SIMULATION OF THE PHYSIOLOGICALLY-BASED PHARMACOKINETIC DISTRIBUTION AND RETENTION OF URANIUM IN RATS

A Thesis

by

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ABSTRACT

The objective of this research project was to create a computer simulation in SIMULINK® to model the distribution and retention of uranium in rats using a compilation of published experimental data that has been acquired over the years by different research groups. This program was created using a general acute baseline model with predefined parameters to account for uranium distribution in rats. The computer simulation response to an ingestion of exogenous uranium material within a rat and the growth in organ size with respect to the age of rat was composed in SIMULINK®.

The results of the baseline model simulation were benchmarked against a research experiment that examined distribution and retention of uranium in rats. The simulation program was tested using various input methods to evaluate the change in program response. The simulation program and implemented methodology indicates that the SIMULINK® program is a user friendly program that allows researchers to customize compartmental functions. Simulation results indicate that a biological experiment that lacks a patterned response is difficult to model in a simulation program with predefined parameters. Using the original baseline acute model simulation results for uranium, the predefined parameters were tested in this research and indicate that a modification to the predefined parameters is essential to properly model biological responses.

A reformulation of compartmental biological parameters such as the removal half-time and/or deposition fraction into various compartments is proposed based on the simulation results. This change to predefined parameters is not excluded by the published data. The simulation results also indicate specific sections of organ response that require additional investigation for effective models. As more research data becomes available, the program can be modified to improve upon this simulation model.

DEDICATION

This is dedicated to my family.

ACKNOWLEDGEMENTS

I would like to thank my committee chair, Dr. John Ford, for his continued patience and guidance throughout my graduate work. Your teaching effort is a value to all students within the Nuclear Engineering department and to any student that has you as an advisor/mentor. Your continued assistance is an asset to the students and the University. You have given me this opportunity that makes me forever grateful and thankful. I would also like to thank my two additional committee members, Dr. John Poston and Dr. Nancy D. Turner, whom have been very helpful and considerate. I truly appreciate your understanding with everything. I am grateful and honored to have all of you on my committee. Thanks also to everyone within the Nuclear Engineering department and to Texas A&M University.

I would like to thank Lippincott, Williams & Wilkins for their approval to use their published data from Paquet et al. 2006, which is referenced in this manuscript.

I would also like to thank my family that has been the support system that gives me the strength to continue. I thank my mom, Margarita Alvarez, and brother, Roy Alvarez, for their continued love and support over the years. I would also like to thank my late father, Roy C. Alvarez, who will always be present in the work that I accomplish.

NOMENCLATURE

A	Amount of Uranium
BBB	Blood Brain Barrier
CINDY®	Computer-based INternal DosimetrY system
CR	Clearance Rate
F	Deposition Fraction
GI	Gastrointestinal Tract
GMM	General Michaelis Menten
I	Intake
ICRP	International Commission on Radiological Protection
IRF	Intake Retention Fraction
PBPK	Physiologically Based Pharmacokinetic
RBC	Red Blood Cells
SAAM II®	Simulation, Analysis and Modeling Software for Kinetic Analysis
SD	Sprague Dawley
SIMULINK®	MATLAB® Computer Component
ST0	Soft Tissues (Rapid Turnover)
ST1	Soft Tissues (Intermediate Turnover)
ST2	Soft Tissues (Slow Turnover)
SW	Skeletal Weight
TC	Transfer Coefficient

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CHAPTER I

INTRODUCTION

The application of radiation and its effects has become a benefit to many, from the medical field to the industrial field. These benefits have also become a burden due to the risks that can occur from exposure to radioactive materials. With the risks from radiation comes the responsibility to quantify, to the extent possible, effects that radiation has on people and the environment. The radioactive material, uranium, has been a cause for concern over the years due to the required use of this material within the nuclear industry and the natural occurrence of this material in nature. Uranium, the heaviest naturally occurring element, is a ubiquitous soil component (Sztajnkrycer 2004), a product found in groundwater and surface water around the world, and a nuclear cycle byproduct. The continued interaction with uranium by occupational workers and by the public supports continued research of this material to refine risk assessments associated with this element.

Biological research is the key to understanding any risks associated with a substance, and there are many published documents that have reviewed the effects of uranium on a biological system. A biological system that is used throughout research is the rat as this species can lead to interspecies scaling and risk assessments for humans. Computer models are being created for a species to examine the radiological effects on the biological system. If a computer simulation program can model the behavior of radionuclides in a rat, the risk assessments can be used for other species, such as

humans. A computer program for the rat species must first be created to model the behavior of a specific radionuclide, such as uranium. Paquet et al. (2006) created a computer model and attempted to match their rat biological research data using this computer program: the simulation, analysis, and modeling software, SAAM II®. This program uses graphical interfaces that are considered user-friendly, so human physiological models are incorporated into this program to address radiation exposure incidents (Callegari et al. 2002). Another program that has been used for internal dose calculations in humans is the computer-based internal dosimetry system, CINDY®. This program calculates bioassay and dose information for fifteen biokinetic models that can be tailored to a specific subject; however, this program is not specific to rats and has not been utilized for rat system parameters. Even though there are programs that have been utilized in the past for various assessments, there are very few models specific to rat parameters. Paquet et al. (2006) could not completely model their biological data using their computer program in SAAM II®; therefore, this research has identified a gap to assess. Since rats are a primary animal model for biological research, including research specific to radioactive materials, the absence of a computer model that can simulate biological data is a gap that is addressed by this research.

This research attempts to close this gap and use available, published data to create a simulation model that can be used as a reference for biological parameters. This research examines the utilization of commercially available software, SIMULINK®, to simulate uranium exposure within a rat biological system using a predefined baseline model that has been adapted for the rat. Previous researchers have utilized this baseline

model and predefined parameters; however, this simulation attempts to expand upon the program model by incorporating the rat organ growth with respect to rat age progression, which can affect material disposition. The simulation results did not match all the biological data; however, the simulation tested new configurations that can lead to improved models.

CHAPTER II

BACKGROUND

Physiologically-based pharmacokinetic (PBPK) models have been utilized by researchers as these models identify the behavior for certain chemicals that have been evaluated for different species, such as rats. This behavior explains the transfer, distribution, and retention of a chemical within a species. Understanding the pharmacokinetic behavior of a substance allows for an accurate description of the distribution and disposition of a substance over time within a species. There is continued research for detailing PBPK models for drugs in humans, which has led to the creation of generic biokinetic models (Taylor 2000). Just as drugs can be monitored when introduced into a biological system, researchers have started to apply PBPK modeling functions for the representation of radioactive materials to "describe the behavior of the radionuclide until it has either been totally eliminated from the body, or until the radioactivity has completely decayed" (Taylor 2000).

A baseline biokinetic model with predefined deposition and clearance rates for rats was adapted for uranium by Leggett and Pellmar in 2003, which was utilized in this manuscript (Leggett et al. 2003). This research group created a model by combining the International Commission on Radiological Protection (ICRP) physiological model for uranium in humans and the published data for commonly studied forms of uranium in rats. This model was based upon research for injected, inhaled, or ingested forms of uranium; therefore, this model was chosen as part of this research project as this is the

only available model for rats. One possible limitation for the use of this model is that this "reference model is based on knowledge of the behavior of uranium in rats after acute exposure" (Paquet et al. 2006).

We examine the compatibility of this acute baseline model using a biological research experiment for a chronic exposure to test this model as a benchmark. Paquet et al. (2006) documented uranium retention within several biological compartments; therefore, their published biological data allowed for a thorough review of major compartments for a rat system. A compilation of research studies evaluating various uranium exposures were utilized to create the initial rat baseline model that is unlike a detailed PBPK model, which "divides the body into anatomically and physiologically meaningful compartments" (Lave et al. 2007). The rat model uses physiological and species-specific parameters to detail the absorption from the gastrointestinal tract and the elimination from the major compartments such as kidney and liver; however, it fails to account for non-eliminating tissues that may be affected (Lave et al. 2007). The limitations of this model are addressed within this report to evaluate the potential of this model as a comparative tool.

CHAPTER III

BASELINE MODELING METHODS

Uranium Biokinetic Model: Biokinetic information was integrated into the simulation program using several published documents that examined the use of exogenous uranium in a rat (Paquet et al. 2006 and Leggett and Pellmar et al. 1999). An evaluation of age progression for rats, the change in organ/tissue size, and the change in skeletal structure was also studied (Mirfazaelian et al. 2007 and Brown et al. 1994). Age progression functions were incorporated in the program to assess the model expansion and to accurately compare the simulation results with the biological data. Roth et al. (2001) state that the "chemical and thus the biological behavior of uranium is the same for all isotopes"; therefore, only a simple model is used for all common isotopes of uranium. If the simulation uranium retention results are comparable to the biological data, this simulation can be adapted to other types of uranium exposure as the biological behavior is considered the same for all isotopes.

Leggett and Pellmar Baseline Model: The baseline model adapted by Leggett and Pellmar (2003) and referenced in this research is shown in Fig. 1. The excretion paths for feces and urine that can be used for bioassays are shown as circles. Bioassays can be used to evaluate the retention of uranium in a system. The arrows identify the entrance and exit routes from one biological compartment to another.

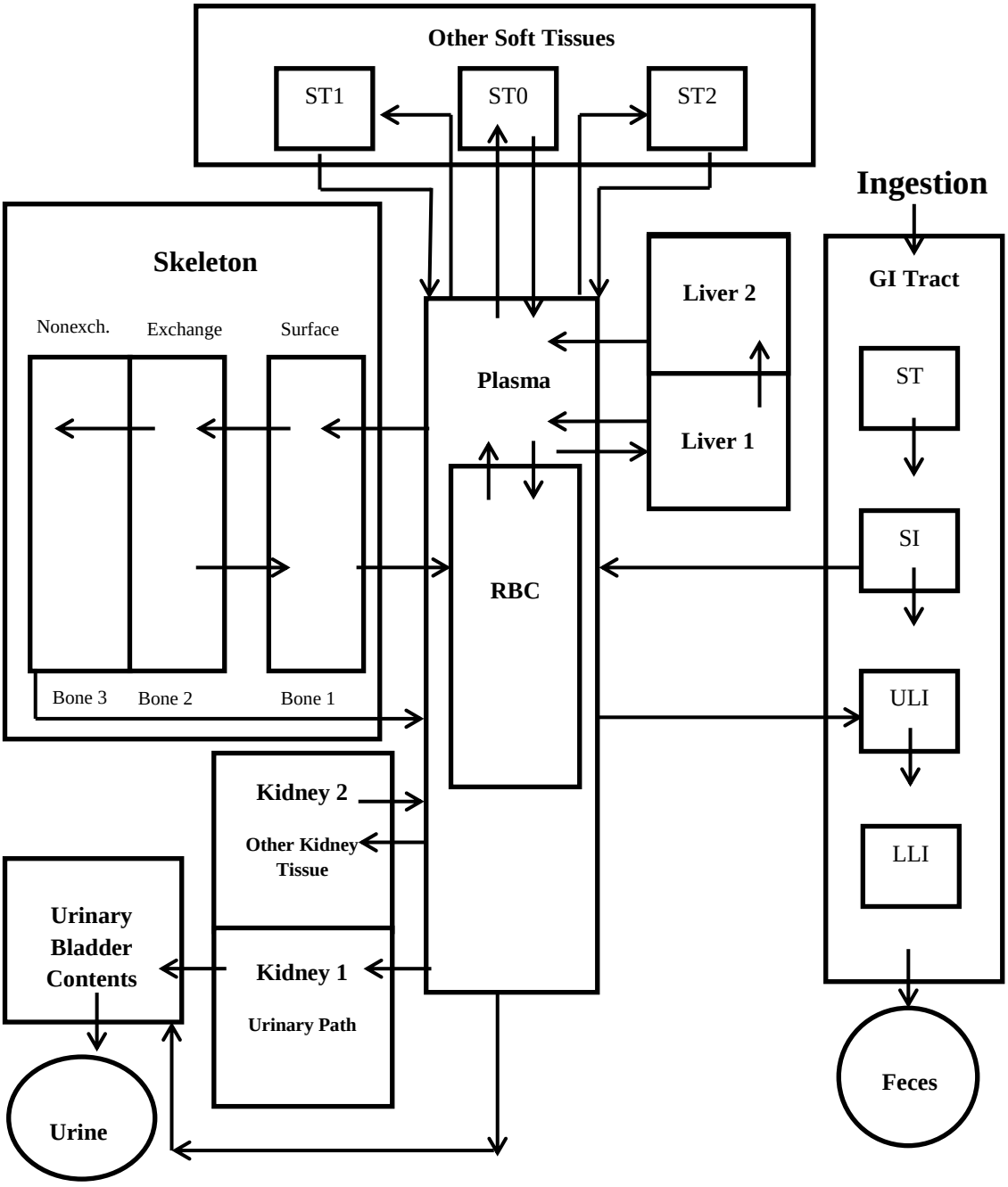

Fig. 1. Uranium Biokinetic Model for Rats

These arrows are considered to have a transfer coefficient/rate coefficient that accounts for the deposition fractions in the compartments and the biological half-time/removal half-time. The baseline transfer rate coefficients from Leggett and Pellmar (2003) and referenced in Paquet et al. (2006) have been placed in Appendix A for reference. These parameters were incorporated into the program. The program is user-friendly; therefore, the subsystems within the program can be accessed easily and modified as more research data becomes available.

In the SIMULINK® model, the biological half-time/removal half-time was calculated using Eqn (1)

$$\textit{Biological half-time } (t_{1/2}) = \frac{\ln 2}{TC} \qquad (1)$$

where *ln 2* is the natural logarithm of 2 and *TC* is the transfer coefficient. The radiological half-time is ignored in the program because uranium has a very long half-life. Sztajnkrycer also states that the principal toxicological effects come from the properties of uranium as a heavy metal rather than the radiological properties; therefore only the biological half-time is applied in the simulation (Sztajnkrycer 2004). The transfer coefficients (TC) are a key component for any simulation. The transfer coefficients "are dependent on the type, amount, and chemical characteristics of the form of the contaminant and on the animal species, sex, age" (Arruda-Neto et al. 2001 and 2004). The transfer coefficients for the Leggett and Pellmar (2003) model were obtained using a review of several animal experiments. The transfer coefficients were calculated assuming no recycling occurred, which accounts for a smaller numerical value than a

real-life biological system that contains continual recycling (Leggett and Pellmar 2003). These transfer coefficients were implemented into the simulation; however, recycling was added to the program for an initial baseline analysis.

Another factor to consider for the simulation analysis is the original creation of the baseline model as this reference model is based on acute exposure data. The direction that was taken by Leggett and Pellmar (2003) to create this baseline model is of great significance to this research since the current simulation is dependent upon their adapted model and this is the only comparative tool currently available for a chronic biological uranium exposure.

ICRP Dose Model and Compartment Review: The Leggett and Pellmar (2003) baseline model was adapted from the International Commission on Radiological Protection (ICRP) human model for uranium. This simulation incorporates the ICRP modeling throughout the compartments. The ICRP dosimetric model for the human gastrointestinal (GI) tract follows first order kinetics; therefore, the rat model follows a similar pattern.

The biological research was based on a chronic ingestion, so the model was created with the main input function within the GI subsystem. The GI subsystem has four sub-compartments: stomach, small intestine, upper large intestine, and lower large intestine. The stomach has the functions that are tested in this research and explained within this manuscript. The small intestine has two transfer routes in the physiological model. The small intestine transfers to the upper large intestine and is the site of

absorption into the blood/plasma. This transfer rate to the blood/plasma affects the other tissue compartments.

The ICRP 30 model states that the absorption to the blood is dependent upon f_1, the fractional absorption coefficient. The uranium absorbed into the blood/plasma through the ingestion process is circulated throughout the system to reach other organs at a specified rate of 200 $days^{-1}$. The transfer coefficients for the uranium model are expressed in $days^{-1}$, which is the transfer per day (Leggett and Pellmar 2003). The uranium that enters into the plasma is assumed to leave at two very different rates. There is a rapid phase and a slower phase of recycling (Leggett and Pellmar 2003). Leggett and Pellmar state that 30% of uranium that enters the blood is assumed to interact with the rapid turnover (ST0) soft tissue compartment. The uranium is assumed to enter the soft tissue compartment within sixteen minutes and return back to the plasma compartment within approximately two hours for continual recycling. When compared to the human model, the time for clearance to the rapid turnover soft tissue compartment and then back to the blood circulation is approximately 3½ hours. The rapid phase compartment in the rat is quicker than one would find in a human model because there is a high rate of kidney filtration. The remaining 70% of uranium in the plasma is assumed to circulate to the remaining sections of the model. Leggett and Pellmar view this 70% as the actual amount of uranium leaving the circulation system.

Equations (2) through (5) illustrate the calculation breakdown for removal half-time that were incorporated into the simulation for uranium transfer

$$\text{Uranium elimination from plasma} = 200 \text{ days}^{-1} \quad (2)$$

$$\text{Rapid phase} = (0.30 \times 200 \text{ days}^{-1}) = 60 \text{ days}^{-1} \quad (3)$$

$$\text{Slower phase} = (0.70 \times 200 \text{ days}^{-1}) = 140 \text{ days}^{-1} \quad (4)$$

$$\text{Remaining compartments } TC = (F \times 140 \text{ days}^{-1}) \quad (5)$$

where *TC* is the transfer coefficient for uranium and *F* is the deposition fraction of uranium into organ/tissue compartments. The slower phase is distributed to all compartments except for the rapid turnover soft tissue compartment, which has already received 30% of the initial uranium amount and considered part of the circulation system. The percentage of uranium distributed to the remaining organs within the baseline simulation model is detailed in Table 1, which is adapted from Leggett and Pellmar (2003). Fifty percent (50.0%) of the uranium that enters into the circulation reaches the urinary bladder within 14 minutes and then transfers to the urine after 16 hours.

Table 1. Biokinetic Compartment Distribution for Uranium in Blood

Compartment Identification	**Uranium Percentage (%)**
Urinary Bladder Contents	50.0
Bone 1	20.0
Kidney 1	20.0
Upper Large Intestines	5.0
ST1	2.50
ST2	1.0
Liver 1	0.80
Kidney 2	0.50
Red Blood Cells	0.20

Bone 1, a retention compartment, receives 20.0% of the uranium content in the blood (Leggett and Pellmar 2003). Bone 1, the surface compartment, transfers uranium to Bone 2, and this uranium is moved further into the skeletal compartment. Bone 1 also loses uranium back to the blood/plasma. The removal rate constants for the Bone 1 are 10 days for both transfer routes. Bone 2 is identified as the exchange section that recycles uranium back to the surface section, which ultimately empties back into the plasma/blood system. Bone 2 also transfers uranium to Bone 3 with a removal rate constant of 119 days. The Bone 3 compartment, although identified as the non-exchange portion of the system in the baseline model, translocates uranium to the plasma. All sections of the skeletal compartment are returning uranium to the plasma for recycling, even though there is one section identified as non-exchange. Bone 3 is releasing uranium back to bloodstream with a removal rate constant of 231 days.

The kidney is considered a target organ for uranium. The kidney compartment received 20.0% of the uranium deposition (Leggett and Pellmar 2003). The kidney subsystem is divided into two compartments, Kidney 1 and Kidney 2. Kidney 1 is the short-term compartment that transfers the uranium to the urinary bladder within 3.99 days. The amount of uranium transferring to the urinary bladder contents through the kidney is minimal compared to the amount of uranium transferring directly to the urinary bladder contents in 0.0099 days from the plasma. Kidney 2 is viewed as a storage compartment that interacts with the urinary bladder, but this section recycles the uranium back to the circulating system. Even though there is uranium in the Kidney 2 compartment, the percentage from the blood is minimal at approximately 0.50%

(Leggett and Pellmar 2003). The kidney is a key component for the excretion of uranium from the body.

The transfer of uranium to the upper large intestines accounts for 5.0% of circulation distribution. There currently is a minimum amount of data for endogenous fecal secretion from the bile duct (Leggett and Pellmar 2003, adapted from Neuman 1948). The 5.0% of uranium transfer from the circulation to the gastrointestinal tract is an assumption with minimal experimental verification and is based on the percentage of uranium that transfers to other compartments.

Several compartments combined together account for the remaining uranium distribution. Of the 5.0% of uranium remaining, the soft tissue compartments, ST1 and ST2, account for 3.5% (Leggett and Pellmar 2003), which is divided and shown in Table 1. These soft tissue compartments maintain the slow and intermediate turnover of uranium for soft tissues other than kidneys and liver. This section includes all organs that are often ignored in research as these organs are lumped together. Due to the minimal research for the additional soft tissues, all organs are placed in both compartments, ST1 and ST2, for initial simulation evaluation and identification as an ST1 or ST2 compartment.

The liver compartment receives an initial distribution of 0.80% (Leggett and Pellmar 2003). The uranium amount that enters initially into compartment one of the liver is redistributed. Three percent (3%) of the initial liver amount enters Liver 2 over a period of 233 days and the remaining 97.0% recycles back to the blood within approximately one week (Leggett and Pellmar 2003).

The red blood cells compartment receives the smallest percentage of uranium that is circulating throughout the blood, only 0.20% (Leggett and Pellmar 2003). The biological half-life of uranium for the RBC is approximately one day; therefore, most of the uranium that enters exiting quickly. This compartment is providing uranium back to the circulation compartment, which is considered the recycling center for the program.

Model Compartments: The compartments created in the SIMULINK® simulation system follow first order kinetics throughout each compartment to calculate the elimination and retention rates. The simulation structure was separated into several major compartments: liver, skeleton, soft tissues, kidneys, plasma and gastrointestinal tract. The SIMULINK® model uses first order kinetics similar to what is found in the ICRP 30 publication (ICRP 1979). Equations (6) and (7) account for the amount of uranium lost over time in addition to the input from previous compartments

$$\frac{dA(t)}{dt} = Intake - \lambda_{clearance1}[A(t)] \quad (6)$$

$$\frac{dA(t)}{dt} = -\lambda_{clearance2}[A(t)] - \lambda_{clearance3}[A(t)] + \lambda_{clearance1}[A(t)] \quad (7)$$

where $A(t)$ is the amount of uranium at specific time (t), dt is the change in time (seconds), and $\lambda_{clearance}$ is the clearance constant calculated from the transfer coefficient.

This baseline modeling was used as an initial starting point for this simulation. There are predefined parameters that are reviewed within the simulation results, which include these predefined model compartments. Understanding the baseline model is important for a thorough review and understanding of the simulation model.

CHAPTER IV

SIMULINK® MODELING METHODS

Introduction: The experiments of Paquet et al. (2006) were utilized as a benchmark for our modified simulation model since this group documented several factors for uranium analysis, such as uranium ingestion amounts and final uranium distribution within rats. The uranium analysis and distribution accounted for the retention organs that are routinely evaluated for uranium as well as soft tissue organs that are often ignored. The work by Paquet et al. (2006) also included data required for the simulation analysis such as initial body and organ weight references to calculate retention quantities. This would allow for research expansion to include organ/tissue weight progression. The published research of Paquet et al. (2006) omitted some critical data for this simulation, so we obtained this information from other sources. For example, Paquet et al. (2006) provided their retention/distribution in ng/gram; however, the exact weights for the organs were not provided. Fortunately, there is a wide variety of alternate sources for the various biological parameters of a rat that are necessary for this simulation. This research attempted to obtain data specific to Sprague Dawley rats since these are the rats that were used by Paquet et al. (2006); however, additional strains of rat were utilized to identify data that was not available for the Sprague Dawley rat. Brown et al. (1994) compiled data, such as organ weights, which have been used as a reference within this simulation. This group has created a reference manual for PBPK modeling that can be utilized when some data from one experiment are not gathered or

omitted from final publication (Brown et al. 1994). This approach was taken as the intention was to produce a model for the distribution and retention of uranium in rats, which could be scaled to other species. The Brown et al. 1994 data was based on the mean values for experimental research studies for the rat.

***SIMULINK®*:** The software utilized in this research is an extension of the computer software package, MATLAB®, and is time-dependent (Wada et al. 1995). SIMULINK® utilizes a graphics interface as well as block diagrams to model the response that would occur within a biological system (Connolly et al. 2000). The SIMULINK® program was utilized to construct biologically-based simulation models to calculate solutions to differential equations that mathematically represent the distribution and retention of exogenous material. The program visually documents the execution of the simulation and the progress of the program that allows the user to break down the entire biological system. With the visual component comes the advantage of debugging possible discrepancies that might occur within a program (Dabney and Harman 2001).

Paquet et al. (2006) adapted their biokinetic data from the previous work of Leggett and Pellmar (2003). The model we have developed also attempts to validate the feasibility of the Leggett and Pellmar (2003) acute exposure model for chronic exposures using SIMULINK®. This research evaluates whether a rat reference model can be used as a comparative tool with biological research and whether additional sections of the program and reference model must be evaluated further. Paquet et al. (2006) attempted to create a computer model using their biological data and this same rat

reference model. Their program results overestimated the accumulation of uranium by more than one order of magnitude (Paquet et al. 2006).

In the Paquet et al. (2006) study, the uranium was consistently given via mineral water in the amount of 0.97 $\pm$ 0.15 mg uranium/(animal×day) to male Sprague Dawley (SD) rats. This amount was calculated using the final concentration of uranium remaining in the bottle, which was 41.3 $\pm$ 1.5 mg uranium/liter, and the consumption of 23.7 $\pm$ 1.7 mL/day. Different groups of rats were examined at days 32, 95, 186, 312, 368, and 570. On each of the specified days, the animals were euthanized and the organs were analyzed for uranium content. Even though published literature states that the kidneys and bone are the main target organs for uranium accumulation, uranium concentrations for several other organs were determined. This additional organ analysis provides data for a more complete simulation. There was constant consumption of water that occurred for the first 368 days. This constant 0.97 mg uranium/day was chosen as the initial modeling for this research, which equaled the amount of uranium that was utilized in the biological research by Paquet et al. (2006). The researchers documented an average beginning weight of 328 $\pm$ 17 grams that increased to a stable weight of 618 $\pm$ 17 grams for the male rats by day 368. This information was used to calculate an approximate age of the rats, from which specific organ weights were calculated within the program using an organ weight function that is dependent on age. Appendix B documents the age and weight functions that were incorporated into the simulation.

Simulation Main Page (Rat Strain Choice): The final SIMULINK® model was expanded further to provide the user an additional choice, which is shown in Fig. 2.

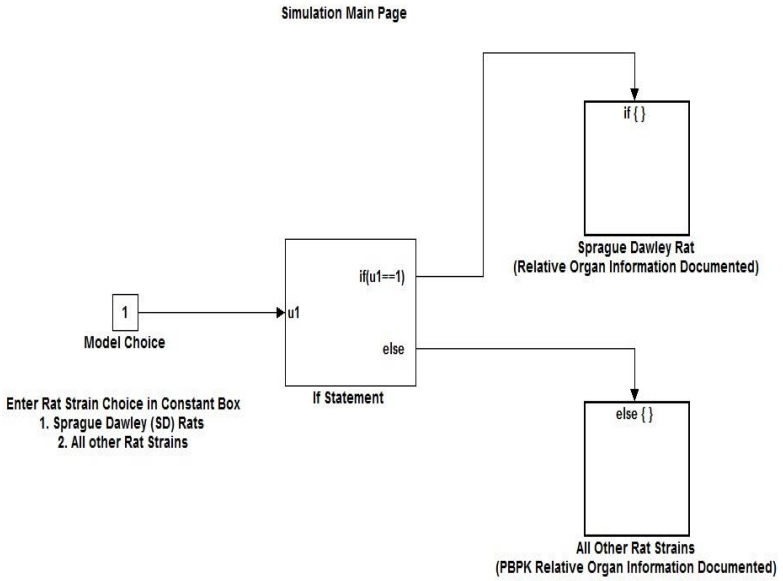

Fig. 2. Simulation Main Page for Rat Strain Choice

This figure illustrates the two options for the user based upon rat strain, which allows the program user to choose the main type of rat strain that is utilized for the comparative evaluation. The first option incorporates the Sprague Dawley (SD) rat data obtained from the Mirfazaelian et al. (2007) research group. Mirfazaelian et al. (2007) measured maturing organ growth in the SD rat strain, and their data is used in this research. The second option for the program user follows the same simulation format as the Sprague Dawley (SD) simulation; however, this second option was formatted with general parameters obtained from Brown et al (1994). The Brown et al. (1994) option references information that has been compiled using several types of rat strains, both sexes, and various age groups. The range of information that was used allows for a broad range of possibilities with this simulation choice.

Although we concentrated our research on using mainly SD data, there were data from other rat strains that had to be incorporated into the simulation to fill gaps where they existed. The organ weights not referenced in Mirfazaelian et al (2007) were calculated using a percentage of whole body weight or using other published research data explained in this manuscript. As data becomes available, the simulation main page can be modified to incorporate additional variables, such as the sex of the rat.

Uranium Biokinetic Display in SIMULINK®: Fig. 3 represents the rat organs/tissues that were compartmentalized as described in the original biokinetic model. The top three horizontally placed boxes represent the soft tissues. The soft tissues were assumed to undergo all forms of turnover, but this can be adjusted as data becomes available for more accurate turnover rates. Leggett and Pellmar (2003) stated that an organ that was not explicitly addressed in the model was derived by assuming a uniform distribution of uranium. This was the approach we followed for the intermediate (ST1) and slow (ST2) soft tissue compartments. The central subsystem in the middle of the program diagram represents the total circulation for the rat and contains the recycling inputs from the other compartments. The subsystem below the soft tissues and at the top left corner of the circulatory system represents the rat skeletal system, which is divided further inside into Bone 1, Bone 2, and Bone 3. The simulation section for the kidneys is found directly below the skeletal system followed by a compartment that represents the urinary bladder contents. The subsystem at the far right of the figure represents the gastrointestinal tract. The gastrointestinal tract compartment has additional sub-compartments.

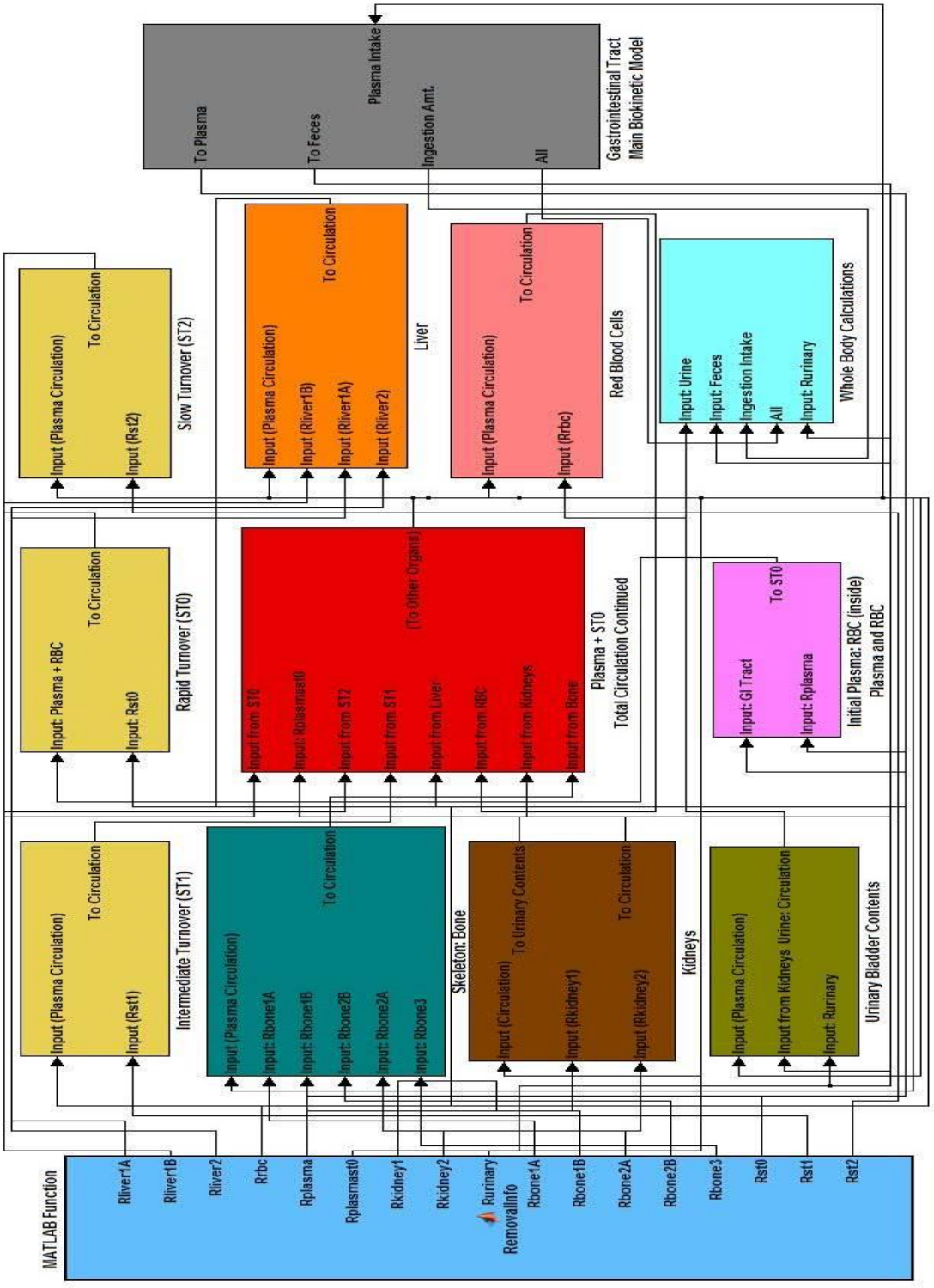

Fig. 3. Uranium Biokinetic Display in SIMULINK®

The compartments for the liver and the red blood cells are placed in between the GI tract and circulation system. The final two compartments at the bottom of the figure were not part of the baseline model but were included in our simulation for whole body retention and circulation calculations. The subsystem, identified as the "initial plasma: RBC" in Fig. 3 accounts for the very fast transfer from the GI tract to the rapid turnover section. Then, the uranium goes to the circulation system that transports the uranium to all the organs modeled in the simulation. The left-most block in the diagram represents a MATLAB® function with equations for the transfer coefficients required for the uranium calculations.

Gastrointestinal Tract (GI) Compartment: The GI subsystems are found in Fig. 4. There are four subsystems that represent the stomach, small intestine, upper large intestine, and lower large intestine. The elimination rates calculated using Eqns (2) through (5) were placed within another function box. The right side of the gastrointestinal compartment model is used to calculate the change in organ size of the GI tract in a rat over time. The digestive tract is the first major system exposed to the radionuclide as the route of entry is ingestion. The GI tract has substantial importance as shown in Fig. 3. There are two major transfers for the GI system, one to the external release section through the fecal matter and one absorption transfer from the GI tract to the blood. The GI tract has specific clearance parameters that were implemented into the simulation; however, the biological system continues to be developed.

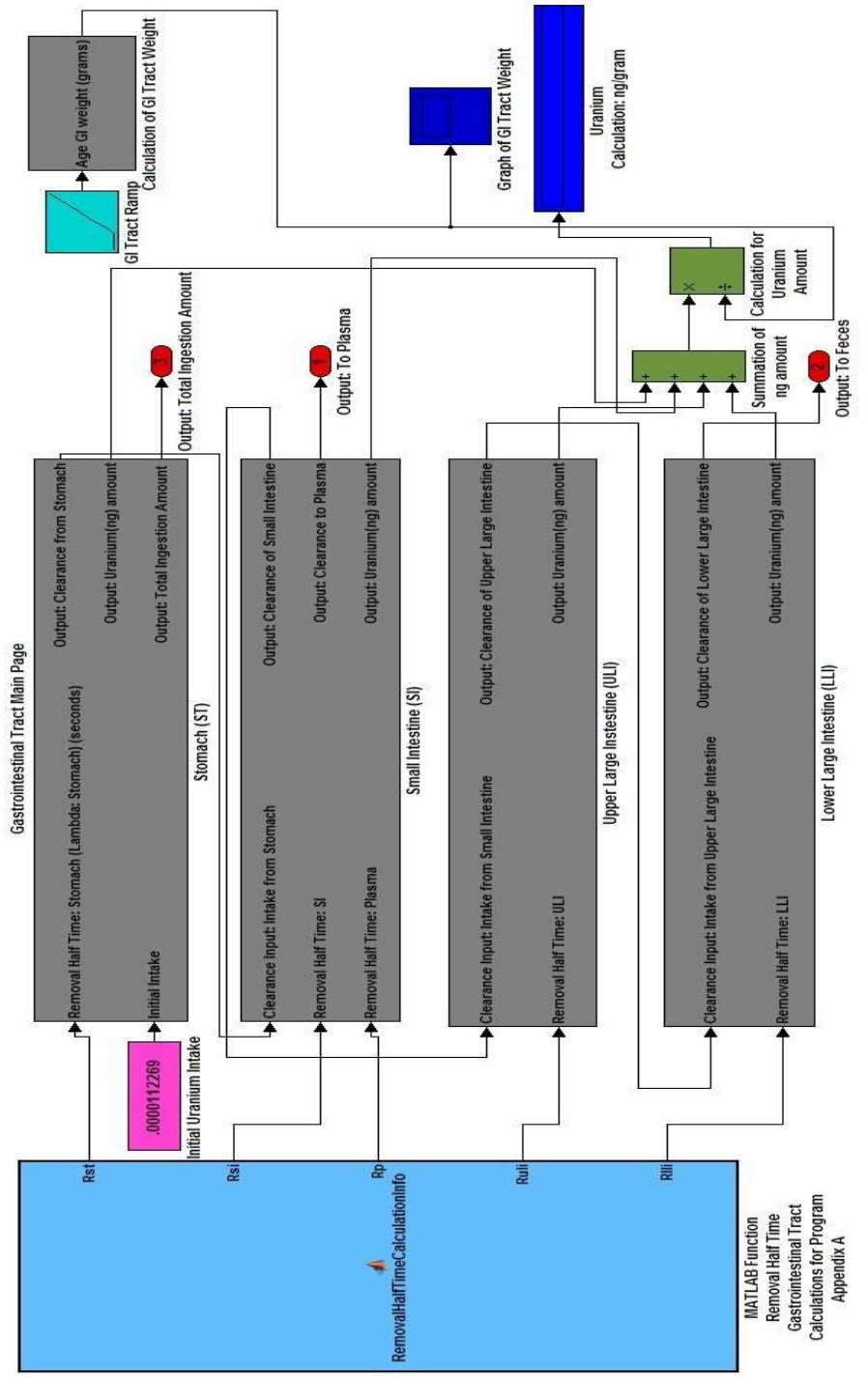

Fig. 4. Simulation Subsystem: Gastrointestinal Tract

Dublineau et al. state that there is no knowledge on the effects of uranium ingestion on the digestive functions (Dublineau et al. 2006). There is minimal research data for the clearance rate values that must be utilized for the simulation. The Dublineau et al. research group did not find a toxic effect on the digestive tract after acute exposure; however, the chronic exposures are still uncertain (Dublineau et al. 2006 and 2007). Even though there is minimal research for the effect of uranium on the GI tract, the functions are integrated into the simulation to incorporate a complete biological system.

Paquet et al. (2006) provides data for the esophagus, but this portion of the GI was eliminated from the current simulation as the food and water remain in the esophagus for only a few seconds. The minimal amount of time within the esophagus is considered negligible. Tugay et al. (2003) state the esophageal properties change with maturation; therefore, additional parameter evaluation is necessary before implementation of this tissue into a simulation (Tugay et al. 2003). A comparative analysis of the GI tract simulation and the Paquet et al. (2006) research is difficult based on the acquisition of data by Paquet et al. (2006). Paquet et al. (2006) document the results of uranium found in the walls of the stomach, large intestine, and small intestine, following a wash of the organs. The simulation program, on the other hand, does not account for the wall contents alone. This biokinetic model accounts for the sections of the GI tract as a whole. The stomach compartment, which can be found in Fig. 5, is the compartment where initial uranium intake occurs. The stomach is a reservoir organ of ingested food before it is subsequently passed into and absorbed by the intestines (Liao 2005).

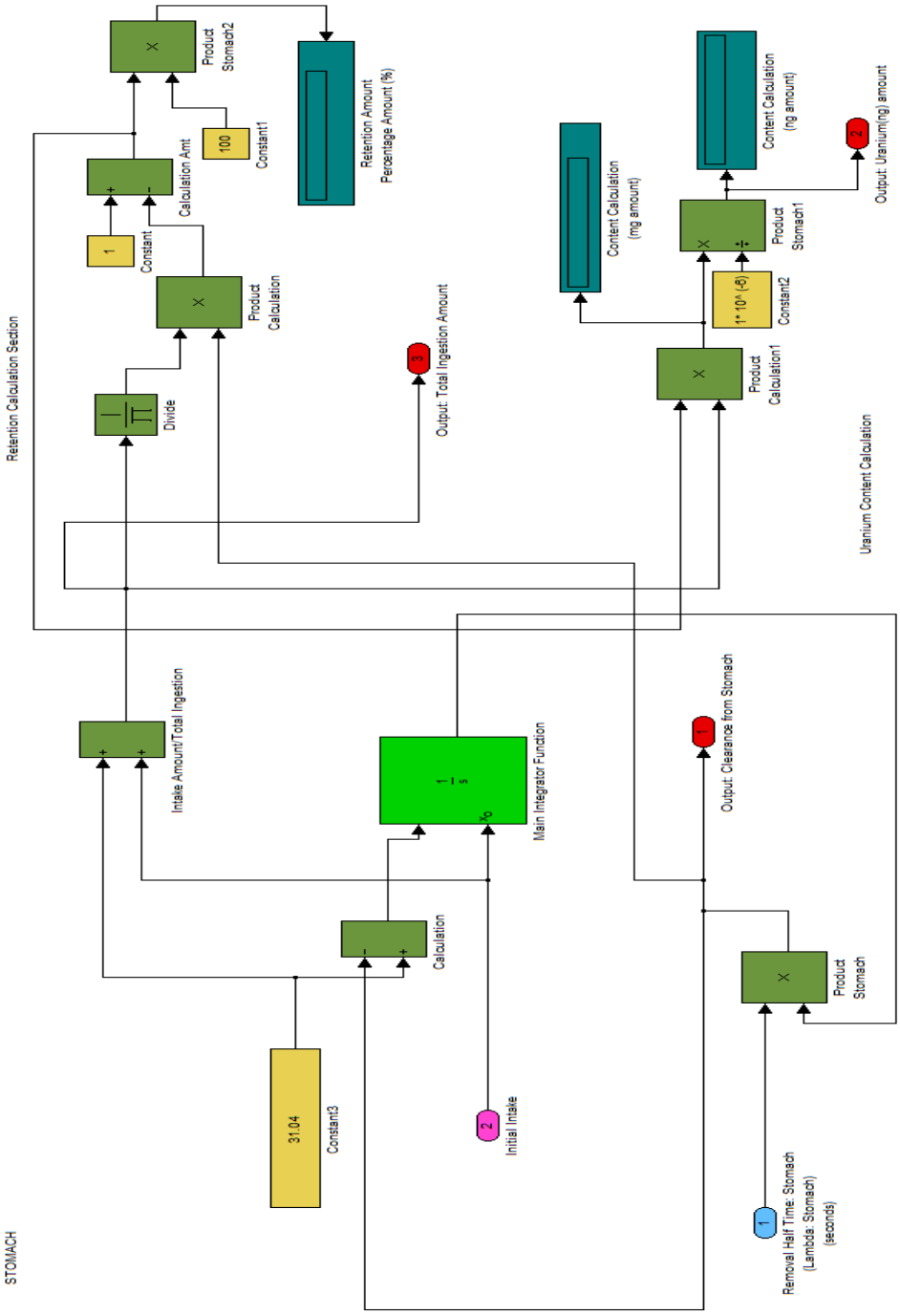

Fig. 5. Simulation Subsystem: Stomach Compartment

As the initial section of the program, the stomach compartment was modified for several test modes in order to evaluate different uptake scenarios. The first method, which is shown in Figure 5, represented a one time/acute intake of all the uranium that would be ingested over the defined time periods. The second approach was an attempt to use the exact same model as the first approach, but to use a slow/chronic intake using a time clock calculation. Both input functions would account for the Paquet et al. (2006) ingestion amounts for the various evaluation times.

In the model, an intake retention equation was placed into the subsystems to calculate the retention of uranium. As the initial physiological compartment, the stomach retention was based on the initial ingestion intake amount. The retention of the other compartments depends on the amount of uranium that enters as a clearance from one or more other compartments. The retention fraction is calculated using Eqn (8)

$$IRF = \frac{CR}{I} \qquad (8)$$

where *IRF* is the intake retention fraction, *CR* is the clearance rate, and *I* is the intake of uranium.

Plasma/RBC Compartment: The plasma/RBC compartment was not represented as a separate compartment in the original baseline model. This compartment was added into our simulation to account for the initial absorption from the gastrointestinal tract and the rapid turnover of uranium that entered the ST0 compartment. The absorption into the plasma/RBC compartment from the gastrointestinal tract is represented by the f_1 parameter for uranium. This plasma uptake is essential to the other compartments as this is the amount of uranium that remains internally and is circulated throughout the body.

Frelon et al. (2005) studied uranium absorption in the gastrointestinal tract. They have documented a variance in the f_1 parameter from 0.38 $\pm$ 0.03 % to 0.45 $\pm$ 0.1%; however, the simulation f_1 parameter was based on the Paquet et al. (2006) f_1 parameter, which equaled 0.38%. The f_1 parameter was not changed within the simulation; therefore, this was the only uptake amount from the GI tract. Biologically, the uranium that is transported through the blood can enter other compartments in various ways such as "a complex either with large proteins or with low molecular weight ions" (Carriere 2004). The advantage of the simulation program is the accessibility to change the f_1 component that would either increase or decrease the amount of uranium excreted to the blood/plasma simulation circulation.

ST0 Compartment: This compartment represents soft tissues that would have a very rapid turnover of uranium, releasing the uranium back to circulation within two hours. There are limited research data for the rapid clearance that occurs in some tissues/organs. To address rapid clearance, this simulation program used a ST0 compartment to represent short-term retention in soft tissues. The ST0 compartment is also necessary to facilitate the modeling of the transfer rate into the total circulation subsystem. Since the simulation results were taken well after the hypothesized clearing occurred, the ST0 compartment does not account for any of the soft tissues/organs that would be compared to the Paquet et al. (2006) data.

Total Circulation Compartment: The total circulation system is considered the recycling center for the entire simulation. Although the original transfer coefficients were calculated without taking recycling into consideration, we wanted to attempt to

model the recycling. This system has several inputs unlike the other main compartments. The total circulation compartment accounted for the transfer of the uranium to the other compartments for uranium distribution and retention. The initial intake for this compartment comes from the uranium that was excreted from the ST0 compartment. This total circulation compartment recycles uranium to the organs and back into the blood to be redistributed throughout the simulation program for the remaining time. The output of the circulation was divided into the organ compartments as explained in baseline modeling methods to continue redistribution of uranium.

Kidney Compartment: A total of 70.5% of uranium circulation clearance enters into the entire kidney subsystem. According to Leggett and Pellmar (2003), the glomerulus filters the uranium, and the uranium that does not get transferred to the urine remains deposited in the kidney (Leggett and Pellmar 2003). The initial intake into the kidney compartments is divided into the Kidney 1 and 2 subsystems. The kidney simulation subsystem in Fig. 6 accounts for 20.5% of the 70.5% uranium that enters from the plasma subsystem. The remaining 50% of extracted uranium enters through the kidney but is assumed to immediately exit to the urinary bladder contents. Of the 20.5% of uranium that enters from the circulation, 20% is entering the kidney short term subsystem, Kidney 1. Only 0.5% of the original 20.5% uranium enters the long-term kidney storage section, Kidney 2, and remains for approximately two months. The excretion rate for both the short-term and the long-term storage combined account for a final uranium accumulation value. The change in mass of the kidney was implemented into this subsystem and calculates the kidney mass based on the age of the rat.

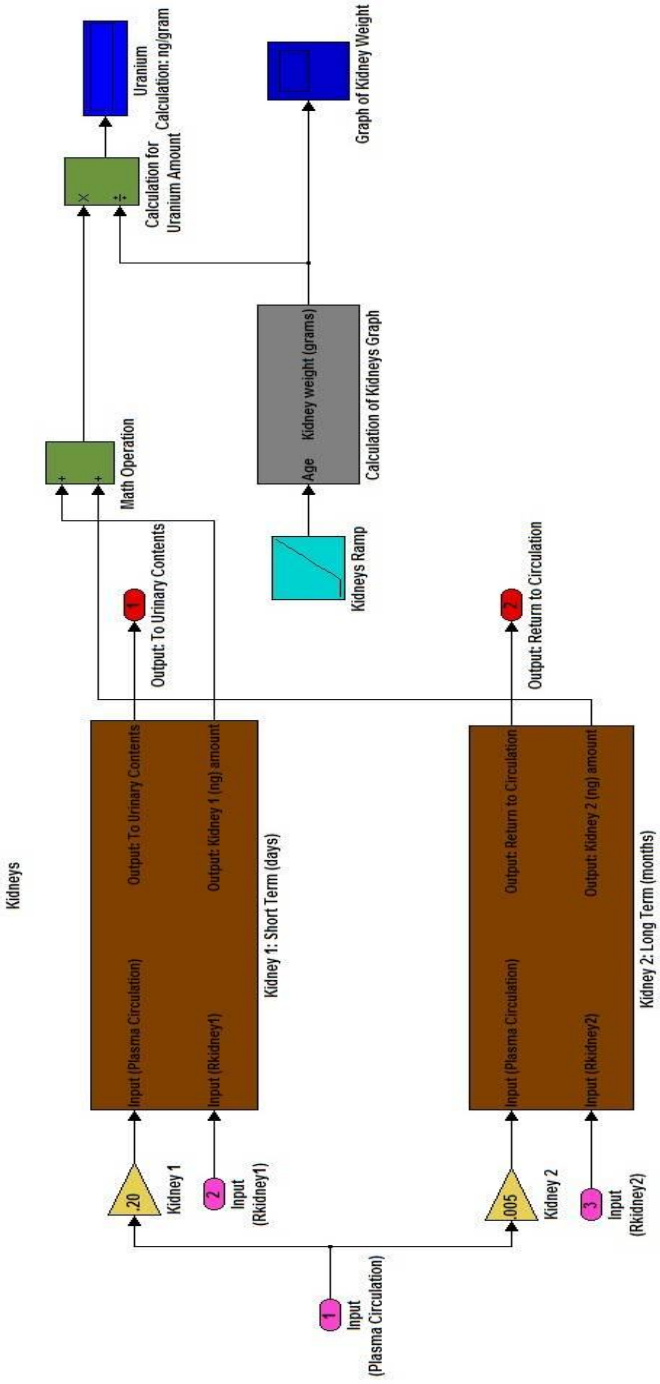

Fig. 6. Simulation Subsystem: Kidneys

The kidneys are of great interest for the simulation due to the direct relation of this organ with a major excretion route. Renal failure occurs biologically due to uranium retention within an animal. Dublineau et al. (2006) found that their experimental results indicated acute renal failure as soon as three days after initial ingestion. The effect of this renal failure cannot be executed in the simulation system without an evaluation of the predefined intake into the kidney compartment and/or a review of the clearance factors. The system is formulated with predefined parameters that follow the same pattern as previously reviewed compartments.

Soft Tissues (ST1 and ST2) Compartment: These two subsystems account for organs that were not included in the original baseline model. The baseline model lumps together all organs that are often ignored in research into the soft tissue compartments and does not identify the organs individually. Several organs examined by Paquet et al (2006) were placed in these subsystems in an attempt to identify the clearance parameter for the organs. Both subsystems are replicated identically and have the spleen, pancreas, brain, heart, and testes. Fig. 7 represents the ST1 and ST2 subsystems. For this simulation, organ masses were calculated using the previously explained reference data from Brown et al. (1994) and Mirfazaelian et al. (2007). The simulation can be used to calculate the organ weight based on a percentage of the body weight. As more data becomes available for organ mass with respect to age, the simulation can be modified.

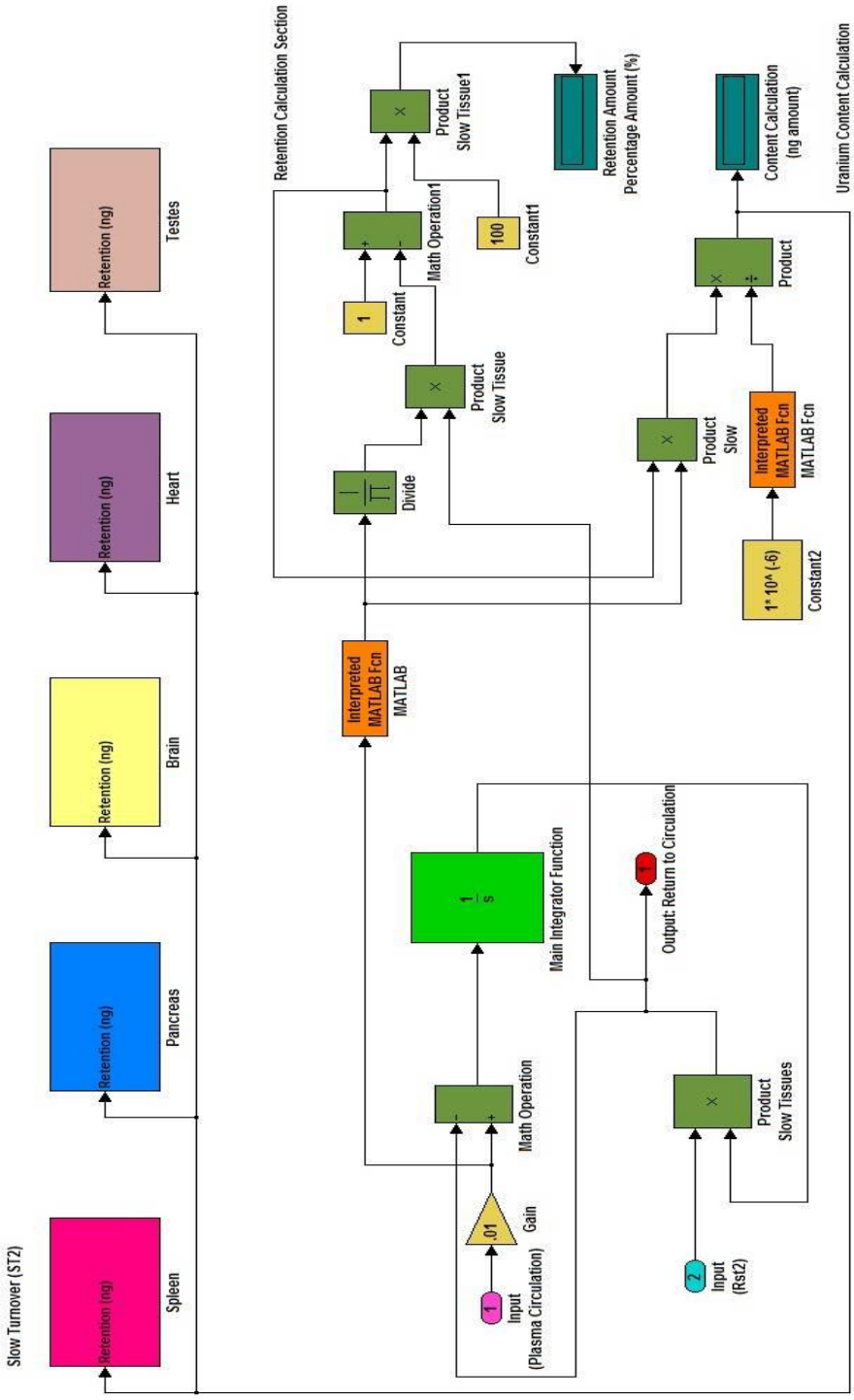

Fig 7. Simulation Subsystem: Soft Tissues (ST1 and ST2)

Several organs were identified by Paquet et al (2006) as retaining uranium in amounts which may seem negligible, but these organs were included to see if their inclusion would increase the accuracy of the modeled deposition and retention of uranium. The soft tissue compartments, which include the testes, spleen, heart, brain, and pancreas, are different from the other target organ compartments. The many years of research on target organs have defined transfer rates for these target compartments. The minimal research for the organs identified in the soft tissue compartment has led to a single transfer coefficient. The soft tissues are not thoroughly investigated in published literature due to the information that has already been established for uranium and the supporting documentation for the target organs. Although uranium has been documented to target two major parts of the body, the skeleton and kidneys, the simulation models the soft tissue organs in an attempt to expand upon original model.

The work by Williams et al. was used in the simulation to approximate a testes size for the research evaluation times (Williams et al. 2000). The development process for the testes differs for this organ. The rat testes development is nearly complete at the age of 70 days (Gayton et al. 1986). Accounting for a change in testes size is not necessary for implementation into the simulation since the program begins with a rat that is approximately 66 days old.

Another soft tissue organ to be analyzed using the simulation model is the brain. The transfer of uranium into the brain has become of interest to several research groups due to the ability of this radioactive material to cross the blood brain barrier (BBB). One research group found that exposed rats had a non-alteration of the BBB (Lemercier

2003). Of the entire brain, the cerebral cortex accounts for the greatest percent volume at 31%, but the cortex was found to have negligible amount of uranium by the Paquet et al. (2006) group. The brain as a whole was accounted for in this simulation.

Skeletal Compartment: The tri-layered model for the skeleton is depicted in Fig. 8 and follows the same first order kinetics as the other compartments. The first two compartments, Bone 1 and Bone 2, have several clearance paths with different rates. This model allows calculations for the teeth, femur, and lumbar vertebrae. The mass data for the skeletal parts were obtained from references that measured the particular structures. The masses for the skeletal section were calculated as a percentage of the whole body weight using the available experimental data. The skeletal compartment is a more complex system for the simulation. The skeletal system is vital to the program as a large recipient of the uranium deposition. The bone is a dynamic part of the anatomy that will "change mechanical properties and structure in response to mechanical stress as a phenomenon of functional adaptation" (Yamamoto et al. 2003). The introduction of uranium into the biological system may affect the growth and remodeling of bone. There is a paucity of data for the behavior of uranium for the skeleton of a rat. The skeleton of the rat is similar to the human model for the simulation as the skeleton remains compartmentalized into three sections; therefore, the division of these sections further is evaluated in this research. The teeth, lumbar vertebrae, and femur were tested in the skeletal compartment.

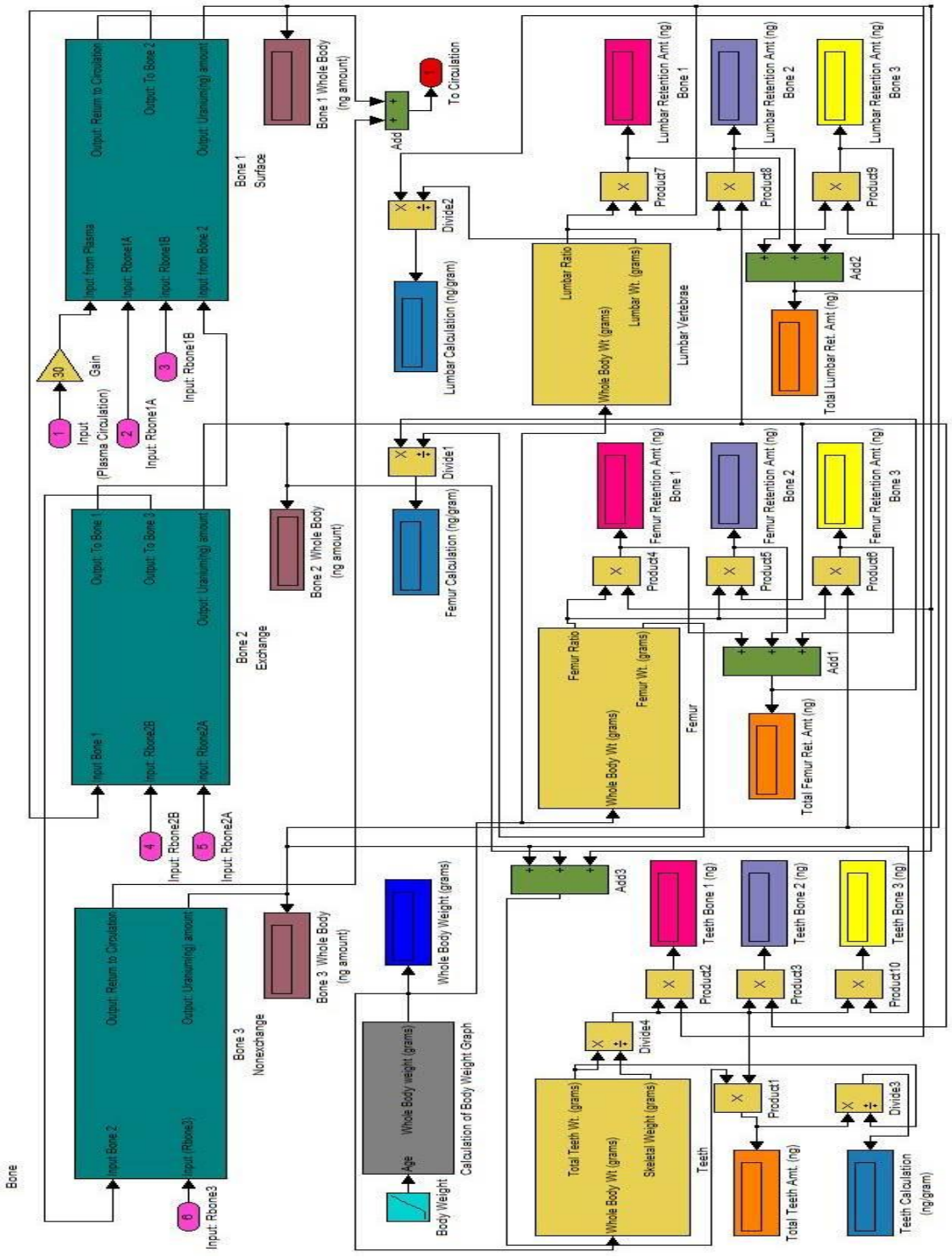

Fig 8. Simulation Subsystem: Skeleton

The three sections of the rat skeleton that were analyzed by Paquet et al. (2006) were the teeth, the lumbar vertebrae, and the femur contents (diaphysis and epiphysis). The diaphysis is the midsection of a long bone, and the epiphysis is the top rounded end of the long bone. The diaphysis and the epiphysis contain marrow and tissue that was not accounted for by the simulation model due to the paucity of research data to account for these sections of the bone. Isolating the diaphysis and the epiphysis would involve information that was not available; therefore, the femur calculation considered the bone as a whole

The simulation attempts to account for the teeth even though the teeth are not comparable to a human. The rat teeth are continually changing. The incisors for rats are continually growing and wearing off. The weight of the teeth for a rat was obtained using published data from O'Day et al. (1962). This research group analyzed the effect of food variation on developing rat molars. The rat strain utilized in their research was the Holtzman rat, which is a sub-line of the Sprague Dawley rat strain. As research for the Sprague Dawley rat strain becomes available, the simulation can be modified. This simulation utilizes the Holtzman rat teeth information as a benchmark. This work by O'Day et al. (1962) is graphically represented in this report to create a function that could be implemented into the simulation. Fig. 9 references the change in molar weight over a 20-day period (O'Day et al 1962). These data were formatted into a linear equation as documented in Eqn (9), with 'u' accounting for time in seconds. The general equation used to calculate the growth of the molars over time as the age of the animal progresses is

$$y = [(0.000021\ mg/sec) * u] + 31.07\ mg \qquad (9)$$

where y is the total mass of molars (mg) and u is the simulation time change (seconds).

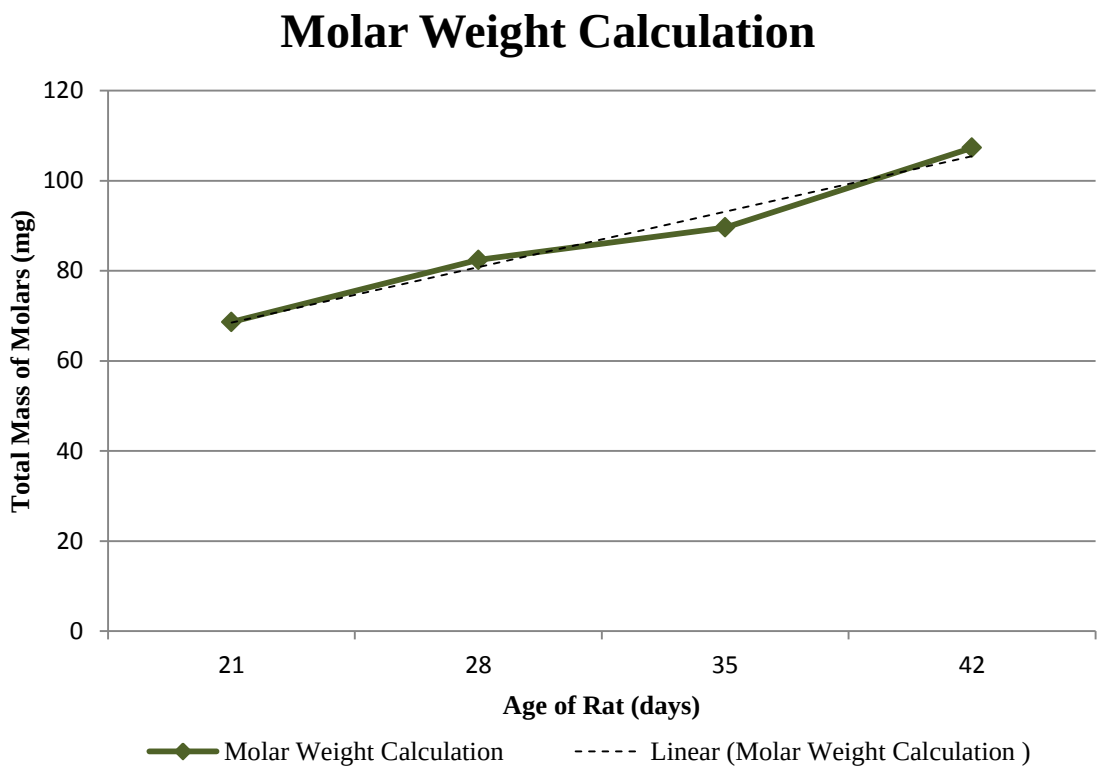

Fig. 9. Molar Weight Calculations for Holtzman Rat (sub-line of SD strain)

The incisor weight calculation was obtained from the biochemical study of tooth growth by Matsuda et al. (1926) that used albino rats to investigate the upper and lower incisors of the male rat. The albino rats are comparatively similar to the SD rats used by Paquet et al. (2006). The SD rats were developed from the Wistar rat, which is an outbred strain of albino rats. Matsuda found that the increase in incisor weight is similar

to the body weight. However, Eqn (10), a linear equation, was utilized rather than a sigmoidal equation as the sigmoidal equation is dependent upon several factors that would require additional reference information (Matsuda et al. 1928). Incisor weight is calculated by

$$y = [(0.000012\ mg/sec) * u] + 4.48\ mg \qquad (10)$$

where y is the final incisor weight (mg) and u is the simulation time change (seconds). This information includes the Hill coefficient that would affect the sigmoidicity for the equation. Eqn (10) ensured the calculations for the teeth were consistent for the simulation and followed a similar format as the molar weight calculation. As additional reference information becomes available for the skeletal structures, the program can be modified. With the equations for the weight of teeth formulated, the percentage of uranium that accumulated in the teeth was calculated using an approximate skeletal mass that was calculated using reference data from Brown et al. (1994). Eqn (11) was placed as a function in the simulation and calculates the skeletal weight information of the rat

$$SW = 0.0801\ (BW)^{0.983} \qquad (11)$$

where SW is the skeletal weight and BW is the body weight. Once the skeletal weight was calculated based on the whole body weight, the teeth weight could be calculated based on the ratio of teeth to skeletal weight (SW).

An additional section of the skeleton model that was implemented into the simulation model was the femur. The femur weights were calculated based on a percentage of the whole body weight. Paniagua et al. (1998) evaluated the bone mass in female Wistar rats and found the mass of the lumbar spine and femur (Paniagua 1998).

Using the experimental measured masses and the body weight of the Wistar rats documented in the biological experiment, a relative percent was calculated. The mean body weight (BW) for the rats in the Paniagua et al. (1998) experiment was 205.08 grams $\pm$ 20.12 grams. The weight of the femur was 237.8 mg $\pm$ 29.18 mg. Using these data, the relative femur weight was calculated to be 0.116% of the body weight. With the calculation of the femur weight included in the program, the uranium retention could be calculated using the ratio of femur to skeleton weight, in a manner similar to other calculated sections.

The final section of the skeleton to be included in the simulation model was the lumbar vertebrae. The mass of the lumbar vertebrae was obtained using the Paniagua et al. (1998) method explained previously. The biological experiment documented a weight of 184.46 mg $\pm$ 24.70 mg for the lumbar vertebrae with a body weight of 205.08 grams $\pm$ 20.12 grams. A ratio could be calculated from the experimental weight.

The uranium model has a range of removal half-times that have been a concern over the years. The bone compartment is similar to the f_1 component due to the broad range of information that is available within the published data (Frelon et al. 2005). An additional concern for evaluation when dealing with the skeletal model is the sex of the rat that is being utilized. The Paniagua et al. (1998) reference data used female rats to isolate the femur mass. Biologically, the sex of the rats should be considered when analyzing the simulation results since the calcification of the bone is better in a female and accounts for a heavier mass at certain body weights (Zucker and Zucker 1946). The sex of the research subject is a concern since the metabolism of uranium resembles that

of calcium in the bone (Kurttio 2005). These additional factors are not accounted for in the simulation with the current data; however, the simulation can easily be modified in the future.

Liver Compartment: The liver compartment is constructed similarly to the kidney compartment and contains two sub-compartments for uranium calculation, Liver 1 and Liver 2. The liver section was divided to separate the longer retention compartment from the immediate turnover portion of the liver. Unlike the kidney compartment that divides the initial input into the system, Liver 1 receives the entire intake amount of uranium that is allocated from the blood/plasma. In an animal, the liver as a whole receives this uranium; however, the program must account for the different clearance rates. In order to accomplish this, the program divides the liver into the two subsystems. Fig. 10 illustrates the uranium transferred from the plasma into this compartment enters directly into Liver 1. Liver 1 is receiving 0.8% of the uranium from the plasma. From this minimal percentage amount, 97% is released back to the plasma for recirculation and 3.0% is ultimately transferred to Liver 2 over long time intervals. The uranium in Liver 2 remains stored in the liver before being returned to the plasma for additional recycling.

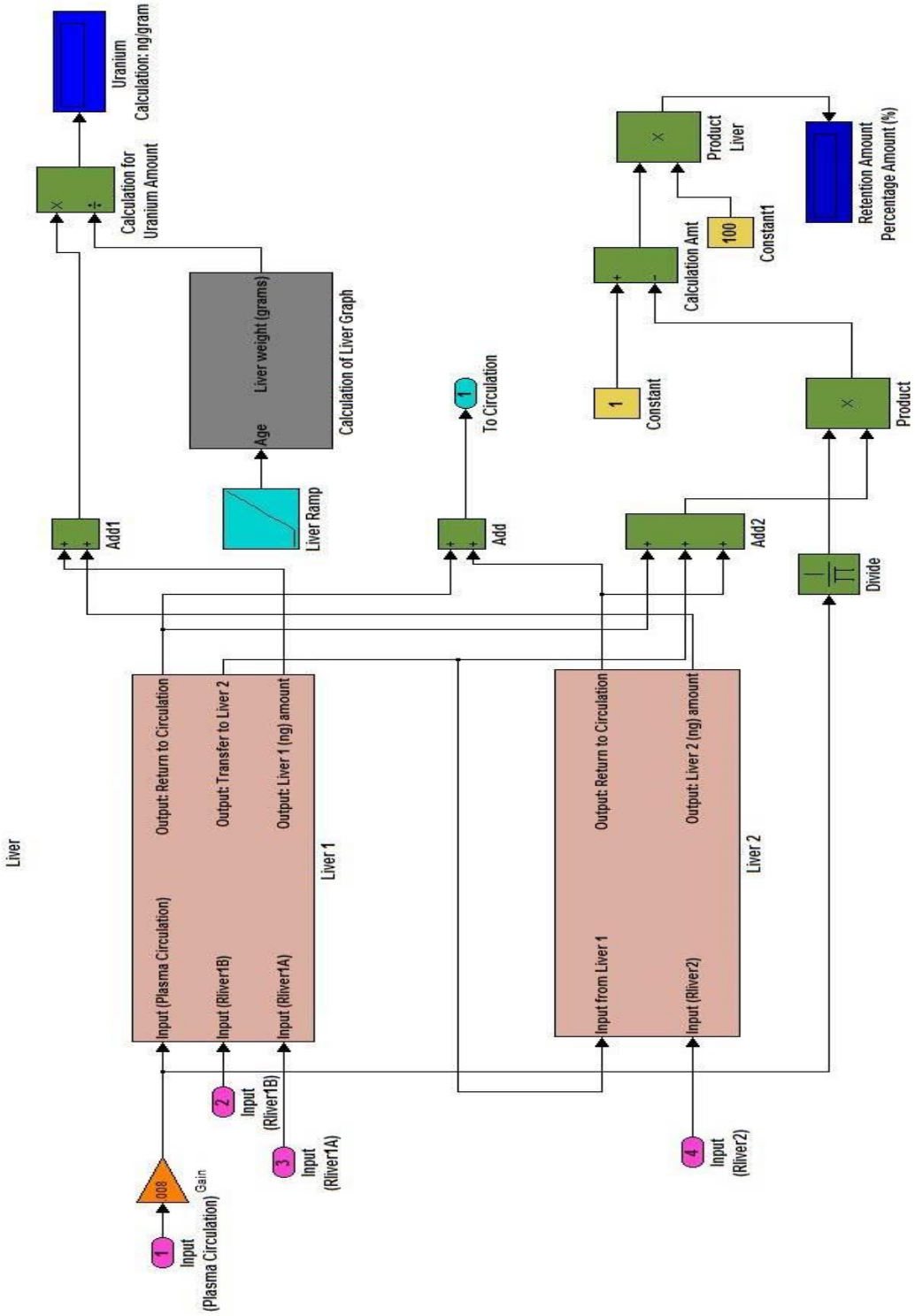

Fig. 10. Simulation Subsystem: Liver

Modifications in the Simulation Model for Organ Growth: With the organ compartments created, the organ growth equations were placed appropriately within the simulation for final organ mass calculations for specific time intervals. This final mass was used to calculate the amount of uranium that remains in the system per unit mass for the specific organ within the subsystem. The calculated organ mass was also utilized to convert the retention information (ng/g) in Paquet et al. (2006) into uranium concentration in nanograms. Mirfazaelian et al. (2007) provided the organ growth equations for the Sprague Dawley rats. These equations were based on measured body weight and age data published by Lopez et al. (2000). Research over the years has evaluated the body weight progression starting with the growth equations by Gompertz in the 1930s and Richards in 1950s. Using their data, Lopez et al. (2000) implemented the Generalized Michaelis-Menten model (GMM) making a flexible animal growth function, improving upon the Richards' model (Lopez et al. 2000). Organ growth modeling is necessary to "reliably predict target organ deposition of chemicals in different age groups of maturing rats" (Mirfazaelian et al 2007). Therefore, the implementation of organ growth and body weight in our simulation is a significant advancement over previous models.

To implement the GMM functions into the simulation, certain parameter values are needed to create the sigmoidal organ functions proposed by Mirfazaelian et al. (2007). The values provided in the Mirfazaelian et al. (2007) reference that must be accounted for are the maximal organ/tissue weight ($W_{t\text{-}max}$), the Hill coefficient (γ), the age of rat at half maximal organ growth (K), and the animal organ/tissue weight of the

rat at birth ($W_{t\text{-initial}}$) documented in Table 2. Table 2 provides the average values obtained from Mirfazaelian et al. (2007).

Table 2. Model Organ Parameter Growth Information

Organ / Tissue	$W_{t\text{-max}}$ (mg)	Hill coefficient(γ)	K (days)	$W_{t\text{-initial}}$ (mg)
Liver	15390.4	2.76	43.49	298.4
Spleen	974	1.53	43.64	8.3
Kidneys	3801.7	1.78	50.83	60.1
Heart	1328.7	1.96	43.69	32.2
Lungs	1972.2	1.47	49.34	101.8
Brain	2073.1	1.44	12.82	175.6
GI Tract	16028	3.16	33.2	279.2
Lipid	37583.82	1.47	95.52	119.9
Body Weight	521026.13	2.01	63.21	7314.7

The final weight for the organ/tissue is calculated using Eqn (12)

$$W_t = \frac{(W_{t\text{-initial}} * K^{\gamma}) + (W_{t\text{-max}} * A^{\gamma})}{K^{\gamma} + A^{\gamma}} \qquad (12)$$

where W_t is the final organ/tissue weight, $W_{t\text{-initial}}$ is the weight of the organ/tissue at rat birth, K is the age of rat at half maximal organ growth, γ is the Hill coefficient, $W_{t\text{-max}}$ is the maximal organ/tissue weight, and A is the age of the rat (Mirfazaelian et al. 2007). The Hill coefficient in Table 2 affects the growth curve sigmoidicity (Mirfazaelian et al. 2007). The Hill coefficient and the K-value were utilized unchanged in our model. Table 2 was utilized as an initial reference for all the organs considered in the calculation. Since the body masses of Paquet et al. (2006) exceed the Table 2 values, the initial weight used in Eqn. 12 was recalculated. The initial weight used for the

simulation was calculated to equal the biological research data. This calculation variation is shown in Appendix B. This reformulation of the Mirfazaelian et al. (2007) functions was done for all organs within the simulation.

SIMULINK® is a time-dependent program, and the system is set to a defined initial time parameter that is controlled by the user. The rat age was integrated into each subsystem initially. The rat age was initially calculated based on available reference data. Fig. 11 shows the example of the age simulation growth subsystem that the user would use to modify the age of rat being referenced in the biological experiment. This subsystem can also be accessed to modify the variables that affect the final weight calculations. Initially, the user accesses the organ subsystem for the Mirfazaelian et al. (2007) function as shown in Eqn (12), which is used to calculate the specific organ weight for a user defined timeframe.

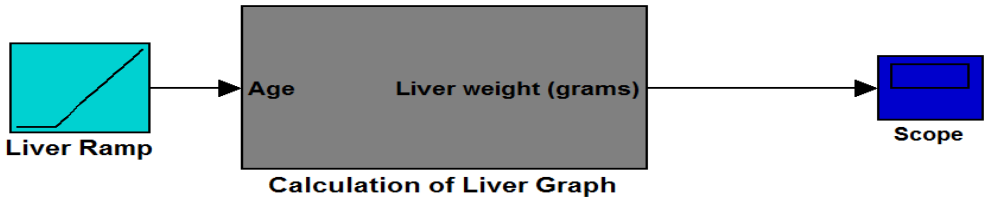

Fig. 11. Organ Size Subsystem in Simulation Program

For this simulation, the rat ages were based on the initial weights, 328 ± 17 grams, reported by Paquet et al. (2006) and then increased to a final weight of 618 ± 79 grams. An approximate age was calculated using these biological data as well as

Mirfazaelian et al. (2007) calculations to account for absent data required for the simulation calculations. Eqn (13) was used to calculate the age of the rat in the simulation

$$Age\ of\ Rat = K_{BW} * [(BW - W_{t\text{-}init(BW)}) / (W_{t\text{-}max(BW)} - BW)]^{(1\ /\ BW\ \gamma)} \qquad (13)$$

where K is the age of rat at half maximal organ growth, BW is the body weight of the rat, $W_{t\text{-}init}$ is the weight of the organ/tissue at rat birth, γ is the Hill coefficient, and $W_{t\text{-}max}$ is the maximal organ/tissue weight (Mirfazaelian et al. 2007). Using the referenced data, the initial age of the rat was calculated to be approximately 66.45 days. The user can verify the calculated age using Eqn (12) to ensure the calculated value, Wt, equals the initial starting weight for the biological experiment. The calculated age of 66.45 days is added to the variable 'u' of the function inside the subsystem to initiate the increase in rat age for the simulation and ultimately the growth of the rat organs, since "organ weights are highly correlated with their respective ages" (Mirfazaelian et al. 2007).

Simulation System Inputs: The main page for the simulation program allows for a choice of the rat parameters to be utilized by the user. The first choice was created using research data from Mirfazaelian et al. (2007) to calculate the organ growth. If an organ/tissue growth equation utilizing Mirfazaelian et al. (2007) was unavailable, the organs were calculated using additional reference data reviewed in this manuscript. The second option was created using the research data from Brown et al. (1994). This would allow the user to calculate uranium retention and distribution using general physiological parameters calculating organ weight as a percentage of the body weight. To test the program, the simulation choice would be the Mirfazaelian et al. (2007) data. The

program was run for 32, 95, 186, 312, and 368 days, which corresponded to the days examined by Paquet et al. (2006). The program was set up to run for predefined times and ingestion amounts. Once the simulation time was chosen, the input was updated in seconds to run within the system. Quantitative display boxes were placed throughout the program to view the changes in uranium retention throughout the system. Various functions were implemented into the simulation to test the program and evaluate potential modifications to accurately model a biological system. One function slowly and consistently input the uranium over time for a specified time frame giving an approximate daily input of 0.97 mg/day, which matched the documented Paquet et al. (2006) ingestion. A second function was a one time, single input for the total uranium ingested. The two approaches were compared to the actual measurements by Paquet et al. (2006). Appendix C details the information used to benchmark the simulation.

CHAPTER V

PROGRAM ANALYSIS: SIMULATION RESULTS

Continuous Ingestion Input: The program can be run using different activity or radionuclide input methods. Since the experiment chosen for the analysis was actually a chronic exposure, we chose to initially test a slow, chronic intake model in the system. The chronic ingestion format was simulated using a function choice that was low activity for a set period of time. The function would ultimately ensure an ingestion input equal to that found in the experiment. The program was run using the equation, (0.000011 * u), where 'u' is the time in seconds that is applied in the system. The function was created using two separate evaluation days with their associated uranium intake. The initial results using this continuous input function format were not successful. Fig. 12 depicts the results that were obtained from this continuous ingestion input. Although the initial results for the chronic intake seem to indicate that the simulation was erroneous, further evaluation was carried out for the simulation. Additional SIMULINK® blocks were integrated into the program to break down the sections of the simulation. The continuous input system was a slower response. Since the simulation can be modified, the initial system function was tested.

Single Ingestion Input: The second test for the simulation used a single input amount that equaled the continuous input function total. The single ingestion amount compares better with the experimental data than the chronic input using the same biokinetic assumptions. The clearance rates are closer to the Paquet et al (2006) data as

shown in Fig. 12. The chronic intake is clearing approximately 4.0% less than the acute intake initially; however, this function within the system begins to progressively increase. The acute intake, on the other hand, is releasing approximately 0.4% less than the Paquet et al. (2006) data initially and maintains a steady clearance. The Paquet et al. (2006) research group documents the urinary volume excreted remained constant, which is comparable to a computer simulation that maintains a patterned response. The error rate for the Paquet et al. (2006) clearance is minimal.

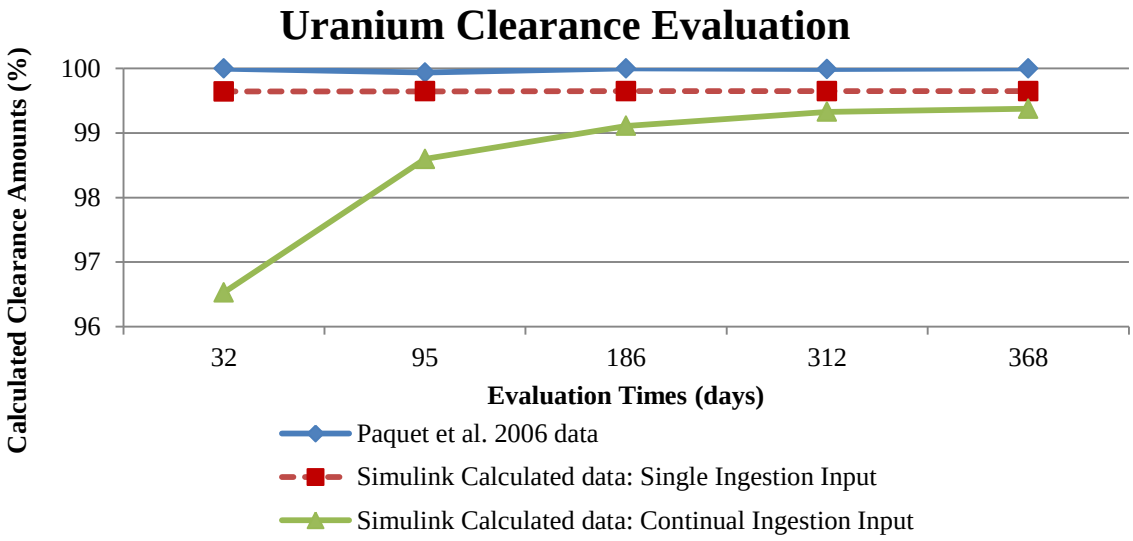

Fig. 12. Uranium Release Percentage

There are no research data on the fecal excretion rate from Paquet et al. (2006). Leggett and Pellmar (2003) state that "although the rats show high biliary secretion of many metals, there appears to be little endogenous fecal excretion of uranium in rats".

Fig. 13 shows the calculated excretion rates for both the urine and the feces from the simulation.

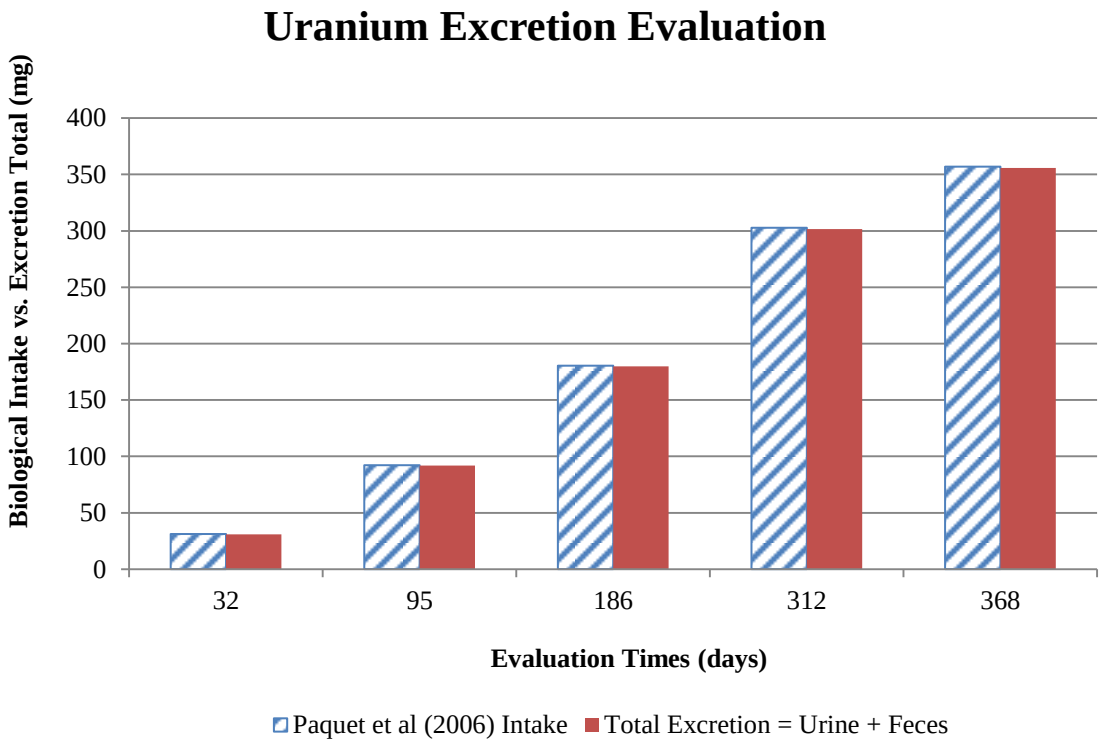

Fig. 13. Single Intake Excretion

Liver Compartment: As stated previously in the report, the liver was divided in the simulation to account for the different retention and clearance rates for Liver 1 and Liver 2. The retention of both compartments of the liver for the simulation was summed for the final retention for the liver as a whole. These retention amounts are given in Fig. 14 and Table 3. The liver retention for the simulation program decreased over time, and

there was consistency throughout the evaluation times for the simulation. Unfortunately, the data for the simulation does not match the Paquet et al. (2006) data.

Table 3. Liver Retention Values

	Amount of Uranium in Organ/Tissue (ng/gram)				
Liver	**32 days**	**95 days**	**186 days**	**312 days**	**368 days**
Acute	6.53	5.35	5.90	4.84	3.86
Paquet	0.12	2.10	0.50	26.70	16.80

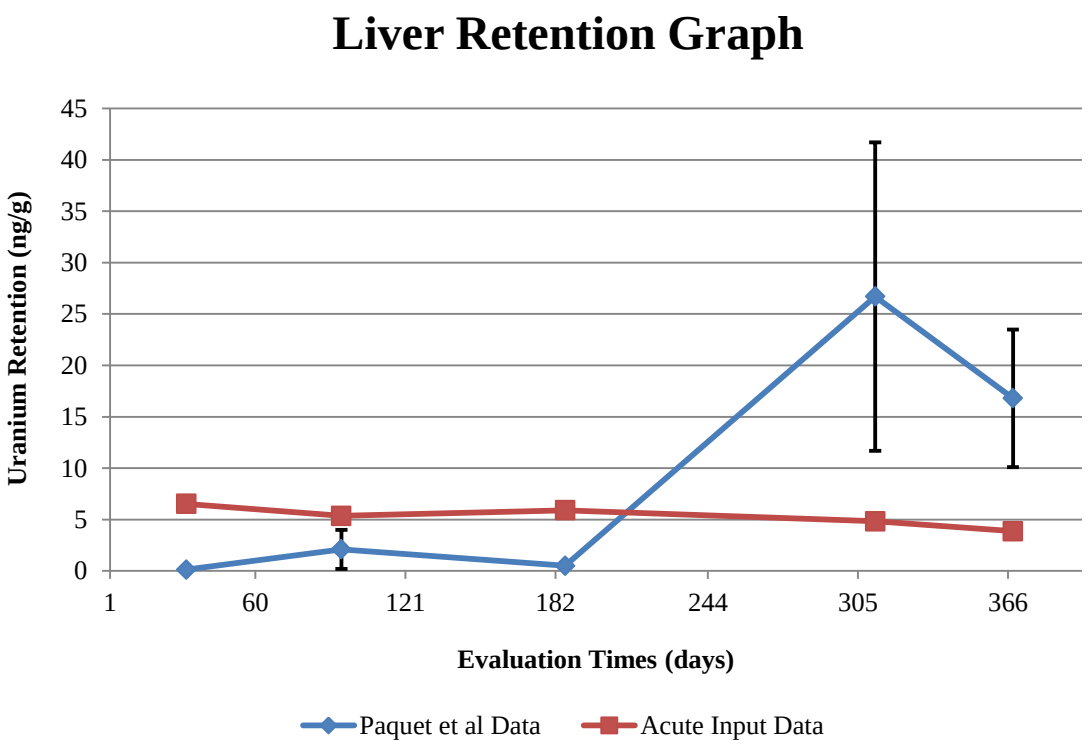

Fig. 14. Uranium Retention in the Liver

The liver results from the biological experiment start with a minimal retention that increases for the final two evaluation points. Using the predefined circulation input

function of 0.8%, the liver results for the simulation, identified in Fig. 14 as "acute input data", were too high and not comparable to the biological data. Using the simulation results, the program was tested to check the organ growth equation that was implemented into the liver compartment. This would determine if the difference in simulation and biological research was based on the organ weight. If the weight is verified, the program could be reviewed further. At the 32 day evaluation time, the rat is approximately 98 days/3 months old. The Mirfazaelian et al. (2007) equation within the program calculated the liver weight to be 16.56 grams in the simulation. The research of Leggett and Pellmar (2003) document the tissue weights for a rat at different ages, specifically three months of age as well. The liver calculation within the program is comparable to the Leggett and Pellmar (2003) liver data at 16 grams. The calculated weight cannot be the reason for the drastic differences in uranium retention. Table 1 states 0.80% of the uranium in the blood enters the liver system. If the initial intake and/or clearance rate remain constant as it does in the simulation, the results cannot model a biological experiment and further evaluation of the variables affecting the uranium retention, such as the biological clearance rate, is necessary. This is evident in Fig. 14. To create a valid subsystem for the liver compartment, a change to the intake function and/or biological clearance rate must be considered.

Kidney Compartment: The simulation results for the kidneys start higher than the Paquet et al. (2006) data and remain consistently higher until the final simulation evaluation point. For the kidney compartment, the simulation clearance parameters were modified as an initial test of the predefined parameters. The clearance rate for the

Kidney 2, long-term section, was modified in the program to reduce the biological clearance rate. This section has a predefined clearance of 50 days, which was reduced to 30 days and 20 days. Although there is currently no data to support the change, the program was tested based upon the initial review of the simulation results. The simulation program cleared the uranium; however, the clearance rate was too slow in the simulation. The reduction to the clearance parameter would reduce the retention of uranium, which is shown in Fig. 15. The uranium retention values documented in Table 4 documents a comparable response with a 20 day clearance rate.

Table 4. Kidney Retention Values

	Amount of Uranium in Organ/Tissue (ng/gram)				
Kidneys	**32 days**	**95 days**	**186 days**	**312 days**	**368 days**
Acute	284.0	274.5	218.9	142.7	78.9
Acute-30 day clearance	251.7	181.4	136.3	101.9	47.5
Acute-20 day clearance	221.8	133.4	113.7	92.8	38.2
Paquet	220.0	97.3	82.0	60.5	72.4

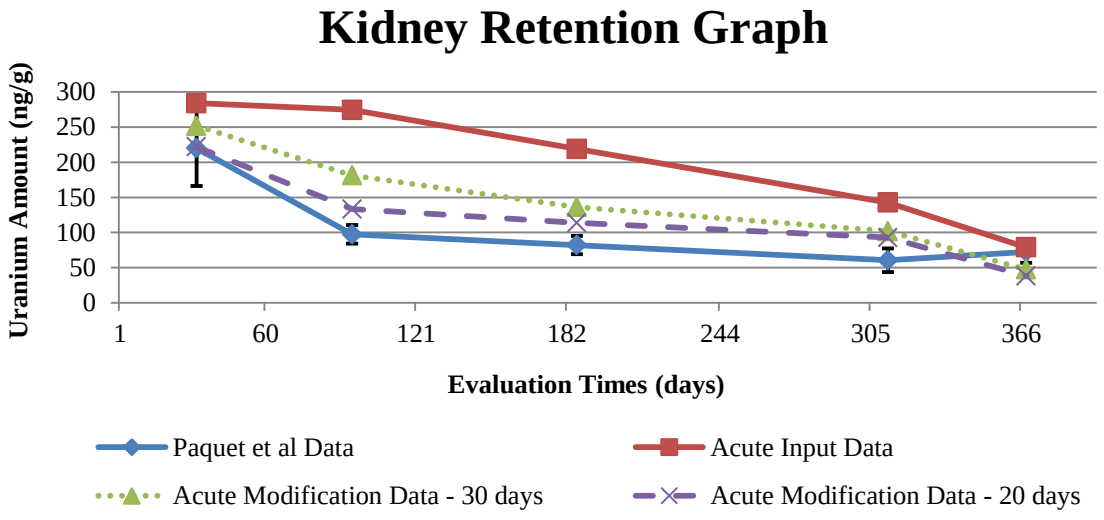

Fig. 15. Uranium Retention in the Kidneys

The clearance rate for the kidneys should be investigated for modification to increase the excretion for the simulation and minimize the retention amount.

Skeletal Compartment: The skeletal compartment was set up in a similar manner as the other compartments documenting the retention of uranium in the ng/g format; however, this section of the program documents the uranium in nanograms, instead of ng/g. Since the weight of the Paquet et al. (2006) data is not published for use in the simulation program, the calculated weight for the program is used to convert the Paquet et al. (2006) data to uranium amount in nanograms. There are several bone sections from the baseline model that are evaluated for uranium retention. The Bone 1 subsystem accounts for the smallest retention percent. The Bone 2 accounts for the greater retention. Compilation of mass weights was vital to the final computation of uranium amount that would be compared to the biological research.

Fig. 16 depicts the first part of the skeletal structure reviewed, the teeth. As the evaluation times progress, the variance in the biological data and the Paquet et al. (2006) data begins to increase. The simulation data, "Acute Input Data", is calculated using Bone 1, Bone 2, and Bone 3. The final "Acute Input Data" amount compares to the Paquet et al. (2006) data for the initial evaluation point only. Bone 1 has a fast clearance rate of 10 days compared to Bone 2 and Bone 3; therefore, this section was eliminated as an initial review for evaluation points, 32 days and 95 days. Evaluation points at 186, 312, and 368 days eliminated both Bone 1 and Bone 2 because Bone 2 has a clearance rate of approximately 119 days. These clearance times are less than the evaluation times; therefore, the program is used to document the elimination of these sections all

together, without any recycling. This allowed an examination of Bone 3, specifically. Even though the Bone 3 data is comparable initially, the results are still higher than the Paquet et al. (2006) data; therefore, the simulation would indicate that the biological system is clearing the uranium at a faster rate than the defined parameters in the program.

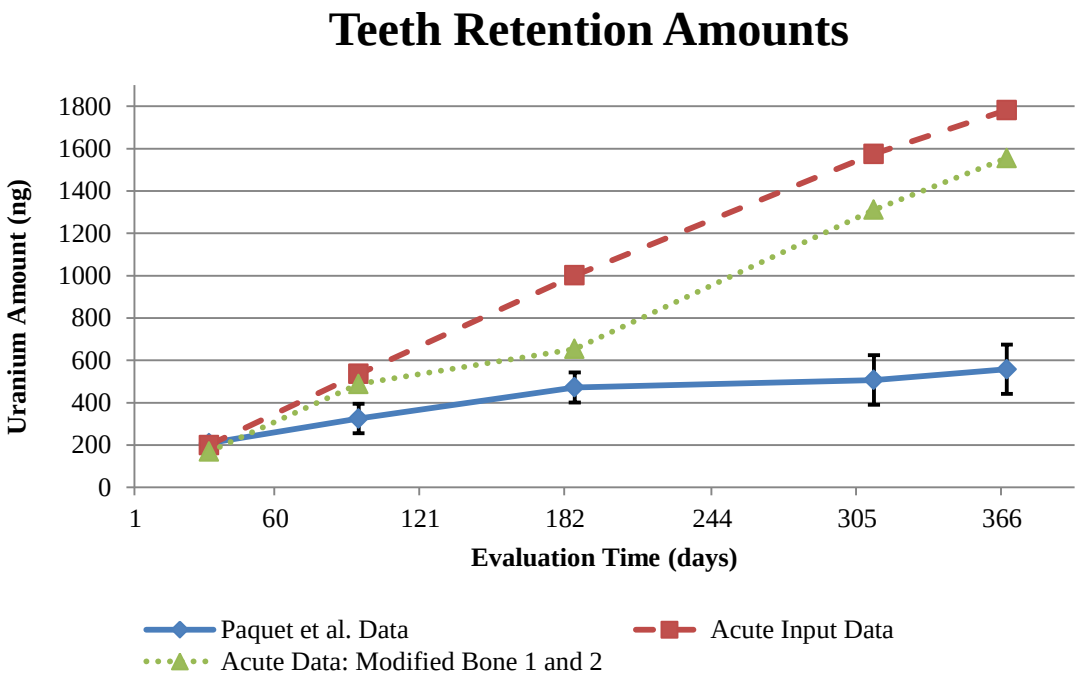

Fig. 16. Uranium Retention in the Teeth

The final calculated values for the femur are shown in Fig. 17. The uranium data from Bone 1, Bone 2, and Bone 3 combined is documented as "Total Acute Input Data". The femur results confirm a skeletal retention site for the system; however, the

simulation results were too high. As with the teeth compartment, the femur section was modified. For evaluation points at 32 and 95 days, only Bone 1 was eliminated from the "Acute Data Modified: Bone 1 and 2" data. The simulation results are slightly higher even with the Bone 1 elimination. Bone 1 and 2 were removed for days 186-368 in the "Acute Data: Modified Bone 1 and 2" data as the clearance rates are less than the evaluation times. Similar to the teeth section, the variance in data increases for evaluation days at 186, 312, and 368. The simulation has identified evaluation times that could support additional biological testing of the clearance rates. There is speculation that uranium is retained more in young animals than in the adult (Forbes et al. 1956); however, the simulation and biological data seem to negate this hypothesis.

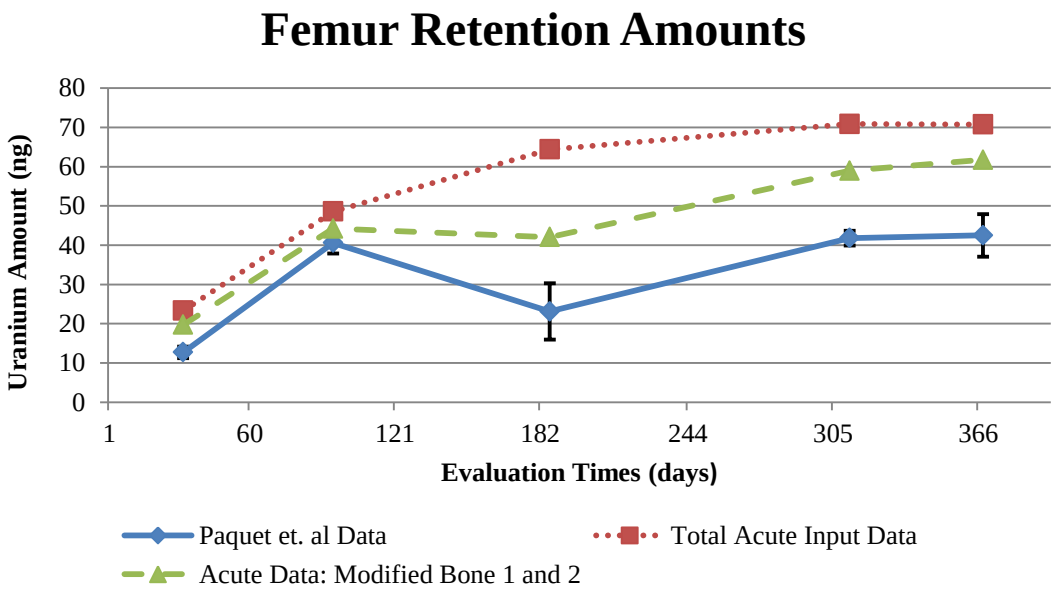

Fig. 17. Uranium Retention in the Femur

The final simulation results for the lumbar vertebrae can be seen in Fig. 18. Using the results of Bone 1, Bone 2, and Bone 3 would account for higher retention amounts compared to the biological experiment. Additional review would be required since only the first evaluation point was comparable. As with the other bone structures, Bone 1 and Bone 2 were adjusted within the simulation. The change to the program did not affect the results. Since the simulation can be modified easily, this section of the program was changed to only consider the Bone 2 data. Once Bone 2 data was graphed, one can see that the information is comparable to the biological data for most of the evaluation times.

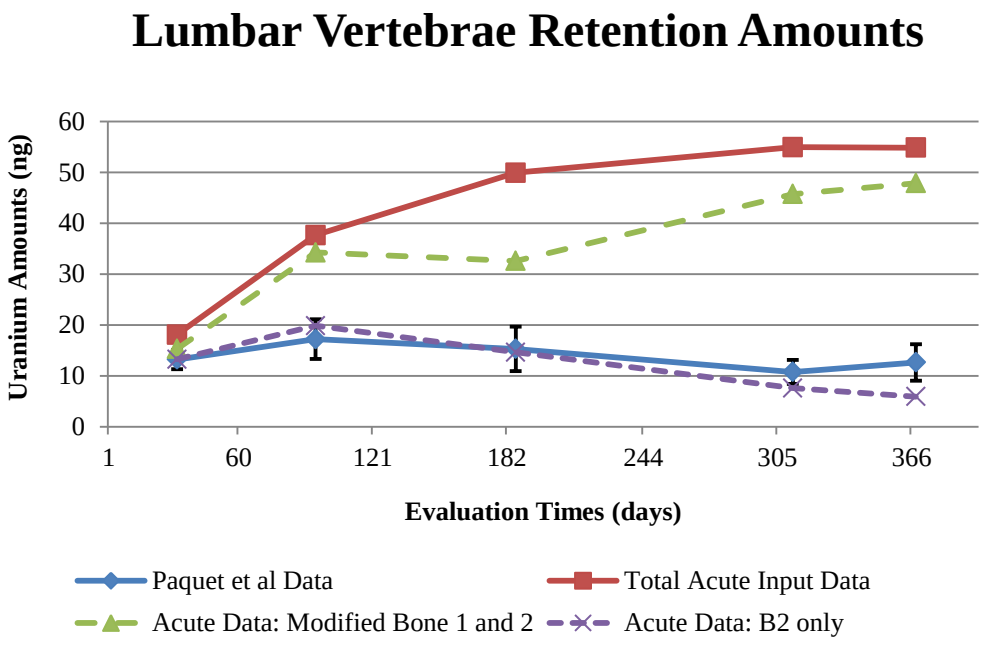

Fig. 18. Uranium Retention in the Lumbar Vertebrae

This is the advantage to the simulation program, the breakdown of the individual compartments that can be graphed. The results of the lumbar indicate a clearance rate equal to Bone 2.

Soft Tissues Compartment: The soft tissues could be evaluated for identification as an ST1 or ST2 compartment. Fig. 19 depicts the results for the heart. The resultant calculations for the heart were near zero for the ST1 compartment and too high for the ST2 compartment. The biological data indicates that the clearance rate for the system might be less than the predefined parameters as there is minimal retention within the organ. There is a 100 day clearance rate for the ST2 compartment that calculated the higher results. ST1 results with a 3 day clearance rate accounted for an extremely fast clearance time for all the evaluation times. In order to increase the ST1 results and minimize the ST2 results, a new clearance parameter was tested. A clearance parameter of 30 days was chosen for initial review and documented in Fig. 19 as the "Acute Input Data: Modified". The results based off this new calculation match better and suggest that additional biological testing is required to confirm the change to the parameter.

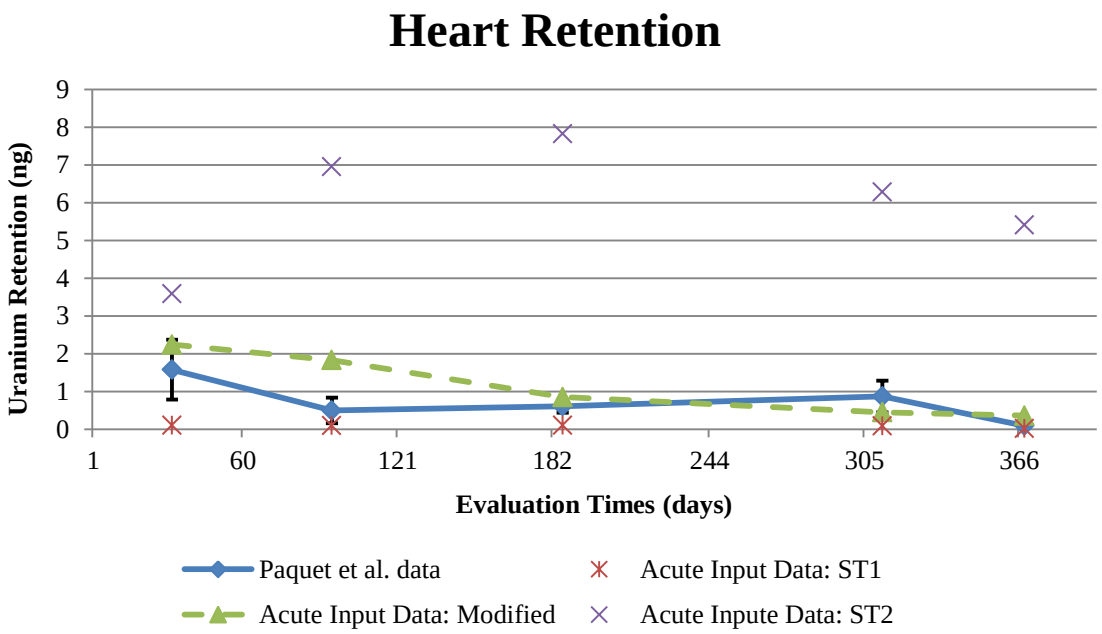

Fig. 19. Uranium Retention in the Heart

The testes were another soft tissue organ examined in the simulation program and compared to the Paquet et al. (2006) research data. The final uranium retention in the testes is depicted in Fig. 20. The Paquet et al. (2006) testes data had an unusual significant peak increase in uranium retention at 95 days. There is no documented reason for the significant increase detected. The retention at 32 days for the simulation is similar to the Paquet et al. (2006) data; however, the simulation continues to retain uranium unlike the biological research data that drastically decreases after 95 days.

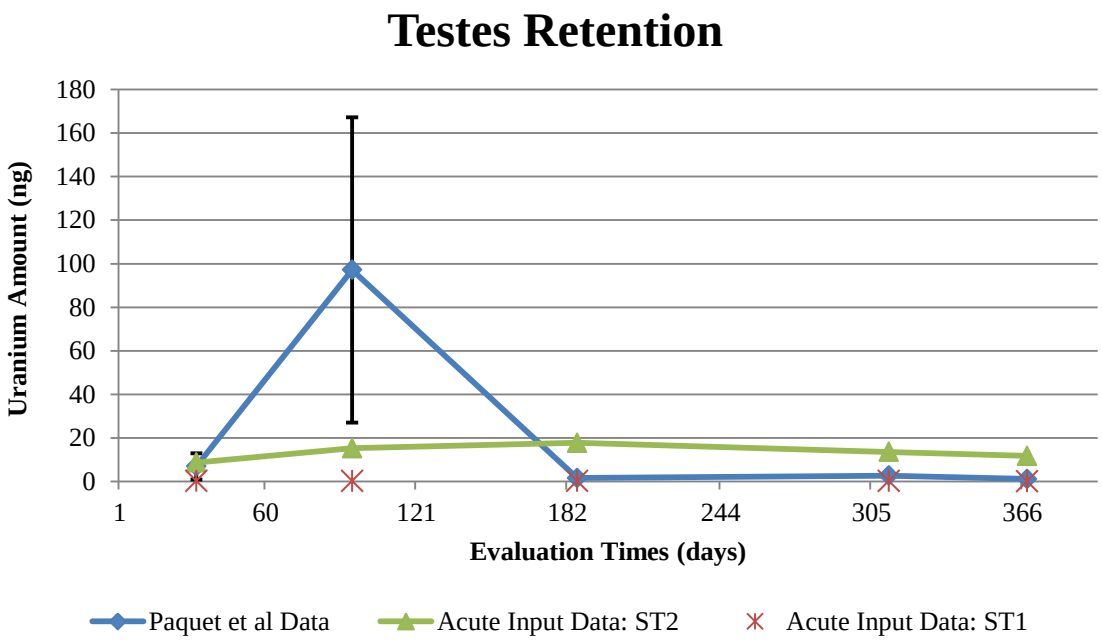

Fig. 20. Uranium Retention in the Testes

The testes organ cannot be identified as solely an ST1 or ST2 clearance organ. This is one compartment that can be considered as a combination of both. The first evaluation point is comparable to Paquet et al. (2006). Since the 95 evaluation point is significantly higher, it is eliminated from comparison. Evaluation points at 186, 312, and 368 days has minimal retention; therefore, ST1 clearance rate could be utilized. The kidney and the liver compartment have several clearance parameters; therefore, the testes are a soft tissue that behaves similarly to these main organs.

Another organ modeled as soft tissues was the spleen, whose retention is shown in Fig. 21. The simulation calculations for the spleen are comparable to the Paquet et al. (2006) data for most of the time points. The results from the simulation support the

identification of the spleen as a slow turnover (ST2) compartment for the soft tissue. The spleen is the soft tissue organ that is comparable for most of the evaluation points without modification.

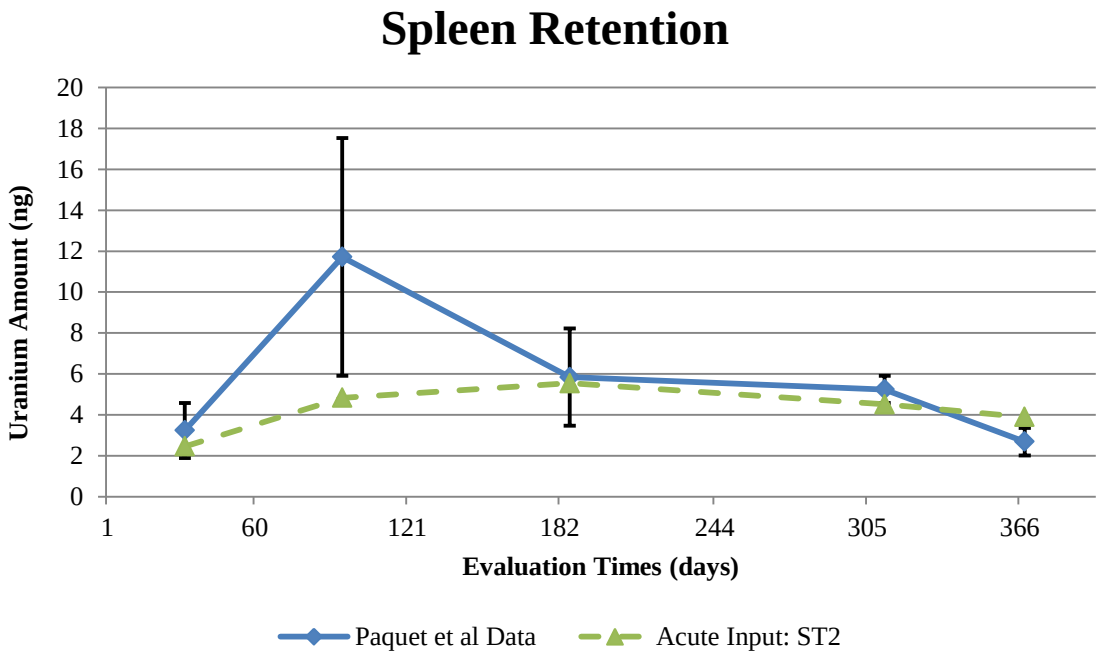

Fig. 21. Uranium Retention in the Spleen

The simulation also examined retention in the pancreas. The pancreas weight was estimated to be approximately 0.33% of the body weight (Lifson et al. 1985). The Paquet et al. (2006) data documents another increase in retention with a minimal retention starting around 312 days. Fig. 22 depicts the pancreas results from the simulation.

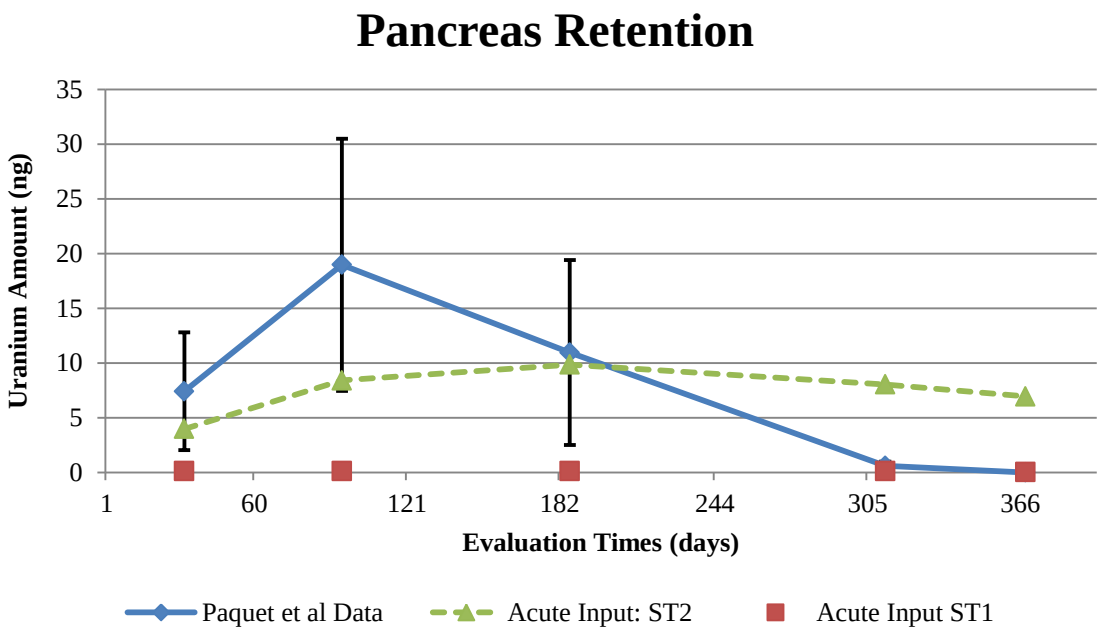

Fig. 22. Uranium Retention in the Pancreas

This organ cannot be defined as solely an ST1 or ST2 compartment. The pancreas response in the simulation starts as an ST2 clearance; however, the simulation results drastically decrease for the Paquet et al. (2006) result, indicating the ST1 clearance rate starting at the 312 evaluation day. The simulation with the consistent predefined parameter cannot model the biological results throughout every evaluation point.

Fig. 23 depicts the results for the final soft tissue organ, the brain. The ST2 compartment calculated a retention that was not comparable to the Paquet et al. (2006) data. Only the first evaluation time at 32 days was comparable to the Paquet et al. (2006) information. The ST1 compartment documents minimal uranium retention as was shown in previous soft tissue compartments.

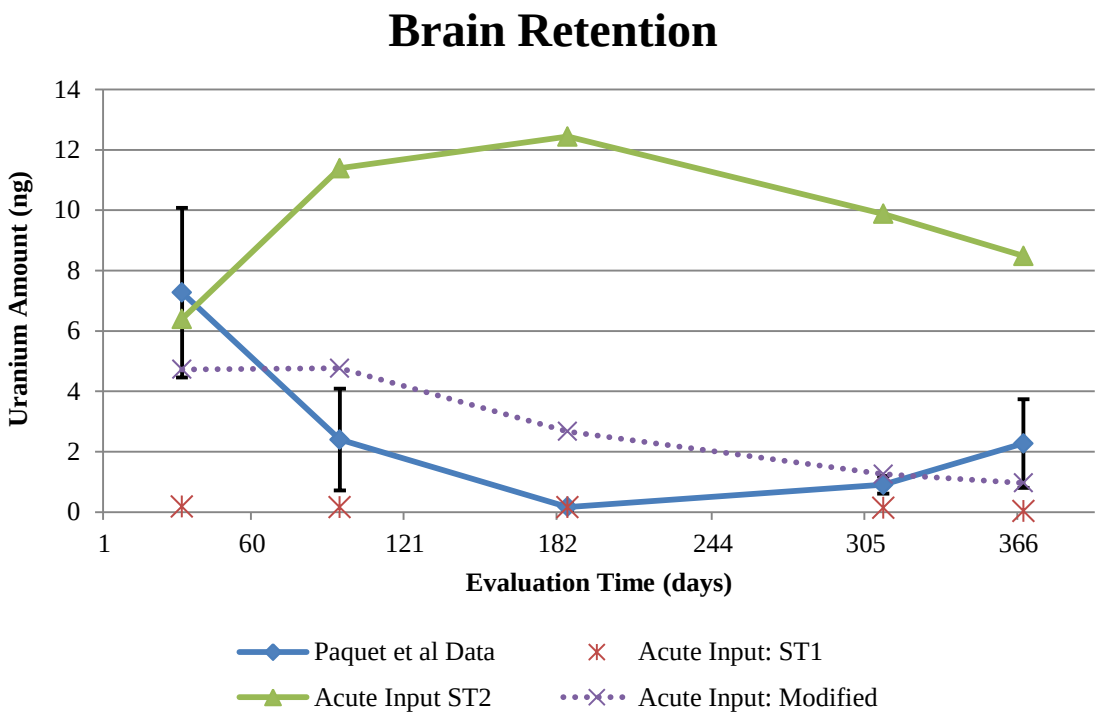

Fig. 23. Uranium Retention in the Brain

The clearance rate for this system was chosen as a parameter to modify and identify the results. The "Acute Input: Modified" data was formatted into Fig. 23. This data accounts for the reduction of the clearance rate to 23 days. The ST2 removal half-time of 100 days documented high retention results and the ST1 removal half-time of 3 days documented a very minimal retention amount. The removal clearance parameter was initially reduced from 100 days to 40 days; however, the results not could be considered comparable to the Paquet et al. (2006) data, so a clearance rate of 23 days was tested and graphed for uranium retention.

With the paucity of research data for the soft tissues, the results of the simulation expand upon the limit in biological research. The simulation has identified organs that require additional investigation. Although the simulation could not model the Paquet et al. (2006) data completely, the simulation did document retention sites within these organs that are often ignored or lumped together in research. The breakdown of the soft tissue organs expands upon the current acute model for the ST1 and ST2 tissues. The model addresses the current clearance rate that is utilized as a whole for the soft tissues. As more research becomes available, this model can be expanded upon to account for the soft tissues as single compartments, similar to the kidneys and liver.

CHAPTER VI

CONCLUSION

The simulation model for this report was created using a compilation of biological rat data, which was incorporated into an acute reference model for transfer and deposition of uranium. This simulation, which was based on the widely used Sprague Dawley rat, attempted to model the biological processes that occur within a rat such as organ growth. The model is user friendly and can be easily modified to account for other experimental parameters. In attempting to initially model chronic ingestion, this research found that a single input of radionuclide fit the data better than a slow continuous input. Modifications to the model are possible in the future as more information on the biokinetics of radionuclides in rat organs becomes available.

The evaluation of uranium effects is a continuing concern from ongoing dispersal of uranium pollutants in the environment (Dublineau 2007) and the natural uranium that continues to surround all living organisms. The computer simulation can be used to focus on parameters that are most important. The simulation accounted for retention of uranium for several organs and simulation points; however, some parts of the model could not replicate the biological data. Paquet et al. (2006) also described a computer program that had little success in modeling their data. The simulation was created using an acute baseline model that is currently available. This simulation, although not comparable throughout the evaluation times, can be utilized as another baseline component because several compartments were modified. The simulation was tested

using various intake deposition amounts and clearance rates, which affected the model response. This research is a starting point to the future expansion as more research data and biological information becomes available.

The simulation utilized a baseline model to implement organ mass change within a rat to evaluate an acute model with predefined parameters. The results from this research can lead to expansion as the model identified several sections of the baseline model that require additional testing of the current biological parameters. The simulation identified that the clearance parameters, if modified, can simulate the biological results. In addition to the clearance parameters that were questioned within this report, there is additional published literature that measures the f_1, the fractional absorption coefficient. This simulation utilized a constant absorption coefficient to initially identify the response from the program. Additional tests modifying the fractional absorption coefficient can be done. The advantage to the simulation program that was created is that these variables, along with others such as the sex of a rat, can be tested to create a new reference model for a chronic intake of uranium.

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APPENDIX A

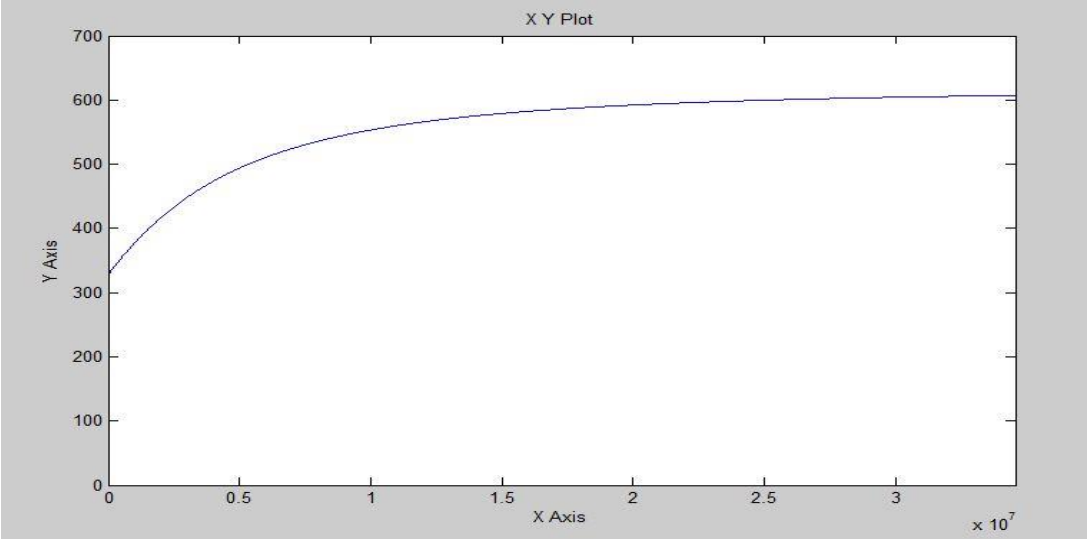
Transfer Rate Coefficient (adapted from Leggett and Pellmar 2003 and Paquet et al. 2006)

Compartment 1	Compartment 2	Trans. Rate: days^{-1}	Trans. Rate: sec^{-1}
Gastrointestinal Tract			
Stomach	Small Intestine	2.64E+01	3.06E-04
Small Intestine	Upper Large Int.	7.20E+00	8.30E-05
Small Intestine	Blood Plasma	2.75E-02	3.18E-07
Upper Large Int.	Lower Large Int.	4.86E+00	5.60E-05
Lower Large Int.	Fecal Excretion	5.50E+00	6.40E-05
Total Circulation			
Plasma	Rapid ST (ST0)	6.00E+01	6.94E-04
Rapid ST (ST0)	Plasma	8.32E+00	9.60E-05
Soft Tissues (ST)			
Plasma	Intermediate (ST1)	3.50E+00	4.10E-05
Intermediate (ST1)	Plasma	2.31E-01	3.00E-06
Plasma	Slow (ST2)	1.40E+00	1.60E-05
Slow (ST2)	Plasma	6.93E-03	8.02E-08
Liver			
Plasma	Liver 1	1.12E+00	1.30E-05
Liver 1	Liver 2	2.97E-03	3.44E-08
Liver 1	Plasma	9.60E-02	1.00E-06
Liver 2	Plasma	6.93E-03	8.02E-08
Red Blood Cells (RBC)			
Plasma	RBC	2.80E-01	3.00E-06
RBC	Plasma	6.93E-01	8.00E-06
Kidneys			
Plasma	Kidney 1	2.80E+01	3.24E-04
Plasma	Urinary Bladder	7.00E+01	8.10E-04
Kidney 1	Urinary Bladder	1.73E-01	2.00E-06
Urinary Bladder	Urine	1.00E+00	1.20E-05
Plasma	Kidney 2	7.00E-01	8.00E-06
Kidney 2	Plasma	1.39E-02	1.60E-07
Bone/Skeleton			
Plasma	Bone 1	2.80E+01	3.24E-04
Bone 1	Plasma	6.93E-02	8.02E-07
Bone 1	Bone 2	6.93E-02	8.02E-07
Bone 2	Bone 3	5.78E-03	6.69E-08
Bone 2	Bone 1	1.73E-02	2.00E-07
Bone 3	Plasma	3.00E-03	3.47E-08

APPENDIX B

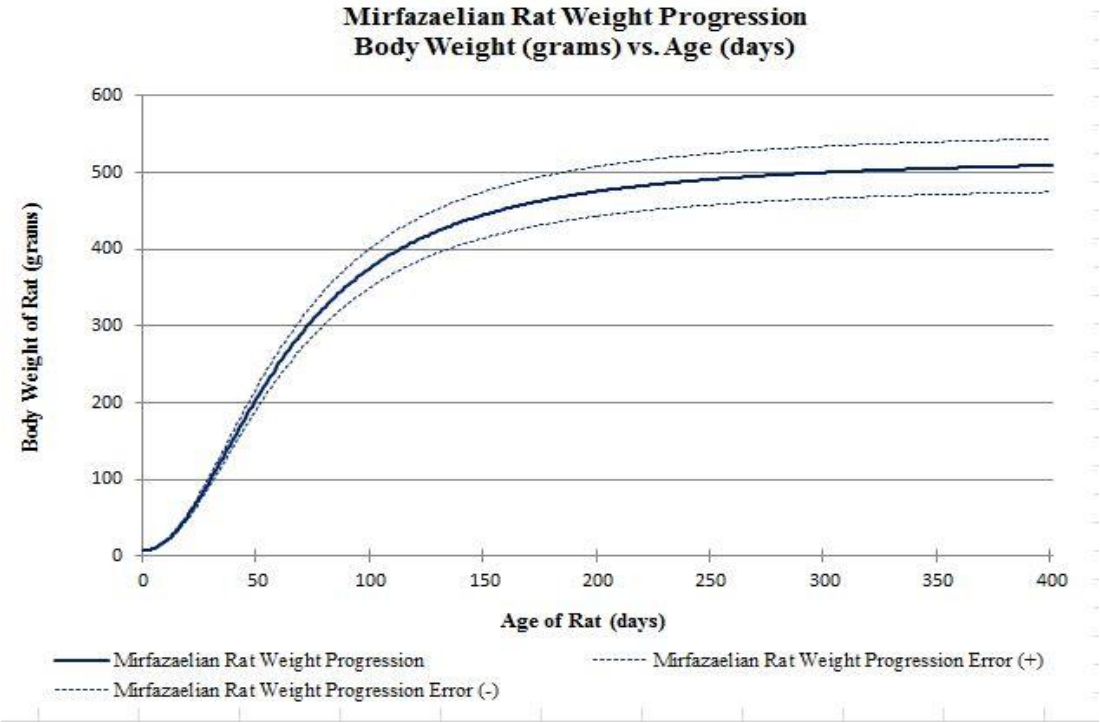
Example of Graphical Representation of Organ Change (Paquet et al. 2006 Data)

Example Simulink Simulation for the Paquet et al. 2006 Graph with Data Input

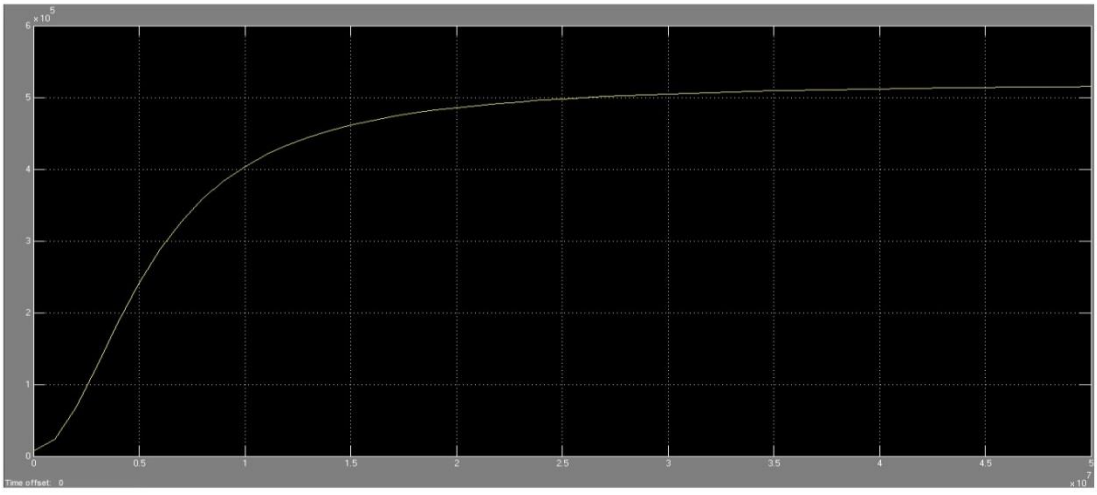


((8676.12*(63.21^2.01)) + (618000 * (((u+(5.74128E6))/86400)^2.01))) / ((63.21^2.01) + (((u+(5.74128E6))/86400)^2.01))

Example of Graphical Representation of Organ Change (Mirfazaelian et al. 2007 Data)



Example of Simulink Simulation for the Mirfazelian et al. 2007Graph with Data Input



((7314.70*(63.21^2.01)) + (521026.13 * (((u+(5.74128E6))/86400)^2.01))) / ((63.21^2.01) + (((u+(5.74128E6))/86400)^2.01))

APPENDIX C

Paquet Data Table (with permission from Paquet et al., 2006)

	Amount of Uranium in Organ/Tissue (ng/gram)				
Tissue/Organ	**32 days**	**95 days**	**186 days**	**312 days**	**368 days**
Teeth	659 ± 56	656 ± 139	625 ± 94	455 ± 105	439 ± 92
Whole Brain	3.1 ± 1.2	1 ± .7	.07 ± .05	.37 ± .12	.93 ± .60
Cerebellum	21.5 ± 9.1	2.2 ± 1.1	.80 ± .61	1.81 ± .20	.53 ± .12
Striatum	1.63 ± .62	.92 ± .57	------	-------	.49 ± .21
Thalamus	54.4 ± 15.4	.79 ± .45	2.54 ± 1.24	34.3 ± 18.2	24.2 ± 14.5
Hippocampus	.92 ± .75	4.5 ± 2.5	5.4 ± 3.1	30.3 ± 12.8	14.6 ± 3.1
Cortex	ND	1.3 ± .98	ND	ND	.32 ± .14
Total Gut	1295 ± 82	1178 ± 221	990 ± 207	1265 ± 250	.05 ± .01
Esophagus	190 ± 21	178 ± 47	22.8 ± 6.8	115 ± 54	.57 ± .36
Stomach *	1167 ± 326	623 ± 315	157 ± 55	524 ± 123	1.7 ± 1.7
Small Int. *	920 ± 114	499 ± 119	279 ± 67	231 ± 51	9.1 ± 5
Large Int. *	2222 ± 102	2777 ± 719	2957 ± 814	3877 ± 911	------
Pancreas	5.1 ± 3.7	10.7 ± 6.5	5.7 ± 4.4	.3 ± .2	------
Spleen	3.6 ± 1.5	11.5 ± 5.7	5.4 ± 2.2	4.7 ± .6	2.4 ± .6
Liver	.12 ± .08	2.1 ± 1.9	.5 ± .2	26.7 ± 15.0	16.8 ± 6.7
Lungs w/trachea	29.6 ± 20.8	42.9 ± 31.3	------	2.4 ± 2.4	------
Heart	1.2 ± .6	.34 ± .23	.40 ± .11	.56 ± .27	.06 ± .06
Muscle	-------	2.0 ± 1.3	1.4 ± 1.11	2.2 ± 1.9	.3 ± .1
Lumbar Vert.	33.3 ± 4.8	35.6 ± 8.1	29.2 ± 8.3	19.9 ± 4.4	23.2 ± 6.6
Total Femurs	24.9 ± 2.9	65.1 ± 4.3	34.2 ± 10.6	59.9 ± 2.7	60.5 ± 7.7
Diaphysis	21.3 ± 4.6	73.5 ± 6.4	48.2 ± 13.1	69.9 ± 2.0	74 ± 7.9
Epiphysis	28.4 ± 2.3	55.4 ± 4.0	22.1 ± 10	52.2 ± 4.0	49.5 ± 7.5
Kidneys	220 ± 54	97.3 ± 13.2	82 ± 13	60.5 ± 16.8	72.4 ± 15.5
Testes	2.2 ± 1.9	30.2 ± 21.8	.48 ± .16	.79 ± .06	.34 ± .09
Head	9.1 ± 4.3	9.3 ± 4.4	11.3 ± 2.1	20.3 ± 14.4	7.3 ± 5.5
Carcass	1.5 ± .5	111 ± 51	2.1 ± .6	51.5 ± 20.5	2.1 ± .9
Whole Body	51 ± 3.0	182 ± 66	41.2 ± 8.9	134 ± 28	3.8 ± .6

* only accounts for wall of organ