energy along its path. The scintillator media and sample are placed in a highly translucent material, usually glass that sits in between two PM tubes, which are used to detect the emitted light from the scintillator medium (Knoll 2000).

Improvements in detection efficiency, especially with low-energy Betas (less than 300 keV), can be attributed to the improvements in photomultiplier tubes over the years. In 1960, an efficiency of 20% was common for tritium (^{3}H), which has an average beta energy of 18 keV. Today, it is reasonable to expect an efficiency of about 90% for a ^{3}H sample with sufficient shielding and modern PM tubes (Knoll 2000).

The LSC works on the same basic principle as crystalline scintillation detectors like sodium iodide (NaI). Energy is deposited inside a detection body, usually from gamma or beta radiation. The incident radiation causes fluorescence in the scintillation medium. The fluorescence (intensity or number of light photons) emitted by the scintillation medium is proportional to the amount of energy deposited in the detector.

The light photons emitted by the scintillator are directed toward a photo-multiplier tube (PM tube). The basic design of a PM tube can be seen in Figure 2.3. The light that leaves the scintillation medium first passes through a photocathode. The photocathode absorbs the light emitted by the scintillator, and in turn emits low-energy (photo-) electrons. The photocathode is coupled to an amplifying device, which collects the electrons and multiplies the signal. The next part of the PM tube is the electron multiplication region. The multiplication region of the PM tube is necessary because, only a few hundred photoelectrons

are associated with an average pulse and the total charge which they would carry is too small for use as an electrical signal. This is where the electron multiplication region of a PM tube comes into the picture, providing for electron amplification to 10^7 - 10^{10} total electrons, sufficient to serve as the charge signal for the original scintillation event (Knoll 2000).



1-12: Dynodes 14: Focusing electrodes

13: Anode 15: Photocathode

Figure 2.3:PM tube Basic Design.

In the case of a LSC detector, the scintillation medium is a liquid typically called a liquid scintillation cocktail. The LSC system is unlike a solid scintillator, where the radioactive material is placed on or adjacent to the detector. For LSC, counting the radioactive material is immersed or dissolved in the cocktail. This cocktail is designed to emit light (fluoresce) when energy is deposited in its

medium (Knoll 2000). The two PMT tubes that are on either side of the LSC vial then collect the light output. Some ultra-low background LSC detectors have a third PM tube connected to a shield minimizing false positive counts in the detector.

The factors that may interfere with the light production within the scintillator medium are referred to as quench. There are two types of quench. The first is a color quench and the second is a chemical quench. A color quench can be anything, which is introduced into the cocktail that interferes with the optical properties of the solution. In the case of this research the color quench would be the digested Artemia. A chemical quench is when a chemical is introduced to the solution, which inhibits or interferes with the solution's ability to emit or absorb light. In the case of the research presented here the chemical quench would be the digestive acid that was used to break down the Artemia and was the cause of the majority of the quench.

The solubilizing or digestion agent used for this research was Solulene-350® a product of PerkinElmer[5]. This agent was chosen over other solubilization agents like nitric acid because of its properties, particularly low color and chemical quench when mixed with liquid scintillation cocktail. The properties of Soluene®-350 are as follows: it is a strong organic base, formulated with toluene,

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[5] PerkinElmer http://www.perkinelmer.com

that has an excellent capacity for the solubilization of wet tissue, aqueous tissue homogenates, proteins, nucleotides, plant material and other substances into a solution compatible with liquid scintillation cocktails. Solulene-350® also has minimum chemiluminescence produced when used with most common liquid scintillation cocktails such as Hionic-Fluor™ and Ultima Gold™[6].

The LSC cocktail used in this study was HiSafe®, a product of PerkinElmer. The chemical used as the base for HiSafe® cocktail is di-isopropyl naphthalene (DIN). Its characteristics include: high flash point (148°C), virtually odorless and colorless, no permeation through plastic vials, biodegradable in accordance with EU directive 79/831 Annex VII, low photo- and chemiluminescence, and good color and quench resistance[7].

2.4 The Physics of Beta decay

Understanding the properties of beta particles and their decay has taken scientists many years. The process of beta minus decay involves a transition within the nucleus were a neutron is converted into a proton through the ejection of an electron and an antineutrino (Nuclides and Isotopes 2002). The process of

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[6] http://las.perkinelmer.com
[7] http://las.perkinelmer.com

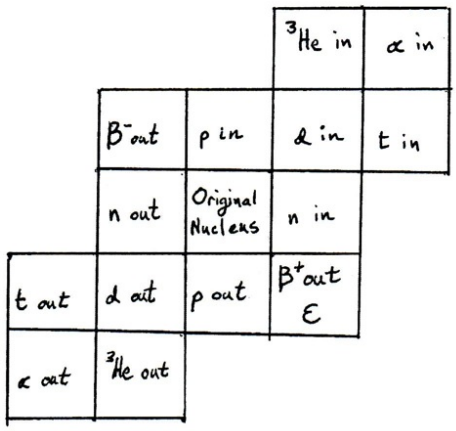
beta plus (β^+) decay involves the transformation of a proton into a neutron and a neutrino. This is commonly called positron decay, where positron decay is a positively charged electron emitted from the nucleus.

Unlike alpha particles that are emitted as mono-energetic, beta particles are emitted in a spectrum of kinetic energies. These range from zero to the maximum energy produced by the radionuclide with an average Beta energy of one-third the maximum beta energy. The spectrum of energies that a beta particle will be emitted at is attributed to sharing the decay energy with an antineutrino, which is simultaneously emitted with the Beta and is responsible for the rest of the energy that the beta does not have.

The basic equation for beta minus decay can be seen in equation 2.1. An illustration of Beta decay, recreated out of the Chart of the Nuclides, can be seen in Figure 2.4.

$$ {}^{A}_{Z}X \rightarrow {}^{A}_{Z+1}Y + \beta^- + \bar{\nu} $$ Equation 2.1: Basic Beta minus decay

Beta decay is favored by neutron-rich radionuclides and positron decay is favored by proton-rich radionuclides.



n = neutron
p = proton
d = deuteron
t = triton

α = alpha
β⁻ = Beta minus (negative electron)
β⁺ = Beta plus (positron)
ε = electron capture

Figure 2.4: Major modes of Decay.

2.5 STELLA® Modeling

For the computer-modeling portion of this research, STELLA® version 9.0.1 from isee systems[8] was used. The software package STELLA® is a useful tool designed to model dynamic systems, which are usually very complex, having many components with involved relationships. STELLA® can be used to model

[8] isee systems makes STELLA® software more can be found at http://www.iseesystems.com

such disparate problems as: competition among species for limited resources, chemical reactions of enzyme kinetics, or as in this case, to model uptake and efflux of a radioactive isotope in a given system.

The best way to describe the STELLA® software is to think about it as a system of buckets and pipes referred to in the software as Stocks (buckets) and Flows (pipes). The buckets are where material is stored or accumulated. In the case of this research: ^{36}Cl was the material, and the "bucket" can represent an environmental compartment (e.g. soil) or body depending on the research. The buckets are connected together by pipes; these pipes allow for the flow of material from the stock. A "converter" controls the rate at which material moves, which along with doing its namesake also stores equations or constants and does not accumulate like a bucket. Converters are connected to pipes and buckets via a "connecter", which appears as an arrow. An example of a basic STELLA® model describing the decay of Cesium – 137 (^{137}Cs) can be seen in Figure 2.4.

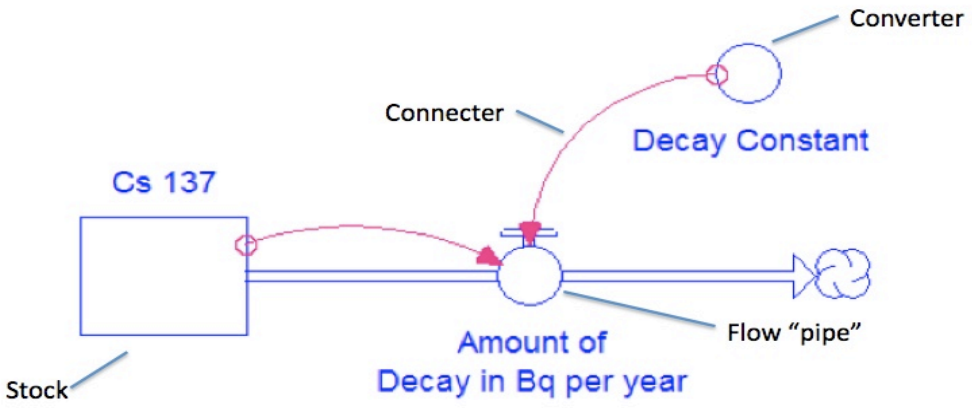


Figure 2.5: Basic STELLA® Model.

In the example of ^{137}Cs, the box represents the source of ^{137}Cs atoms. For this example an initial ^{137}Cs source strength of 10,000 Bq was assigned. The decay constant of ^{137}Cs 0.0230511 $year^{-1}$ was assigned to the converter. This can be solved directly with Equation 2.2, or it can be approximated in a step-wise fashion (Equation 2.3), which is the method employed by STELLA® to solve such problems. In the case of both equations: N is the number atoms, N_0 is the initial number of atoms, λ is the decay constant, and t is the amount of time that has passed.

$$N = N_0 e^{-\lambda t} \qquad \text{Equation 2.2: Decay equation}$$

$^{137}Cs(t) = {}^{137}Cs(t-dt) + (-\text{decay rate})dt$ Equation 2.3: Basic STELLA® routine

Using the previously provided ^{137}Cs source information the model was run to illustrate the loss of activity over 210.5 years or in seven half-lives of ^{137}Cs. After setting the run specs in STELLA® time to years, delta time (DT) to 0.05 (the incremental time steps taken for every iteration), and the total time to run to 220 years, the activity left after 210.5 years is found to be 77.89 Bq. This value was found using STELLA®, the visual representation of the software display of the decay of a 10000 Bq sources over 220 years can be seen in Figure 2.6.

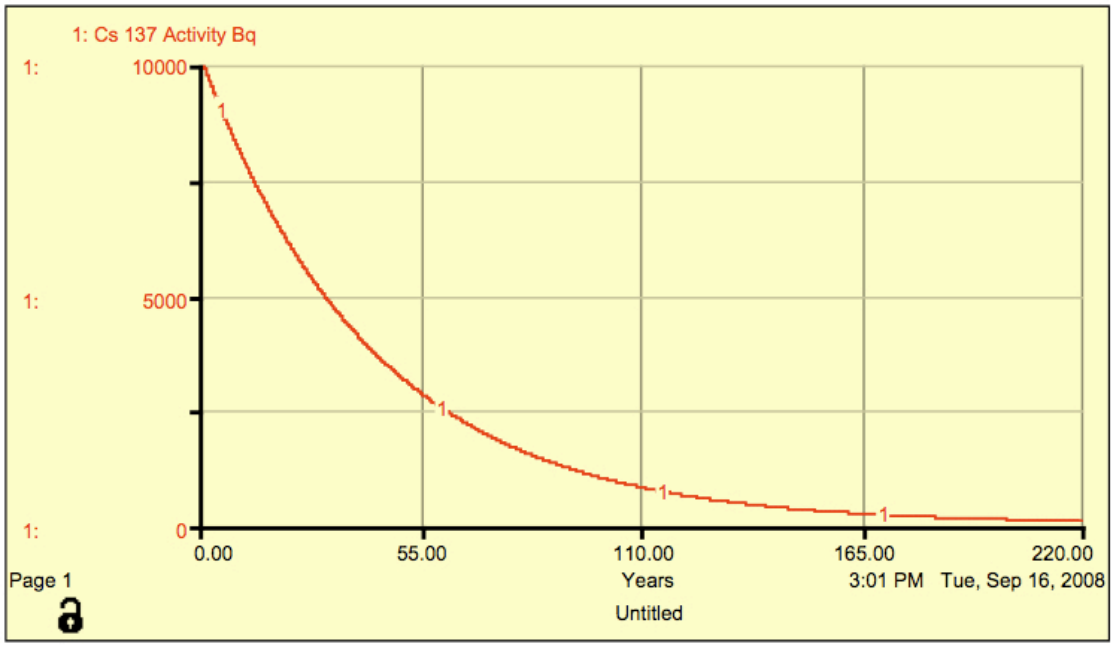


Figure 2.6: STELLA® ^{137}Cs Activity Vs. Time.

Chapter 3

Methods and Materials

3.1 Scientific Design

In this section the scientific design of the research is described in three portions. The overall system setup can be seen in Figure 3.1. The first portion of the design discussed will be the cooling loop, which is a closed loop designed to maintain a constant temperature while precluding contamination of the cooling system. Next is the containment design, which includes both the primary and secondary barriers, which were designed as a safety precaution to contain ^{36}Cl throughout the course of research. Finally, the methodology employed to determine the Artemia ^{36}Cl concentration will be presented.

1. Cooling loop:

Due to the need to maintain a constant temperature of 288 K in two separate tanks, a closed cooling loop was designed. This system had to maintain a constant temperature, but also had to prevent the transfer of radioactive material

outside the system. Cost was also a factor. Consequently, it was decided that it was necessary to design a system from scratch for this project.

Figure 3.1: Research setup.

The following parts where incorporated into the design: a cold well consisting of a refrigeration coil inside a sealed container, piping, fluid reservoir, thermostat and a pump. The completed assembly maintained an average temperature in tank #1 and #2 of 288.8 +/- 0.35 K and 288.6 +/- 0.34 K respectively for the duration of the research (Figure 3.2).

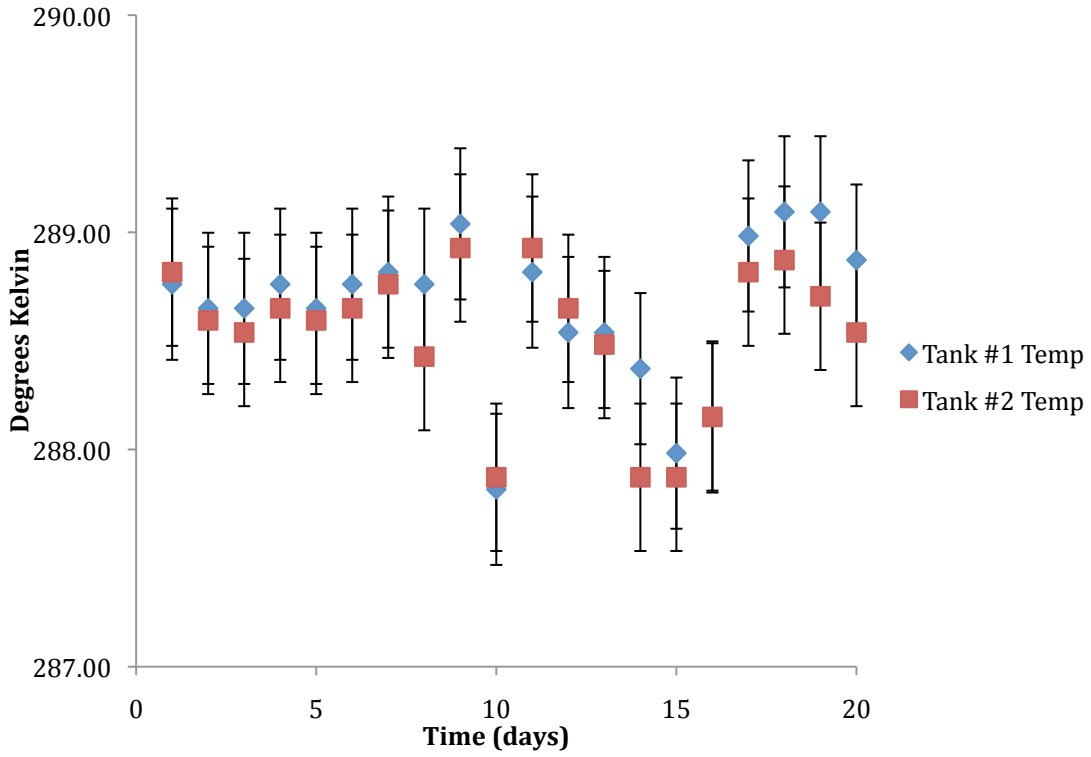


Figure 3.2: Temperature Tanks 1 and 2 versus time.

The cooling loop was pressure tested and leak checked prior to the spiking of the systems with ^{36}Cl. After the systems were spiked, the cooling loop reservoir was sampled daily, checking for contamination of ^{36}Cl. No contamination in the cooling loop was detected at any time. Because the solution in the cooling loop was at a higher pressure than both tanks #1 and #2, the solution levels were checked daily ensuring no cooling solution was entering either tank.

2. Containment:

A secondary barrier was used as a spill guard and backup containment in case of a failure in the primary solution containment. The primary containment system acted as a containment and secondary Beta shield. The primary Beta shield was the solution itself followed by the primary containment.

3. Determining Artemia ^{36}Cl concentrations:

The Artemia were to be removed from a contaminated environment by net. This would allow as much excess tank solution as possible to drip back into the tank. It was, therefore, necessary to dry them in an oven at a temperature of 333 K. A drying time of 60 minutes was established in trials before the beginning of the research. The drying time was established by removing 4 approximately equal samples of Artemia and drying them in identical pre-weighed small weighing dishes then checking their weight every 15 minutes. After 3 hours the data were analyzed. In this analysis, a plateau of sample mass was noticed after 55 minutes. After this, the benefits from further drying were minimal and became counter-effective by cooking them onto the weighing dishes after 70 minutes making it difficult to remove them from the weighing dishes and placed them in the counting vials.

Radiochemical analysis required the Artemia to be digested prior to their addition to the LSC. Ideal digesting time was established for the Artemia in a trial before the beginning of the research. This was done by counting 10 samples, which had approximately the same mass (+/- 0.04 g) of Artemia added to each vial and all had the same volumes of 2 ml of Solulene-350® and HiSafe® liquid scintillation cocktail. The samples were digested over time periods between 1 and 36 hours. It was found that the color quench decreased with time and counting efficiency increased up to 24 hours of digestion. After that there was no noticeable benefit from further digestion.

Digestion was used to minimize beta attenuation in the tissue of the multiple Artemia taken in each sample. In their un-digested state, their tissue would have lowered the efficiency of the LSC by moderating some of the beta particles emitted by ^{36}Cl. Solulene-350® was used due to its ability to digest organic material and its nonreactive nature when mixed with High-Safe LSC cocktail®.

LSC beta counting efficiency can be affected by multiple factors. Due to the increased quench the assumed 100% efficiency for betas over 300 keV was not used for any digested Artemia sample. The new counting efficiency of 63%, was established using a known spike of ^{36}Cl, digested Artemia samples, 2 ml of Solulene-350®, and 5 ml of High-Safe Liquid Scintillation Cocktail®.

3.2 Research Methods

After designing and setting up the cooling loop to maintain the temperature in both tanks at approximately 288 K throughout the study (Figure 3.1) temperature was monitored by two CORALIFE® digital thermometers calibrated to an accuracy of +/- 0.1 K. Both tanks where brought to approximately 288 K to maximize the life span of the Artemia[9].

Artemia were introduced to both tanks in approximately equal quantities of $15x10^3$ Artemia. The number is based on weight-to-population approximation from the vendor. After a week of establishing a stable system (which meant that the Artemia had not died off and that the turbidity of the tanks was clear and constant) all controls for the systems were removed from both tanks including Artemia, tank solution, and cooling loop solution. The purpose was to determine a base line in case of contamination to the cooling loop. One gallon of the uncontaminated tank solution was set aside to mix with the Artemia food for daily feeding. This solution was identical to that in both systems, prior to spiking them with ^{36}Cl.

[9] Temperature was recommended by e-mail from vendor http://livebrineshrimp.com

After the controls where removed from both tanks, ^{36}Cl was added to both systems. In tank #1, $1.24x10^4$ Bq was added to yield 1.64 Bq/ml or 1.62 Bq/g[10]. In tank#2, $1.24x10^5$ Bq was added to yield 16.40 Bq/ml or 16.17 Bq/g. These amounts where chosen to represent a low concentration and high concentration of ^{36}Cl, respectively.

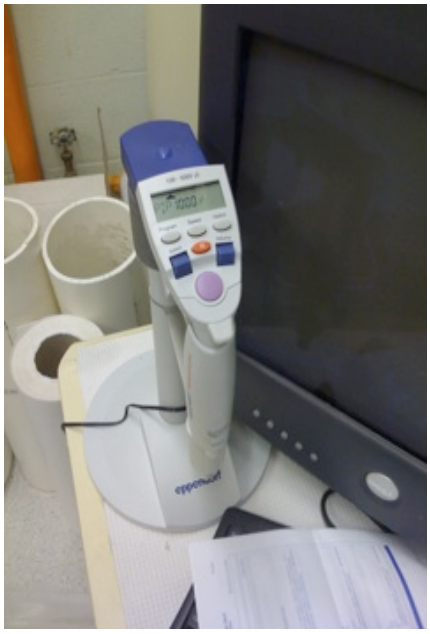


Figure 3.3: Automatic pipette.

[10] So that everything is in the same units for easier statistical comparison a solution density of 1.014 g/ml (density of saltwater) was used.

Daily samples were taken at approximately the same time every day. The sampling protocol required a 1 ml sample of tank solution and cooling loop solution to be taken[11] (Figure 3.3). A sample of Artemia was taken from each tank using a brine shrimp net moving from bottom to the top of the tank. An average dry mass of 0.212 +/- 0.111 g and 0.181 +/- 0.120 g of Artemia was taken daily representing the low and high ^{36}Cl tanks respectively.

After being removed from the tanks Artemia were placed into the drying oven on individual weighing dishes, which were weighed prior to introducing the sample for later weight calculation. They were dried for a period of 60 minutes.

[11] Samples taken using a calibrated 5000 μL capacity Eppendorf® Research Pro automatic pipette

Figure 3.4: BECKMAN LS 6500® LSC.

Once the drying time was complete:

1. The Artemia were removed from the drying oven and placed into 7 ml PerkinElmer® LSC glass counting vials.
2. 2 ml of Solulene-350® was added to each 7 ml vial containing Artemia and allowed to digest overnight.
3. Once digestion was complete, 4 ml of Hi-Safe Liquid Scintillation Cocktail® was added to each 7 ml vial for counting.
4. Each sample was counted for 20 minutes in a BECKMAN LS 6500® (Figure 3.4) along with three background blanks for statistical analysis.

3.3 Radiation Safety

When designing shielding for the purpose of radiation safety with a beta emitter like ^{36}Cl, the most important consideration is the density of the material in which the electron will travel. The higher the density of the material, the greater the attenuation or affect that material will have on the beta particles. When calculating the attenuation of Beta particles as they travel through the primary containment, it is important to understand that the density is more a function of electron density in the material in which the Beta is traveling than the atomic number of the material when considering beta radiation (Cember 1996). The best shield materials are of low electron density or Atomic number, this is due to the principle that as the electron density increases to anything over that of Aluminum, the electron density becomes high enough where the possibility of Bremsstrahlung production becomes important (Cember 1996). The density of Aluminum is 2.7 g/cm^3, where as the density of the primary containment material of commercial Lucite is approximately 1.16 g/cm^3.

3.4 Safety Analysis

A safety analysis of adequate shielding and safety was completed before the research was conducted and was based on a conservative model, assuming a point source next to the surface of the primary containment system equal to the total concentration of ^{36}Cl added to the system. This conservative model was chosen as a worse possible scenario in the course of this research.

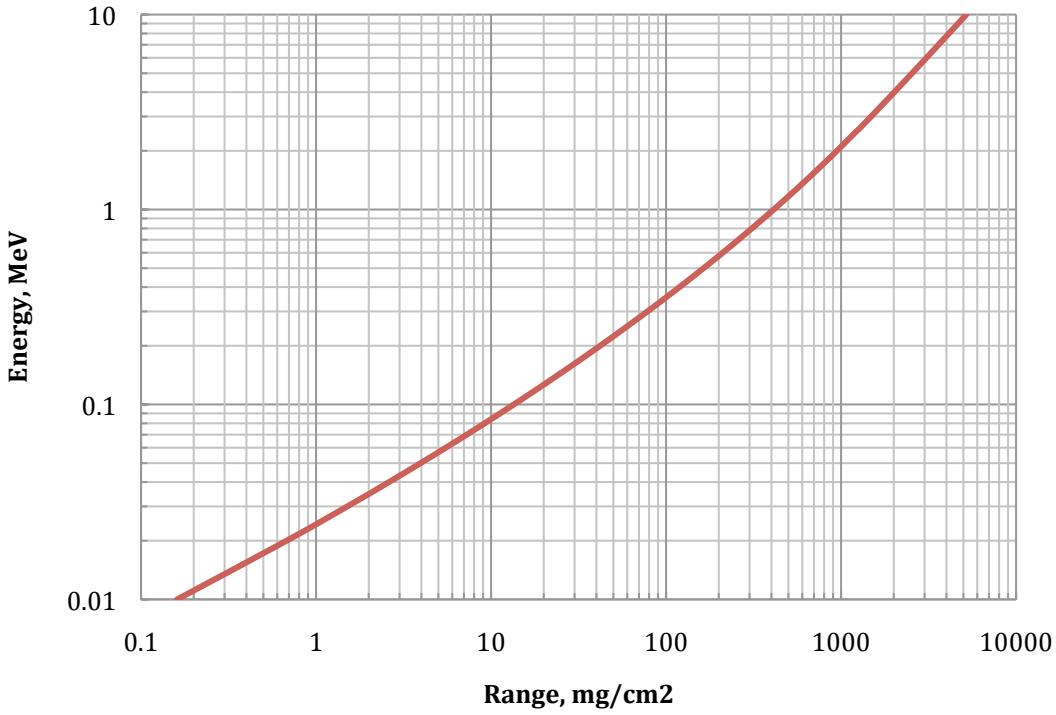


Figure 3.5: Recreated Range Energy Curve[12].

[12] Figure recreated from Cember (1996).

The range of the Beta flux that would penetrate the primary containment was analyzed by looking at the Beta range energy curve (figure 3.5), which was recreated from Cember 1996. Then, using the Beta max of ^{36}Cl of 709 KeV, the range of that beta particle was found to be 264.167 mg/cm^2. In this case, the primary containment is made of commercial grade Lucite, which has a density conservatively assumed to be 1.16 g/cm^3. The actual range of Lucite densities in commercial products lies between 1.16 g/cm^3 and 1.20 g/cm^3. The range of Beta particles in Lucite as well as other materials can be seen in Figure 3.6.

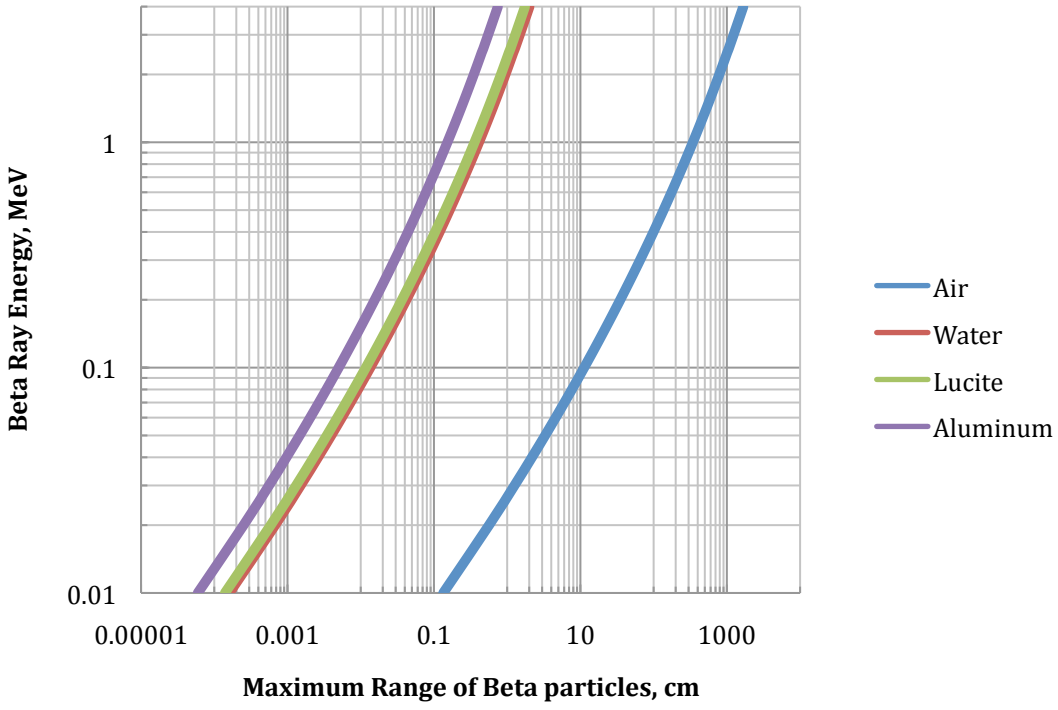


Figure 3.6: Energy-Range Curves for Various Substances.

The final range for a 709 KeV Beta particle in Lucite with a density of 1.16 g/cm^3 was found to be 0.229 cm and the thickness of the primary containment was measured to be 0.34 cm. Therefore the 0.34 cm thick primary containment barrier will shield the ^{36}Cl source and the secondary barrier is in place in case a leak or failure of the primary containment.

3.5 Conceptual Model

For the two tank systems that were previously discussed (low and high ^{36}Cl concentration) a conceptual model was built in STELLA®. Although different parameter values were used, the same conceptual model map (Figure 3.7) and initial inputs with the exception of initial concentration, uptake, and efflux were used for both systems. This simple model was designed to predict uptake to Artemia or fraction of activity removed at any point in time of ^{36}Cl from a tank into the Artemia. It captures both mechanisms of exchange diffusion and active transport in the efflux rate. Uptake is presumed to be via exchange. Based on this model design the expected response would be a simple uptake response of the form that can be seen in Figure 3.8 for both high and low ^{36}Cl systems. This response is based on the tank concentration and the uptake and efflux rates being constant. All parameters and values can be seen in table 3.1.

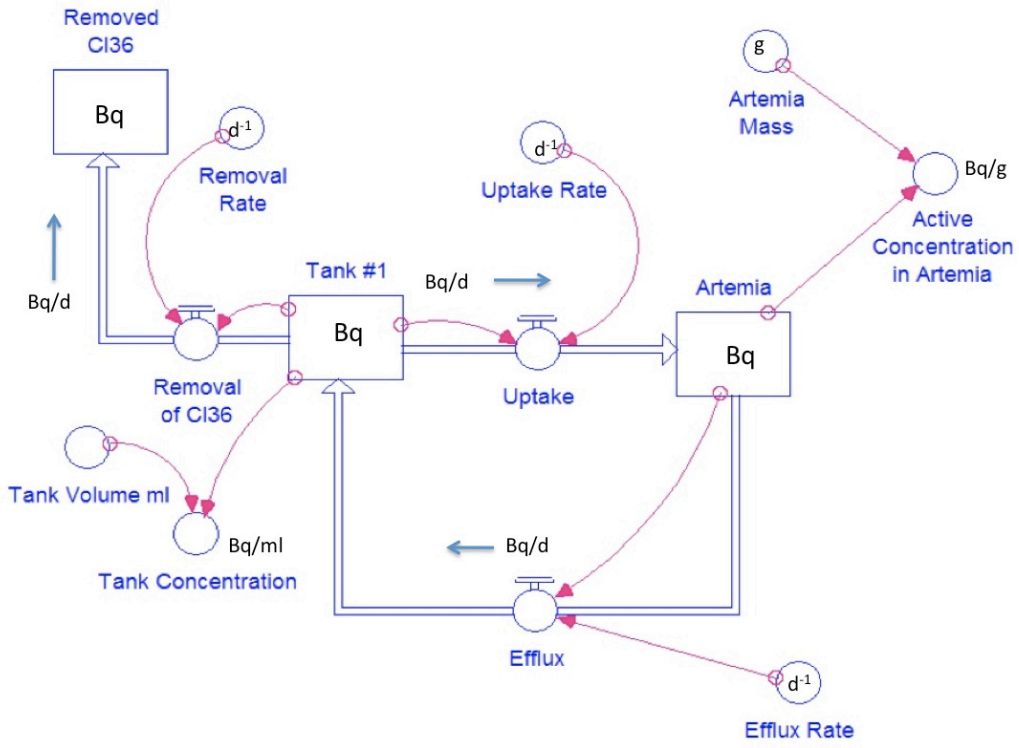


Figure 3.7: Conceptual model for the input and loss of chlorine in Artemia in an aquarium system.

Table 3.1: Conceptual Model parameters and description.

Parameter Name	Description	Parameter value for low ^{36}Cl system	Parameter value for high ^{36}Cl system
Tank (Bq)	Total Activity in Tank	$1.24x10^{4}$*	$1.24x10^{5}$*
Uptake (Bq/day)	Product of Uptake Rate and Tank Activity	0*	0*
Uptake Rate (day^{-1})	Rate of Artemia Uptake	0.20	0.20
Artemia (Bq)	Total Activity in Artemia Compartment	0*	0*
Active Concentration in Artemia (Bq/g)	Activity in the Artemia compartment divided by mass of Artemia in the compartment	0*	0*
Artemia Mass (g)	The mass of Artemia	100	100
Efflux (Bq/day)	Product of Efflux Rate and Artemia Activity	0*	0*

Efflux Rate (Bq/day)	The Rate at which the ^{36}Cl Leaves the Artemia compartment	0.5	0.5
Removed ^{36}Cl (Bq)	The amount of ^{36}Cl removed during sampling	0*	0*
Removal Rate (day^{-1})	Rate of removal during sampling	0.005	0.005
Removal of ^{36}Cl (Bq/day)	Product of Removal Rate plus Removal of ^{36}Cl	0*	0*
Tank Volume (ml)	Volume of Solution in Tank	7526	7526
Tank Concentration (Bq/ml)	Tank Activity divided by Tank Volume	1.65*	16.50*

*Initial values.

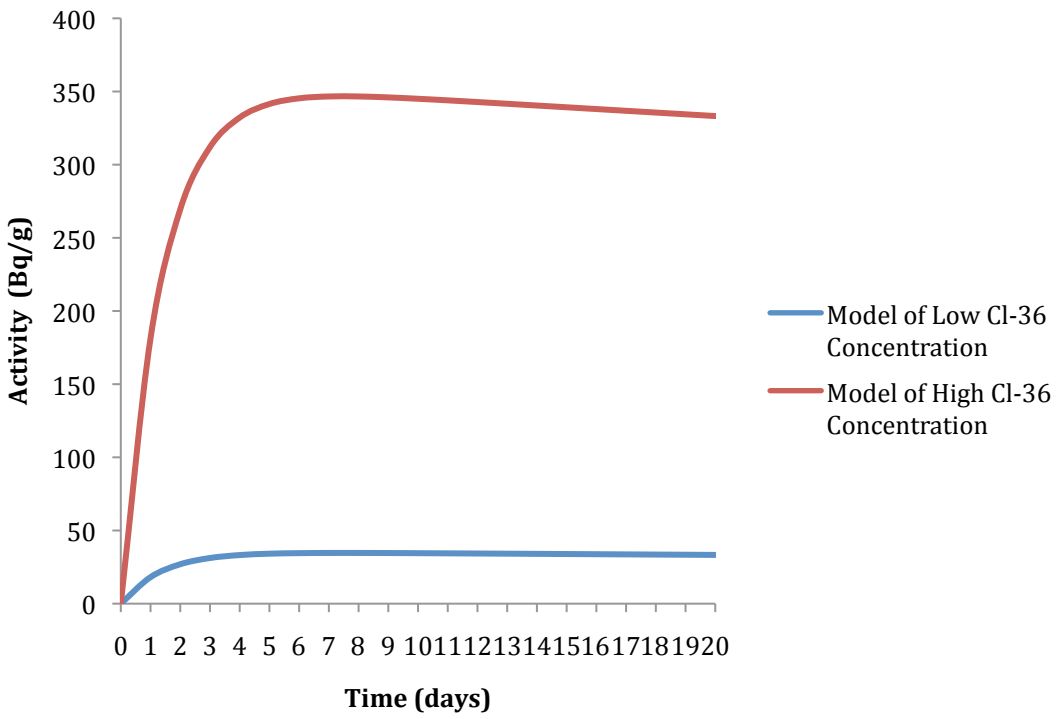


Figure 3.8: Activity concentration versus time in Artemia as predicted by the conceptual models of high and low ^{36}Cl concentration systems.

Chapter 4

Results

4.1 Artemia

The low ^{36}Cl concentration system received a single spike of 1.24×10^4 Bq of ^{36}Cl. This resulted in an initial tank concentration of 1.64 +/- 1.28 Bq/g. Artemia sampled 24 hours after the initial spike had an activity concentration on day 1 of 17.22 +/- 1.38 Bq/g. The results over the 20-day experiment are shown in Figure 4.1. The error in the count or standard deviation (σ) that can be seen in all figures were calculated using a single count x minus the background as can be seen in equation 4.1.

Equation 4.1: Standard Deviation of a net count rate (r)

$$\sigma_{net} = \sqrt{\sigma_{gross}^2 + \sigma_{Background}^2}; \sigma_{gross}^2 = \sqrt{\frac{Gross\_Count\_Rate}{Time}}$$

$$\sigma_{Background}^2 = \sqrt{\frac{Background\_Count\_Rate}{Time}}$$

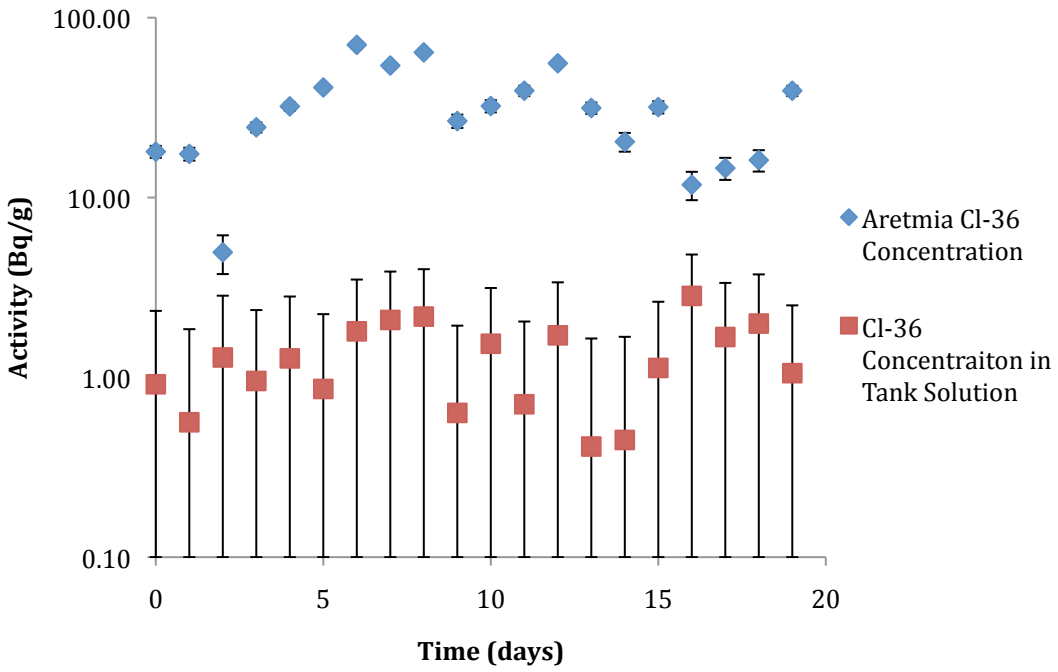


Figure 4.1: ^{36}Cl Activity concentration in Artemia as a function of time in tank #1, error is 1 standard deviation.

The maximum concentration of ^{36}Cl in Artemia in the low concentration tank occurred on day 6. The dry weight concentration was 70.62 +/- 8.40 Bq/g, which was approximately 34 times the initial tank concentration. The minimum concentration of ^{36}Cl occurred on day 2 and was 4.96 +/- 2.23 Bq/g or approximately 2 times the initial tank concentration.

Tank #2, the high concentration system of ^{36}Cl (figure 4.2), had an initial spike of $1.24x10^5$ Bq. This resulted in an initial tank concentration of 16.40 +/- 4.04 Bq/g. The Artemia had a concentration of 71.41 +/- 1.47 Bq/g on day 1, 24 hours after the initial spike.

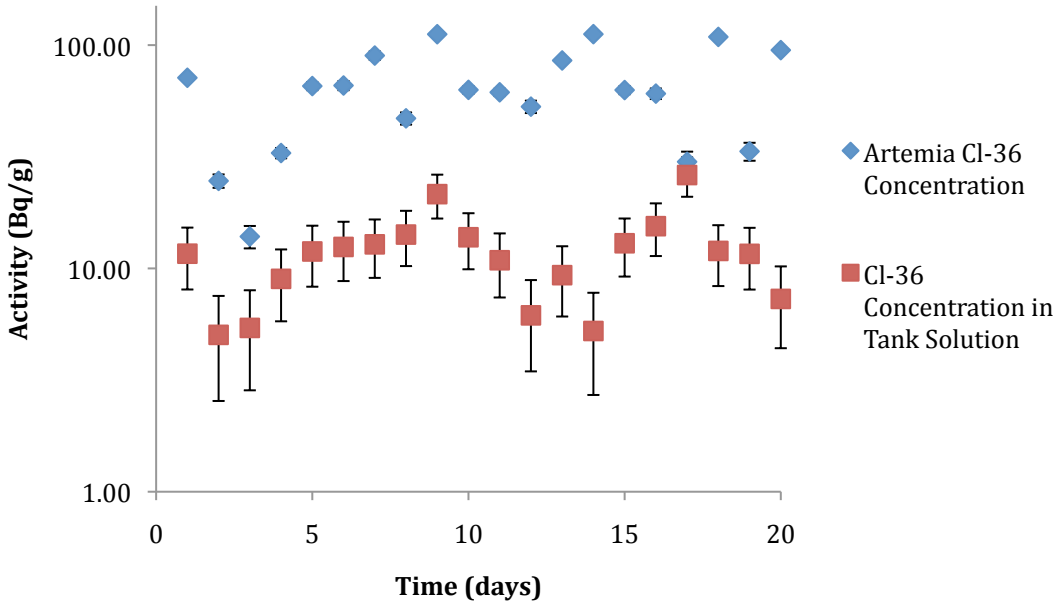


Figure 4.2:^{36}Cl Activity concentration in Artemia as a function of time in tank #2, error is 1 standard deviation of each count.

The maximum concentration of ^{36}Cl in the Artemia in tank #2 occurred on day 8 with a concentration of 111.89 +/- 10.58 Bq/g or approximately 5.00 times the initial concentration of the tank, the minimum concentration of ^{36}Cl occurred on day 3 and was 4.96 +/- 2.23 Bq/g or approximately 20 % of the original concentration of the tank.

4.2 Conceptual Model

No independent uptake and efflux rates were found for chlorine in Artemia in the literature. Because of this, arbitrary starting values were chosen for both uptake and efflux for both the high and low concentration systems (Figure 3.7, Table 3.1). A concentration of approximately 3.40×10^{1} Bq/g in Artemia was predicted for the end of the 20-day trial run of the conceptual model with these initial inputs for the low concentration system. This concentration was roughly half the maximum value observed in Artemia in tank #1.

Measured concentrations of ^{36}Cl in Artemia were used to establish uptake and efflux rates using a buildup curve equation[13]. Equation 4.2 was used to calculate the uptake and efflux from the research data. The highest concentration (day 6) was used as an estimate of q(eq). A linear regression was done on points 1-6 to obtain an estimate of efflux (λ), as the slope. The slope of the linear regression (λ) was then used to solve for the uptake (Equation 4.3). Using this method, it was found that the uptake and efflux rates were low, especially the efflux rates for both systems. The inability to use the Schultz/Whicker approach

[13] Schultz/Whicker, "Radioecology: Nuclear Energy and the Environment".

was due to the large amount of variability in the research data, and because a stable equilibrium was not reached during experiment.

Equation 4.2: Linear regression between points 1-6 yields an estimation of efflux (λ).

$$\ln[q(eq) - q(t)]$$

Equation 4.3: Calculation of uptake.

$$uptake = q(eq) * \lambda$$

where:

q(eq)=the point where the data reaches a steady state, λ =efflux rate, and

t = time

The model inputs were adjusted to see how closely the conceptual model could mimic the low ^{36}Cl concentration system research data (Table 4.1). It was found that within the conceptual model it was necessary to only change the uptake value. After the uptake value was adjusted, a maximum concentration of approximately $7.14x10^{1}$ Bq/g was found. The comparison between the adjusted conceptual model and the Research-based Artemia data can be seen in Figure 4.3 as well as the changes made to the inputs can be seen in Table 4.1.

An alternate method was used to estimate uptake and efflux. The average uptake rates were calculated by taking all of the periods during which there was a positive gain between points and then averaging them over the time of the research. The average uptake was estimated to be 35.77 (Bq/day)/g. The average efflux rate was estimated to be 35.79 +/- 7.26 (Bq/day)/g over the course of the research. This was calculated in the same way as the uptake except taken over periods of efflux. Both trends of uptake and efflux can be seen in Figure 4.1.

The same process was applied to the high concentration system for the conceptual model since the only difference between the two systems was the initial spike. The same initial uptake and efflux values were used as the low concentration system. Unlike the low concentration system a high final concentration of $3.45x10^{2}$ Bq/g was shown on day 20.

After the inputs were adjusted, a maximum concentration of $1.13x10^2$ Bq/g was predicted for the period of 20 days (Figure 4.3). A comparison between the conceptual model and that of the Artemia based research data can be seen in Figure 4.4 as well as the changes made to the inputs can be seen in Table 4.1.

Table 4.1: Values used in the model for both high and low ^{36}Cl systems

Parameter Name	Adjusted Value for Low [^{36}Cl], Tank #1	Adjusted Value for High [^{36}Cl], Tank #2
Tank (Bq)	$1.24x10^4$*	$1.24x10^5$*
Uptake Rate (day^{-1})	0.69	0.053
Efflux Rate (day^{-1})	0.5	0.5
Removal Rate (day^{-1})	0.005	0.005

*Initial value

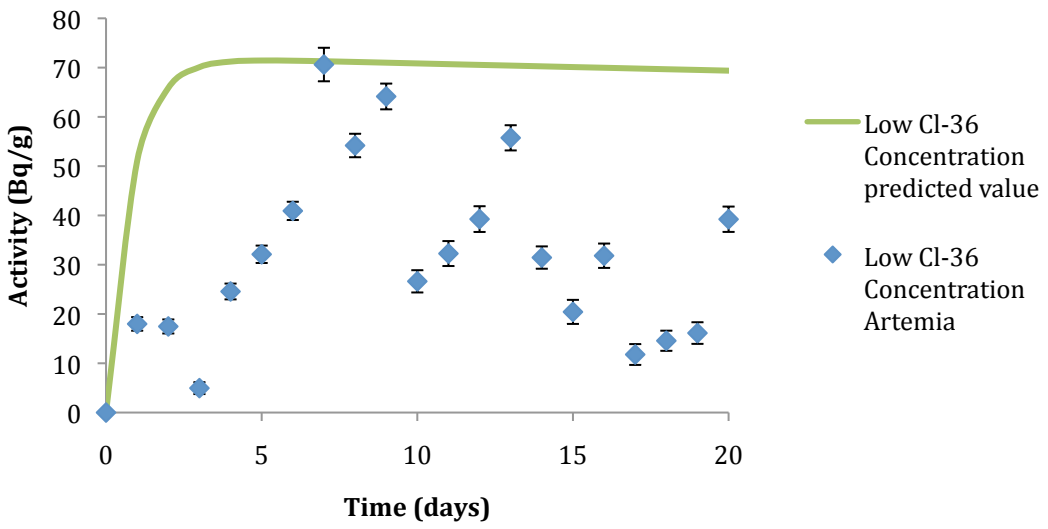


Figure 4.3: Comparison between the revised model prediction and observed values for the low ^{36}Cl concentration system, error is 1 standard deviation of each count.

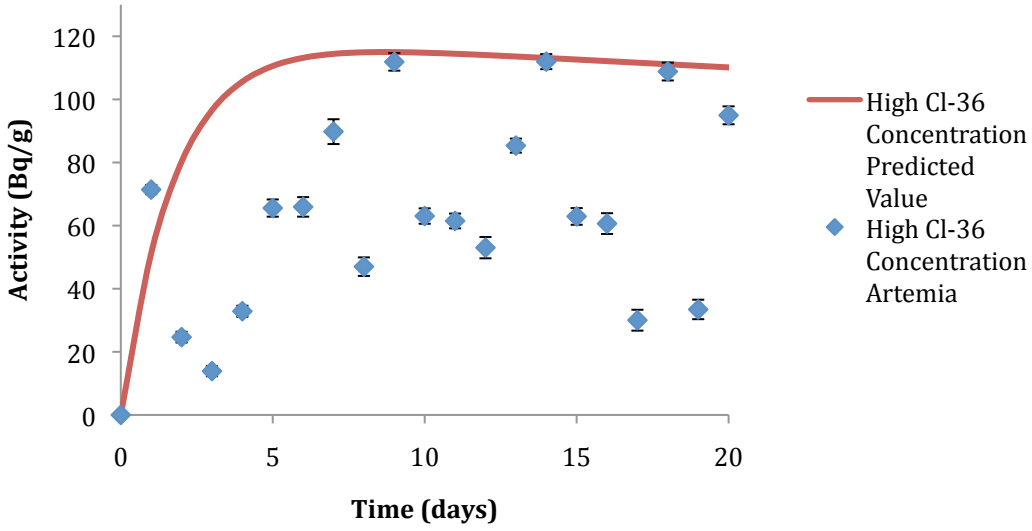


Figure 4.4: Comparison between the predicted and observed values for the high ^{36}Cl concentration system, error is 1 standard deviation of each count.

Chapter 5

Discussion

5.1 Artemia Research

When looking at figures 4.1 and 4.2 there appears to be a cycling pattern that appears in both systems. In an attempt to understand the similarities and differences between both systems the concentration ratios between Artemia and the solution for both systems were compared (figure 5.1). Concentration ratios were calculated (Equation 5.1) for both systems. The expectation was that the concentration ratios should be constant and similar for both systems.

$$CR = \frac{\left[{}^{Bq}/_{g}\ Artemia \right]}{\left[{}^{Bq}/_{g}\ Tank \right]}$$

Equation 5.1: Concentration Ratio (CR) calculation

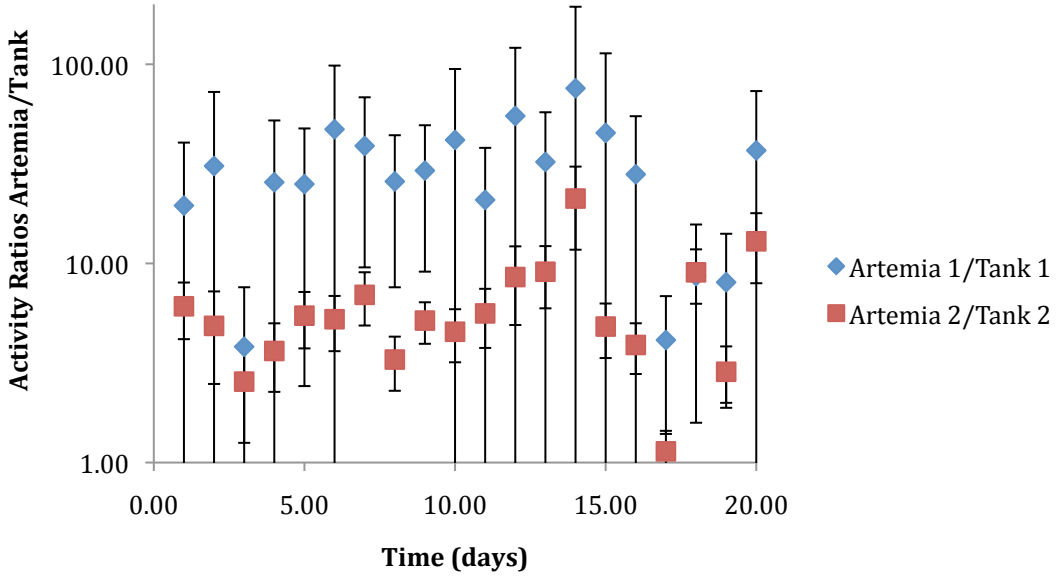


Figure 5.1: A comparison of calculated concentration ratios for Artemia to Tank Concentrations (ml/g).

After calculating the ratios as well as performing the appropriate error propagation (Equation 5.2) it was found that the low and high ^{36}Cl concentration systems were not similar. This suggests that there is something else going on, which could possibly be due to several different factors. First, because replicates were not collected for every sample period, a more accurate analysis was not possible. Second, the addition of non-radioactive solution, which was removed before the tanks were spiked, was added to each tank on a daily basis mixed with their food, which could have effected the concentrations in both tanks. Third, once the Artemia reach egg-producing stage, gestation of nauplii and newborn

nauplii growth was not taking into account. There could be possible differences in sampling techniques between the two systems. The addition of more stable, Chlorine in the high concentration tank could have affected the Artemia's ability to hyper-regulate causing uptake and efflux rates not indicative of a healthy Artemia, or over saturation in the Artemia in the high concentration system may have affected proportional difference between the two systems. In Artemia it is considered that fluid only travels in one direction from the mouth to the anus, it is possible that it is coming in from both ends causing a build up in the gut. Variations in light conditions within the lab could have affected Artemia feeding patterns, and finally, possible over feeding of the Artemia in both systems.

$$\left(\frac{\sigma_R}{R}\right)^2 = \left(\frac{\sigma_{N_1}}{N_1}\right)^2 + \left(\frac{\sigma_{N_2}}{N_2}\right)^2$$ Equation 5.2: Error propagation for activity ratios

$R = \frac{N_1}{N_2}$ N = Count rate σ = Standard deviation

In an attempt to test for temporal cycling in the data a simple moving average was calculated. This was done to reduce the amount of random noise that may be present in the data. A simple moving average is an average of a

subset of a data set[14]. The comparison between the simple moving average and the original data set can be seen for both the low and high concentration system in figures 5.2 and 5.3 respectively. This comparison was done to get a clearer picture of any apparent cycling that may be taking place without the presence of as much random noise as possible.

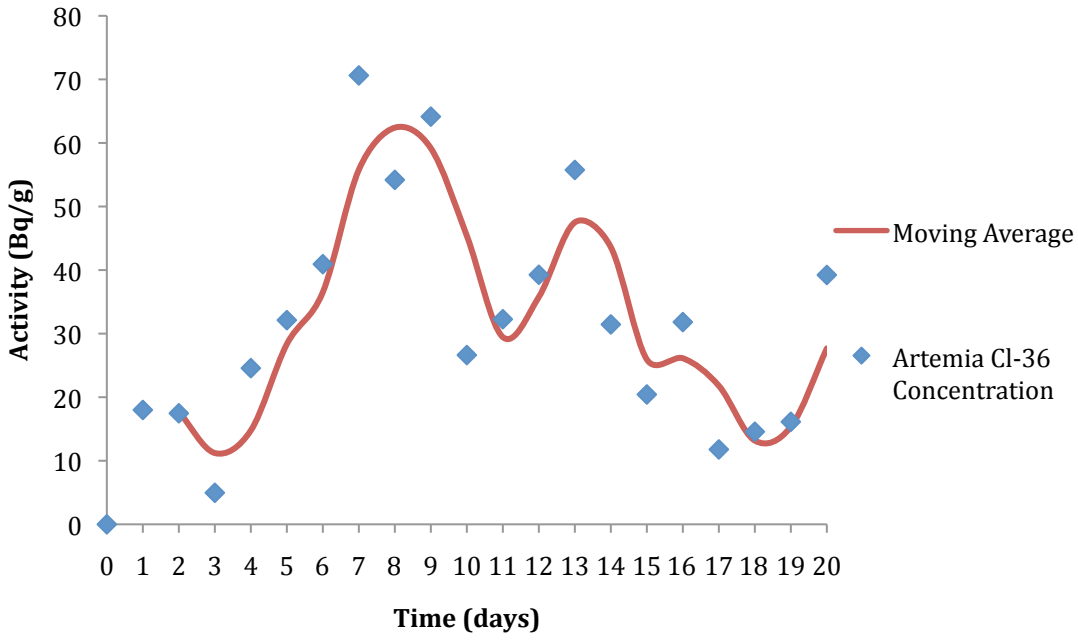


Figure 5.2: A comparison between the moving average and the low ^{36}Cl concentration system.

[14] Engineering Statistics Handbook
http://www.itl.nist.gov/div898/handbook/pmc/section4/pmc4.htm

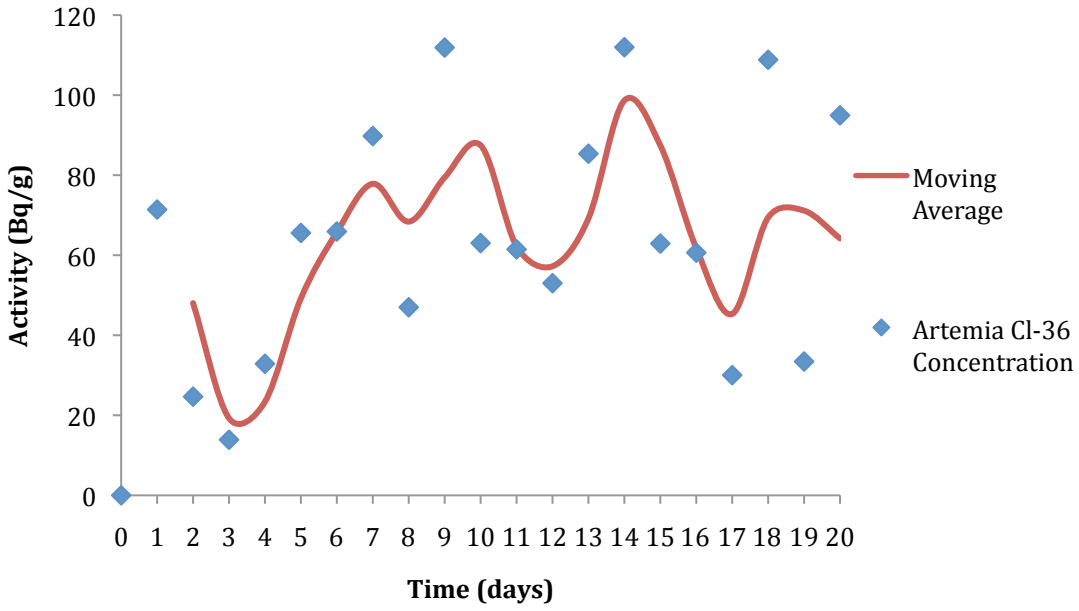


Figure 5.3: A comparison between the moving average and the high ^{36}Cl concentration system.

The percent of activity in the Artemia as compared to the initial activity found in their environment (Bq) was also looked at in the low and the high concentration systems (Figures 5.4 & 5.5, respectively). The transfer of total activity from tank #1 to Artemia ranged from a low of approximately 5 percent to a high of approximately 100 percent. In Tank #2 the transfer ranged from a low of approximately 3 percent to a high of 33 percent. In both systems there was a substantial movement of activity from the tank solution (volume approximately 7500 ml (approximately 7800 g)) to Artemia (approximately 100g).

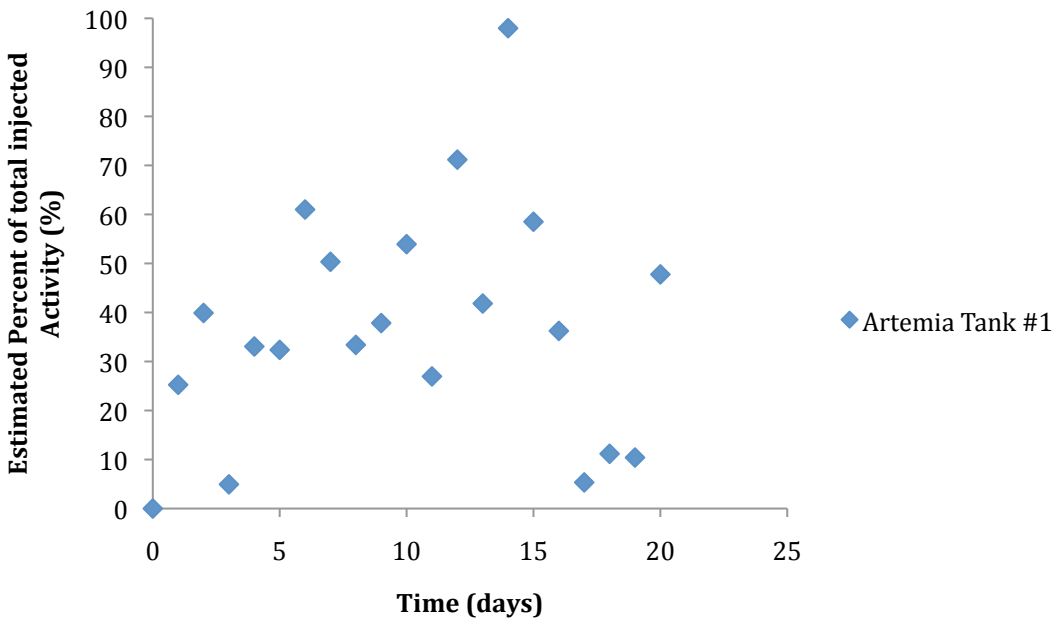


Figure 5.4: Estimated percent of total injected activity transferred to Artemia over time in Tank #1.

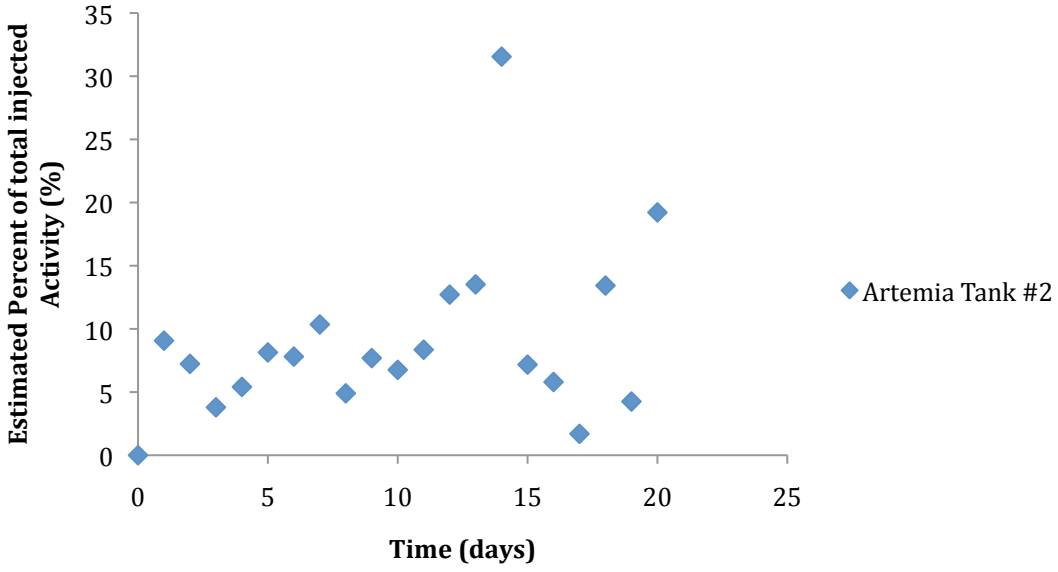


Figure 5.5: Estimated percent of total injected activity transferred to Artemia over time in Tank #2.

An interesting thing showed up in the comparison of activity percentages between the low and high systems. Even though the low ^{36}Cl concentration systems total activity levels in the Artemia were never as high as that of the high ^{36}Cl concentration systems, its overall uptake by percentage was noticeably higher.

The 98 percent maximum percent concentration seen in the Artemia in the low ^{36}Cl system may be an artifact from the original system setup, Artemia were divided into approximately equal halves. Because the vendor did not specify mass an approximate value of 100g of Artemia was used for both systems. This value is based on vendor specifications regarding total number of Artemia in the order and multiplied by an assumed mass per organism.

The high concentration system in tank #2 had a maximum percent concentration in the Artemia of approximately 32 percent and an average of approximately 9 percent of the total initial concentration.

After the research had been concluded and the data was analyzed, it was realized that it would have been very beneficial if multiple replicates of daily samples had been taken. This would have given greater insight into the system variability and/or uncertainty. It may have also shown that the cycling seen in this research was an artifact of variability and uncertainty in the measurements.

Showing that the actual data was much closer to the expected uptake and plateau similar to that pictured in Figure 4.4.

The apparent cycling pattern could also be due to the process of birth and growth of Artemia. As the Artemia grow they molt several times (approximately 15). Presumably the are taking in more nutrients to support this process, potentially allowing for more uptake of ^{36}Cl. Originally this factor was discounted because all Artemia were received from the vendor at the same life stage: adult pre-egg producing [15](Figure 2.1). However it is possible that the female Artemia began producing eggs shortly after arriving at OSU. If true, then 4 days after they began producing eggs, nauplii (or cysts) would have been born. There is also some work that has been done that shows that the gestation cycle is increased at lower temperatures when the water falls below approximately 293 K (Dhaheri 2003). In both systems from this study the temperature was below 293 K. This was done in an attempt to maximize the life span of the Artemia. The average of both systems was approximately 289 K. It is possible that as the nauplii were born at a higher then normal rate, then grew and molted. This could have caused an increase in the uptake and efflux of ^{36}Cl in both systems.

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[15] They are shipped just after they reach a mature size and have finished molting as this is when they are of the highest nutritional value.

Another factor could have been when replacement fluid was added to the system on a daily basis (with the daily feeding). The adding of fluid with the Artemia food was necessary to properly mix the Artemia food before adding it to the system. This factor has been discounted. The water concentrations of ^{36}Cl in both systems, (seen in figure 5.6 & 5.7) was relatively constant over the course of the research. No noticeable changes occur due to the addition of 20 ml of previously removed non-radioactive fluid[16] mixed with Artemia food to the total tank volume of $7.56x10^3$ ml. The total ^{36}Cl levels for both tanks stayed constant through out the research maintaining the mass/activity balance. The tank solution should have also had multiple counts pulled to give a clearer picture or the changes in ^{36}Cl concentrations over time.

[16] Fluid was removed prior to spiking the two systems with ^{36}Cl insuring no changes in overall fluid chemistry throughout the course of research.

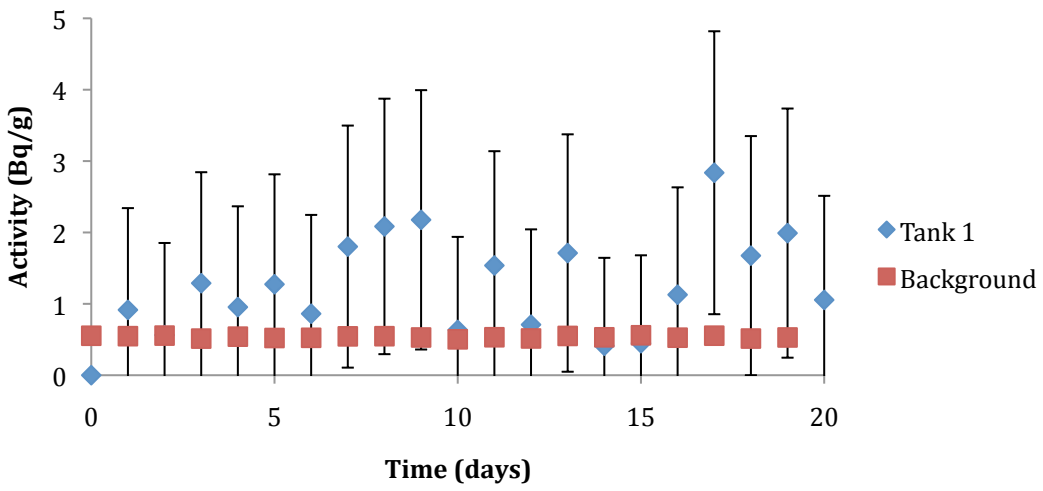

Figure 5.6: Tank 1 ^{36}Cl activity concentration versus time, error is 1 standard deviation of each count.

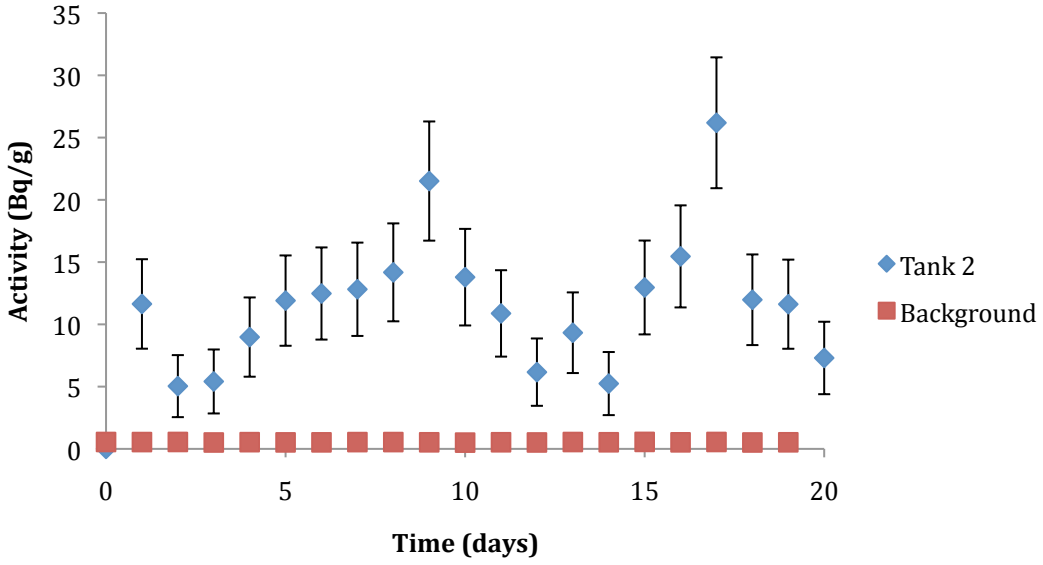

Figure 5.7: Tank 2 ^{36}Cl activity concentration versus time, error is 1 standard deviation of each count.

Possible changes in sampling technique throughout the research could be another factor causing the appearance of cyclical activity. The same procedure was used every time a sample was taken during the research. The primary investigator took all the samples. The procedure was that the lights were shut off and a flashlight was used to draw the Artemia to the surface. With a sampling net moving from the bottom of the tank to the top in a single pass, a sample of Artemia was removed, which was then placed on tared weighing dishes. The dishes were then placed in a drying oven for one hour, after which samples were placed in an LSC vial along with a digestint then counted. No cross-contamination between samples occurred throughout the research, this was achieved by using separate but identical tools for both systems, that were housed separately when not in use and properly labeled.

The addition of more chlorine to the two systems in the form of stable chlorine carrier for the ^{36}Cl could have impacted the function of the Artemia's hyper-regulation system. Additional Chlorine could result in an increase in the total concentration of chlorine in the system beyond what the Artemia were capable of regulating. If this were the cause of the cycling, one would expect to see greater changes in the system with the highest concentrations of ^{36}Cl, the changes should be proportional to the increase in ^{36}Cl concentration. As shown in Figure 5.1, the comparison of ratios between the two systems, there was no

proportional difference shown. If the addition of ^{36}Cl was the reason for the cycling one would also expect to see a greater difference in the average rates of uptake and efflux between the two systems representative of the initial amount added to the systems. That is unless the Artemia's hyper-regulation system was so overwhelmed by the total level of chlorine that it caused the difference between the two systems proportionality.

In the high ^{36}Cl tank, one would have expected to see an increase in the maximum concentration proportional to the increase in the initial spike concentration, which was an order of magnitude larger (10 times) than that of the low ^{36}Cl tank. A comparison between the two systems can be seen in Figure 5.8. The lack of an increase in the proportional change of uptake and efflux as well as maximum concentration could be due to a saturation of chlorine in the Artemia. Since no known chlorine limit for proper body function in a mature Artemia or Artemia at any life stage was found and a lethal level of chlorine[17] was not found in the literature or established[18] in this research, it is not possible to discount this possibility for the differences between the two systems.

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[17] Chlorine a key ion in most biological systems but it can become a poison as it acts as an oxidizer.

[18] Throughout the course of research no noticeable die off of Artemia occurred in either system.

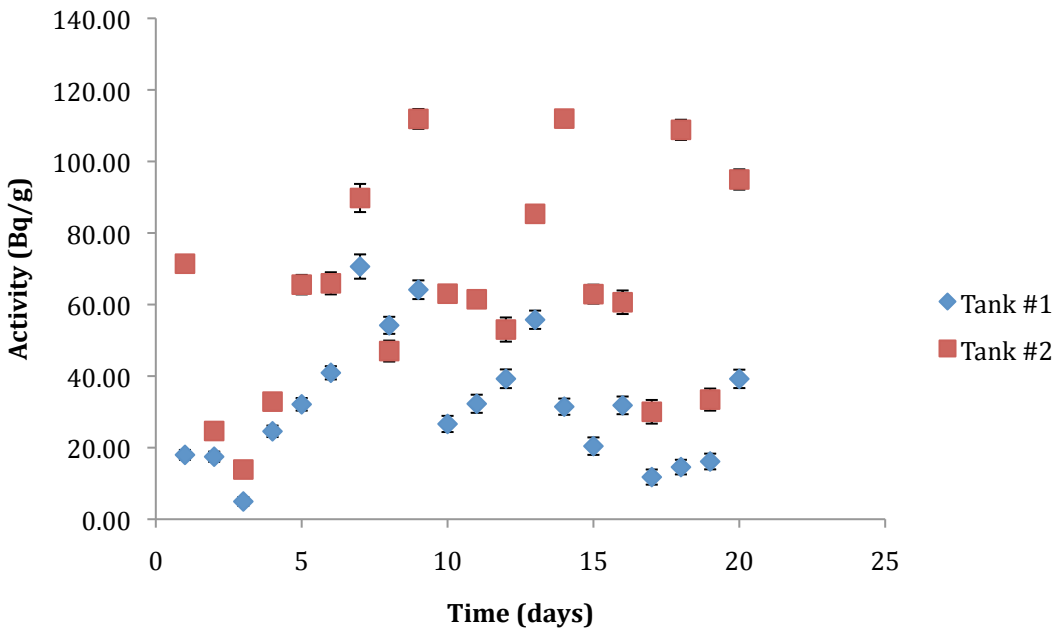

Figure 5.8: Tank#1 Artemia Vs. Tank #2 Artemia, error is 1 standard deviation of each count.

The possibility of radiation damage to Artemia was also investigated. The dose to the Artemia in both systems was calculated using equation 5.3. It was assumed that the Artemia were completely surrounded by a uniform spiked aqueous environment. The internal dose was estimated where it was conservatively assumed that 100 percent of the betas emitted were absorbed in the Artemia for both systems. The conservative calculated dose to the Artemia over the 20 days of the study in the low ^{36}Cl concentration system was 2.61×10^{-3}

Sv and the dose to the Artemia in the high ^{36}Cl system was $5.20x10^{-3}$ Sv. These dose rates should not be sufficient to cause an adverse impact on the Artemia[19].

Equation 5.3: Calculation for dose to Artemia (External Dose + Internal Dose)

$$Dose(Sv) = ((2(2.59x10^{-9})E_{\beta^-_{\max}} W_R)(Tank\_Concentration(\frac{Bq}{m^3})) + (((\frac{Bq}{g})(E_{\beta^-_{avg}} + E_\gamma))$$
$$(3.156x10^7 \frac{\sec}{year})(1.00x10^3 \frac{g}{kg})(1.602x10^{13} \frac{MeV}{J})^{-1}))((\frac{1year}{365.242days})(20days))$$

$E_{\beta^-_{\max}}$ = 0.709 MeV, $E_{\beta_{avg}} = 0.236.33MeV$, $E_\gamma = 0.511MeV$ [20], W_R = 1 for Beta/electrons

$Tank\_Concentration(\frac{Bq}{m^3})$= System 1=$1.64x10^2$ $(\frac{Bq}{m^3})$, System 2=$1.64x10^3$ $(\frac{Bq}{m^3})$

$Artemia\_Concentration(\frac{Bq}{g})$ = System 1=$3.23x10^1(\frac{Bq}{g})$, System 2=$6.44x10^1(\frac{Bq}{g})$

In the conceptual model, Artemia are considered to have only one compartment. In that compartment fluid only travels one way from the mouth to the anus. It is possible that it is coming in from both ends causing a buildup in the gut. Croghan (1958, C) described a study were Artemia were exposed to a red

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[19] Personal communication with K.A. Higley.
[20] Gamma energy was taken from www.nndc.bnl.gov

dye then after a few hours they were pulled and their internal organs were analyzed. It was found that the dye was retained in the gut lumen for prolonged periods. Even after a period of 72 hours an appreciable amount was still present in the lumen. He concluded that fluid had to be swallowed from both ends of the gut. This process would lead to a buildup of concentrated solution in the gut, which could lead to the process of uptake and retention in the Artemia.

Another possible factor is the amount of light and frequency of light variation throughout the research. The lab setup had a light that would come on between the hours of 8 a.m. and 8 p.m. giving a constant light to dark schedule. Although, it should be noted, that the primary investigator was not the only individual using the lab. Variations in light intensity as others came and went could have affected the biological function of the Artemia, which in theory could have lead to the apparent cycling, which was seen.

There is also a possibility that variations in the feeding practices could have affected the uptake and efflux rates of the Artemia. This has been discounted because prior to spiking the system, a feeding regimen was established. It was found that by feeding 0.180 – 0.250 g of food a day mixed with 20 ml of previously removed solution, the levels of turbidity in the water were minimized 3 to 4 hours after feeding.

5.2 Conceptual Model Revision

Previously a comparison had been made between the collected data and the conceptual model (section 3.5 & 4.2). The comparison suggested that the conceptual model might be an inaccurate depiction of the actual system. In the revised model an "oven" was included in an attempt to mimic the apparent cycling patterns in the research data (Figure 5.9). An oven can be thought of as a processor of discrete batches of material. It opens its doors and allows uptake until it hits a load limit or until a pre-set time. Then it closes its doors for a pre-set period, it then opens and discharges. The process repeats causing a lag in the outflow from a compartment. It was thought that this might mirror the retention function of the gut as found by Croghan (1958, B). In the conceptual model the oven appears as a box within a box as seen in Figure 5.10 labeled as Artemia. The model was designed to include three cycles for both the high and the low concentration systems, which closely mimics the patterns in the research data for both the low and high ^{36}Cl concentration systems.

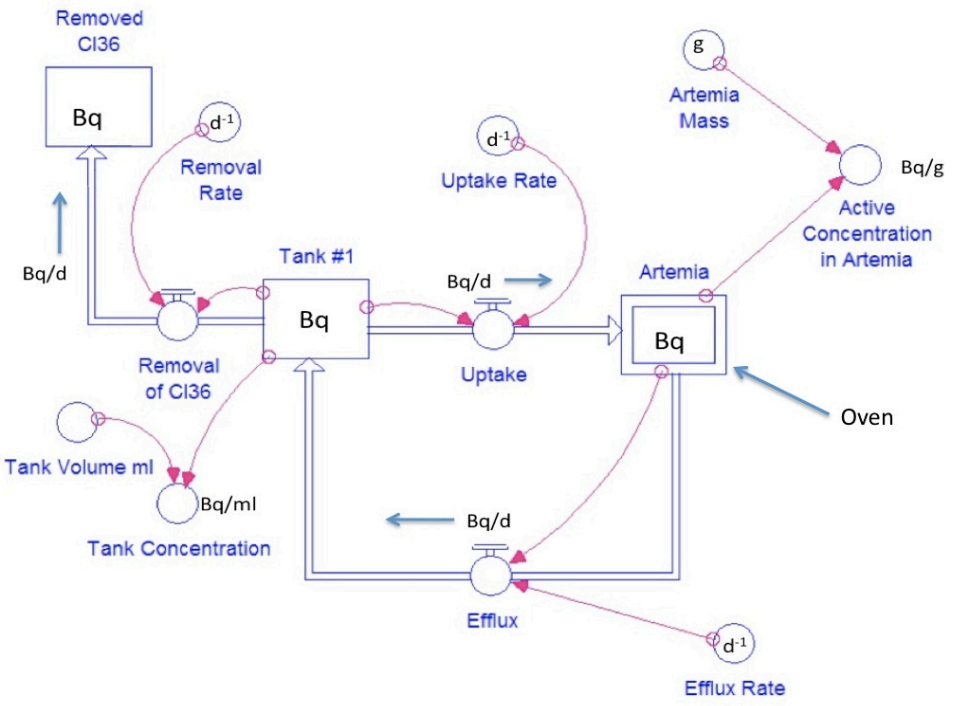

Figure 5.9: Revised conceptual model of ^{36}Cl uptake and efflux in Artemia

After running the revised conceptual model with the same data from the adjusted conceptual model for the high ^{36}Cl system, a maximum concentration of $3.35x10^2$ Bq/g with a minimum activity of 5.39 Bq/g was predicted. The maximum predicted concentration of ^{36}Cl, as well as the cycling effect of the oven compared to the research results can be seen in figure 5.10.

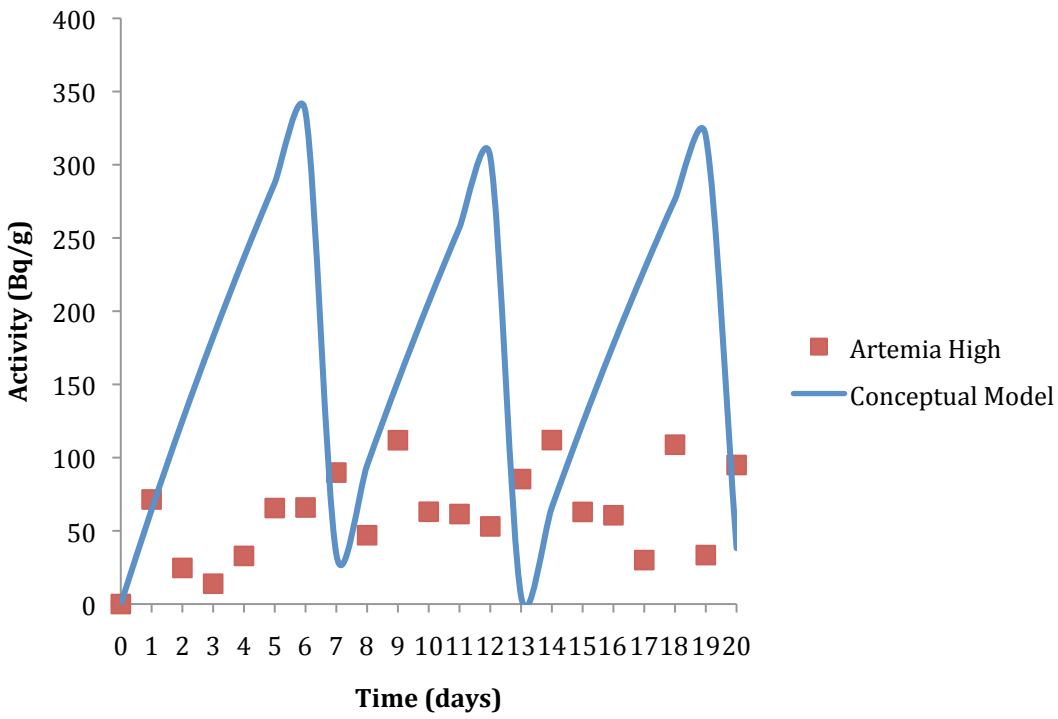

Figure 5.10: Revised conceptual model (with oven) versus Artemia activity concentration in tank #2.

As can be seen in Figure 5.10, the revised conceptual model exhibits cycling, but the predicted concentrations were much greater than that of the observed data. It was found that by decreasing the uptake rate in the conceptual model to 1.60×10^{-2} (day^{-1}), the maximum activity that the conceptual model calculated ^{36}Cl concentration dropped to 1.13×10^{2} Bq/g. This result much more closely followed what was seen throughout the course of this research in the high activity system. A comparison can be seen between the high concentration system and the data from the adjusted conceptual model results (figure 5.11) and

inputs into the revised model (table 5.1). A shift in the conceptual model to start the oven on day 3 was made because it appears that the apparent Artemia cycling started a new cycle on day 3(figure 5.12).

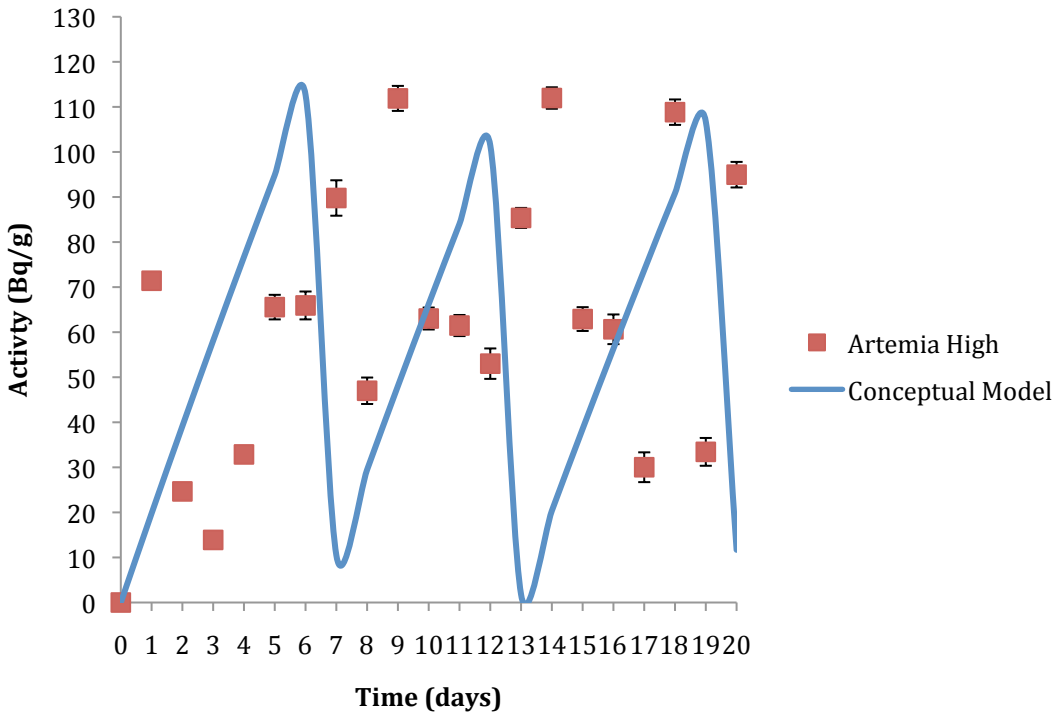

Figure 5.11: The performance of the revised conceptual model (including adjusted parameter values) versus measured Artemia concentration in tank #2, error is 1 standard deviation of each count.

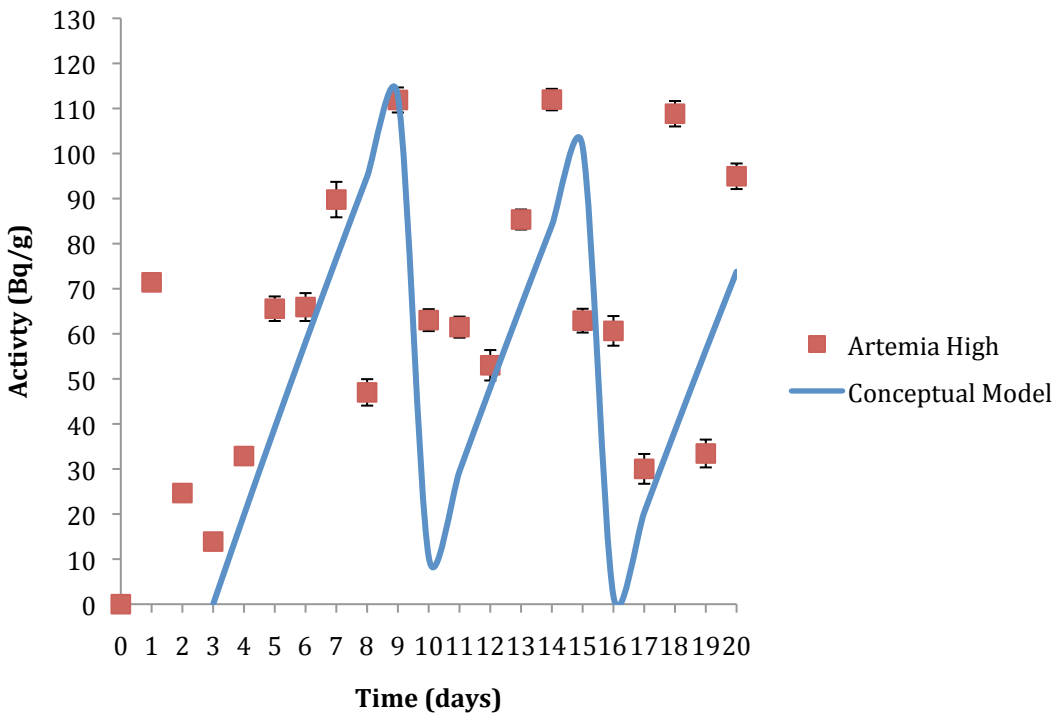

Figure 5.12: The performance of the revised conceptual model (including adjusted parameter values and time delayed onset) versus measured Artemia concentration in tank #2, error is 1 standard deviation of each count.

For the second part of the conceptual modeling, the low activity system was reconstructed in the conceptual model and plotted against the Artemia low ^{36}Cl concentration data using the adjusted input values from table 4.1 (figure 5.13). The results of the lab-based Artemia research showed different rates of uptake for the low concentration system than the high concentration system. This held true for the conceptual model as well. The conceptual model with the

adjusted values from table 4.1 predicted a maximum concentration in the low ^{36}Cl concentration conceptual model of $1.22x10^{2}$ Bq/g and a minimum activity of 4.31 Bq/g.

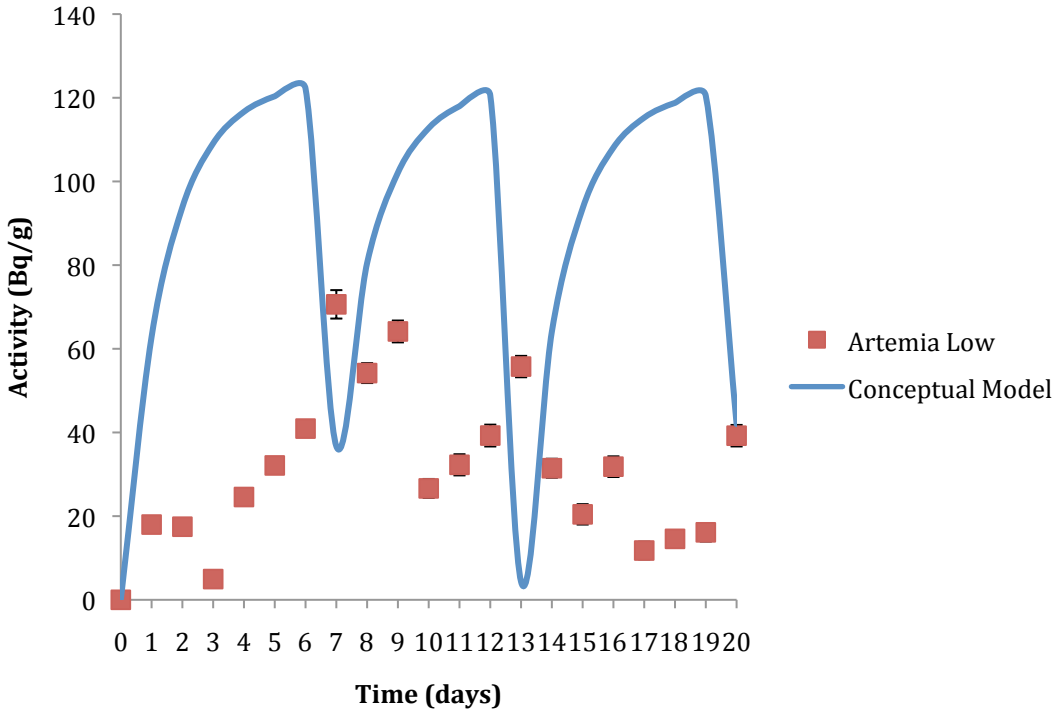

Figure 5.13: Revised conceptual model (with oven) versus Artemia activity concentration in tank #1, error is 1 standard deviation of each count.

Like the high concentration tank, it was found that within the model setup the only adjustment needed to maintain appropriate number of cycles and the approximate maximum concentration of $7.33x10^{1}$ Bq/g was the uptake rate, which was found to be $1.50x10^{-1}$ (day^{-1}) for the low concentration system.

A comparison between the research data and the adjusted conceptual model can be seen in figures 5.14. A shift the conceptual model starting point to day 3 to more closely resemble the beginning of the apparent period of Artemia cycling (figure 5.15). The adjustments made to the inputs to both systems can be seen in table 5.1.

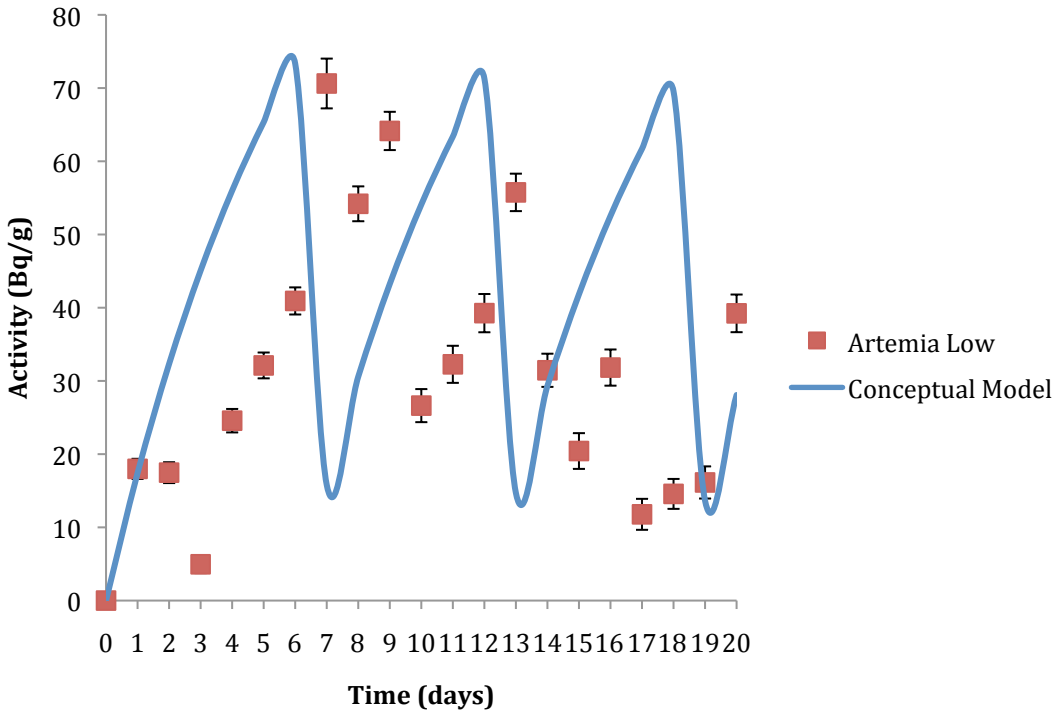

Figure 5.14: The performance of the revised conceptual model (including adjusted parameter values) versus measured Artemia concentration in tank #1, error is 1 standard deviation of each count.

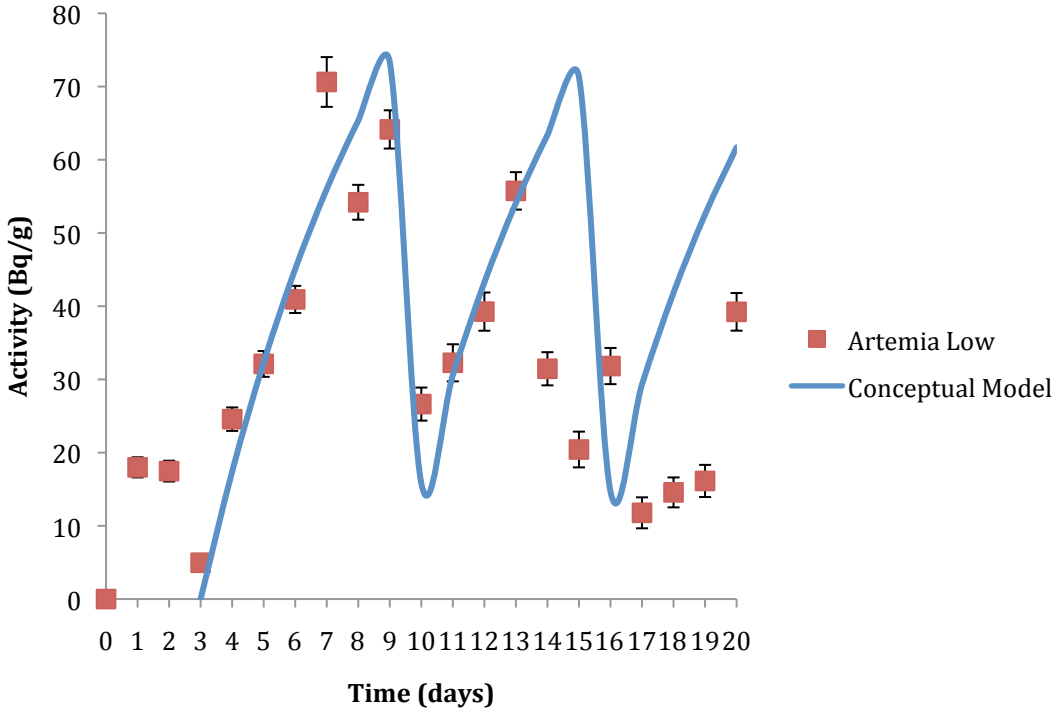

Figure 5.15: The performance of the revised conceptual model (including adjusted parameter values and time delayed onset) versus measured Artemia concentration in tank #1, error is 1 standard deviation of each count.

Table 5.1: Changes in revised inputs for conceptual model

Parameter	Value used for Low ^{36}Cl concentration	Value used for High ^{36}Cl concentration
Initial Tank Spike (Bq)	$1.24x10^4$	$1.24x10^5$
Initial Artemia Concentration (Bq/g)	0	0
Uptake Rate (day^{-1})	0.15	0.016
Efflux Rate (day^{-1})	0.05	0.05
Removal Rate (day^{-1})	0.005	0.005

The main issue when modeling the low ^{36}Cl concentration system in the conceptual model was getting the maximum concentration to decrease or dampen with time (Figure 5.14). The proposed hypotheses to explain the decrease in the maximum active concentration with time as seen in the research data was that the initial spike of ^{36}Cl was very low, approximately $1.24x10^4$ Bq. Over the course of the research an average of 32.32 +/- 17.90 (Bq/day)/g of Artemia when doing daily sampling approximately 1 % of the total initial spike was removed over the course of the research, which was enough to decrease the overall total ^{36}Cl concentration in the system. This decrease was to a point that it was first noticed in the Artemia sample count rate around day 7 as a decreasing maximum ^{36}Cl activity concentration in Artemia (figure 4.1).

The decrease in maximum concentration over time was not seen in the Artemia from the high ^{36}Cl concentration system. This was attributed to the much larger initial concentration of ^{36}Cl, which was an order of magnitude larger (10 times) than that of the lower ^{36}Cl concentration system. So, that the removal of 64.43 +/- 29.28 (Bq/day)/g of Artemia was insufficient to bring the overall concentration down enough to be noticeable in the maximum active concentration of ^{36}Cl in the Artemia.

Another possible reason for the decrease in or damping affect in the low concentration system is that all Artemia were received from the vendor as a

synchronized population at the same life stage. As time moves forward they lose that synchronization and the total population moves to different stages of the Artemia life cycle[21]. The dampening affect may be moving toward a true constant that can't be seen because the time frame for this research was not long enough to capture that phenomenon.

After getting the conceptual model to cycle and hit the appropriate maximum concentration similar to that in the research data, it was found that the conceptual model did not show a noticeable drop in maximum concentration with time like the low ^{36}Cl concentration system. When the initial spike was lowered in the conceptual model to see if that would produce a dampening effect, another issue appeared, which was that as the initial spike was decreased the maximum concentration began to noticeably decrease at the same time. This problem was attributed to the fact that at low spike concentrations the conceptual model has a maximum concentration that it can give to any compartment defined by equation 5.4.

[21] Personal communication with K.A. Higley

Equation 5.4: Calculation for the concentration in a compartment at a given point in time (t).

$$Artemia = Artemia(t - dt) + (uptake - eflux)dt$$

Getting the conceptual model to mimic the research data has shown that with this setup and model inputs mimicking the uptake and efflux, it could not accurately predict the dampening effect at the necessary maximum concentrations. This is attributed to the initial concentration in the model, which must be low enough in the conceptual model so that with any reasonable constant removal rate a decline in maximum ^{36}Cl concentration will be seen with time. The lower initial concentration needed in the conceptual model is due to the fact that it is a computer model and requires very specific initial values for reasons previously mentioned and not a highly complex and not completely understood biological system as was dealt with in the Artemia.

Chapter 6

Conclusions

This study has shown variations in the uptake and efflux of ^{36}Cl over a twenty day period of time that has not been previously described in any scientific studies. Then the Artemia results found in the lab were modeled using STELLA® v9.0.1.

In the lab portion of the research the Artemia exposed to the 1.64 +/- 1.28 Bq/g in ^{36}Cl solution had an average uptake rate of 35.77 +/- 7.26 (Bq/day)/g with an average efflux rate of 35.79 +/- 6.77 (Bq/day)/g. Both the uptake and efflux rates for the low ^{36}Cl concentration systems can statistically be considered the same. The average concentration in the Artemia exposed to low concentrations of ^{36}Cl was 32.32 +/- 17.90 Bq/g.

The Artemia exposed to a concentrations of 16.40 +/- 4.04 Bq/g ^{36}Cl had an average uptake rate of 70.02 +/- 11.16 (Bq/day)/g with an average efflux rate of 62.98 +/- 11.81 (Bq/day)/g. Like the low ^{36}Cl concentration system the uptake and efflux rates are statistically very similar. The average concentration in the Artemia exposed to high concentrations of ^{36}Cl was 64.43 +/- 29.28 Bq/g.

Both the low and the high ^{36}Cl concentration systems demonstrated similar patterns of uptake and efflux over the period of this study. After ruling out effects due to human or other outside error, several possibilities remain as to what could have caused the apparent patterns of uptake and efflux. It was also not possible to state with any significance that these oscillating patterns in both the low and high concentration systems were occurring due to the low number of observations that were recorded. With a larger data set, time-series analysis might be used to find structure like cycling or oscillating patterns[22].

An alternative to time-series analysis was also used. A moving average was calculated for the low and high concentration systems taking out some of the random noise from the data (figure 5.2 & 5.3 respectively), in an attempt to give a clearer picture of the data. It showed an oscillating pattern but there was not enough data to definitively say if there was or was not cycling present.

There was also a comparison done between the initial activity of ^{36}Cl in the tank and the activity in the Artemia. It was observed that the low concentration system had a much higher average percent concentration (approximately 39 percent) as compared to the total initial spike than that of the high concentration system, which had an average percent concentration in Artemia much smaller

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[22] Engineering Statistics Handbook
http://www.itl.nist.gov/div898/handbook/pmc/section4/pmc4.htm

than that of the low concentration system. This would be expected if the level of chlorine that a healthy adult Artemia maintains lies between the low and high initial chlorine concentrations.

The unexplained phenomenon of the uptake and efflux is possible attributed to a complex biological mechanism or mechanisms, which are most probably linked to the hyper-regulation mechanism of the Artemia used to maintain ionic levels of chlorine and other biologically key ions. In the other studies that were reviewed, no study was found that was conducted over this long of a period. Most were done over a period of a few days at a maximum, which is not sufficient amount of time to observe this phenomenon. Another possibility is that this phenomenon can't be attributed to any single factor like the hyper-regulation mechanism and is instead the result of several combined factors. Of these is a combination of adult female gestation newly–born nauplii molting (rapid growth), a function of possible buildup in the lumen from filter feeding through both ends of the digestive track, and excessive levels of chlorine throwing off the proportionality that should exist between the low and high ^{36}Cl concentration systems.

The apparent pattern of uptake and efflux (or cycling) in both the high and low ^{36}Cl concentration systems were closely demonstrated in the conceptual model. Although, it was also shown that in order to use the conceptual model, the

uptake rate must be much smaller in the model parameters than what was seen in the research to achieve approximately the same maximum concentrations in both the high and low ^{36}Cl concentration systems. As the uptake rate in the conceptual model was the determining factor to the maximum concentration.

It was also found after the research data analysis that almost all (approximately 99%) of the Artemia exposed to low concentrations of ^{36}Cl had higher concentrations of ^{36}Cl than that of the solution in the tank throughout the research. Whereas a slightly smaller percent (approximately 95%)of the Artemia exposed to high concentrations of ^{36}Cl had higher concentrations of ^{36}Cl than that of the solution throughout the course of this research.

It would be helpful to repeat this study over a longer period of time with a third system containing between $4.40x10^4$ Bq – $6.20x10^4$ Bq of ^{36}Cl representing a middle level of contamination testing possible chlorine saturation in the Artemia. This study would also more thoroughly test for cycling. It would also be necessary to pull multiple replicates of each sample and a larger total number of samples/observations to give a more statistically accurate daily count. This would increase the accuracy of the count as well as minimizing any possible variance of the daily count and providing more data to test for cycling. It could also be helpful to increase the total length of the research to 30 to 40 days. A more reliable system of light control would also be beneficial for any future

studies. It could also be very beneficial to look at uptake throughout the life–cycle of Artemia as different phases of life, the uptake and efflux rates will more than likely vary.

As no study has been conducted on Artemia over this long of a time frame looking at concentrations of ^{36}Cl. This research is a first step in attaining a greater understanding of the various mechanisms involved with the uptake of isotopes particularly ^{36}Cl, which resemble biologically essential ions in hyper-regulated invertebrate species in a marine system.

It should be mentioned that the correlation between the conceptual model data and the Artemia-based research data should be used cautiously while the true source of the oscillating pattern of uptake and efflux remains unknown. An accurate model of the cycling is not possible without understanding the underlying factors affecting the uptake and efflux rates in Artemia.

Bibliography

BIOPROTA. (2006). Report of an International Forum on Cl-36 in the Biosphere Chatenay-Malabry, France. 27-28 September 2006. Published on behalf Of the BIOPROTA Sponsering Committee by ANDRA(Agence nationale pour la gestion des dechets radioactifs), Chatenay-Malabry, France

Cember, H. (1996). Introduction To Health Physics (3rd ed.). McGraw-Hill Companies Inc..

Croghan P.C. (1958) A. IONIC FLUXES IN ARTEMIA SALINA (L.). Journal of Experimental Biology, 35. 425–436.

Croghan P.C. (1958) B. THE OSMOTIC AND INONIC REGULATION OF ARTEMIA SALINA (L.). Journal of Experimental Biology, 35, 425-436.

Croghan P.C. (1958) C. THE MECHANISM OF OSMOTIC REGULATION IN ARTEMIA SALINA (L.): THE PHYSIOLOGY OF THE GUT. Journal of Experimental Biology, 35, 243-249.

Davis, Stanley N., Cecil, DeWayne, Zreda, Marek, Sharma, Pankaj (1998). Chlorine-36 and the initial value problem. Hydrogeology Journal, 6:104-114

Dhaheri, Al, Drew, C., (2003). Guidelines for the management of the Brine shrimp (Artemia franciscana) at Al Wathba wetland reserve. Terrestrial Environment Research Centre, Abu Dhabi.

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Kirschner, L. (2004). The mechanism of sodium chloride uptake in hyperregulating aquatic animals. Journal of Experimental Biology, 207, 1439-1452.

Knoll, G. (2000). Radiation Detection and Measurement (3rd ed.). John Wiley & Sons, Inc.

Nuclides and Isotopes, Chart of Nuclides (16th ed.). (2002) KAPL, Inc., Lockheed Martin.

SELINUS O., ALLOWAY B., CENTENO J. A., FINKELMAN R. B., Fuge, R., LINDH U. & SMEDLEY P.(eds) (2005). Essentials of Medical Geology. Impacts of the Natural Environment on Public Health. Elsevier Academic Press. Boston. 2005

Smith P.G. (1969). The Ionic Relations of Artemia Salina (L.). Journal of Experimental Biology, 51, 739-757.

Stappen, G. V. (1996). Food and Agriculture Organization of the United Nations. Manual on the Production and use of Live Food for Aquaculture. 4.1. Introduction, Biology and ecology of Artemia. Rome.

Appendices

Appendix A

Figures

Figure 1: STELLA® equation: The predicted conceptual model activity in low ^{36}Cl concentration Artemia system unadjusted.

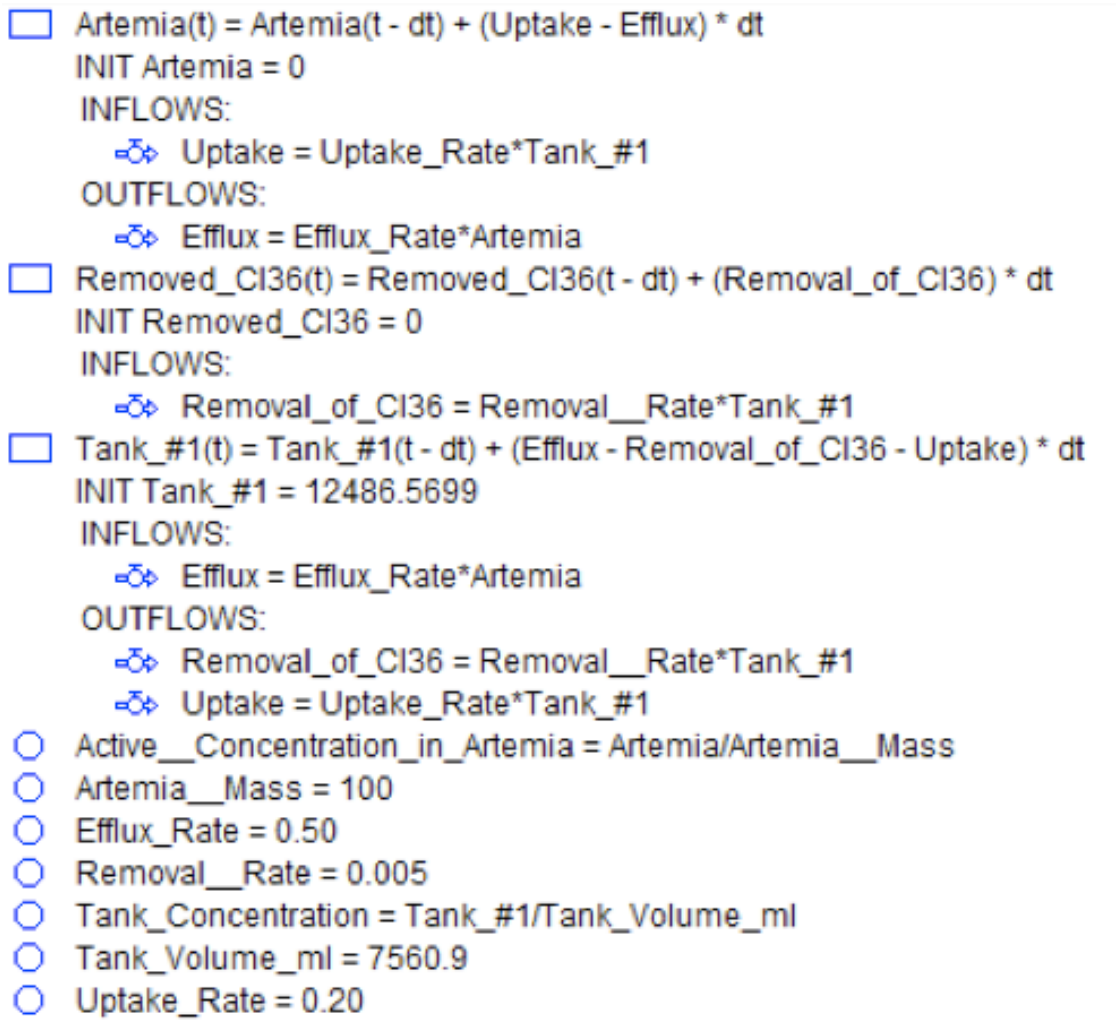


Figure 2: STELLA® equation: The predicted conceptual model activity in low ^{36}Cl concentration Artemia system adjusted.

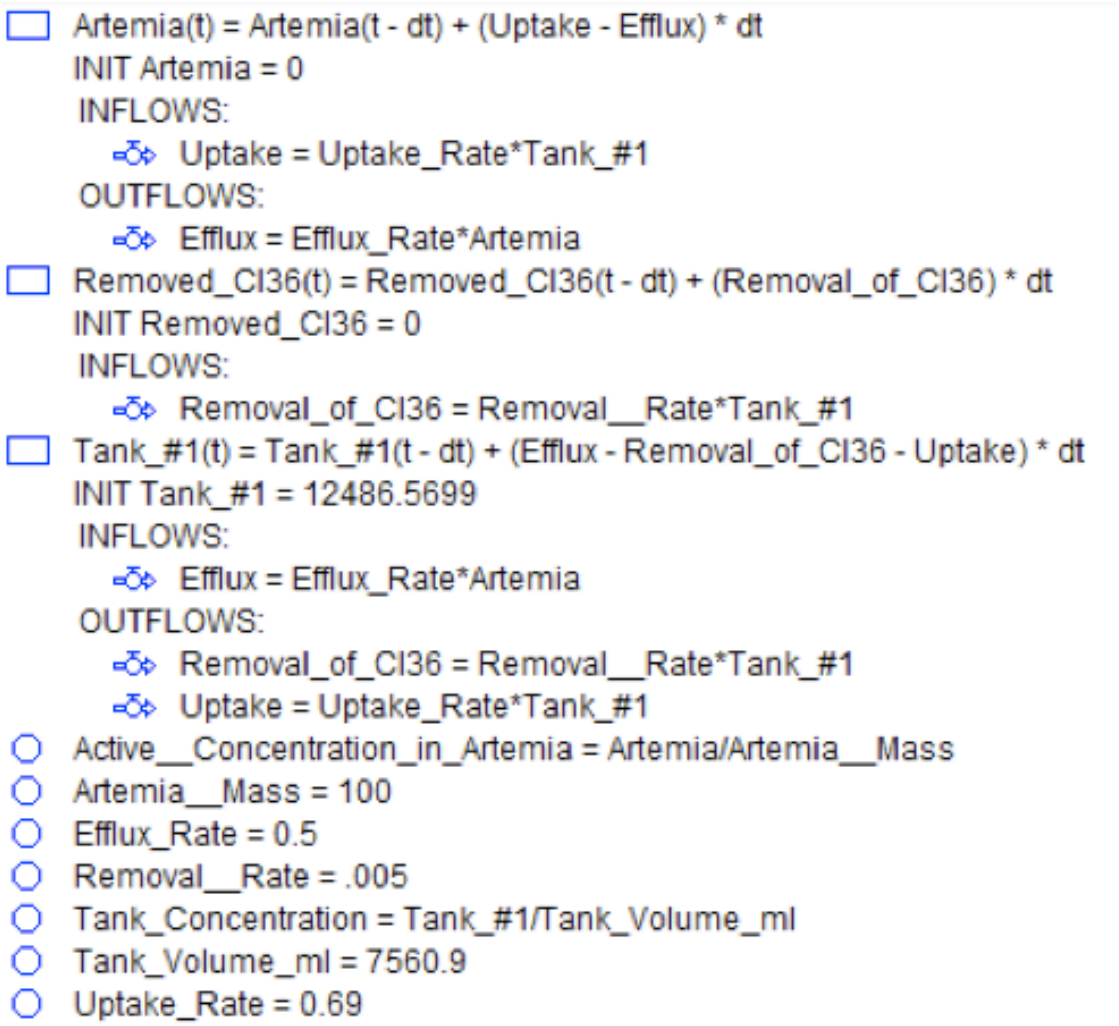


Figure 3: STELLA® equation: The predicted conceptual model activity in high ^{36}Cl concentration Artemia system unadjusted.

Figure 4: STELLA® equation: The predicted conceptual model activity in high ^{36}Cl concentration Artemia system adjusted.

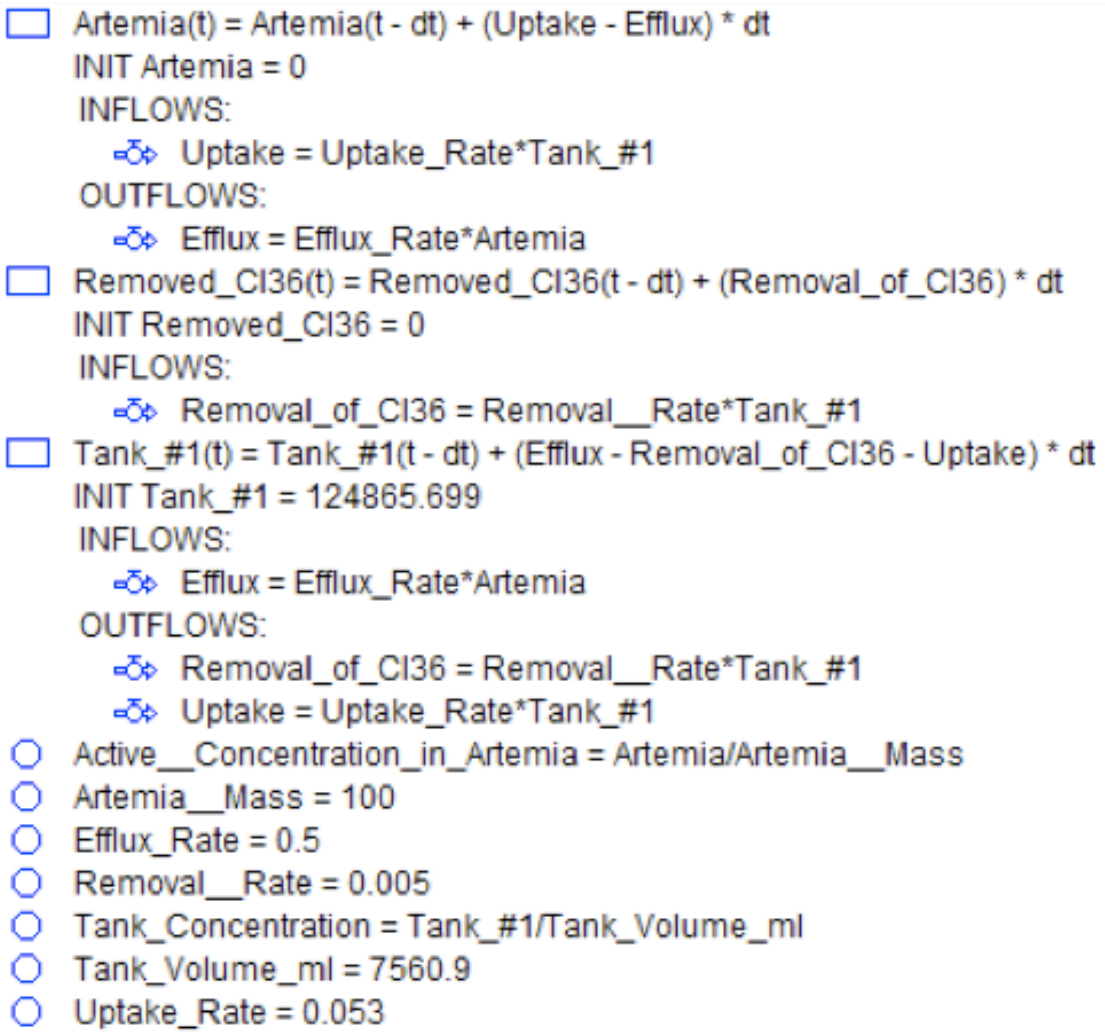


Figure 5: STELLA® equation: Unadjusted conceptual model low ^{36}Cl concentration system with oven.

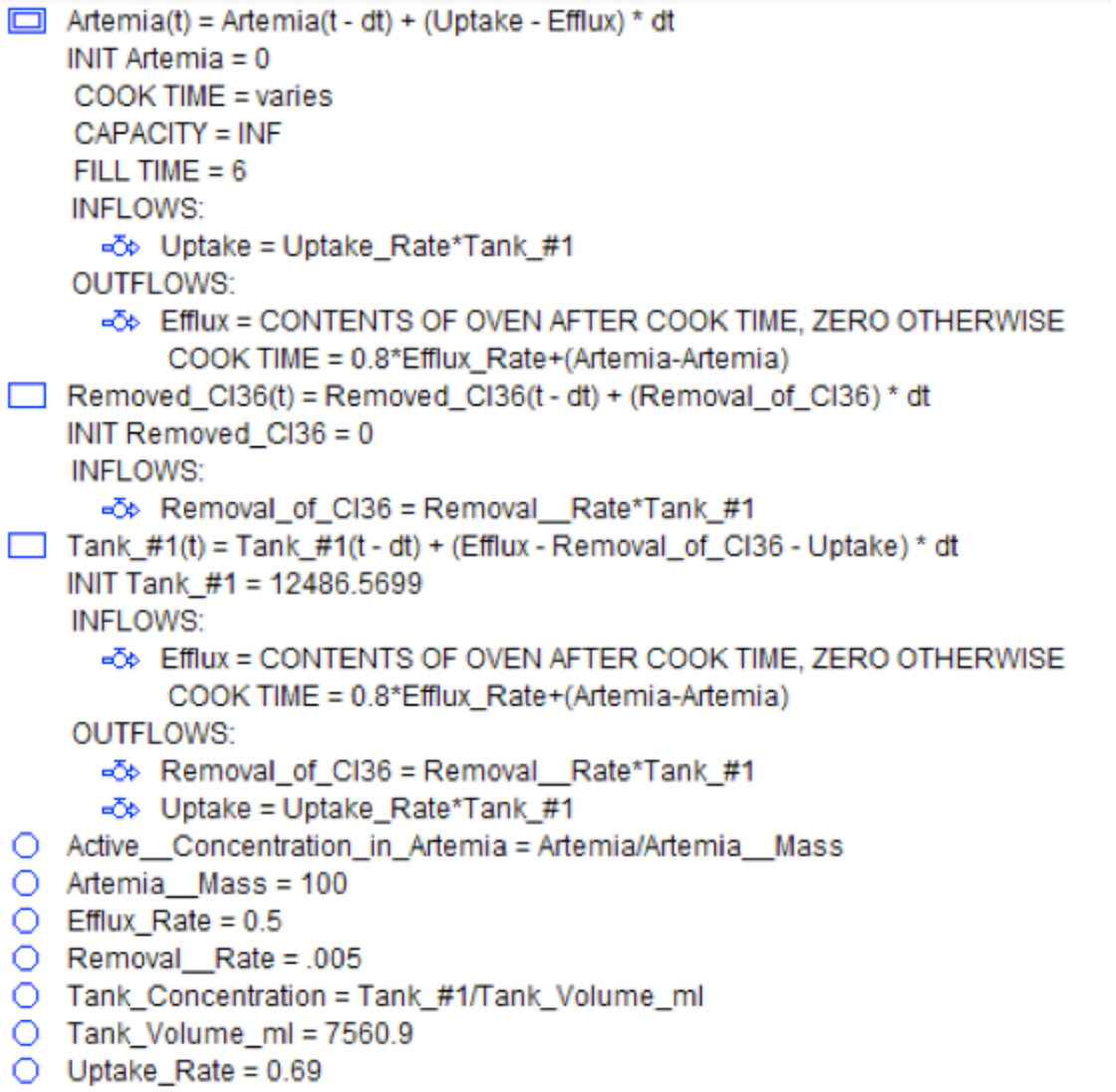


Figure 6: STELLA® equation: adjusted conceptual model low ^{36}Cl concentration system with oven.

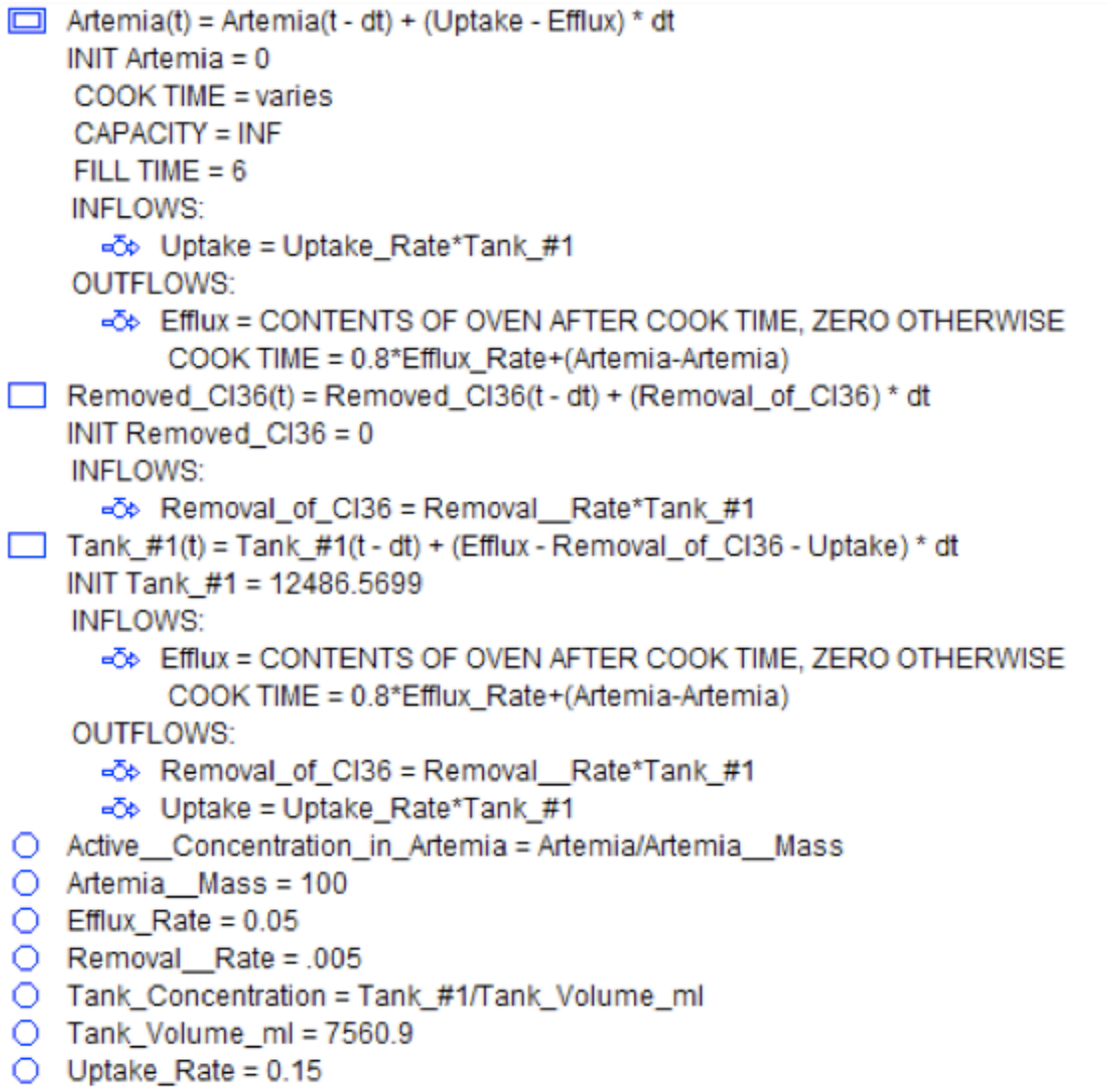
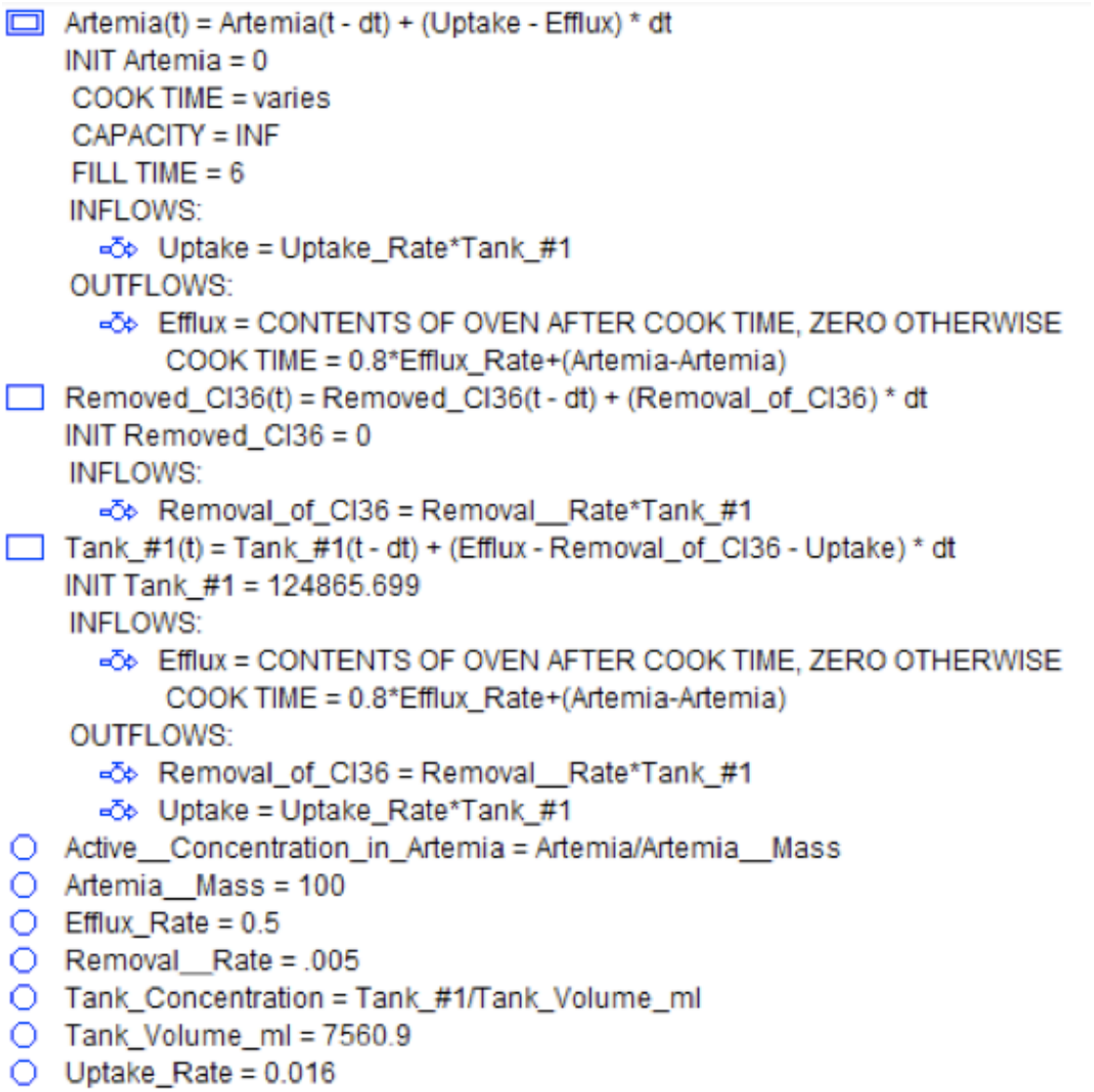


Figure 7: STELLA® equation: Unadjusted conceptual model high ^{36}Cl concentration system with oven.

Artemia(t) = Artemia(t - dt) + (Uptake - Efflux) * dt
INIT Artemia = 0
COOK TIME = varies
CAPACITY = INF
FILL TIME = 6
INFLOWS:
Uptake = Uptake_Rate*Tank_#1
OUTFLOWS:
Efflux = CONTENTS OF OVEN AFTER COOK TIME, ZERO OTHERWISE
COOK TIME = 0.8*Efflux_Rate+(Artemia-Artemia)
Removed_Cl36(t) = Removed_Cl36(t - dt) + (Removal_of_Cl36) * dt
INIT Removed_Cl36 = 0
INFLOWS:
Removal_of_Cl36 = Removal__Rate*Tank_#1
Tank_#1(t) = Tank_#1(t - dt) + (Efflux - Removal_of_Cl36 - Uptake) * dt
INIT Tank_#1 = 124865.699
INFLOWS:
Efflux = CONTENTS OF OVEN AFTER COOK TIME, ZERO OTHERWISE
COOK TIME = 0.8*Efflux_Rate+(Artemia-Artemia)
OUTFLOWS:
Removal_of_Cl36 = Removal__Rate*Tank_#1
Uptake = Uptake_Rate*Tank_#1
Active__Concentration_in_Artemia = Artemia/Artemia__Mass
Artemia__Mass = 100
Efflux_Rate = 0.5
Removal__Rate = 0.005
Tank_Concentration = Tank_#1/Tank_Volume_ml
Tank_Volume_ml = 7560.9
Uptake_Rate = 0.053

Figure 8: STELLA® equation: adjusted conceptual model high ^{36}Cl concentration system with oven.



Appendix B

Artemia Research Data Tables

Table 1: Controls removed prior to spiking the system

Controls:	B.S. (cps)	BKGD (cps)	B.S. Mass (g)	SA (Bq/g)	STD (+/-)
Tank #1	0.01	0.55	0.10	0.14	1.05
Tank #2	0.05	0.55	0.11	0.43	1.07

Table 2: Tank #1 solution samples

Day	#1 H2O (cps)	BKGD (cps)	SA (Bq/g)	STD (+/-)
0	0.93	0.55	0.92	1.43
1	0.57	0.55	0.56	1.29
2	1.31	0.56	1.29	1.55
3	0.97	0.51	0.95	1.41
4	1.29	0.54	1.27	1.54
5	0.87	0.52	0.86	1.38
6	1.83	0.52	1.80	1.70
7	2.11	0.54	2.08	1.79
8	2.21	0.55	2.18	1.82
9	0.64	0.53	0.63	1.30
10	1.56	0.50	1.54	1.60
11	0.72	0.53	0.71	1.34
12	1.74	0.52	1.71	1.66
13	0.42	0.55	0.41	1.23
14	0.45	0.53	0.45	1.23
15	1.14	0.56	1.13	1.50
16	2.88	0.53	2.84	1.98
17	1.70	0.55	1.68	1.68
18	2.02	0.51	1.99	1.75
19	1.07	0.53	1.05	1.46

Table 3: Tank #1 Artemia samples

Day	#1 B.S. (cps)	BKGD (cps)	Efficiency	B.S. Mass (g)	SA (Bq/g)	STD (+/-)
0	0.81	0.54	0.63	0.072	17.99	1.38
1	0.92	0.56	0.63	0.084	17.47	1.42
2	0.37	0.54	0.63	0.120	4.96	1.20
3	1.51	0.53	0.63	0.098	24.57	1.60
4	2.07	0.52	0.63	0.103	32.12	1.76
5	2.38	0.52	0.63	0.093	40.93	1.85
6	10.47	0.54	0.63	0.237	70.62	3.40
7	4.58	0.55	0.63	0.135	54.19	2.38
8	5.77	0.53	0.63	0.144	64.14	2.61
9	4.10	0.50	0.63	0.246	26.63	2.26
10	5.35	0.53	0.63	0.265	32.27	2.53
11	5.80	0.52	0.63	0.236	39.26	2.61
12	5.44	0.55	0.63	0.156	55.75	2.56
13	4.07	0.53	0.63	0.207	31.45	2.27
14	4.84	0.56	0.63	0.379	20.43	2.44
15	5.06	0.53	0.63	0.254	31.82	2.47
16	3.37	0.55	0.63	0.457	11.78	2.12
17	3.15	0.51	0.63	0.346	14.57	2.04
18	3.76	0.53	0.63	0.373	16.13	2.20
19	5.57	0.52	0.63	0.227	39.23	2.57

Table 4: Tank #2 Solution samples

Day	#2 H2O (cps)	BKGD (cps)	SA (Bq/g)	STD (+/-)
0	11.80	0.55	11.64	3.59
1	5.11	0.55	5.04	2.49
2	5.49	0.56	5.41	2.57
3	9.10	0.51	8.98	3.18
4	12.07	0.54	11.91	3.63
5	12.65	0.52	12.48	3.70
6	13.00	0.52	12.82	3.75
7	14.38	0.54	14.18	3.93
8	21.81	0.55	21.51	4.79
9	13.98	0.53	13.79	3.88
10	11.03	0.50	10.88	3.47
11	6.25	0.53	6.16	2.70
12	9.46	0.52	9.33	3.24
13	5.32	0.55	5.25	2.53
14	13.14	0.53	12.96	3.77
15	15.68	0.56	15.46	4.10
16	26.56	0.53	26.19	5.25
17	12.14	0.55	11.98	3.64
18	11.78	0.51	11.62	3.58
19	7.40	0.53	7.30	2.91

Table 5: Tank #2 Artemia samples

Day	#2 B.S. (cps)	BKGD (cps)	Efficiency	B.S. Mass (g)	SA (Bq/g)	STD (+/-)
0	1.07	0.54	0.63	0.024	71.41	1.47
1	1.79	0.56	0.63	0.116	24.66	1.70
2	1.48	0.54	0.63	0.170	13.89	1.60
3	1.99	0.53	0.63	0.097	32.87	1.75
4	6.40	0.52	0.63	0.156	65.56	2.73
5	8.54	0.52	0.63	0.207	65.93	3.10
6	14.38	0.54	0.63	0.256	89.78	3.93
7	7.53	0.55	0.63	0.256	47.00	2.94
8	6.65	0.53	0.63	0.095	111.89	2.78
9	4.93	0.50	0.63	0.125	63.03	2.44
10	4.42	0.53	0.63	0.115	61.47	2.34
11	10.38	0.52	0.63	0.313	53.01	3.38
12	3.84	0.55	0.63	0.072	85.36	2.22
13	4.62	0.53	0.63	0.066	111.97	2.38
14	5.86	0.56	0.63	0.149	62.91	2.64
15	9.79	0.53	0.63	0.258	60.64	3.29
16	9.82	0.55	0.63	0.523	30.03	3.31
17	6.94	0.51	0.63	0.102	108.83	2.82
18	8.47	0.53	0.63	0.405	33.44	3.09
19	7.01	0.52	0.63	0.118	94.95	2.84

Table 6: Cooling Loop solution samples

Day	C.L. Water (cps)	BKGD (cps)	SA (Bq/g)	STD (+/-)
0	0.04	0.55	0.58	0.14
1	-0.02	0.55	0.52	0.13
2	-0.04	0.56	0.51	0.13
3	0.01	0.51	0.52	0.13
4	0.02	0.54	0.55	0.14
5	-0.02	0.52	0.49	0.13
6	0.05	0.52	0.56	0.14
7	0.01	0.54	0.55	0.14
8	0.02	0.55	0.56	0.14
9	0.03	0.53	0.55	0.13
10	0.04	0.50	0.53	0.13
11	0.01	0.53	0.53	0.13
12	-0.01	0.52	0.50	0.13
13	-0.01	0.55	0.54	0.14
14	-0.01	0.56	0.55	0.14
15	0.05	0.53	0.57	0.14
16	0.02	0.55	0.57	0.14
17	0.03	0.51	0.53	0.13
18	0.00	0.53	0.52	0.13
19	0.07	0.53	0.59	0.14