DOSE-RESPONSE ANALYSIS OF HYPOTHALAMIC-PITUITARY-THYROID (HPT) AXIS PERTURBATIONS IN THE ADULT RAT USING STATISTICAL METHODS FOR THE BINARY MIXTURE OF PCB126 AND PERCHLORATE AND COMPUTATIONAL MODELING FOR IODIDE DEFICIENCY AND PERCHLORATE

by

EVA DANEKE MCLANAHAN

(Under the Direction of Jeffrey W. Fisher)

ABSTRACT

Some environmental chemicals affect endocrine function and may alter hormone systems at low doses. The hypothalamic-pituitary-thyroid (HPT) axis controls many physiologic functions, including metabolism, growth, development, and reproduction. Two studies were conducted to evaluate the low dose effects of ammonium perchlorate (ClO$_4^-$) on the HPT axis of adult male rats pretreated with 3,3',4,4',5'-pentachlorobiphenyl (PCB126). Both compounds are widespread environmental contaminants and have well characterized primary modes of action for disruption of the HPT axis. Results indicated that for rats pretreated with PCB126 and then placed on drinking water containing ClO$_4^-$ the effects on the HPT axis endpoints examined were less than additive, and PCB126 appeared to mask the effects of ClO$_4^-$. The TSH stimulated thyroid created a condition where the effect of ClO$_4^-$ on inhibition of thyroidal iodide uptake was diminished. In addition, no synergistic or greater than additive responses were observed when animals were dosed at concentrations at or near the no-observed-effect-level (NOEL).
A biologically based dose-response (BBDR) model of the adult male rat HPT axis was also constructed. The model for the adult male rat includes sub-models for dietary iodide, thyroid stimulating hormone (TSH), as well as thyroid hormones, thyroxine (T₄) and 3,5,3′-triiodothyronine (T₃). First, the individual sub-models were developed independently of one another using radiolabeled tracer studies to estimate various model parameters. Then, the models were combined to form one endogenous model that includes (1) feedback of T₄ on TSH production, (2) stimulation of T₄/T₃ production and thyroidal iodide uptake by TSH, and (3) the use of thyroidal iodide in hormone production. Model application included prediction of perturbations in the thyroid axis that result in iodide deficient conditions, as well as linking the BBDR-HPT axis model with a physiologically based pharmacokinetic (PBPK) model for ClO₄⁻ by the primary mode of action, competitive competition of thyroidal iodide uptake. Model exercises revealed the distinct possibility of an additional mode of action for ClO₄⁻ perturbation of the system. These models demonstrate the ability of the BBDR-HPT axis model to be integrated with other PBPK models for thyroid toxic compounds to predict changes based on the mode of action of the compound.

INDEX WORDS: PCB126, Perchlorate, Thyroid, Rat, BBDR Model, Iodide, Thyroid hormones, HPT Axis
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BSEH, The University of Georgia, 2003

A Dissertation Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

DOCTOR OF PHILOSOPHY

ATHENS, GEORGIA

2007
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December 2007
DEDICATION

This work is dedicated to my inspiration and first mentor in the field of toxicology, “Rambo” Randy Manning. I admire him for his integrity, dedication, and wealth of knowledge. He has truly been a phenomenal role model and cherished friend.

I also dedicate this work to my husband, Paul, whose immeasurable patience and understanding is invaluable. I am eternally grateful for his unwavering faith in me and willingness to make many sacrifices, thereby providing the means for me to complete my degree. In addition, my four-legged friends provided comfort, love, and always brightened my day. Coltrane was a steadfast companion, greeting me no matter what time I arrived home and always willing to listen. And dear little Charlie (♂ GDF, 5496), her excitement for life, passion for adventure, and unconditional love enriched my life like never before.
ACKNOWLEDGMENTS

“The future belongs to those who believe in the beauty of their dreams.”

- Eleanor Roosevelt

All my life I have been fortunate to have a strong foundation of support provided by my friends and family. My parents have always encouraged me to pursue my dreams and persevere to accomplish my goals, and for that I express my utmost appreciation. I am grateful for the love, encouragement, and patience provided by my immediate and extended family, as well as all my friends throughout this endeavor.

Numerous people have assisted with the completion of this research, and I am very appreciative of all their contributions to make this work a success. Researchers at AFRL/HEPB, USEPA/NHEERL, Boston Medical Center, and UGA laboratories of Drs. James V. Bruckner and Duncan C. Ferguson provided analytical assistance. Special thanks to Srinivasa Muralidhara (SM) for his patience and hours of training and laboratory assistance. My sincere gratitude goes to my graduate committee members, Drs. Marsha C. Black, James V. Bruckner, Deborah A. Keys, and David R. Mattie, who guided me, believed in me, and held me to the high standards necessary to succeed as a scientist.

I am indebted to my advisor and friend, Dr. Jeffrey W. Fisher, who was always available to provide academic and emotional support. He instilled his faith in me and had confidence in my ability to accomplish the exciting, yet extremely challenging, and monumental task of modeling the thyroid axis. However, I could not have completed the research without the many hours he spent collaborating and reviewing my project. Thank you.
Finally, the financial support for this research was provided in many forms. Primary funding for this work was provided by the Agency for Toxic Substances and Disease Registry (ATSDR) (U61/ATU472105) and the United States Environmental Protection Agency (U.S. EPA) Science to Achieve Results (STAR) grant (RD83213401-0). I was fortunate to have been the recipient of two Fellowships, including a National Science Foundation GK-12 Graduate Teaching Fellowship (DGE0229577) and an U.S. EPA STAR Fellowship (FP916793). Special thanks to Dr. David A. Knauft (PI – DGE0229577) for three busy years, a plethora of learning, and an unforgettable excursion to Costa Rica.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ACKNOWLEDGMENTS</td>
<td>v</td>
</tr>
<tr>
<td></td>
<td>LIST OF TABLES</td>
<td>xi</td>
</tr>
<tr>
<td></td>
<td>LIST OF FIGURES</td>
<td>xii</td>
</tr>
<tr>
<td>1</td>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Purpose of Study</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Scope of Dissertation</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>LITERATURE REVIEW</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Modeling of the HPT Axis</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Mixture Studies of Thyroid Active Chemicals</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>References</td>
<td>12</td>
</tr>
<tr>
<td>3</td>
<td>LOW-DOSE EFFECTS OF AMMONIUM PERCHLORATE ON THE HYPOTHALAMIC-PITUITARY-THYROID (HPT) AXIS OF ADULT MALE</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>Abstract</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>Introduction</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>Materials and Methods</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>Results</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>Discussion</td>
<td>31</td>
</tr>
</tbody>
</table>
Acknowledgments ...................................................................................................36
References ...............................................................................................................37
Supplementary Data ..............................................................................................52

4 A BIOLOGICALLY BASED DOSE-RESPONSE MODEL FOR DIETARY IODIDE
AND THE HYPOTHALAMIC-PITUITARY-THYROID (HPT) AXIS IN THE
ADULT RAT: EVALUATION OF IODIDE DEFICIENCY ......................................57
Abstract ...................................................................................................................58
Introduction .............................................................................................................59
Materials and Methods ............................................................................................62
Results .....................................................................................................................78
Discussion ...............................................................................................................84
Funding ....................................................................................................................89
Acknowledgments ...................................................................................................89
References ...............................................................................................................90
Supplementary Data ..............................................................................................127

5 THE USE OF A BIOLOGICALLY BASED DOSE-RESPONSE MODEL OF THE
HYPOTHALAMIC-PITUITARY-THYROID (HPT) AXIS TO EVALUATE
PERCHLORATE INDUCED PERTURBATIONS OF THE HPT AXIS IN
ADULT RATS ......................................................................................................129
Abstract .................................................................................................................130
Introduction ...........................................................................................................131
Materials and Methods ..........................................................................................133
Results ...................................................................................................................137
Discussion .............................................................................................................143
Acknowledgments .................................................................................................147
References .............................................................................................................147

6 CONCLUSIONS........................................................................................................173

PCB126/Perchlorate Mixtures Study .................................................................173
BBDR-HPT Axis Iodide Deficiency Model ............................................................174
BBDR-HPT Axis Model of Perchlorate Perturbation ...........................................175
Future Applications of the BBDR-HPT Axis Model ...........................................176

APPENDICES .............................................................................................................................177

A The acslXtreme (version 2.4.0.11) .csl file for BBDR-HPT axis radiolabeled model
code (Chapter 4) is contained within this Appendix .................................................177
B The acslXtreme (version 2.4.0.11) .m file for BBDR-HPT axis radiolabeled model
simulations (Chapter 4) is contained within this Appendix ........................................188
C The acslXtreme (version 2.4.0.11) .csl file for BBDR-HPT axis iodide deficiency
model code (Chapter 4) is contained within this Appendix. .....................................195
D The acslXtreme (version 2.4.0.11) .m file for BBDR-HPT axis iodide sufficient and
deficient model simulations (Chapter 4) is contained within this Appendix ......210
E The procedure (PROCED) commands used in the BBDR-HPT axis model manuscript
(Chapter 4) to set the initial values at each starting body weight are included in this
Appendix. .........................................................................................................................219
F The acslXtreme (version 2.4.0.11) .csl file for BBDR-HPT axis and perchlorate
PBPK model code (Chapter 5) is contained within this Appendix ......................226
The acslXtreme (version 2.4.0.11) .m files for BBDR-HPT axis and ClO₄⁻ PBPK model simulations (Chapter 5) is contained within this Appendix.
LIST OF TABLES

Table 3.1: Study design and dosing schedule for Dosing Study I .................................................43
Table 3.2: Study design and dosing schedule for Dosing Study II ................................................44
Table 3.3: The liver/body weight (BW) ratios for all animals in Dosing Study I were multiplied
by 100 and are shown in the Table ± SEM ...................................................................45
Table 3.1S: Thyroid Histopathology C/EFC data for Dosing Study I ...........................................53
Table 3.2S: Thyroid Histopathology C/EFC data for 1 Day Perchlorate Exposure (Dosing Study
II) ...................................................................................................................................53
Table 3.3S: Thyroid Histopathology C/EFC data for 4 Day Perchlorate Exposure (Dosing Study
II) ...................................................................................................................................53
Table 3.4S: DOSING STUDY I ....................................................................................................54
Table 4.1: Model Boundaries for Parameter Estimation ...............................................................99
Table 4.2: Physiological Parameters for the Adult Rat .................................................................100
Table 4.3: Compound-Specific Parameters .................................................................................101
Table 4.1S: Compound-Specific Parameters for Radiotracer Sub-models ..................................128
Table 5.1: Physiological Parameters for the Adult Rat .................................................................153
Table 5.2: Perchlorate and Iodide Parameters .............................................................................154
Table 5.3: Thyroid Hormone and TSH Production Parameters ...................................................154
## LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 3.1</td>
<td>Dosing Study I</td>
<td>46</td>
</tr>
<tr>
<td>Figure 3.2</td>
<td>Dosing Study II</td>
<td>48</td>
</tr>
<tr>
<td>Figure 3.3</td>
<td>Stable endogenous iodide ($^{127}\text{I}$) measured in one lobe of the thyroid from a portion of the rats in Dosing Study II</td>
<td>50</td>
</tr>
<tr>
<td>Figure 3.1S</td>
<td>Thyroid axis response up to 5 days following PCB126 oral gavage dose of either 0.075 (●), 0.75 (○), or 7.5 (▼) μg/kg</td>
<td>55</td>
</tr>
<tr>
<td>Figure 4.1</td>
<td>Sub-model structure for radiotracer compounds used in model development and preliminary estimation of kinetic parameters</td>
<td>102</td>
</tr>
<tr>
<td>Figure 4.2</td>
<td>BBDR-HPT axis model structure for the adult rat hypothalamic-pituitary-thyroid (HPT) axis, including sub-models (areas shaded in gray) for dietary iodide ($^{127}\text{I}$), TSH, T₄, and T₃</td>
<td>104</td>
</tr>
<tr>
<td>Figure 4.3</td>
<td>Relationship between total amount of thyroidal iodide and fraction of total thyroid hormone production that is T₃</td>
<td>106</td>
</tr>
<tr>
<td>Figure 4.4</td>
<td>Model simulations (lines) compared with literature data (points) for iv doses of radiolabeled compounds used in HPT axis model development</td>
<td>108</td>
</tr>
<tr>
<td>Figure 4.5</td>
<td>Steady-state, iodide sufficient, model simulations (lines) shown with literature data (points) for HPT axis model calibration</td>
<td>112</td>
</tr>
<tr>
<td>Figure 4.6</td>
<td>Short term effects of LID (0.35μg I/day) on serum thyroid hormones and total thyroid iodide of adult male Holtzman Sprague-Dawley rats</td>
<td>115</td>
</tr>
</tbody>
</table>
Figure 4.7: Long term effects of LID (0.33μg I/day) on serum thyroid hormones and total thyroid iodide of adult male (A) Simonsen Albino and (B) Holtzman Sprague-Dawley rats. 117

Figure 4.8: Long term effects of LID (1.14 μg I/day) on serum thyroid hormones and total thyroid iodide of adult male Holtzman Sprague-Dawley rats. .............................119

Figure 4.9: Recovery from ID in adult male Sprague-Dawley rats fed a LID for seven months. 121

Figure 4.10: Normalized sensitivity coefficient (NSC) graph for parameters that yielded a NSC of 0.90 or greater for at least one response (serum T4, T3, and TSH or total thyroid iodide) examined under iodide sufficient (20 μg I/day) (left) and iodide deficient (1 μg I/day) (right) steady-state conditions. ..............................................................123

Figure 4.11: Iodide dose-response plot for serum T4 and TSH. ..........................................................125

Figure 5.1: Perchlorate PBPK model structure for the adult rat. ..........................................................155

Figure 5.2: BBDR-HPT axis model structure as described previously (McLanahan et al., submitted). .................................................................157

Figure 5.3: Model simulations (lines) of serum (——) and thyroid (——) concentrations following 3.3 mg 36ClO4⁻/kg bw iv dose in adult male Sprague-Dawley rats compared with dta (serum: ● ± SD and thyroid: ○ ± SD). ..............................................................159

Figure 5.4: BBDR-HPT axis and ClO4⁻ PBPK integrated model predictions with ClO4⁻ inhibition of NIS thyroidal iodide transport. .................................................................161

Figure 5.5: BBDR-HPT axis model predictions following exposure to ClO4⁻ in drinking water, including inhibition of thyroidal iodide uptake and suppression of thyroid hormone production by ClO4⁻. .................................................................164

Figure 5.6: Serum (A) and thyroid (B) concentrations of ClO4⁻ in adult rats following exposure to ClO4⁻ in drinking water. .................................................................167
Figure 5.7: HPT axis perturbations in serum T₄ (_____ ) and TSH (___ ...) following drinking water exposure to 15 mg ClO₄⁻/kg-day in adult rats .................................................................169

Figure 5.8: Dose-response plot for serum T₄ and TSH after exposure to ClO₄⁻ in drinking water for 14 days ......................................................................................................................171
CHAPTER 1

INTRODUCTION

Many environmental chemicals have been shown to affect the hypothalamic-pituitary-thyroid (HPT) axis of vertebrates and invertebrates. Two widespread environmental chemicals, 3,3’,4,4’,5-pentachlorobiphenyl (PCB126) and perchlorate (ClO₄⁻), were evaluated based on their primary, well-defined modes of action to disrupt the HPT axis in rodents. Laboratory studies with the adult rat were conducted to gain insights into the kinetics of these chemicals and their ability to disrupt homeostasis. In many cases rodent toxicity data are used to extrapolate to humans and make predictions of human health effects. One method of extrapolating the kinetics and potential effects of chemicals on laboratory animals is through the use of mathematical modeling, such as physiologically based pharmacokinetic (PBPK) or biologically based dose-response (BBDR) modeling. This dissertation includes the statistical analyses of the effects of ClO₄⁻ on adult male rats pretreated with PCB126 and the development of BBDR models of the HPT axis in the adult rat that predict perturbations resulting from iodide deficient conditions and ClO₄⁻ exposures.

Purpose of Study

The purpose of this study was to evaluate the hypothalamic-pituitary-thyroid (HPT) axis dose dependent effects of ClO₄⁻ on adult male rats pretreated with varying doses of PCB126, using knowledge of their primary modes of action. Two studies were conducted to determine the dose-response characteristics for binary mixtures of ClO₄⁻ and PCB126. Adult rats were
pretreated with a wide range of single oral bolus doses of PCB126 and then placed on drinking water containing ClO₄⁻, also over a range of concentrations known to cause minimal to no effect on the HPT axis. This exposure scenario allowed for: 1) the evaluation of ClO₄⁻ effects on the HPT axis in rats that were in various hypothyroid states when ClO₄⁻ treatment was initiated, 2) the evaluation of the dose-response characteristics for a binary mixture of chemicals that act on the HPT axis by two different modes of action, and 3) the collection of kinetic data sets for future development of a BBDR model to describe the interactions of a binary mixture on the HPT axis.

Finally, the ultimate goal was to develop a biologically based dose-response (BBDR) model of the adult rat HPT axis (BBDR-HPT axis model) that could be linked to physiologically based pharmacokinetic (PBPK) models of thyroid active compounds to predict perturbations in the thyroid system. However, because ClO₄⁻ (a thyroid active chemical) is thought to cause an iodide deficient condition in the thyroid by competitive inhibition of iodide uptake, it was necessary first to mathematically describe the changes induced by iodide deficiency using literature derived datasets. Next, a PBPK model for ClO₄⁻ was constructed and linked to the BBDR-HPT axis model to predict perturbations in serum thyroid hormone concentrations. The BBDR-HPT axis model was developed with the idea that it can be integrated with PBPK models for additional thyroid active chemicals to predict disturbances from exposure alone (e.g. PCB126) or in combination (e.g. PCB126 and ClO₄⁻). Furthermore, the BBDR-HPT axis model, presented in Chapter 4, will be used as the foundation for expansion and description of the maturing and developing HPT axis in rodents and humans.
Scope of Dissertation

This dissertation includes a brief literature review (Chapter 2) of approaches and models developed previously for the HPT axis. Due to the clinical significance of the HPT axis, many models have been created, although none have included dietary iodide, an indispensable element for thyroid hormone production. The approaches, successes, and problems associated with the models are discussed. Research published on the HPT axis effects that result from chemical mixtures are also reviewed.

Following the literature review, Chapter 3 describes the experiments and conclusions of the laboratory studies to determine binary mixture effects of PCB126 and ClO₄⁻ on the adult male rat HPT axis. This chapter was published in Toxicological Sciences (McLanahan et al., 2007). Portions of Chapter 3 were presented at several national scientific meetings, including poster presentations at the Society of Toxicology Contemporary Concepts in Toxicology special meeting (February 2005; Atlanta, GA), Society of Toxicology annual conference (March 2005; New Orleans, LA), and an invited oral presentation at the Toxicology and Risk Assessment Conference (April 2006; Cincinatti, OH). Chapter 4 reports the development of a BBDR-HPT axis model for the adult rat and application to iodide deficient conditions and was submitted to Toxicological Sciences (McLanahan et al., submitted October 2, 2007). A portion of Chapter 4 was presented in poster format at the U.S. EPA Graduate Fellowship Conference (September 2006; Washington, D.C.) and the Society of Toxicology annual conference (March 2007; Charlotte, NC). In Chapter 5, the BBDR-HPT axis model presented in Chapter 4 is combined with a PBPK model for ClO₄⁻ to simulate the ClO₄⁻ induced perturbations on serum thyroid hormones and will be submitted to Environmental Health Perspectives. The final chapter,
Chapter 6, summarizes the conclusions and importance of this research project and future work that will benefit from, and build upon the BBDR-HPT axis model developed.

Two invited book chapters are in progress based on the research reported in this dissertation and are co-authored with Dr. Jeffrey W. Fisher. A chapter in “Principle and Practice of Mixtures Toxicology” (M. Mumtaz, ed.) focusing on the effects of chemical mixtures on the HPT axis and a case study of the research in Chapter 3. Also, a chapter on HPT axis mathematical models in “Quantitative Modeling in Toxicology” (K. Krishnan and M.E. Andersen, eds.) is under development based upon the research presented in Chapters 4 and 5.
CHAPTER 2
LITERATURE REVIEW

Many clinical and research endocrinologists have reviewed the details of the thyroid axis over the past several decades (Bianco et al., 2002; Boas et al., 2006; Carrasco et al., 1993; Dohan et al., 2003; Hennemann et al., 2001; Morley, 1981; Vassart et al., 1992, Yen et al., 2001; and Zoeller et al., 2007). However, no reviews focus on the mathematical models of the thyroid axis or how mixtures of compounds affect the homeostasis and control mechanisms of the thyroid axis. Therefore, the aim of this literature review is to evaluate models of the hypothalamic-pituitary-thyroid (HPT) axis that have been developed, as well as several studies on how chemical mixtures disrupt the HPT axis.

Modeling of the HPT Axis

In the past, multiple mathematical descriptions of HPT axis components have been reported. However, to date, the most common approaches used to describe production, distribution, metabolism, and elimination of thyroid hormones involve multiexponential, multicompartamental, and noncompartmental analyses. DiStefano and Landaw (1984) presented a review and explanation of these types of models.

Briefly, multiexponential models are often described as models of data and are composed of polynomial functions and sums-of-exponential functions, which do not require hypotheses about the physiological processes. These models have been used and developed for many areas of science (e.g. classical pharmacokinetics and engineering); however, without including
mechanisms or physiology in the mathematical descriptions, they cannot be used reliably for extrapolation or predictive purposes.

Multicompartmental models and noncompartmental models are models of systems and often have two or more compartments interconnected, to describe an exchange of material. Exchange may occur by movement across a barrier (e.g. membrane transport) or undergoing metabolic transformation. Multicompartmental models involve mass balance and rates of transfer from one compartment (pool) to another. Physiologically based pharmacokinetic (PBPK) models involve mass balance and transfer of masses from one compartment to another. PBPK models often have physiologically analogous compartments, while the pools in classical pharmacokinetic (PK) multicompartmental models are equivalent distribution volumes, although sometimes they can be assigned physiological identity. Noncompartmental models are often used to estimate steady-state whole organism parameters including volume of distribution ($V_D$), plasma clearance rate (PCR), and whole-body mean residence time (MRT). Noncompartmental models are useful when measurements are made in a single pool and only one endogenous or exogenous source feeds the pool, but is not desired to describe data collected from systems where there is more than on source (e.g. endocrine systems including the thyroid) (DiStefano, 1982).

Landmark classical PK multicompartmental models for the thyroid hormones (thyroxine, $T_4$, and 3,5,3’-triiodothyronine, $T_3$) and their derivatives (3,3’,5’-triiodothyronine, $rT_3$; 3,3’-diiodothyronine, 3,3’-T$_2$; 3’,5’-diiodothyronine, 3’,5’-T$_2$; and 3’-monoiodothyronine, $T_1$) have been developed for the rodent by DiStefano and colleagues (DiStefano et al., 1982; DiStefano and Feng, 1988). In general, these models were based upon radiolabeled injections of the compounds and consisted of three or more compartments (plasma, fast, and slow pools). The PK
models developed by DiStefano and colleagues, employing multiexponential and multicompartamental techniques, were used to estimate kinetic parameters of thyroid hormone production, transport, and metabolism. The models were capable of reproducing kinetics of the iv doses of radiolabeled T3 and T4 but have little utility for exploration of perturbations on the axis due to exposure to thyroid active compounds. These models provided quantitative insights into the HPT axis, but are not models for endogenous forms of thyroid hormones and are therefore of limited usefulness for toxicological research.

Li (1995) and colleagues also employed a PK compartmental approach to model the dynamic and pulsatile nature of the human thyroid axis, including estimates for thyroid releasing hormone (TRH), thyroid stimulating hormone (TSH), T4, and T3. This model is different from previously developed models in that it described the pulsatile secretions of hormones, as well as feedback (e.g. TRH stimulation of TSH production and free T3 and T4 inhibition of TSH production), and serum binding of hormones in plasma and tissues. Unfortunately the model presented by Li et al. (1995) employs an approach that resulted in 54 unknown coefficients, which results in significant uncertainty and the inability to associate the coefficients with biological processes. However, comparing coefficients for a few selected parameters associated with the feedback control of the hormones, the authors suggest that the feedback effects of thyroid hormones on the hypothalamus (control of TRH secretion) are much smaller than effects on the pituitary (control of TSH secretion). Furthermore, the authors also suggest that thyroid hormone negative feedback control on TSH secretion has a significant impact when TRH concentrations are low, and this influence disappears when TRH concentration is high, resulting in a condition of maximal secretion of TSH where thyroid hormones have little influence on TSH secretion in humans. The utility of this model is to explore the magnitude and frequency of
the pulsatile nature of the HPT axis. This model cannot be readily incorporated or linked with PBPK models for compounds that disrupt the HPT axis because of the theoretical basis of the model (lacking data for model validation).

In 1996, Kohn et al. developed a physiological dosimetric model of the distribution of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in the rodent that also included a HPT axis sub-model. The very complex model presented for thyroid hormone regulation was comprised of five compartments (liver, thyroid, pituitary, rapidly perfused tissues, and blood), including the description of TSH stimulation of $T_4$ and $T_3$ production in the thyroid and regulation of TSH production by hypothalamic peptides (somatostatin, SS; and thyroid releasing hormone, TRH). However, the model did not describe the primary negative feedback loop of the HPT axis, inhibition of TSH secretion by $T_4$. This feedback was implicitly described by $T_4$ inhibiting the synthesis and secretion of the hypothalamic peptides that, in turn, regulated TSH secretion. Regardless, the TCDD model integrated with the HPT axis model was utilized to predict decreases in serum $T_4$, via a TCDD-dependent increase in phase II conjugation of $T_4$, and alterations in serum $T_3$ and TSH after 31 weeks of biweekly oral dosing of TCDD (Kohn et al., 1996).

In 2002, Dietrich et al., applied an engineering approach to describe the $T_4$/TSH negative feedback loop in humans and the pulsatile nature of TSH secretion. Four approaches were evaluated for their ability to describe the pulsatile nature of TSH secretion by the pituitary. One of the four models was able to replicate the fractal (complex, repetitive mathematical description) behavior of TSH oscillations seen in the empirical data observed in human subjects. Using these models the authors were able to provide insight into the mechanism of TSH release, suggesting that the regulation of TSH release is more complex than a simple non-competitive
inhibition of TRH mediated activation of TSH release (Dietrich et al., 2002). In addition, authors suggest that TSH may also play a role in an ultra-short feedback loop upon its own release from the pituitary gland (Dietrich et al., 2002).

More recently, Mukhopadhyay and Bhattacharyya (2006) implemented discrete time delays for hormone transport and feedback mechanisms in order to describe the pulsatile nature of TSH secretion in humans. This simple mathematical representation described the T₄/TSH negative feedback based upon serum T₄ concentration, and assumed that secreted TSH activated an enzyme in the thyroid to produce T₄. This model has limited applications, but did provide some insights into the thyroid axis malfunction causing periodic catatonic schizophrenia, which is thought to be associated with the periodic variations in TSH.

In addition to the aforementioned models, PBPK models for radiolabeled iodide in rodent and human have been developed for different life stages (Merrill et al., 2003, 2005; Clewell et al., 2003a, 2003b). These were based on ¹²⁵I or ¹³¹I kinetic studies and did not include dietary iodine (¹²⁷I) or thyroid hormones.

Although several types of kinetic models, as reviewed above, have been constructed to describe the thyroid axis, to date none have taken into account dietary iodide, TSH, T₄, and T₃. Therefore, one goal of this research was to develop a biologically based dose-response (BBDR) model of the adult male rat HPT axis, which includes the utilization of dietary iodide in the thyroid gland for thyroid hormone production.

Mixtures Studies of Thyroid Active Chemicals

Challenges exist to improve chemical risk assessment practices, particularly with the reliance of human health risk assessments on laboratory animal toxicology studies. Many individual chemicals are known to disturb the hypothalamic-pituitary-thyroid (HPT) axis in
rodents by fairly well understood mechanisms of action (Capen, 2001). Numerous studies on the effects of individual chemicals on the HPT axis have been reported. However, few HPT axis toxicity studies have been designed to evaluate the interactions that may occur from chemical mixtures. Assessing toxic effects of chemical mixtures is important because humans are exposed to mixtures of chemicals, not individual chemicals.

Perchlorate (ClO\(_4^-\)), a thyroid active chemical and an environmental contaminant, has received much attention over the last decade because of its prevalence in water systems (Motzer, 2001). The primary well-defined mode of action for ClO\(_4^-\) disruption of the thyroid axis is by blocking thyroidal uptake of iodide (Wolff, 1998). The ClO\(_4^-\) inhibition of thyroidal iodide uptake can result in a disease state called hypothyroidism, characterized by low circulating levels of T\(_4\) and increased levels of TSH. Recently, research efforts have been expanded to better understand the contributions of other common pollutants that share a similar mode of action as perchlorate on the thyroid gland, namely nitrate and thiocyanate (Braverman et al., 2005 and Tonacchera et al., 2004). Nitrate is found in food and water and thiocyanate in food and tobacco products. These authors report ‘response’ additivity based on the affinity (Km) of the anion for the sodium-iodide symporter (NIS) protein using a non-linear Michaelis Menten equation to describe competitive inhibition of thyroidal uptake of radiolabeled iodide (Tonacchera et al., 2004).

A few binary mixture studies have been conducted in rats that include perchlorate as one of the compounds. Khan et al. (2005) reported synergistic interactions occurred when rats ingested the binary combination of ammonium perchlorate (NH\(_4\)ClO\(_4\)) and sodium chlorate (NaClO\(_3\)) in drinking water for 7 days, as evidenced by greater decreases in serum T\(_4\) levels than seen with the individual chemicals. Interestingly, Khan et al. (2005) also noted that male Fischer
rats appear less sensitive than male Sprague-Dawley rats to NH₄ClO₄ induced alterations in the HPT axis. In a more recent study, perchlorate was administered alone or in combination with ethanol to female Myers’ rats to examine the effects on plasma thyroid hormones and brain catecholamine concentrations (James-Walke et al., 2006). However, perchlorate doses administered (300 µg/L and 3000 µg/L; yielding average intake of 0.06 and 0.6 mg/kg bw) for 21 days were not high enough to alone result in statistically significant effects on serum T₄ and T₃ concentrations. Furthermore, when these doses of perchlorate were administered with 10% ethanol, no further reductions in total serum T₄ or T₃ were observed (James-Walke et al., 2006).

In complex mixture studies with a different class of chemicals, namely organochlorines, Desaulniers et al. (2003) and Crofton et al. (2005) administered rats cocktails of mixtures containing polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p-dioxins (PCDDs), and polychlorinated dibenzofurans (PCDFs) and evaluated serum thyroxine (T₄) concentrations. A primary mechanism of action by which these chemicals act on the HPT axis is through activation of the aryl hydrocarbon receptor (AhR), resulting in enhanced hepatic metabolism and clearance of T₄, although several other mechanisms are possible (Crofton et al., 2005).

Additive decreases in serum T₄ concentrations were observed by Desaulniers et al. (2003) after administration of mixtures of either 6 PCDDs, 3 non-ortho-PCBs, or 7 PCDFs to prepubertal female Sprague-Dawley rats. Crofton et al. (2005) evaluated serum T₄ levels after four consecutive days of oral gavage dosing with a mixture of eighteen polyhalogenated aromatic hydrocarbons, including PCB126. There was no deviation from additivity at the lowest mixture dose, but a greater-than-additive decrease in serum T₄ was observed at the three highest mixture doses.
Many studies have been conducted to determine the effect of complex mixtures of thyroid active compounds; however, only the few mentioned above were conducted such that the contribution of each component of the mixture could be ascertained. For example, Zhou et al. (2002) evaluated the effect of a complex mixture of polybrominated diphenyl ethers on the thyroid axis; however, the contribution of each component of the mixture was not analyzed, so determination of the mixture effect relative to the individual compounds was not available. It is important to determine the ability of complex mixtures to affect the HPT axis, but also equally important is to determine the contributions of the mixture components in order to determine if the mixture behaves in a less than additive, additive, or greater than additive fashion.

References


CHAPTER 3

LOW-DOSE EFFECTS OF AMMONIUM PERCHLORATE ON THE HYPOTHALAMIC-PITUITARY-THYROID (HPT) AXIS OF ADULT MALE RATS PRETREATED WITH PCB1261

Abstract

The objective of this research was to characterize the disturbances in the hypothalamic-pituitary-thyroid (HPT) axis resulting from exposure to a binary mixture, 3,3’,4,4’,5-pentachlorobphenyl (PCB126) and perchlorate (ClO$_4^-$), known to cause hypothyroidism by different modes of action. Two studies were conducted to determine the HPT axis effects of ClO$_4^-$ on adult male Sprague-Dawley rats pretreated with PCB126. In Dosing Study I, rats were administered a single oral dose of PCB126 (0, 7.5, or 75 $\mu$g/kg) on Day 0 and nine days later ClO$_4^-$ (0, 0.01, 0.1, or 1 mg/kg-day) was added to the drinking water until euthanasia on Day 22. Significant dose-dependent trends were found for all thyroid function indices measured following ClO$_4^-$ in drinking water for 14 days. 75 $\mu$g PCB126/kg resulted in a significant increase in hepatic T$_4$-glucuronide (T$_4$-G) formation, causing a decline in serum T$_4$ and fT$_4$, and resulting in increased serum TSH. Serum TSH was also increased in animals that received 7.5 $\mu$g PCB126/kg; no other HPT axis alterations were found in these animals. When pretreated with PCB126 the ClO$_4^-$ dose trends disappeared, suggesting a less than additive effect on the HPT axis. In Dosing Study II, animals were given lower doses of PCB126 (0, 0.075, 0.75, or 7.5 $\mu$g/kg) on Day 0, and followed with ClO$_4^-$ (0 or 0.01 mg/kg-day) in drinking water beginning on Day 1 and continuing for several days to explore transient HPT axis effects. No statistical effects were seen for PCB126 or ClO$_4^-$ alone, and no perturbations were found when administered sequentially in Dosing Study II. In conclusion, these studies demonstrate that HPT axis disturbances following exposure to ClO$_4^-$ are less than additive when pretreated with relatively high doses of PCB126. At relatively low doses, at or near the NOEL for PCB126 and ClO$_4^-$, no interactions between the chemicals occur.

Key Words: PCB126, perchlorate, rat, thyroid, T$_4$, TSH, UDPGT
Introduction

Several important scientific challenges exist to improve chemical risk assessment practices, particularly with the reliance of human health risk assessments on laboratory animal toxicology studies. For example, most toxicology studies are conducted with a single chemical; however, humans are exposed to complex mixtures of chemicals. Environmental exposures to chemicals are typically much lower than the doses administered to laboratory animals. For toxicants that indirectly disturb endocrine system homeostasis, the interpretation of laboratory animal findings is confounded by significant species differences in endocrine physiology, such as the hypothalamic-pituitary-thyroid (HPT) axis (Capen, 1996).

To begin to understand some of the scientific challenges for mixtures toxicology and endocrine disrupting chemicals, we designed studies to evaluate HPT disturbances in rats administered low to moderate binary doses of two thyroid active chemicals that induce hypothyroidism by dissimilar mechanisms. Perchlorate (ClO$_4^-$) and 3,3’,4,4’,5-pentachlorobiphenyl (PCB126) were selected because of their widespread distribution in the environment (ATSDR, 2000; NRC, 2005; and NTP, 2006), detection in human tissues (CDC, 2005 and Blount et al., 2006), and their well characterized mode of action on the HPT axis in rats (Craft et al., 2002; Fisher et al., 2006; NRC, 2005; and Yu et al., 2002). PCB126 is a potent coplanar (non-ortho) dioxin-like PCB congener with a toxic equivalency factor of 0.1 (Safe, 1994) and generally exists with mixtures of multiple PCB congeners in the environment. PCBs are no longer used by industry, but are ubiquitous in the environment with detectable concentrations found across all media, including air, soil, water, sediment, and biota (NTP, 2006). The primary mode of action for PCB126 mediated disruption of the HPT axis is through increased phase II metabolism of the thyroid hormone, thyroxine (T$_4$). PCB126 binds to and
activates hepatic aryl hydrocarbon receptors (AhR). AhR activation results in the upregulation of several hepatic enzymes, including uridine diphosphate glucuronyl transferases (UDPGTs). An increase in phase II conjugation of T\(_4\) (formation of T\(_4\)-glucuronide, T\(_4\)-G) results in increased biliary excretion of T\(_4\)-G (Craft et al., 2002) and decreased circulating T\(_4\), leading to hypothyroidism. Dose-response characteristics for PCB126 and HPT disturbances in the rat were recently characterized in our laboratory (Fisher et al., 2006).

Perchlorate has been the subject of several toxicology studies targeting the HPT axis in wildlife, laboratory animals and humans because of its presence in water and food supplies (NRC, 2005). The ammonium perchlorate (AP) salt is used as an oxidizer in pyrotechnics, solid rocket fuels, and air bags. AP is highly water soluble and dissociates in water forming the perchlorate anion (Motzer, 2001). Apparently perchlorate is also formed naturally (Dasgupta et al., 2005). Perchlorate acts on the HPT axis by competing for thyroidal uptake of dietary iodide (I\(^-\)), resulting in a decline in available iodide for synthesis of the thyroid hormones (Wolff, 1998 and Yu et al., 2002) and the onset of hypothyroidism. A specialized transporter protein, referred to as the sodium/iodide symporter (NIS), located on the basolateral side of the follicular cell actively transports iodide and possibly perchlorate from the blood supply into the thyroid gland. Competitive inhibition of thyroidal uptake of radiolabeled iodide by perchlorate in the adult rat has been carefully characterized, along with the subsequent perturbations in serum thyroid hormones and thyroid stimulating hormone (TSH) (Yu et al., 2002).

Very few studies with mixtures of either PCBs or anions have been conducted to ascertain mixture composition contributions to disruption of the HPT axis in the rat. In one study, Crofton et al. (2005) evaluated serum T\(_4\) levels after four consecutive days of oral gavage dosing with a mixture of eighteen polyhalogenated aromatic hydrocarbons, including PCB126.
There was no deviation from additivity at the lowest mixture dose, but a greater-than-additive decrease in serum T4 was observed at the three highest mixture doses. Khan et al. (2005) reported that synergistic interactions occurred when rats ingested binary combinations of perchlorate and chlorate in drinking water for 7 days, as evidenced by greater decreases in serum T4 levels. Interestingly, Khan et al. (2005) also noted that male Fischer rats appear less sensitive than male Sprague-Dawley rats to ClO4\(^-\) induced alterations in the HPT axis. In vitro competitive inhibition studies using FTRL-5 and COS NIS-6 cells have been undertaken with several anions to estimate the affinity of anions for the NIS protein (Van Sande et al., 2003).

In the present study, we evaluated the combined effects of two chemicals, both of which induce hypothyroidism by different mechanisms in the rat. Rats were pretreated with a single oral bolus dose of a potent and persistent thyroid active chemical (PCB126) that is cleared slowly from the body. Dose- and time-dependent perturbations of the HPT axis are well characterized for PCB126. At a specified time after dosing, the PCB126 pretreated rats were given drinking water containing a second thyroid active chemical, ClO4\(^-\), for different periods of time. Dose- and time-dependent perturbations of ClO4\(^-\) on the HPT axis have also been characterized. Our working hypothesis was that for rats in which serum TSH was elevated by pretreatment with PCB126, the blocking effects of ClO4\(^-\) on thyroidal uptake of iodide would be diminished, resulting in less than additive perturbations in the HPT axis. TSH stimulates the production of the NIS protein, which results in increased thyroidal uptake of iodide (Eng et al., 2001) and the formation and secretion of thyroid hormones. If increases in TSH associated with PCB126 leads to increases in the NIS protein, perchlorate may be less effective at blocking the thyroidal uptake of iodide.
Materials and Methods

Dose Selection and Design

To study the interactions of PCB126 and ClO$_4^-$, doses of each chemical were selected that are known to cause moderate disturbances in the HPT axis and those that are thought to cause minimal or no disturbances in the HPT axis in the adult male rat. PCB126 dose selection (single oral bolus administration of 7.5 and 75 µg/kg) was based on previous research in our laboratory with single oral bolus doses of PCB126 and resulting perturbations in the HPT axis of the male Sprague-Dawley rat (Fisher et al., 2006). Rats were given ClO$_4^-$ in drinking water for two weeks at dose rates of 0.01, 0.1, and 1.0 mg/kg-day; the two highest doses (0.1 and 1.0 mg/kg-day) were previously reported to cause moderate disturbances of the HPT axis (Yu et al., 2002).

Dosing Study I. The average weight on Day 0 for 128 Sprague-Dawley rats used in this study was 216 ± 11 g. This study was divided into two groups (Table 3.1) with group 1 dosed one day prior to group 2. On Day 0 a portion of the rats were administered single oral bolus doses of PCB126 dissolved in corn oil (7.5 or 75 µg/kg) or corn oil alone (controls), while others remained on house supplied water. On Day 9 after dosing with PCB126, rats were administered ClO$_4^-$ (0, 0.01, 0.10, or 1.00 mg/kg-day) in their drinking water for an additional 14 days. Rats were euthanized on Day 22 between 7 and 10 A.M. and tissues collected for analysis (See Methods: Tissue collection and preparation).

Dosing Study II. The purpose of this study was to examine the interactions of lower doses of PCB126 with perchlorate. The time on treatment for Dosing Study II was shortened to five days or less after administration of a single oral gavage, lower dose of PCB126 (0, 0.075, 0.75, or 7.5 µg PCB126/kg) on Day 0 to capture the transient perturbations in the HPT axis. The average weight on Day 0 for 192 adult male Sprague-Dawley rats used in this study was 250 g ±
16 g. One day following PCB126 dosing, rats were administered ClO$_4^-$ in drinking water to obtain doses of 0 or 0.01 mg/kg-day. A portion of the rats received only PCB126 and were euthanized and tissues collected 12 hr, 1 day, 2 days, and 5 days post dosing. In addition to PCB126, a subset of rats also received ClO$_4^-$ in drinking water that began one day post PCB126 dose, and these animals were euthanized 2 days and 5 days post PCB126 dosing, respectively (Table 3.2).

**Chemicals and Reagents**

PCB126 (100 µg/mL in isoctane) was obtained from Accustandard Corporation (New Haven, CT). Dosing solutions (target doses 0.0, 0.075, 0.75, 7.5, and 75 µg/kg) were prepared as detailed in Fisher *et al.* (2006) having final concentrations of 0, 0.02, 0.2, 1.2, and 12.0 µg PCB126/ml corn oil, respectively. Ammonium perchlorate salt (99.8% pure) was obtained from Aldrich (Milwaukee, WI). Final ClO$_4^-$ drinking water concentrations for target doses of 0, 0.01, 0.1, and 1.0 mg/kg-day were 0, 0.09, 0.9, and 9.0 mg/L.

**Animals**

Adult male Sprague-Dawley (SD) rats were obtained from Charles River Laboratories (Wilmington, MA) weighing 161-180g on arrival. Rats were housed individually in a “shoe-box” style cage at an accredited American Association for Accreditation of Laboratory Animal Care (AAALAC) facility with humidity/climate-control and a 12-h light/dark cycle. Rats were fed Purina PMI Certified Rodent Chow #5001 and provided water (with or without perchlorate) *ad libitum*. Rats were allowed to acclimate for one week prior to dosing. The studies were conducted in accordance with the National Institutes of Health (NIH) guidelines for the care and use of laboratory animals. Body weights and food and water consumption were determined at the time of dosing, every three days during the study, and at euthanasia.
**Tissue Collection and Preparation**

Rats were euthanized by CO₂ asphyxiation and exsanguinated from the inferior vena cava as described in Fisher *et al.* (2006). Whole blood was collected in serum separator tubes, allowed to clot, centrifuged, and the serum removed. Serum aliquots were stored at -80°C until analysis of TSH and thyroid hormones. Livers were excised, weighed, and divided for analysis. Five grams of the liver were used to make microsomes for UDPGT activity (Fisher *et al.*, 2006). Liver microsomes were also stored at -80°C until analysis. Thyroids were excised free of fat and connective tissue and weights recorded. Both thyroid lobes from Dosing Study I animals and one lobe from animals in Dosing Study II were placed in 10% formalin for histomorphometric determination of the colloid/follicular volume ratio. The other lobe in Dosing Study II was frozen at -80°C until analysis of iodide content.

**Hepatic Enzyme Analysis**

Thyroxine-glucuronide (T₄-G) formation rates catalyzed by uridine diphosphate glucuronyl transferases (UDPGTs) and glucuronic acid were determined for all doses and dose combinations based on the method of Visser *et al.* (1993) as modified by Zhou *et al.* (2001). The calculated activity of hepatic UDPGTs was reported as pM T₄-G formed/mg protein/min. The minimal UDPGT activity detection using T₄ as the substrate was 0.05 pM T₄-G/mg protein/min. Hepatic microsomal protein was determined using the Folin phenol reagent method published by Lowry *et al.* (1951).

**Serum Hormone and TSH Analyses**

Serum aliquots of 0.5 mL were stored at -80°C until analysis, which occurred less than four months post collection. A previously unfrozen serum aliquot was used for each assay. Serum free T₄ (fT₄) concentrations were measured by equilibrium dialysis using a radioisotopic
assay kit (No. 40-2210, Nichols Institute Diagnostics, San Juan Capistrano, CA). Serum total T4 was determined by radioimmunoassay as in Fisher et al. (2006) using T4-15 antisera obtained from Endocrine Sciences (Calabasas, CA). Serum TSH was measured using a purchased (commercially available) rat TSH radioimmunoassay kit (MPBiomedicals #07C-90102, Orangeburg, NY).

The intra-assay coefficients of variation were 4.8%, 9.4%, and 6.0% for Dosing Study I measurements of fT4, T4, and TSH, respectively. For Dosing Study II, the intra-assay coefficients of variation were 19.4%, 7.7%, and 14.6%.

**Thyroid Histopathology and Iodide Content**

Thyroid glands were collected and prepared for histomorphometric analysis with a hematoxylin and eosin stain (Fisher et al., 2006). Two sections, for each lobe of the thyroid when available, were examined microscopically and two photographs of each section were taken for digital analysis of area ratios. Images with average area of 0.55 mm² were analyzed using the computer software Image-Pro Plus (MediaCybernetics, Silver Spring, MD). Follicular epithelial cell area was contrasted with colloid area (black versus white, respectively) and the total black versus white area was computationally determined. These values were then used to determine the colloid volume to epithelial follicular cell volume (C/EFC) ratios. An average of four ratios (4 images) was determined for each animal.

In Dosing Study I, both lobes of the thyroid from each animal were used for histomorphometric analysis; however, in Dosing Study II, one lobe from each thyroid, alternating right and left, was reserved for iodide analysis. Total thyroidal endogenous iodide content (¹²⁷I) was determined using the method of Benotti et al. (1965) for a portion of Dosing Study II animals.
**Statistical analyses**

All statistical analyses were performed using the statistical software package SAS (Statistical Analysis System) V8.2 (SAS Institute Inc., Cary, NC). Dosing Study I data were transformed by taking the square root prior to analysis. Analysis of variance (ANOVA) was used to determine if there were differences between the measurements taken on the two days of collection for controls or animals treated with only perchlorate. No statistical differences ($p \leq 0.05$) were determined between days of euthanasia for the control or ClO$_4^-$ only dose groups. Therefore, results from these rats were grouped together, which resulted in a total of 16 rats in the control and ClO$_4^-$ only dose groups for Dosing Study I.

Subsequently, the transformed data were evaluated by ANOVA to determine treatment related effects (PCB126 or ClO$_4^-$) and followed by Tukey’s multiple comparison (MC) test ($p \leq 0.05$) to compare treatment means (individual compounds and mixtures) to control means. In addition, Tukey’s MC test was used for comparison of the mixture means to the responses of the individual chemicals. ANOVA followed by Tukey’s MC test, was also utilized for analysis of Dosing Study II data. Data presented are expressed as percent of control (100%) ± SEM.

The Tukey-Ciminera-Heyse (TCH) trend test (Tukey et al., 1985) was used, in addition to ANOVA and Tukey’s MC test, in order to detect nonzero trends in response to the test compounds. The TCH test was conducted sequentially, using contrast coefficients calculated using SAS v8.2 and equations and methods described by Antonello et al. (1993), to determine the no-statistical-significance-of-trend (NOSTASOT) dose. For Dosing Study I, contrast coefficients were calculated for ClO$_4^-$ doses (0, 0.01, 0.10, and 1.0 mg/kg-day), and the TCH test was used to determine ClO$_4^-$ trends at different dose concentrations of PCB126 (0, 7.5, and 75 µg/kg) pretreatment for each variable. The contrast coefficients for Dosing Study II were
calculated for PCB126 doses (0, 0.075, 0.75, and 7.5 µg/kg) and used to determine PCB126 trends at each time point, with (2 and 5 day) and without ClO₄⁻ (0.5, 1, 2, and 5 day). A significant non-zero trend was identified when \( p \leq 0.05 \). The NOSTASOT dose was determined when \( p > 0.05 \).

Results

**Dosing Study I**

*Body and organ weights.* There were no significant differences in cumulative mean body weight gain over the 22 day study period after either a single oral bolus dose of PCB126 (7.5 or 75 µg/kg), 14 day exposure to ClO₄⁻ in drinking water (0.01, 0.10, or 1.00 mg/kg-day), or a combination of the two after a nine day pretreatment period with PCB126. The average weight gain for all animals was 120 ± 24 g. In addition, food and water consumption were not altered during the course of the study with daily intakes averaging 24.9 ± 1.0 g and 38.9 ± 2.2 mL, respectively. Rats administered 75 µg PCB126/kg had a significant increase of 18% in mean liver to body weight ratio (Table 3.3). No treatment related differences in thyroid weights were found (data not shown).

*Individual chemical treatment.* Administration of 7.5 and 75 µg PCB126/kg resulted in a dose-dependent increase in the rate of hepatic T₄-glucuronide (T₄-G) formation, although only the 75 µg PCB126/kg dose group was significantly elevated by 166% above control (Figure 1). Seventy-five but not 7.5 µg PCB126/kg resulted in a significant decrease in serum T₄ (49%) and fT₄ (51%) concentrations compared to controls at 22 days post treatment. Elevated serum TSH concentrations were detected for both PCB126 dose groups (Figure 1). However, the 7.5 µg/kg
dose group TSH concentrations were slightly higher than the 75 µg/kg dose group concentrations, although not statistically different from one another.

When the TCH trend test was employed to evaluate trends in the data across the three ClO$_4^-$ dose groups, dose-dependent trends were detected for all thyroid function indices measured. A dose-dependent increase in mean serum TSH concentrations was observed in rats that received 0.01, 0.1, or 1.0 mg/kg-day of ClO$_4^-$ in drinking water for 14 days (Figure 1). The NOSTASOT dose, or lowest dose of ClO$_4^-$ that did not cause a statistical alteration in serum TSH, was 0.01 mg/kg-day. An increasing trend was also determined for rate of T$_4$-G formation, while a decreasing trend was seen in serum T$_4$ and fT$_4$ concentrations with a NOSTASOT dose of 0.10 mg ClO$_4^-$/kg-day for these indices (Figure 1). Histopathology analysis of thyroids from animals that received either PCB126 or ClO$_4^-$ did not result in any statistical differences from control. The mean colloid:epithelial follicular cell (C/EFC) ratio for vehicle control animals that received no test compounds was $1.19 \pm 0.25$ (see supplemental data).

*Binary mixture treatment.* No dose-dependent trends were detected for the effect of ClO$_4^-$ on the rate of T$_4$-G formation when animals were pretreated with 7.5 or 75 µg PCB126/kg. Thus, PCB126 masked the effect of exposure to ClO$_4^-$ for 14 days in drinking water on the rate of T$_4$-G formation (Figure 1). Results from Tukey’s MC test also support this finding, since no statistical differences between the co-exposed animals and animals that received only PCB126 were seen.

PCB126 also masked the effect of ClO$_4^-$ on serum fT$_4$, T$_4$, and TSH measured after a nine day pretreatment period with either 7.5 or 75 µg PCB126/kg and followed with exposure to ClO$_4^-$ in drinking water for 14 days (0.01, 0.1, or 1.0 mg/kg-day). As previously stated, ClO$_4^-$ dose-dependent trends were observed for changes in serum T$_4$, fT$_4$, and TSH concentrations.
when ClO$_4^-$ was administered alone for 14 days in drinking water; however, no ClO$_4^-$ trends were found when it was administered to rats that were pretreated with PCB126.

No changes in the volume of colloid or follicular cells (C/EFC ratio) were seen in animals that received both PCB126 and ClO$_4^-$; similar C/EFC ratios were determined for animals that received only one test compound (see supplemental data).

In summary the binary mixture of 7.5 or 75 µg PCB126/kg and either 0.01, 0.1, or 1.0 mg ClO$_4^-$/kg-day resulted in the disappearance of the ClO$_4^-$ dose-dependent HPT axis effects (Figure 1) indicating a less than additive response for the binary mixture.

**Dosing Study II**

Dosing Study II provided information on binary mixtures for lower doses of PCB126 determined in this study to be at or near NOSTASOT doses for PCB126, combined with a NOSTASOT ClO$_4^-$ dose of 0.01 mg/kg-day determined in Dosing Study I. To carry out these studies in the same fashion as Dosing Study I, shorter treatment periods were selected as described in the Methods.

*Body and organ weights.* No significant difference in body weight gain was observed over the 5 day period after a single oral gavage dose of 0.075, 0.75, or 7.5 µg PCB126/kg. Also, no significant changes were found in liver or thyroid weights (data not shown). Food consumption was not monitored in this study because no differences were found at the higher doses in Dosing Study I. Water consumption was monitored to calculate ClO$_4^-$ intake. The average daily intake of water was 41.5 ± 6.6 mL.

*Individual chemical treatment.* A dose-dependent increase in rate of T$_4$-G formation was observed for animals treated with PCB126 at 2 and 5 days post-dosing (Figure 2). The rate of T$_4$-G formation peaked at day 2 and began to return to control values by day 5 (see supplemental
The NOSTASOT dose for increase in rate of T₄-G formation was found to be the lowest dose of PCB126 (0.075 µg/kg) administered.

Interestingly, these low doses of PCB126 resulted in a dose-dependent decrease in serum fT₄ at 12 and 24 hours post dosing with NOSTASOT doses of <0.075 and 0.75, respectively (see supplemental data). No trend or statistically significant differences from controls were detected at later time points of 2 or 5 days after dosing (Figure 2). No PCB126 dose-dependent trends were determined for serum T₄ (Figure 2). An increasing trend in serum TSH due to PCB126 exposure (NOSTASOT dose of 0.75 µg PCB126/kg) was found at 12 hours post-dosing (see supplemental data), but no trends were evident at later time points.

There were no statistical differences (Tukey’s MC test) in the rate of T₄-G formation or serum T₄, fT₄, or TSH concentrations in rats administered 0.01 mg ClO₄⁻/kg-day for one or four days (Figure 2).

**Binary mixture treatment.** For the binary mixture in Dosing Study II, animals were pretreated with PCB126 (0.075, 0.75, or 7.5 µg/kg) for one day and exposed to 0.01 mg ClO₄⁻/kg-day in drinking water for one or four days. The TCH trend test failed to find any significant PCB126 dose trends in the co-exposure data for Dosing Study II (Figure 2). The serum TSH concentrations were not statistically different from control values (Tukey’s MC test) and the TCH test did not detect a trend across PCB126 doses.

No statistical significant differences in thyroid morphology were observed in these animals (see supplemental data). Total thyroidal iodide content was not significantly altered by treatment. Stable iodide (¹²⁷I) content ranged from 10-15 µg per thyroid gland (Figure 3).
**Discussion**

The objective of these experiments was to characterize the low-dose interactions between two thyroid active compounds, 3,3’,4,4’,5-pentachlorobiphenyl (PCB126) and perchlorate (ClO₄⁻), which act via different modes of action to disturb the hypothalamic-pituitary-thyroid (HPT) axis. PCB126 is thought to act primarily by binding to the Ah-receptor (AhR) to induce hepatic UDPGT enzymes which increase the metabolism of T₄; ClO₄⁻ acts by inhibiting iodide uptake into the thyroid gland, resulting in decreased thyroid hormone production. The studies were designed to evaluate HPT axis disturbances caused by low doses of ClO₄⁻ on rats with modest preexisting disturbances in the HPT axis as a result of PCB126. The serum half-life ($t_{1/2}$) of PCB126 in rats is approximately 17 days (Yoshimura et al., 1985), while the plasma $t_{1/2}$ of intravenously administered $^{36}$ClO₄⁻ is 7.3 hours (Yu et al., 2002). The long $t_{1/2}$ of PCB126 allowed for administration of a single dose of PCB126 several days before treatment with ClO₄⁻ was initiated. This experimental design may mimic human exposures to these chemicals.

PCB126 human exposure occurs from contaminated diet and its $t_{1/2}$ is approximately 4.5 years (Ogura, 2004), while ClO₄⁻ exposure occurs primarily from ingestion of water and food with a $t_{1/2}$ of 6-8 hours (NRC, 2005).

Generally speaking, the dose-response characteristics of PCB126 on the HPT axis were similar to those obtained previously at higher doses (Fisher et al., 2006). That is, serum TSH concentrations were elevated, serum thyroid hormones were either unchanged or decreased and hepatic T₄-G production rates were increased. Interestingly, in this study, serum TSH concentrations in animals dosed with 7.5 µg/kg were similar to the 75 µg/kg dose group. Fisher et al. (2006) reported that 75 µg/kg of PCB126 resulted in elevated serum TSH concentrations greater than the 275 µg/kg dose group. Other PCB126 studies report variable findings for
treatment related changes in serum TSH concentrations (Martin, 2002 and NTP, 2006) suggesting that PCB126 may be disturbing the HPT axis by more than one mechanism of action. Further evidence for this suggestion comes from the present study. Serum fT₄ concentrations declined by 12 hrs after dosing with PCB126, but a corresponding increase in hepatic T₄-G formation was not observed (see supplemental data). Similar findings have been reported for other PCBs in which declines in serum T₄ were not accompanied by UDPGT induction (Hansen, 1998 and Li and Hansen, 1996). This may suggest PCB126 disturbs the thyroid axis by another mechanism that has yet to be elucidated.

After fourteen days of ClO₄⁻ treatment in drinking water, serum thyroid hormone concentrations were similar to control values across the dose groups that received only ClO₄⁻, suggesting that the modest thyroid upregulation by TSH provided adequate compensation for thyroidal iodide uptake and thyroid hormone synthesis. The possible induction of hepatic T₄-G formation by ClO₄⁻ deserves further study, since rate of T₄-G formation has not been reported previously for ClO₄⁻ treated animals. In the present study, the no-statistical-significance-of-trend (NOSTASOT) dose, also considered to be the no-observed-effect-level (NOEL), for alterations in serum TSH was 0.01 mg ClO₄⁻/kg-day. The alterations in serum T₄, fT₄, and TSH for rats exposed to ClO₄⁻ alone in Dosing Study I agree with previously published data for the 0.10 and 1.00 mg/kg-day exposures (Yu et al., 2002). The determination of 0.01 mg ClO₄⁻/kg-day as a no-statistical-significance-of-trend (NOSTASOT) dose based on serum TSH extended the dose-response curve for ClO₄⁻ established in Yu et al. (2002) into the low-dose region.

The binary mixtures data collected from Dosing Study I support our hypothesis that ClO₄⁻ is less effective as a thyroid axis disruptor in rats pretreated with PCB126. In the data from animals co-exposed with the high PCB126 (75 μg/kg) dose from Dosing Study I, it is evident
that PCB126 dominated the HPT axis responses in these co-exposed animals. Serum total and free T4 in animals co-administered 75 μg PCB126/kg and 1.0 mg ClO4⁻/kg-day were significantly below control values by about 40%, which corresponded to the decrease seen in PCB126 (75 μg/kg) only animals (50%). At this highest dose of ClO4⁻ (1.0 mg/kg-day) administered in Dosing Study I, a dose-dependent decrease in serum total and free T4 (8% and 13%, respectively) and subsequent increase in TSH of 100% was found. This suggests that the upregulation and stimulation of the thyroid by TSH at this dose of ClO4⁻ was not sufficient to maintain normal thyroidal iodide levels for hormone production. However, in animals pretreated with 75 μg PCB126/kg prior to administration of the high dose of ClO4⁻ (1.0 mg/kg-day), ClO4⁻ was unable to exacerbate the hypothyroid condition further. That is, there was no further decrease in serum total or free T4, and no ClO4⁻ dose related statistical trend in serum TSH was found in animals pretreated with PCB126.

Additionally, it is speculated that due to prior exposure to PCB126, the degree to which ClO4⁻ is able to block thyroidal iodide uptake (and subsequently disturb the HPT axis) is diminished in the presence of elevated TSH, which is known to stimulate NIS protein expression and activity (Dohan et al., 2003). PCB126 appeared to mask the effect of ClO4⁻ in these animals, which is supported by the lack of ClO4⁻ dose-response trends in the binary mixture studies conducted in Dosing Study I. Since ClO4⁻ dose-response trends were found when the compound was administered alone, the disappearance of these trends in PCB126 and ClO4⁻ co-exposed animals suggests that the effect is less than additive at the dose combinations tested in Dosing Study I. This is also supported by evaluating expected additive responses based on the absolute mean percent change from control (supplemental data). The response as percent of control for hepatic rate of T4-G formation averaged 39% less than additive. On average for the
binary mixture combinations tested in Dosing Study I, TSH was 35% less than expected under the response additivity assumption for chemicals of dissimilar modes of action, and total T₄ averaged 5% less than additive. The free T₄ deviation from additivity was different for each dose of PCB126. At the low dose of PCB126 (7.5 μg/kg) animals co-exposed had mean serum free T₄ levels on average 17% greater than expected under the additivity assumption; however, animals co-exposed with 75 μg PCB126/kg had free T₄ levels 13% less than the predicted additive response. The reason for the difference at these two PCB126 doses is not known, but may be related to displacement of the hormone from carrier proteins in the blood. PCBs and their hydroxyl metabolites have been shown to displace T₄ from the serum binding protein transthyretin (TTR) in rats (Brower and van den Berg, 1986; Chauhan et al., 2000; and Cheek et al., 1999); at low concentrations, the M-1 metabolite of PCB126 (Koga, 1990) may play a similar role to PCB metabolites already identified to have this behavior.

In animals that are hypothyroid, as indicated by elevated serum TSH and decreased serum T₄ concentrations prior to ClO₄⁻ exposure, the apparent dose-response curve for ClO₄⁻ inhibition of iodide uptake is shifted to the right. The ClO₄⁻ dose-response curve shift to the right in hypothyroid, or TSH stimulated animals, suggests that a higher dose of ClO₄⁻ is needed to result in the same degree of inhibition of iodide uptake at the NIS that is seen in TSH normal, euthyroid rats.

In Dosing Study II, the objective was to dose rats with low doses of PCB126 and monitor transient changes in the HPT axis to determine a NOEL dose for PCB126. Also, binary experiments were conducted at low doses of both PCB126 and ClO₄⁻ to further characterize HPT axis responses. This study resulted only in a few statistically significant trends for PCB126 up to one day post dose and no statistical differences from control for animals treated with 0.01 mg
ClO$_4$^-/kg-day for one or four days. A NOEL for PCB126, based on its well-defined primary mode of action of PCB126 (phase II conjugation of T$_4$) was found to be 0.075 µg/kg. Results from Dosing Study II demonstrate that doses of PCB126 and ClO$_4$-, which do not cause alternations in the HPT axis when administered alone, will not result in HPT axis disturbances when administered sequentially. Thus, it appears no interaction (synergism or potentiation) occurs at relatively low doses between PCB126 and ClO$_4$- for the thyroid axis indices measured in this study.

No statistically significant differences in thyroid morphology were determined for either study. Changes in thyroid gland have been seen in studies of the individual compounds. Fisher et al. (2006) found a statistically significant change in the ratio of colloid volume to epithelium volume 22 days post-dose at the highest dose of PCB126 administered (275 µg/kg). In addition, female rats exposed to 30 mg ClO$_4$^-/kg-day for two weeks prior to mating through lactation day 22 exhibited altered thyroid morphology, measured by colloid depletion, follicular hyperplasia and hypertrophy (York et al., 2005). To a much lesser extent, animals exposed to 0.1 and 1.0 mg ClO$_4$^-/kg-day for the same length of exposure exhibited some colloid depletion and follicular hyperplasia, while no colloid depletion was found in animals exposed to 0.01 mg ClO$_4$^-/kg-day and follicular hyperplasia was not different from controls (York et al., 2005). Thus, since no differences in thyroid colloid volume and follicular epithelial cell volume ratios were found for the PCB126 and ClO$_4$- experiments presented in this paper, either the treatment period was too short or the doses too low to result in structural changes within the thyroid gland itself.

One issue confronting toxicologists today is accurately extrapolating data from high-dose toxicology studies to low-dose exposures seen in the environment. In many cases, low-dose studies are needed to simulate more realistic human environmental exposures, and to provide
information to minimize uncertainty in the low-dose area of the dose-response curve. However, challenges exist when implementing studies in the laboratory to explore endocrine effects in the low-dose region. Minor changes in hormone levels that result from low-dose exposures are difficult to discern because of hormone inter-individual and intra-assay variability between laboratories. Since the statistical power to detect differences in treated groups can be affected by this variability, future experiments, with a greater number of rats and more refined assays for hormone determination, could be conducted to support the conclusion that PCB126 masked effects of ClO$_4^-$ at these low-dose rates examined.

In conclusion, these studies demonstrate that in animals treated with relatively high doses PCB126 prior to ClO$_4^-$ exposure, HPT axis disturbances are less than additive and the ClO$_4^-$ dose-response curve appears shifted to the right. In addition, when animals are co-exposed with doses at or near the NOEL for each compound, no interaction between the compounds is observed for the thyroid indices measured.

The data from this study and previously published individual chemical studies will be utilized in the development of biologically-based pharmacokinetic models for the adult male rat HPT axis. These models will be used to characterize and further the understanding of dose-response relationships for exposure to mixtures of thyroid disrupting chemical mixtures. In addition, HPT axis mathematical models will help to interpret the non-linear dose response based on the primary well defined modes of action.

**Acknowledgments**

Primary funding for this research was kindly provided by ATSDR grant U61/ATU472105-(02, 03, 04, and 05). Additional research support was provided by the National
References


Table 3.1. Study design and dosing schedule for Dosing Study I. Animals were dosed and euthanized in two groups, which were separated by one day, as indicated by Groups 1 and 2. A single oral gavage dose of PCB126 in corn oil was administered on Day 0. Ammonium perchlorate was added to the drinking water to obtain the target doses indicated, beginning on Day 9 and continuing until the end of study, Day 22. Eight animals (N) were used for each dose combination.

<table>
<thead>
<tr>
<th>Group</th>
<th>PCB126 Dose (µg/kg)</th>
<th>Perchlorate Dose (mg/kg-day)</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
<td>Day 9-22</td>
<td></td>
</tr>
<tr>
<td>1</td>
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<td>0</td>
<td>8</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>0.01</td>
<td>8</td>
</tr>
<tr>
<td>1</td>
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<td>0.1</td>
<td>8</td>
</tr>
<tr>
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<td>7.5</td>
<td>1.0</td>
<td>8</td>
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<tr>
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<td>7.5</td>
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<td>8</td>
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<td>8</td>
</tr>
<tr>
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<td>0</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
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<td>0</td>
<td>0.01</td>
<td>8</td>
</tr>
<tr>
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<tr>
<td>2</td>
<td>75</td>
<td>1.0</td>
<td>8</td>
</tr>
</tbody>
</table>
Table 3.2. Study design and dosing schedule for Dosing Study II. A single oral gavage dose of PCB126 in corn oil was administered on Day 0. Ammonium perchlorate was added to the drinking water to obtain the target doses indicated, beginning on Day 1 and continuing until the end of study. Eight animals (N) were used for each dose combination and time point.

<table>
<thead>
<tr>
<th>PCB126 Dose (µg/kg)</th>
<th>Perchlorate Dose (mg/kg-day)</th>
<th>End of Study (Day)</th>
<th>N</th>
</tr>
</thead>
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<tr>
<td>Day 0</td>
<td>Day 1 – End of Study</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
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<td>8</td>
</tr>
<tr>
<td>0.075</td>
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<td>8</td>
</tr>
<tr>
<td>0.75</td>
<td>NA</td>
<td>0.5</td>
<td>8</td>
</tr>
<tr>
<td>7.5</td>
<td>NA</td>
<td>0.5</td>
<td>8</td>
</tr>
<tr>
<td>0</td>
<td>NA</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>0.075</td>
<td>NA</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>0.75</td>
<td>NA</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>7.5</td>
<td>NA</td>
<td>1</td>
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</tr>
<tr>
<td>0</td>
<td>0</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>0.075</td>
<td>0</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>0.75</td>
<td>0</td>
<td>2</td>
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<tr>
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</tr>
<tr>
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<td>0.01</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>0.075</td>
<td>0.01</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>0.75</td>
<td>0.01</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>7.5</td>
<td>0.01</td>
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<td>8</td>
</tr>
<tr>
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<td>0</td>
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<td>8</td>
</tr>
<tr>
<td>0.075</td>
<td>0</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>0.75</td>
<td>0</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>7.5</td>
<td>0</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>0</td>
<td>0.01</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>0.075</td>
<td>0.01</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>0.75</td>
<td>0.01</td>
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</tr>
<tr>
<td>7.5</td>
<td>0.01</td>
<td>5</td>
<td>8</td>
</tr>
</tbody>
</table>

* Not applicable (NA). Animals were euthanized before ClO₄⁻ treatment began in order to obtain transient disturbances in the HPT axis due to PCB126 alone.
**Table 3.3.** The liver/body weight (BW) ratios for all animals in Dosing Study I were multiplied by 100 and are shown in the Table ± SEM. Animals that received 75 µg PCB126/kg had statistically increased (18%) relative liver weight compared to controls.

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>N</th>
<th>Liver/BW ratio (x 100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle Control</td>
<td>16</td>
<td>4.08 ± 0.27</td>
</tr>
<tr>
<td>ClO₄⁻ 0.01 mg/kg-day</td>
<td>16</td>
<td>4.22 ± 0.39</td>
</tr>
<tr>
<td>ClO₄⁻ 0.10 mg/kg-day</td>
<td>16</td>
<td>4.12 ± 0.35</td>
</tr>
<tr>
<td>ClO₄⁻ 1.00 mg/kg-day</td>
<td>16</td>
<td>4.14 ± 0.43</td>
</tr>
<tr>
<td>PCB126 7.5 µg/kg</td>
<td>8</td>
<td>4.43 ± 0.24</td>
</tr>
<tr>
<td>PCB126 7.5 µg/kg and ClO₄⁻ 0.01 mg/kg-day</td>
<td>8</td>
<td>4.46 ± 0.36</td>
</tr>
<tr>
<td>PCB126 7.5 µg/kg and ClO₄⁻ 0.10 mg/kg-day</td>
<td>8</td>
<td>4.51 ± 0.30</td>
</tr>
<tr>
<td>PCB126 7.5 µg/kg and ClO₄⁻ 1.0 mg/kg-day</td>
<td>8</td>
<td>4.48 ± 0.24</td>
</tr>
<tr>
<td>PCB126 75 µg/kg</td>
<td>8</td>
<td>4.74 ± 0.36ᵃ</td>
</tr>
<tr>
<td>PCB126 75 µg/kg and ClO₄⁻ 0.01 mg/kg-day</td>
<td>8</td>
<td>4.68 ± 0.37ᵃ</td>
</tr>
<tr>
<td>PCB126 75 µg/kg and ClO₄⁻ 0.10 mg/kg-day</td>
<td>8</td>
<td>4.92 ± 0.25ᵃ</td>
</tr>
<tr>
<td>PCB126 75 µg/kg and ClO₄⁻ 1.0 mg/kg-day</td>
<td>8</td>
<td>4.90 ± 0.23ᵃ</td>
</tr>
</tbody>
</table>

ᵃ Significantly different from control ($p \leq 0.05$).
Figure 3.1. DOSING STUDY 1. Hepatic and thyroid axis responses (% of control) in adult rats: 22 days after a single oral bolus gavage of 7.5 or 75 µg/kg of PCB126 (clear bar), 14 days after ingesting of either 0.01, 0.1 or 1.0 mg/kg of ClO₄⁻ in drinking water (solid circle ± SEM connected by sold lines (▬●▬)), or co-exposed to both PCB126 and ClO₄⁻ (shaded bars) as described in Methods. Statistically significant trends associated with dose of PCB126 only is indicated with an italicized a, (clear bar), and for ClO₄⁻ only, with an italicized b, (▬●▬). In rats co-administered PCB126 and ClO₄⁻ (shaded bars), a pound sign (#) indicates a significant difference from corresponding rats dosed with only ClO₄⁻ and an asterisk (*) indicates the co-administered rats were significantly different from controls. Control (100%) indicated by dashed line (-----). Control mean values ± SEM (n=16) for each assay (fT₄: 2.27 ± 0.14 ng/dL, T₄: 4.58 ± 0.14 µg/dL, TSH: 4.60 ± 0.49 ng/mL, and T₄-G: 0.72 ± 0.05 pmol T₄-G formed/mg protein/min).
Figure 3.1
**Figure 3.2.** DOSING STUDY II. Hepatic and thyroid axis response (% of control) in adult rats: Two (left column of panels) or five (right column of panels) days post PCB126 dose (0, 0.075, 0.75, or 7.5 µg/kg; solid circle ± SEM connected by solid lines (●)); one (left column of panels) or four (right column of panels) day exposure to ClO₄⁻ in drinking water (0.01 mg/kg-day; clear bar), or co-exposed animals (shaded bars) as described in Methods. Statistically significant trends associated with dose of PCB126 only are indicated with an italicized *a*, (●). No statistical differences from control were found for animals treated with 0.01 mg ClO₄⁻/kg-day alone (clear bar). In rats co-administered PCB126 and ClO₄⁻ (shaded bars), a pound sign (#) indicates a significant difference from corresponding rats dosed with only ClO₄⁻. No significant differences from control for co-exposed animals were detected. Control (100%) represented by dashed line (- - - -). Control mean values ± SEM (n=8) for one day ClO₄⁻ exposure: (fT₄: 2.46 ± 0.16 ng/dL, T₄: 4.41 ± 0.49 µg/dL, TSH: 7.80 ± 0.83 ng/mL; and T₄-G: 0.62 ± 0.04 pmol T₄-G formed/mg protein/min) and four day ClO₄⁻ exposure: (fT₄: 2.32 ± 0.16 ng/dL, T₄: 3.32 ± 0.26 µg/dL, TSH: 8.73 ± 0.81 ng/mL, and T₄-G: 0.72 ± 0.08 pmol T₄-G formed/mg protein/min).
Figure 3.2
Figure 3.3. Stable endogenous iodide ($^{127}$I) measured in one lobe of the thyroid from a portion of the rats in Dosing Study II. The total thyroidal iodide content (μg) was calculated using the total thyroidal weight determined. The plot shows total thyroidal $^{127}$I for control rats (clear bar) and treated rats (dark bars) exposed to (1) 0.01 mg ClO$_4$-/kg-day for four days, as well as co-exposed rats pretreated with PCB126 doses of (2) 0.075, (3) 0.75, and (4) 7.5 μg PCB126/kg for one day, followed by 0.01 mg ClO$_4$-/kg-day drinking water exposure for four days. There were no significant differences ($p \leq 0.05$) between total thyroidal $^{127}$I content in treated versus control rats.
Figure 3.3
Supplementary Data

“Low-Dose Effects of Ammonium Perchlorate on the Hypothalamic-Pituitary-Thyroid (HPT) Axis of Adult Male Rats Pretreated with PCB126”


The supplementary data provided includes the results from histomorphometric analysis of the thyroid glands for all dose groups in Dosing Study I (Table 3.1S) and Dosing Study II (Tables 3.2S and 3.3S). Table 3.4S shows the HPT axis responses as percent of control for Dosing Study I for the individual compounds, the calculated additive response, the binary mixture response, and the deviation from additivity. Finally, the HPT axis responses for animals dosed with 0, 0.075, 0.75, or 7.5 μg PCB126/kg in Dosing Study II is shown in Figure 3.1S.
### Table 3.1S. Thyroid Histopathology C/EFC data for Dosing Study I.

<table>
<thead>
<tr>
<th>ClO$_4^-$ dose (mg/kg-day)</th>
<th>PCB126 dose (µg/kg)</th>
<th>% Colloid and Follicular Volume Fraction ± SD</th>
<th>Ratio C/EFC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Colloid Volume (C)</td>
<td>Follicular Volume (EFC)</td>
</tr>
<tr>
<td>0.0</td>
<td>0.0</td>
<td>53.85 ± 4.90</td>
<td>46.15 ± 4.90</td>
</tr>
<tr>
<td>0.0</td>
<td>7.5</td>
<td>53.02 ± 5.58</td>
<td>46.98 ± 5.58</td>
</tr>
<tr>
<td>0.0</td>
<td>75</td>
<td>52.66 ± 5.04</td>
<td>47.34 ± 5.04</td>
</tr>
<tr>
<td>0.01</td>
<td>0.0</td>
<td>52.96 ± 5.10</td>
<td>47.04 ± 5.10</td>
</tr>
<tr>
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<td>0.0</td>
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<td>48.44 ± 4.25</td>
</tr>
<tr>
<td>1.00</td>
<td>0.0</td>
<td>53.47 ± 4.30</td>
<td>46.53 ± 4.30</td>
</tr>
<tr>
<td>0.01</td>
<td>7.5</td>
<td>52.64 ± 4.88</td>
<td>47.36 ± 4.88</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>57.53 ± 4.93</td>
<td>42.47 ± 4.93</td>
</tr>
<tr>
<td>0.10</td>
<td>7.5</td>
<td>52.83 ± 3.34</td>
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<tr>
<td></td>
<td>75</td>
<td>50.98 ± 3.96</td>
<td>49.02 ± 3.96</td>
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<td>1.00</td>
<td>7.5</td>
<td>50.25 ± 7.12</td>
<td>50.21 ± 7.12</td>
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<tr>
<td></td>
<td>75</td>
<td>49.79 ± 4.25</td>
<td>49.75 ± 4.25</td>
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</table>

*No significant differences were detected ($p \leq 0.05$).

### Table 3.2S. Thyroid Histology C/EFC data for 1 Day Perchlorate Exposure (Dosing Study II).

<table>
<thead>
<tr>
<th>ClO$_4^-$ dose (mg/kg-day)</th>
<th>PCB126 dose (µg/kg)</th>
<th>% Colloid and Follicular Volume Fraction ± SD</th>
<th>Ratio C/EFC</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Colloid Volume (C)</td>
<td>Follicular Volume (EFC)</td>
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<tr>
<td>0.0</td>
<td>0.0</td>
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<td>42.84 ± 4.93</td>
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<td>49.73 ± 5.74</td>
<td>40.27 ± 5.74</td>
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<tr>
<td>0.0</td>
<td>7.5</td>
<td>59.22 ± 6.18</td>
<td>40.78 ± 6.18</td>
</tr>
<tr>
<td>0.01</td>
<td>0.0</td>
<td>56.40 ± 7.39</td>
<td>43.60 ± 7.39</td>
</tr>
<tr>
<td></td>
<td>0.075</td>
<td>48.77 ± 6.39</td>
<td>51.23 ± 63.9</td>
</tr>
<tr>
<td></td>
<td>0.75</td>
<td>47.34 ± 6.26</td>
<td>52.66 ± 6.26</td>
</tr>
<tr>
<td></td>
<td>7.5</td>
<td>43.76 ± 5.31</td>
<td>56.24 ± 5.31</td>
</tr>
</tbody>
</table>

*No significantly different ratios.

### Table 3.3S. Thyroid Histology C/EFC data for 4 Day Perchlorate Exposure (Dosing Study II).

<table>
<thead>
<tr>
<th>ClO$_4^-$ dose (mg/kg-day)</th>
<th>PCB126 dose (µg/kg)</th>
<th>% Colloid and Follicular Volume Fraction ± S.D.</th>
<th>Ratio C/EFC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Colloid Volume (C)</td>
<td>Follicular Volume (EFC)</td>
</tr>
<tr>
<td>0.0</td>
<td>0.0</td>
<td>47.27 ± 6.75</td>
<td>52.73 ± 6.75</td>
</tr>
<tr>
<td>0.0</td>
<td>0.075</td>
<td>50.25 ± 7.51</td>
<td>49.75 ± 7.51</td>
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<tr>
<td>0.0</td>
<td>0.75</td>
<td>44.65 ± 8.17</td>
<td>55.35 ± 8.17</td>
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<tr>
<td>0.0</td>
<td>7.5</td>
<td>45.18 ± 6.66</td>
<td>54.82 ± 6.66</td>
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<tr>
<td>0.01</td>
<td>0.0</td>
<td>50.89 ± 5.92</td>
<td>49.11 ± 5.92</td>
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<tr>
<td></td>
<td>0.075</td>
<td>48.25 ± 5.73</td>
<td>51.75 ± 5.73</td>
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<tr>
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<td>0.75</td>
<td>50.51 ± 6.21</td>
<td>49.49 ±6.21</td>
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<tr>
<td></td>
<td>7.5</td>
<td>47.70 ± 53.7</td>
<td>52.30 ± 53.7</td>
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</tbody>
</table>

*No significantly different ratios.
**Table 3.4S.** DOSING STUDY I. Table displays the individual chemical responses for hepatic and thyroid function indices as percent of control. The expected response for the co-exposed animals based on the assumption of additivity is shown, as well as the actual mean percent of control for co-exposed animals. The final column shows the deviation from the additivity estimate.

<table>
<thead>
<tr>
<th>ClO₄⁻ Dose</th>
<th>ClO₄⁻ only response</th>
<th>PCB126 7.5 μg/kg</th>
<th></th>
<th></th>
<th>Deviation from Additivity¹</th>
<th></th>
<th></th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>mg/kg-day</td>
<td>% Control</td>
<td>% Control</td>
<td>% Control</td>
<td>% Control</td>
<td>% Control</td>
<td>% Control</td>
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<td>Free T₄</td>
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<td>76</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>109</td>
<td>102</td>
<td>111</td>
<td>92</td>
<td>19</td>
<td>17</td>
<td>24</td>
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<td>84</td>
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<tr>
<td>Total T₄</td>
<td>0.01</td>
<td>102</td>
<td>98</td>
<td>104</td>
<td>-5</td>
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<td>1</td>
<td>92</td>
<td>88</td>
<td>93</td>
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<td>TSH</td>
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<td>118</td>
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<td>124</td>
<td>129</td>
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<td>272</td>
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<td>-92</td>
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</tr>
</tbody>
</table>

¹ Additive response predicted using the equation: % Control\textsubscript{Additive} = (%ClO₄⁴⁻ − 100) + (%PCB126 − 100) + 100

² A negative (-) number indicates a less than additive response, and a positive number indicates a greater than additive response.
Figure 3.1S. Thyroid axis response up to 5 days following PCB126 oral gavage dose of either 0.075 (●), 0.75 (○), or 7.5 (▼) µg/kg. Control (0 µg/kg) indicated by dashed line (100%). A) Hepatic rate of T₄-G formation plotted as percent of control ± SEM (0.5 day: 0.85 ± 0.09; 1 day 0.75 ± 0.08; 2 day: 0.62 ± 0.04; and 5 day: 0.72 ± 0.08 pmol T₄-G formed/mg protein/min). B) Serum fT₄ concentration displayed as percent of control ± SEM (0.5 day: 3.75 ± 0.31; 1 day 2.52 ± 0.11; 2 day: 2.46 ± 0.16; and 5 day: 2.32 ± 0.16 ng/dL). C) Serum T₄ concentrations shown as percent of control ± SEM (0.5 day: 3.92 ± 0.51; 1 day 3.86 ± 0.52; 2 day: 4.41 ± 0.49; and 5 day: 3.32 ± 0.26 µg/dL). D) Serum TSH concentrations plotted as percent of control ± SEM (0.5 day: 3.07 ± 0.47; 1 day 6.53 ± 0.56; 2 day: 7.80 ± 0.83; and 5 day: 8.73 ± 0.81 ng/mL). Doses of significant trend (p ≤ 0.05) indicated by asterisk (*).
Figure 3.1S
CHAPTER 4

A BIOLOGICALLY BASED DOSE-RESPONSE MODEL FOR DIETARY IODIDE AND
THE HYPOTHALAMIC-PITUITARY-THYROID AXIS IN THE ADULT RAT:
EVALUATION OF IODIDE DEFICIENCY

\[\text{Submit to Toxicological Sciences on October 2, 2007.}\]
Abstract

A biologically based dose-response (BBDR) model was developed for dietary iodide and the hypothalamic-pituitary-thyroid (HPT) axis in adult rats. This BBDR-HPT axis model includes sub-models for dietary iodide, thyroid stimulating hormone (TSH), and the thyroid hormones thyroxine (T₄) and 3,5,3’-triiodothyroine (T₃). The sub-models are linked together via key biological processes, including: 1) the influence of T₄ on TSH production (the HPT axis negative feedback loop); 2) stimulation of thyroidal T₄ and T₃ production by TSH; 3) TSH upregulation of the thyroid sodium/iodide symporter (NIS); and 4) recycling of iodide from the metabolism of thyroid hormones. The BBDR-HPT axis model was calibrated to predict steady-state concentrations of iodide, T₄, T₃, and TSH for the euthyroid rat whose dietary intake of iodide was 20 μg/day. Then the calibrated BBDR-HPT axis model was used to predict perturbations in the HPT axis caused by insufficient dietary iodide intake and simulation results were compared to experimental findings. The BBDR-HPT axis model was successful in simulating dietary iodide induced hypothyroid conditions for perturbations in serum T₄, TSH, and thyroid iodide stores for low iodide diets of 0.33 to 1.14 μg/day. Model predictions of serum T₃ concentrations were inconsistent with some reported experimental findings. BBDR-HPT axis model simulations revealed a very steep dose-response relationship between dietary intake of iodide and perturbations in the HPT axis when dietary iodide intake becomes insufficient (less than 2 μg/day) to sustain the HPT axis. This research demonstrates that biologically based models can be successfully developed to predict complex responses in endocrine systems such as the HPT axis.

Key Words: iodide, BBDR model, HPT axis, thyroxine, TSH, rat
Introduction

The hypothalamic-pituitary-thyroid (HPT) axis regulates many physiologic functions, including metabolism, growth, development, and reproduction. HPT axis homeostasis is maintained by complex feedback controls; however, this system may be altered and unable to compensate for changes resulting from exposure to thyroid active environmental contaminants or ingestion of insufficient or excessive amounts of iodide. Iodine, an essential nutrient, is a constituent required for formation of thyroid hormones and is involved in autoregulation of the thyroid gland. During critical periods of development, alterations in the thyroid axis can result in improper development with lifelong consequences. Iodine deficiency, which leads to hypothyroidism, ranks among the highest preventable causes of mental retardation and brain damage throughout the world (Delange, 2001). Insufficient iodine intake is still prevalent in almost one third of world population (Hamann et al., 2006).

The process of thyroid hormone formation is highly regulated. The thyroid gland actively sequesters iodide via the sodium (Na⁺)/iodide(I⁻) symporter (NIS). Iodide is then available for incorporation and use in thyroid hormone production. The normal thyroid gland produces thyroxine (T₄) in greater quantities than the biologically active hormone 3,5,3’-triiodothyronine (T₃) (Greer et al., 1968). Thyroid hormones are secreted from the thyroid gland into systemic circulation, where T₄ can be metabolized to T₃ in peripheral tissues by a family of enzymes called 5’-deiodinases. T₃ binds to nuclear receptors in virtually every cell of the body to regulate gene expression. When circulating blood levels of T₄ and T₃ are low, the anterior pituitary gland is stimulated to produce more thyroid stimulating hormone (TSH), a classical negative feedback loop. TSH is carried to the thyroid gland by blood, where TSH binds to receptors on the plasma membrane of thyroid follicular cells. This receptor-TSH complex
regulates second messenger cascades, which stimulates the increase in NIS expression and activity, and increased production of thyroid peroxidase (TPO) and thyroglobulin (Tg) (Kogai et al., 2006). These orchestrated biochemical events ultimately allow for increased thyroidal uptake of iodide and production and secretion of T₄ and T₃.

Several investigators have quantitatively described selected aspects of the HPT axis using mathematical tools. Classical pharmacokinetic (PK) models for the thyroid hormones T₄ and T₃ have been developed for the rodent (DiStefano et al., 1982; DiStefano and Feng, 1988). Three compartment (blood, fast, and slow pools) models for radiolabeled T₄ and T₃ were used to estimate thyroid hormone production rates, kinetic compartment parameters for the thyroid hormones, and their metabolic clearance rates. Li (1995) and colleagues also employed a more theoretical PK compartmental approach to simulate the dynamic and pulsatile nature of the human thyroid axis, which included serum TSH, T₄, and T₃ concentrations. In 1996, Kohn et al. developed a physiological dosimetric model of the distribution of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in the rodent that also incorporated a HPT axis sub-model. The HPT axis sub-model included the negative feedback loop and production and metabolism of T₄ and T₃. The TCDD model integrated with the HPT axis model was utilized to predict decreases in serum T₄, via a TCDD-dependent increase in hepatic phase II conjugation of T₄ (Kohn et al., 1996).

More recently, Dietrich et al. (2002) used a complex engineering approach to describe biofeedback (e.g. T₄/TSH negative feedback loop in humans) and the pulsatile nature of TSH secretion. Mukhopadhyay and Bhattacharyya (2006) also employed an engineering control system approach, including time delays to describe the pulsatile nature of the human HPT axis for thyroxine and TSH feedback and plasma distribution. In addition to the aforementioned models, physiologically based pharmacokinetic (PBPK) models for radiolabeled iodide (¹²⁵I) in
rodent and human have been developed for different life stages (Merrill et al., 2003, 2005; Clewell et al., 2003a, 2003b). These were $^{125}$I kinetic models and did not include dietary iodide ($^{127}$I) or thyroid hormones.

Although several mathematical models have been constructed to describe the thyroid axis, to date none have taken into account TSH and dietary iodide ($^{127}$I) linked to T$_4$ and T$_3$ formation and secretion. Therefore, the objective of this research was to develop a biologically based dose-response (BBDR) model of the adult male rat HPT axis (BBDR-HPT axis model), including description of dietary iodide and its utilization in the thyroid gland for hormone production. DeVito et al. (1999) concluded serum hormone levels (T$_4$, T$_3$, and TSH), along with thyroid weight and histology are the most critical endpoints for determination of xenobiotic effects on thyroid toxicity. Thus, a quantitative BBDR-HPT model was developed to include the most informative serum hormones, namely, T$_4$ and T$_3$, and the signaling molecule, TSH. Several novel features included in our model are the active transport and regulation of iodide uptake into the thyroid by the NIS, T$_4$/TSH negative feedback loop, extrathyroidal metabolism of T$_4$ to form the biologically active T$_3$, fecal excretion of T$_4$-glucuronide, and recycling of metabolically derived iodide from extrathyroidal metabolism of thyroid hormones.

Many environmental contaminants have been shown to disrupt thyroid hormone homeostasis (Brucker-Davis, 1998). The BBDR-HPT axis model reported in this paper can be integrated with PBPK models of thyroid disrupting chemicals using chemical specific mode(s) of action, to predict changes in serum T$_4$, T$_3$, and TSH and total thyroidal iodide content. However, as a first step in this direction, iodide sufficiency and insufficiency were evaluated with the BBDR-HPT axis model in the adult rat to better understand the role of dietary iodide in HPT axis homeostasis.
Materials and Methods

The BBDR-HPT axis sub-models for the adult rat were constructed using simple model structure that allowed us to focus on an empirical ‘system based evaluation’ of key biochemical features of the HPT axis, such as the negative feedback loop. For example, the production of thyroid hormones (Equation 15) is controlled, in part, by the model predicted serum TSH concentration, while the maximal rate of active sequestration of iodide into the thyroid (Equation 2) is also controlled by the serum TSH concentration. Other investigators have recently described endocrine systems, using serum levels of signaling molecules to control feedback loops such as the adult male rat hypothalamic-pituitary-gonadal (HPG) axis (Barton and Andersen, 1998) and the human HPG axis/menstrual cycle (Schlosser and Selgrade, 2000 and Rasgon et al., 2003). Future modeling efforts focused on expanding the BBDR-HPT axis model to include other tissues of interest (e.g. brain for correlation of tissue concentrations to developmental effects) can be readily integrated into our model structure.

Models were coded using acslXtreme version 2.4.0.11 (Aegis Technologies, Huntsville, Alabama) and solved with the Gear algorithm for stiff systems. Standardized units of nanomoles (nmol), liters (L), kilograms (kg), and hours (hr) were used in the sub-models. The approach for the development of the BBDR-HPT axis model was to first create simple and independent sub-model structures for radiolabeled –iodide, -TSH, -T4, and -T3 using radiotracer studies reported in literature for the adult rat. This provided a number of BBDR-HPT axis model parameter values, although sometimes preliminary, and helped to evaluate the adequacy of using the proposed structure for each sub-model. Boundary conditions were implemented in the development of each radiolabeled sub-model, such that estimated model parameter values could
not result in simulation outcomes that deviated from the boundary conditions for the HPT axis reported in Table 4.1.

Literature derived datasets with endogenous information for the sub-models (iodide, TSH, T4, and T3) were gathered and the sub-models were linked as a system to simulate the HPT axis in the euthyroid adult rat. Key features of the BBDR-HPT axis model included the negative feedback loop, thyroid hormone production using available dietary iodide, and the metabolism of thyroid hormones with release of free iodide available for reuse in thyroid hormone production or urinary excretion. The euthyroid steady-state BBDR-HPT axis model relied on dietary iodide as the only exogenous input. Finally, the calibrated euthyroid, iodide sufficient adult rat BBDR-HPT axis model was tested for its ability to predict perturbations in the system under iodide deficient conditions.

Datasets used for Sub-Model Development

Key datasets were selected for development of the radiotracer sub-models for 125I, 125I-TSH, 131I-T4, and 125I-T3. Data for 125I published by Yu et al. (2002) was selected for use in development and calibration of the iodide sub-model. Adult male Sprague-Dawley rats were administered 33 μg 125I/kg bw via tail vein injection and serum and thyroid concentrations were determined up to 96 hours post dose, as well as cumulative urinary 125I excretion over a 24 hr period following iv dose. Concentrations of 125I in plasma up to 24 hrs post dose were published in Yu et al. (2002); however, additional data for thyroid bound and total concentration of 125I, urinary excretion, and serum concentrations 30-96 hr post dose were kindly provided by Dr. Yu (personal communication). The plasma disappearance of 125I-TSH was reported in male Hebrew University rats (80-100g) for up to 2 hours post iv dose of 5 ng 125I-TSH (Spira et al., 1979). Schroder van der Elst et al. (1997) reported 131I-T4 concentrations 0.25-6 hr post iv dose in
female Wistar rats (180g) as percent of 1.7ng $^{131}$I-T$_4$ dose in several tissues, including blood and liver. In normal adult male Sprague-Dawley rats (300-375g), DiStefano et al. (1993) characterized the distribution of a 0.83 ng $^{125}$I-T$_3$ iv dose in liver and plasma up to 1.2 hr post dose.

Sub-Model Structure and Key Equations

Iodide. A simple sub-model structure was implemented to predict iodide kinetics. Iodide was described as distributing into a volume of distribution (Vd) and a thyroid gland (Figure 4.1A). Iodide is rapidly absorbed to the bloodstream from the digestive tract and quickly diffuses into extracellular spaces throughout the body. Iodide fate is largely determined by a competition between thyroidal sequestration and urinary excretion (Verger et al., 2001). Urinary excretion of iodide is described as a first order clearance from the Vd. Uptake of iodide into the thyroid compartment is described assuming active uptake by the sodium(Na$^+$)/iodide(I$^-$) symporter (NIS) and diffusion (Figure 4.1A).

Iodide processing by the thyroid is multi-faceted. Free iodide enters the thyroid two ways: 1) active uptake by NIS and 2) diffusion via ion channels. NIS is a plasma membrane protein that actively transports two sodium molecules with one iodide molecule down the sodium ion gradient, which is generated by sodium-potassium ATPases (Kogai et al., 2006). TSH has been shown to stimulate NIS mRNA production, NIS protein expression, and retention in the plasma membrane (Carrasco, 1993; Kogai et al., 2000; Levy et al., 1997; Riedel et al., 2001; and Sherwin and Tong, 1974). Sherwin and Tong (1994) found that TSH-induced stimulation of iodide transport increased the rate of iodide uptake and did not affect the affinity (K$_m$) of iodide for the transporter. Thus, iodide uptake into the thyroid via the NIS (dTNIS$/dt$, nmol/hr) and TSH stimulation of the NIS iodide transport rate (V$_{maxT_{i,TSH}}$, nmol/hr) was described as follows:
\[
\frac{dTNIS_i}{dt} = \frac{V \text{ max } T_i^{TSH} \times Cvt_i}{Km_i + Cvt_i}
\]  

\[V \text{ max } T_i^{TSH} = \frac{V \text{ max } T_i \times Ca_{TSH}^{TSH}}{K_{NIS}^{TSH} + Ca_{TSH}}\]  

where \(Cvt_i\) is the free concentration of iodide in thyroid blood (nmol/L), \(Km_i\) is the affinity constant of iodide for the NIS (nmol/L), \(V\text{max}_i\) is the TSH stimulated maximum rate of NIS iodide uptake (nmol/hr), \(Ca_{TSH}\) is the serum concentration of TSH (nmol/L), and \(K_{TSH}^{NIS}\) is the concentration of TSH that gives rise to half-maximal rate of NIS transport of iodide (nmol/L).  

For the purpose of the estimating parameters using the radiolabeled iodide (^125I) sub-model, \(Ca_{TSH}\) was set to a value equal to the average control, euthyroid serum concentration (6.5 ng/mL or 0.232 nmol/L) from McLanahan et al. (2007).

Once iodide enters the thyroid by NIS active uptake or diffusion, iodide is incorporated (organified) by binding to tyrosine residues present in thyroglobulin (Tg) via a thyroid peroxidase (TPO) mechanism (Degroot and Niepomiszcze, 1977). TSH increases the expression of many genes involved in thyroid hormone synthesis, including Tg and TPO (Kogai et al., 2006). The rate of incorporation of iodide (\(d\text{RIB}/dt\), nmol/hr) into thyroid hormone precursors and TSH stimulation (\(V\text{max}\_B^{TSH}\), nmol/hr) of the organification process is simplified and described by:

\[
\frac{d\text{RIB}}{dt} = \frac{V \text{ max } B_i^{TSH} \times CTF_i}{Kb_i + CTF_i}
\]

\[V \text{ max } B_i^{TSH} = \frac{V \text{ max } B_i \times Ca_{TSH}}{K_{B_{TSH}}^{TSH} + Ca_{TSH}}\]

where \(CTF_i\) (nmol/L) is the free concentration of iodide in the thyroid, \(Kb_i\) (nmol/L) is the fitted concentration of free iodide in the thyroid when binding rate is half-maximal, \(V\text{max}_B\) (nmol/hr)
is the TSH stimulated maximum rate of organification of iodide, and \( K_{bTSH} \) (nmol/L) is the concentration of serum TSH that produces half-maximal organification rate of iodide. In the \(^{125}\)I sub-model the concentration of TSH in serum (\( C_{aTSH} \)) was also set to constant as previously described.

Loss of free iodide from the thyroid by outward diffusion was described using an estimated permeability cross-product (\( P_{ATi} \)) and loss of bound iodide as thyroid hormones is described in Equations 15-17. Thus, the thyroid tissue compartment for iodide was described for free (\( dATF_i/dt \), nmol/hr), bound/thyroid hormone incorporated (\( dATB_i/dt \), nmol/hr), and total (\( AT_i \), nmol) iodide by the following equations:

\[
\frac{dATF_i}{dt} = \frac{dTNIS_i}{dt} + P_{ATi} \times (Cvt_i - CTF) - \frac{dRIB}{dt}
\]

\[5\]

\[
\frac{dATB_i}{dt} = \left[ \frac{dRIB}{dt} - \left( \frac{dT4_{prod}}{dt} \times T4_{eq} \right) - \left( \frac{dT3_{prod}}{dt} \times T3_{eq} \right) \right]
\]

\[6\]

\[
AT_i = \int \frac{dATF_i}{dt} + \int \frac{dATB_i}{dt}
\]

\[7\]

where \( dT4_{prod}/dt \) is the secretion rate of \( T4 \) from the thyroid (nmol \( T4/hr \)), \( T4_{eq} \) is the molar fraction of iodide in a \( T4 \) molecule (0.6534), \( dT3_{prod}/dt \) is the secretion rate of \( T3 \) from the thyroid (nmol \( T3/hr \)), and \( T3_{eq} \) is the molar fraction of iodide in a \( T3 \) molecule (0.5848).

**Thyroid Stimulating Hormone (TSH).** Since TSH does not distribute into tissues, a one-compartment sub-model for TSH was constructed using a \( Vd \) (Figure 4.1B). The amount of \(^{125}\)I-TSH in the serum is determined by an iv dose to the \( Vd \) and first order clearance as shown below:

\[
\frac{dAVd_{TSH}}{dt} = RIV_{TSH} - (kel_{TSH} \times AVd_{TSH})
\]

\[8\]
where \( RIV_{TSH} \) is the \(^{125}\text{I}-\text{TSH} \) infusion rate (nmol/hr), \( kel_{TSH} \) is the clearance rate of TSH (hr\(^{-1}\)), and \( AVd_{TSH} \) is the amount of TSH in the Vd (nmol), which is representative of the serum concentration. This model is amended in the endogenous description of TSH in the BBDR-HPT axis model to include endogenous production of TSH, by an empirical description based on serum T\(_4\) concentrations (the T\(_4\)/TSH negative feedback loop described later, Equation 14).

**Thyroxine (T\(_4\)) and 3,5,3'-Triiodothyronine (T\(_3\)).** Each thyroid hormone sub-model was developed with a Vd and liver compartment (Figure 4.1C and 4.1D). Bidirectional diffusion of T\(_4\) in the liver was included in the description of hepatic influx and efflux. T\(_4\) has also been shown to be actively transported into the liver by a high affinity, low capacity transporter, as well as a low affinity, high capacity transporter (Krenning et al., 1981). However, hepatic uptake of T\(_4\) was simplified and described using a single Michaelis-Menten equation:

\[
\frac{dLU_{T4}}{dt} = \frac{V_{max}^{LU} \times (Cvl_{T4} \times 0.01)}{Km_{T4}^{LU} + (Cvl_{T4} \times 0.01)} \tag{9}
\]

where \( V_{max}^{LU} \) is the maximal rate of active uptake of T\(_4\) into the liver (nmol/hr), \( Km_{T4}^{LU} \) is the affinity constant for T\(_4\) active transport (nmol/L), and \( Cvl_{T4} \) is the concentration of T\(_4\) in the liver venous blood (nmol/L). Since at least ninety-nine percent of T\(_4\) is bound to serum proteins in rodents (Mendel et al., 1992), the sub-model code was modified to reflect only free serum T\(_4\) (1\% of total serum T\(_4\)) available for active transport and diffusion into the liver. Phase II metabolism of T\(_4\) in the liver was described using Michaelis-Menten metabolism equations for glucuronidation (formation of T\(_4\)-glucuronide, T\(_4\)-G) and type I 5’-deiodination of T\(_4\), forming T\(_3\) and free iodide. The diffusion limited liver blood compartment for T\(_4\) was described using the equations:

\[
\frac{dALb_{T4}}{dt} = Q_L \times (Ca_{T4} - Cvl_{T4}) + PAL_{T4} \times (CL_{T4} \times (Cvl_{T4} \times 0.01)) - \frac{dLU_{T4}}{dt} \tag{10}
\]
\[
C_{vl_{T4}} = \frac{\int \frac{dAL_{T4}}{dt}}{V_{Lb} \times PL_{T4}}
\]

where \(dAL_{T4}/dt\) is the rate of change of T4 in the liver blood (nmol/hr), \(Q_L\) is the blood flow to the liver (L/hr), \(Ca_{T4}\) is the arterial blood concentration of T4 perfusing the liver (nmol/L), \(PAL_{T4}\) is the permeability area cross-product for liver bidirectional diffusion of T4 (L/hr), \(Cvl_{T4}\) is the concentration of T4 in the liver venous blood (nmol/L), \(V_{Lb}\) is the volume of liver blood (L), and \(PL_{T4}\) is the T4 liver:blood partition coefficient (unitless).

The liver tissue compartment for T4 was described as follows:

\[
\frac{dAL_{T4}}{dt} = PAL_{T4} \times ((Cvl_{T4} \times 0.01) - CL_{T4}) + \frac{dLU_{T4}}{dt} - \frac{dDIL_{T4}}{dt} - \frac{dUGT_{T4}}{dt}
\]

where \(dAL_{T4}/dt\) is the rate of change of T4 in the liver tissue (nmol/hr), \(CL_{T4}\) is the concentration of T4 in the liver (nmol/L), \(dLU_{T4}/dt\) is the rate of active uptake of T4 into the liver from liver blood (nmol/hr, Equation 9), \(dDIL_{T4}/dt\) is the rate of T4 conversion to T3 and free iodide by type I 5’-deiodinating enzymes (nmol/hr), \(dUGT_{T4}/dt\) is the rate of formation of T4-G formation (nmol/hr), and \(V_L\) is the volume of the liver (L). T4 has also been shown to undergo other hepatic metabolic processes, such as sulfation (T4-S formation), however this route accounts for a small fraction (6%) of overall T4 metabolism (Rutgers et al., 1989). Furthermore, T4-S is rapidly deiodinated in the liver (Visser et al., 1990) and so the T3-S metabolic route was not included in this model. To account for the rest of the body metabolism of T4 to T3, a first order metabolism of T4 was included as a loss from the Vd compartment.

Similar to T4, transport of T3 into the liver compartment was described by bidirectional diffusion and active uptake by a transporter protein (Figure 4.1C). Experimental evidence for
hepatic transporter uptake of T₃ from blood suggests that T₃ uptake is not saturable at physiological conditions (Blondeau et al., 1988), thus the active uptake was described as a first-order process. Hepatic metabolism of T₃ in the liver was described as a first-order process also, with the assumption that a percentage of the metabolized T₃ is excreted in feces as T₃ conjugates (e.g. T₃-G, T₃-S, etc.). The remainder is metabolized to free iodide, assuming T₃ metabolism to T₂ is the rate limiting step in releasing free iodide. The fraction of T₃ metabolism excreted in feces (FT₃feces) was fit to provide an approximation (26%) of the a priori boundary condition of 30% (DiStefano et al., 1993) for percent of T₃ dose excreted in feces (Table 4.1). First-order metabolism of T₃ was included in the Vd to account for rest of body metabolism of T₃ to T₀, also assuming that T₃ to T₂ is the rate limiting step.

**Linking the Sub-Models to Create a BBDR-HPT Axis Model**

The sub-models described above for iodide, T₄, T₃, and TSH are linked as shown in Figure 4.2. All compartments for each sub-model were assigned steady-state derived masses at the onset of the simulations. The mass of TSH, iodide, or thyroid hormones was established by running the simulations to steady-state with a dietary iodide intake of 20 μg per day. Dietary intake of iodide was assumed to take place over a 12-hr period, with food/iodide consumption occurring during the night hours (7 pm - 7 am).

TSH is secreted by the anterior pituitary and is found in systemic circulation. Briefly, the TSH one-compartment model in the linked BBDR-HPT axis model was modified from Equation 8 to include an endogenous production term. The production of TSH is based on the primary negative feedback loop of the thyroid axis; that is adequate levels of serum thyroid hormones result in a normal secretion of TSH from the pituitary, but when serum thyroid hormone levels decrease, the feedback control is diminished and TSH production rate increases. Several
researchers have shown a negative correlation between serum T4 and TSH concentrations (Fukuda et al., 1975; Riesco et al., 1977; and Pedraza et al., 2006). This is a primary experimental observation reported by several laboratories and used in the development of the negative feedback loop for the BBDR-HPT axis model. Since total serum T4 is a common measurement in most thyroid disruptor studies, as opposed to free T4, the TSH/T4 negative feedback loop was described using total serum T4 as shown in Equation 14. The empirical description of TSH production is regulated by the model predicted total serum T4 concentration ($Ca_{T4}$). The complete equation used to determine the amount of TSH in the Vd is as follows:

$$\frac{dAVd_{TSH}}{dt} = \frac{k_0^{TSH} \times K_{inh}^{T4}}{K_{inh}^{T4} + Ca_{T4}} - ke_{TSH} \times Ca_{TSH}$$  \hspace{1cm} [14]$$

where $k_0^{TSH}$ (nmol/hr) is the maximal production rate of TSH in the absence of T4, $K_{inh}^{T4}$ (nmol/L) is the estimated concentration of T4 in the serum which results in half-maximal production rate of TSH, $Ca_{T4}$ (nmol/L) is the total T4 serum concentration, and $Ca_{TSH}$ (nmol/L) is the TSH serum concentration calculated by dividing the integral of Equation 14 by $Vd_{TSH}$ (L).

The rate of total thyroidal production and secretion of thyroid hormones (T4 and T3) is determined by a fitted rate constant ($k_{IB}^{TSH}$) times the model predicted serum concentration of TSH and concentration of available thyroidal iodide in the form of hormone precursors:

$$\frac{dTH_{prod.}}{dt} = k_{IB}^{TSH} \times Ca_{TSH} \times CTB_i$$  \hspace{1cm} [15]$$

where $k_{IB}^{TSH}$ (L$^2$/nmol/hr) is a linear rate term, $Ca_{TSH}$ (nmol/L) is the serum concentration of TSH, and CTB$_i$ (nmol/L) is the concentration of bound thyroidal iodide as thyroid hormone precursors. The proportion of thyroid hormones produced as T3 and T4 is then described as a fraction of the total production rate, using the following equations:
where $\frac{dT_3^{\text{prod}}}{dt}$ is the rate of thyroidal $T_3$ production (nmol/hr), and $\frac{dT_4^{\text{prod}}}{dt}$ is the rate of thyroidal $T_4$ production (nmol/hr). The ratio of $T_3/T_4$ secretion increases modestly during iodide deficiency. To account for this, Equation 18 was derived from Pedraza et al. (2006), who collected experimental data on total thyroidal iodide stores and thyroidal $T_3/T_4$ ratios for different rates of iodide intake (Figure 4.3). $FT_3$ (unitless) is the fraction of overall thyroid hormone production within the thyroid that is $T_3$ and was modeled as:

$$FT_3 = 0.2652 \times AT_i^{-0.4684}$$  \hspace{1cm} [18]$$

where $AT_i$ is the total amount of iodide in the thyroid (μg), as calculated in Equation 7 and converted to μg. In iodide deficient conditions, a shift from primarily $T_4$ to $T_3$ production in the thyroid occurs (Greer et al., 1968; Pedraza et al., 2006). This may be due to the increase in deiodination of $T_4$ in the thyroid, or simply the formation of less $T_4$ because less iodide is needed to make $T_3$. However, no instances have been reported where the thyroid synthesizes only $T_3$ at the cost of zero $T_4$ production. A MIN command was implemented in acslXtreme to ensure that the exponential $FT_3$ function (Equation 18) did not exceed 0.90.

**Datasets used in Steady-State Euthyroid BBDR-HPT axis model calibration.**

Serum $T_4$, $T_3$, and TSH, along with total thyroid iodide data from adult male Sprague-Dawley rats published by McLanahan et al. (2007) were used to calibrate the model for steady-state euthyroid conditions in the adult rat (320g). It was also important to include liver $T_4$ and $T_3$ concentrations for calibration; however, there are few datasets with tissue concentrations of thyroid hormones. Morreale de Escobar et al. (1994) reported concentrations of $T_4$ and $T_3$ in
several different tissues, including the liver of control, euthyroid adult female Wistar rats. This study was used in the linked BBDR-HPT axis model calibrations. Furthermore, the only study found to contain free iodide serum concentrations was Eng et al. (1999) in which they reported the data for euthyroid (control) adult male Sprague-Dawley rats.

**Model Parameters**

Model parameters were derived from the published literature whenever possible. Default assumptions for allometric scaling were employed. Thus, blood flows (Q), maximum velocities ($V_{\text{max}}$)$^3$, and permeability area cross-products (PA), were multiplied by $\text{BW}^{0.75}$ and clearance rates ($\text{Cl}$ and $\text{kel}$) were divided by $\text{BW}^{0.25}$. Volumes of distribution (Vd) were scaled linearly with BW.

*Physiological Parameters.* Growth equations developed by Mirfazaelian et al. (2007) were used to account for body weight changes for simulations that were longer than one month. Otherwise, the terminal body weight reported for the study was used in simulation. Blood flows and tissue volumes (V) were obtained from literature (Brown et al., 1997; Malendowicz and Bednarek, 1986; McLanahan et al., 2007). Physiological parameters are shown in Table 4.2.

*Literature Derived Compound-Specific Parameters.* When possible, compound-specific parameters for each sub-model were derived from literature. Parameters for iodide, T4, T3, and TSH are shown in Table 4.3. Liver partition coefficients for T4 ($PL_{T4}$, 1.27) and T3 ($PL_{T3}$, 4.47) were determined from steady state serum and liver concentrations reported by Escobar-Morreale et al. (1996) for female euthyroid, control rats. These values are similar to the values used by Kohn et al. (1996) for T4 and T3 liver partition coefficients (1.632 and 2.22, respectively) that

---

3 An evaluation of literature for total thyroid iodide ($^{127}\text{I}$) concentrations for the range of body weights simulated in this study (120-500g) showed slight change in total amount of thyroidal $^{127}\text{I}$. The model parameter maintaining the stores in the thyroid is $V_{\text{maxBc}}$ ($V_{\text{max}}$ for iodide incorporation into thyroid hormone precursors). Thus, to empirically describe total thyroid $^{127}\text{I}$ concentrations, the value of $V_{\text{maxBc}}$ is divided by $\text{BW}^{0.75}$. 


were estimated from $K_{ow}$ values and the use of various regression equations. No partition coefficients were explicitly incorporated into the endogenous iodide sub-model. Implicitly, the tissue/blood values could be considered to have a value of 1.0. Organification (binding of iodide in the thyroid) of iodide to form thyroid hormone precursors accounted for the large NIS-dependent ratio of iodide in the thyroid compared to serum levels.

The volume of distribution (Vd) for $T_4$, $T_3$, and TSH were obtained from literature, as shown in Table 4.3, and the volume of the liver was subtracted from the Vd for $T_4$ and $T_3$ (Table 4.3). The Vd for $T_4$ (15.6 L/kg) was obtained from the thyroid hormone model developed by Kohn et al. (1996), while the Vd for $T_3$ of 18.6 L/kg was estimated and used by DiStefano et al. (1986) in a simple compartmental model for $T_3$. A TSH Vd (5.54 L/kg) was used as reported by Connors et al. (1984). These authors intravenously dosed female Sprague-Dawley rats (170-220g) with $^{125}$I-TSH.

Clearance terms to account for metabolism in the Vd for TSH, $T_4$, and $T_3$ were calculated from literature values using the relationship

$$k_{el} = \frac{\ln 2}{t_{1/2}}$$

[19]

where $t_{1/2}$ is the serum half-life of the compound (hr) reported as 0.3667 hr for $^{125}$I-TSH (Lemarchand-Beraud and Berthier, 1981), and 6 and 12hr for $T_3$ and $T_4$, respectively (Abrams and Larsen, 1973).

Affinity constants, $K_m(s)$, for metabolism and active transport of iodide and $T_4$ were obtained from the literature (Table 4.3). The affinity constant for thyroid iodide transport by the NIS ($K_m$) of $3.1 \times 10^4$ nmol/L was the average value reported by Gluzman and Niepomniszcze (1983), using radiolabeled iodide and euthyroid human and porcine thyroid cells. The affinity constant for active uptake of $T_4$ into the liver ($K_{m LU}^{T_4}$) of 650 nmol/L was reported by Blondeau.
et al. (1988) using rat hepatocytes. Michaelis-Menten saturable metabolism of T₄ in the liver was described for the phase II glucuronidation and deiodination pathways. The saturable metabolism of T₄, by type I 5'-deiodination, was described assuming that one molecule of T₃ and iodide are formed for each molecule of T₄ metabolized. Phase II metabolism of T₄ (T₄-glucuronide formation, T₄-G) occurs by a reaction catalyzed by uridine diphosphate glucuronyl transferases (UDPGTs). The Km value for the type I 5'-deiodinase metabolism of T₄ (Kmᵩ₄⁻¹, 2300 nmol/L) was obtained from Leonard and Visser (1986) from in vitro metabolic studies, and Km for the formation of T₄-G (Kmᵩ₄⁻¹, 1 × 10⁵ nmol/L) was taken from Visser et al. (1993) in vitro studies in Wistar rat liver microsomes. For each of these saturable metabolic processes the Km values were derived from the literature and Vmax values were optimized to fit serum kinetics of T₄ that resulted in values that were close to the boundary conditions for fraction of T₄ metabolized to T₃ and fraction of T₄ excreted in feces (Table 4.1).

When sub-models were combined for the BBDR-HPT axis model, endogenous production of TSH was described as shown in Equation 14. The maximal rate of TSH production (k₀ᵩ₄) was set to the value (6 nmol/hr) of TSH secretion reported by Connors et al. (1984) 14 days after thyroidectomy in adult female Sprague-Dawley rats (Table 4.3).

Parameter optimization. Model parameters not available in literature were first optimized to fit each radiotracer dataset (¹²⁵I, ¹³¹I-T₄, and ¹²⁵I-T₃), then when the models were linked to form the BBDR-HPT axis model parameters were re-optimized to fit euthyroid, steady-state, iodide sufficient (20 μg iodide/day) conditions. Optimization of model parameters was performed using acslXtreme Parameter Estimation version 2.4.0.11 (Aegis Technologies, Huntsville, Alabama).
Two parameters that were determined from visual fits were kept constant throughout model optimization, including the volume of distribution of iodide (Vdc, 0.5 L/kg BW) and the linear rate term for thyroid hormone production (k^TSH, 5 \times 10^{-7} \text{ L}^2/\text{nmol/hr}). Setting these model parameters to a constant value was determined necessary in order for the optimization to successfully converge upon a maximum log-likelihood function.

A global optimization of model parameters for the BBDR-HPT axis model was performed as well. During this optimization, all estimated model parameters were optimized to steady-state, euthyroid, iodide-sufficient (20 μgI/day) measurements that included serum and liver T₄ and T₃, serum TSH, serum free iodide, and total thyroidal iodide. Boundary conditions (Table 4.1) were also included in the optimization process.

**Sensitivity Analysis**

An analysis of model parameter sensitivity under steady-state conditions was determined for predicted serum concentrations of T₄, T₃, and TSH and total thyroidal iodide content. Normalized sensitivity coefficients (NSC) were calculated that represent a fractional change in output corresponding to a fractional change in the parameter as described previously (Clewell et al., 2000; Merrill et al., 2003; and Tornero-Velez and Rappaport, 2001). Model parameters were increased by 1% and the model executed using iodide sufficient (20 μg/day) and iodide deficient (1 μg/day) intakes. The NSCs were calculated using the equation

\[
\text{Normalized Sensitivity Coefficient} = \frac{(A - B)}{(B - D)}
\]

where \( A \) equals the model response (serum T₄, T₃, TSH, or total thyroidal iodide) with a 1% increase in parameter value, \( B \) is response with original parameter value, \( C \) is parameter value increased by 1%, and \( D \) is original parameter value.
Application of BBDR Model to Iodide Deficiency

Studies that provided a time-course for iodide deficiency (ID) induced HPT axis alterations (Riesco et al., 1977; Okamura et al., 1981a; and Okamura et al., 1981b) and one study evaluating recovery from ID (Fukuda et al., 1975) were available in published literature. These papers contained the most complete experimental datasets which included iodide content of the diet, serum T4, T3, TSH, thyroid iodide. Many other studies prior to 1970 have been conducted; however, they were considered incomplete for modeling purposes.

Average daily iodide intake was calculated by multiplying food consumption (20 g/day assumed when not reported for the study) by the iodide content in the diet (μg/g). To compare across studies we have reported the intakes as μg iodide per day. The iodide deficiency datasets simulated using our BBDR-HPT axis model are briefly described below.

Riesco et al. (1977) provided adult male Holtzman Sprague-Dawley (120g) rats a low iodide diet (LID) resulting in intake of 0.3-0.4 μg I/day for a short term ID study. They determined serum T4, T3, TSH and total thyroid iodide after 0, 2, 4, 6, 8, 11, 15, and 26 days of feeding the LID. An average intake of 0.35 μg I/day was used in model simulation.

A longer time course for HPT response of rats maintained on a LID was reported by Okamura et al. (1981a). Adult male Simonsen Albino and Holtzman Sprague-Dawley rats were divided by strain and provided a LID of 0.3-0.36 μg I/day (15-18 μg I/kg chow). Average intake of 0.33 μg I/day was used in model simulation. Measurements of serum T4, T3, TSH and total thyroid iodide were obtained after 0, 14, 28, 56, and 84 days of feeding the LID. Simonsen Albino rats appeared to display a greater sensitivity or degree of HPT axis response to the LID than the Holtzman Sprague-Dawley rats.
Another study by Okamura and workers (1981b) examined the opposing effects of iodide and nutritional deficiency, by administering two different LID diets (ICN Remington and Teklad Remington). For modeling purposes, the nutritionally deficient ICN Remington diet was not considered. Adult male Holtzman Sprague-Dawley rats (139g) were administered the Teklad Remington (57ng I/g or 1.14μg I/day, nutritionally adequate) diet beginning on day 0 were killed following 19, 33, 63, and 96 days of treatment. Measurements of serum T4, T3, TSH, and total thyroid iodide were obtained. However, for some unknown reason the serum TSH concentrations reported in this study were much greater than other studies and no measurements for baseline TSH at day 0 were provided. For these reasons, a fold change was not calculated for comparison to our model nor was the data compared to model simulations.

Fukuda et al. (1975) evaluated the recovery of the HPT axis in rats that were placed on an ID diet and then followed with iodide supplementation. Adult male Sprague-Dawley rats (400-500g) were placed on a LID of 0.6 μg I/day (30μg I/kg chow) for seven months, and then to study the recovery phase they were provided iodide supplementation in drinking water. Iodide supplementation provided an additional intake of 2 or 8 μg I/day for four days, yielding an average total intake during the supplementation or refeeding period of 2.6 or 8.6μg I/day. Serial blood samples were taken and measurements of serum T4 and TSH were obtained 0, 1, 2, 3, 6 and 9 days during supplementation. Due to a wide range in serum TSH concentrations at the onset of iodide supplementation, as well as serum T4 concentrations reported as non-detectable, the data model simulations and data were expressed as percent of baseline at the onset of the recovery phase.
Results

Radiotracer Sub-Model Development

The unlinked radiotracer sub-models (Figure 4.1) for each component of the BBDR-HPT axis model were optimized and used to predict published kinetic datasets described in previously. The use and development of these models provided support and validation of the model structure to be linked to form the complete BBDR-HPT axis model. The sub-models used physiological parameters shown in Table 4.2 and compound-specific parameters (supplementary data) for each sub-model that were optimized by fitting to their respective kinetic datasets (Figure 4.4).

Iodide. Model simulations of administered radiolabeled iodide ($^{125}$I) in the adult rat (Yu et al., 2002 and Dr. Yu personal communication) are shown in Figures 4.4A and 4.4B. To obtain optimized parameter values for these kinetic datasets, the concentration of TSH was held at a constant euthyroid value (0.232 nmol/L). The volume of distribution ($V_{dc,i}$, 0.5L/kg BW) was visually fit to the serum time-course kinetics of an iv dose of 33 μg $^{125}$I/kg reported by Yu et al. (2002) (Figure 4.4A). Additionally, the first-order urinary clearance rate ($Cl_{Uc,i}$) of 0.02 hr$^{-1}$ was set during parameter optimization of thyroid iodide constants in order to provide a fit to the total amount of $^{125}$I excreted in urine over a 24-hr period (Figure 4.4A).

The estimated parameter values for Equations 2-4 for iodide processing in the thyroid were simultaneously optimized to total and bound thyroid $^{125}$I concentrations obtained (Dr. Yu, personal communication). Specifically, the Vmax of NIS thyroidal iodide uptake ($V_{maxTc,i}$, 1119.4 nmol/hr) and TSH concentration that produced a half-maximal uptake rate of iodide at the NIS ($K_{NIS,TSH}^{NIS}$, 1.15 nmol/L), maximal rate of iodide incorporation into thyroid hormone precursors ($V_{maxBc,i}$, 2243.6 nmol/hr), and concentration of free thyroid iodide ($K_{b,c,i}$, 221.1 nmol/L) that results in half-maximal $V_{maxBc,i}$ were optimized. The permeability area cross-
product for bidirectional diffusion of iodide into and out of the thyroid gland (PATc) was insensitive to predicting the total and bound thyroidal iodide stores, thus the value of 0.0001 L/hr reported by Merrill et al. (2003) was retained. The simple iodide model structure and optimized parameters provide model simulations of the Yu et al. (2002 and personal communication) $^{125}$I iv dosing study that adequately predicted total serum $^{125}$I concentration (Figure 4.4A), cumulative amount of $^{125}$I excreted in urine (Figure 4.4A), and the bound and total thyroidal $^{125}$I concentrations (Figure 4.4B).

3,5,3’-Triiodothyronine (T$_3$). The radiolabeled T$_3$ ($^{125}$I-T$_3$) sub-model structure was developed and first optimized using $^{125}$I-T$_3$ kinetics following an iv dose of 0.83ng (DiStefano et al., 1993). The uptake of $^{125}$I-T$_3$ into the liver after iv administration of 0.83 ng of $^{125}$I-T$_3$ (DiStefano et al., 1993) in adult male Sprague-Dawley rats could not be described assuming blood-flow limited kinetics (simulations not shown). Thus, a first order active uptake term was implemented along with simple bidirectional diffusion to describe the $^{125}$I-T$_3$ liver kinetics. The first order uptake rate ($k_{LU}^{T3}$, 1.5 hr$^{-1}$) and diffusion constant (PALc$_{T3}$, 0.0683 L/hr) were simultaneously optimized to the $^{125}$I-T$_3$ liver kinetic dataset of DiStefano et al. (1993) (Figure 4.4C). In addition, first order rate of T$_3$ metabolism in the liver (kmetLc$_{T3}$, 1.15 nmol/hr) was optimized to the same dataset and fraction of this metabolism of T$_3$ (FT$_3$feces, 0.30 unitless) resulting in an estimated fecal elimination using 30% of $^{125}$I-T$_3$ dose excreted in feces as a guideline (Table 4.1). The rate of clearance of $^{125}$I-T$_3$ from the serum was slightly under predicted, while the fitted uptake and clearance in the liver was adequately characterized by the sub-model simulation (Figure 4.4C).

Thyroxine (T$_4$). T$_4$ model parameters not available in literature ($V_{maxc}^{LJ}_{T4}$, $V_{maxc}^{UGT}_{T4}$, $V_{maxc}^{DI}_{T4}$, and PALc$_{T4}$) were first estimated by simultaneous optimization of these
parameter values with $^{131}\text{I}-\text{T}_4$ kinetic data reported in the adult female Wistar rat by Schroder van der Elst et al. (1997) using Equations 9-13. Rats were administered an iv dose of 1.7 ng $^{131}\text{I}-\text{T}_4$ and the distribution was characterized up to 6 hrs after dosing, in blood, liver, and other tissues. The Vmax values for liver uptake ($V_{\text{max}}^{\text{LU}}_{\text{T}_4}$, 10552 nmol/hr), type I 5’-deiodination ($V_{\text{max}}^{\text{DL}}_{\text{T}_4}$, 15.1 nmol/hr), $\text{T}_4$-G formation ($V_{\text{max}}^{\text{UGT}}_{\text{T}_4}$, 1080.32 nmol/hr), and the permeability area cross-product for diffusion of $\text{T}_4$ into the liver ($\text{PAL}_{\text{T}_4}$, 0.0488 L/hr) were obtained by optimization of serum and liver $\text{T}_4$ kinetics after an iv dose. In addition, these metabolic Vmax ($V_{\text{max}}^{\text{DL}}_{\text{T}_4}$ and $V_{\text{max}}^{\text{UGT}}_{\text{T}_4}$) parameters were optimized such that the percent of $^{131}\text{I}-\text{T}_4$ metabolized was similar to reported values in the literature (Table 4.1). In this case, the sub-model predicted percent of metabolized $^{131}\text{I}-\text{T}_4$ as 40% for $\text{T}_4$ conversion to $\text{T}_3$ and 24% for $\text{T}_4$ excreted in feces as $\text{T}_4$-G. The $\text{T}_4$ sub-model reproduced the serum and liver $^{131}\text{I}-\text{T}_4$ time-course with slight over prediction of serum and liver concentrations reported as percent dose (Figure 4.4D).

*Thyroid Stimulating Hormone (TSH).* Spira et al. (1979) administered 5ng $^{125}\text{I}-\text{TSH}$ via tail vein injection to adult male Hebrew University rats 5 days post thyroidectomy and determined serum concentrations 0.033 to 2hr post dose. This time course was simulated using the literature derived Vd$_{\text{TSH}}$ (Connors et al., 1984) value of 5.54 L/kg and an elimination constant, kel$_{\text{TSH}}$ (Lemarchand-Beraud and Berthier, 1986) equal to 1.8899 hr$^{-1}$. The sub-model for $^{125}\text{I}-\text{TSH}$ was able to adequately predict serum $^{125}\text{I}-\text{TSH}$ up to 2hr post dose (Figure 4.4E).

*Dietary Iodide BBDR-HPT Axis Model – Model Calibration and Simulation of Steady-State Euthyroid, Iodide Sufficient Conditions.* Many toxicology studies using rats in the laboratory are conducted in iodide sufficient conditions. Common laboratory diets contain 0.8 mg/kg iodide (e.g. LabDiet 5001 and 5008) and thus iodide intake averages 15-20 μg iodide per
day for the normal lab rat assuming 20-25g chow intake per day. Thus, the iodide sufficient model compound-specific parameter optimizations (Table 4.3) and simulations (Figure 4.5) were determined for the common intake for laboratory rats of 20μg I/day.

When the radiotracer sub-models were linked to create the BBDR-HPT axis model by including the production of thyroid hormones (Equations 15-18), metabolism of thyroid hormones, recycling of freed iodide, and the T₄/TSH negative feedback loop (Equation 14), as shown in Figure 4.2, an adequate description of the euthyroid, steady-state iodide sufficient (20μg I/day) condition was not readily achieved. For example, predictions of serum iodide were too low, liver concentrations of T₃ and T₄ were too high, and serum T₄ concentrations were too high, which resulted in under-predicted serum TSH concentrations (simulations not shown). Therefore, the sub-model parameter values obtained to predict serum clearance kinetics of trace amounts of radiolabeled iodide, T₄, T₃, and TSH (supplemental data) were adjusted. This was not completely unexpected for describing endogenous masses of thyroid hormones, dietary iodide, and TSH. Thus, a global optimization of model parameters for the BBDR-HPT axis model was performed and final model parameters are shown in Table 4.3.

Using the BBDR-HPT axis model, the optimized urinary clearance constant (Clucᵢ) was decreased to 0.0046 hr⁻¹ from 0.02hr⁻¹ to predict the free plasma iodide levels (Figure 4.5A) reported by Eng et al. (1999). The maximum rate of thyroidal uptake of iodide by the NIS (VmaxTcᵢ) was increased to a value of 5738.267 nmol/hr compared to a radiotracer derived value of 1119.4 nmol/hr. While the optimized maximum rate of incorporation of iodide into thyroid stores or binding as thyroid hormone precursors (VmaxBcᵢ) was decreased from a radiotracer derived value of 2243.6 to 1005.9 nmol/hr. Figure 4.5A shows the simulated and measured total amount of iodide in the thyroid (McLanahan et al., 2007).
The maximum rate of active uptake of T4 into the liver ($V_{\text{maxc}_{T4}}^{LU}$) decreased in the global optimization of the BBDR-HPT axis model to a value of 4384.73 nmol/hr compared to a radiotracer value of 10552 nmol/hr. The maximum rate of T4 glucuronidation ($V_{\text{maxc}_{U\text{GT}_{T4}}}$) increased from a radiotracer value of 1080.32 to 3435.89 nmol/hr. The calibrated steady-state euthyroid, iodide sufficient model predictions for a 320g rat are shown in Figure 4.5. Total thyroid and free serum iodide (Figure 4.5A), serum TSH (Figure 4.5B), serum and liver T4 (Figure 4.5C), and serum and liver T3 (Figure 4.5D) model predictions fall within the range for normal rats reported in literature.

**Iodide Deficiency HPT Axis Simulations**

Using the BBDR-HPT axis model parameter values, globally optimized for euthyroid iodide sufficient steady-state conditions, the ability of the model to predict temporal changes in serum thyroid hormones (T4 and T3), TSH, and total thyroidal iodide was tested for iodide deficient conditions.

HPT axis disturbances from an iodide deficient diet of 0.35 μg I/day for 26 days (Riesco *et al.*, 1977) is depicted in Figure 4.6 for adult male Holtzman-Sprague Dawley (HSD) rats. Serum T4 concentrations gradually decreased in parallel fashion with thyroidal iodide stores, while only a slight change occurred in serum T3 concentrations. Serum TSH concentrations increased over 10-fold during the study period. After 15 days of administration of the LID, the thyroidal iodide stores were severely depleted. The BBDR-HPT axis model predictions of serum thyroid hormones were in agreement with observed values. The predicted thyroidal iodide stores were slightly underpredicted initially and near the end of the study. Serum TSH increases were predicted during the first 11 days and then moderately over predicted by day 15. In severe iodide deficient conditions, when thyroidal iodide stores were predicted to be below 1 μg, oscillations in...
serum TSH and T₄ and thyroidal iodide occurred because of assumptions about dietary intake of iodide.

Next, the capability of the BBDR-HPT axis model to predict changes during administration of 0.33 μg I/day administered for 84 days to adult male Simonsen Albino (SA) and HSD rats (Okamura et al., 1981a) was tested (Figure 4.7). The SA strain (Figure 4.7A) exhibited a greater sensitivity, shown by the rapid increase in TSH compared to the HSD strain (Figure 4.7B). The BBDR-HPT axis model predicted the change in TSH better for the SA rats than the HSD rats. Serum T₃ concentrations were predicted to be lower than suggested by the data.

BBDR-HPT axis model simulations for a LID of 1.14 μg I/day administered to adult male HSD (Okamura et al., 1981b) are depicted in Figure 4.8. The initial decrease in thyroidal iodide stores and the apparent recovery after 60 days suggests adaptive response(s), such as the negative feedback loop. The BBDR-HPT axis model predictions also suggest this as evidenced by an increase in predicted thyroidal iodide stores and little decline in serum thyroid hormones after 25 days. At a dietary intake of 1 μg/day, this strain of adult rat has some ability to compensate for low iodide intake. Predictions of serum T₃ were slightly under-predicted.

Finally, the BBDR-HPT axis model was used to simulate recovery of the HPT axis in rats rendered iodide deficient for seven months with an average daily iodide intake of 0.6 μg/day (Figure 4.9). On Day 0 of the recovery phase, the rats were supplemented with iodide in drinking water to provide total intake of either 2.6 or 8.6 μg I/day. Serial blood samples were obtained for measurement of serum T₄ and TSH (Fukuda et al., 1975) for 9 days of recovery. The BBDR-HPT axis model slightly over-predicted day 1 increases in serum T₄ following iodide
supplementation for both doses, while the remaining predicted serum T₄ and TSH concentrations agreed with observations.

**Sensitivity Analysis**

The sensitivity analysis of the BBDR-HPT axis model was carried out assuming steady-state serum concentrations of T₄, T₃, and TSH, and total thyroid iodide content for an iodide sufficient (IS) intake of 20 µg I/day and iodide deficient (ID) intake of 1 µg I/day. None of the parameters are associated with normalized sensitivity coefficients (NSCs) greater than 1.0, suggesting that there is minimal amplification of error from the inputs to the model outputs (Clewell *et al.*, 2000). Figure 4.10 shows the NSCs for the model parameters that resulted in an NSC of 0.90 or greater for at least one model output (serum T₄, T₃ and TSH or total thyroidal iodide). Total amount of thyroidal iodide predictions were most affected by a 1% change in the volume of the thyroid (NSC = 0.99) under IS conditions and a NSC of 0.98 under ID conditions. A one percent change in the thyroid hormone production constant \( k^{IB}_{TSH} \) also reflected similar sensitivity of the total amount of iodide in the thyroid with NSCs of -0.95 and -0.96 under IS and ID conditions, respectively.

**Discussion**

The intent was to develop a first generation biologically based dose-response (BBDR) model for the hypothalamic-pituitary-thyroid (HPT) axis in the adult rat to describe the negative feedback loop parsimoniously using serum thyroxine (T₄) and thyroid stimulating hormone (TSH) levels to control the TSH mediated thyroidal uptake of dietary iodide, and the production and secretion of thyroid hormones. The use of ‘macroscopic’ kinetic properties of the HPT axis and simple model parameters that represent composite and complex ‘microscopic’ biochemical
reactions appear to successfully describe many datasets from several laboratories. We first described the HPT axis under steady-state, euthyroid conditions using simple model structures and equations. The dominant negative feedback control of $T_4$ on TSH was described (Equation 14), along with the stimulation of TSH on thyroidal iodide uptake (Equations 1-2) and subsequent thyroid hormone production (Equation 15) using simple and empirical relationships of critical events of the HPT axis. As mentioned in results, a few of the BBDR-HPT axis predictions deviated from observations for rats fed low iodine diets (LID). It is unclear if strain differences or assay methods played a role in the reported literature results.

Adult rats excrete approximately 95% of a daily iodide sufficient intake (normal laboratory intake of 20 $\mu$g I/day), according to our model simulations. Urinary iodide levels arise from metabolism of thyroid hormones, as well as excess iodide provided in the diet. The normal adult rat stores 10-15 $\mu$g iodide (McLanahan et al., 2007) and model predictions estimate that rats utilize about 1.4 $\mu$g I/day in thyroid hormone production under normal, euthyroid conditions. Furthermore, under iodide sufficient conditions our model predicts that 85% of the daily $T_3$ production is derived from $T_4$ metabolism, with the remaining (15%) produced in the thyroid. This is in agreement with others who suggest that at least 80% of the daily $T_3$ production occurs as a result of $T_4$ metabolism in a euthyroid system (Burger, 1986).

Many current studies that employ updated and revised thyroid hormone assay techniques to examine HPT axis effects resulting from iodide deficiency only report data for single time points, and do not evaluate the time component of HPT axis perturbations (Pedraza et al., 2006; Hotz et al., 1997). Only a handful of kinetic studies examining multiple indices of thyroid function in relationship to iodide intake have been reported (Okamura et al., 1981a, 1981b;
Riesco et al., 1976, 1977). Our model was developed for iodine sufficient (euthyroid) and iodine deficient (hypothyroid) conditions, both which were effectively described.

Using optimized model parameters for the euthyroid, iodide sufficient model, the BBDR-HPT axis model was tested for its ability to predict changes in serum T₄, T₃, TSH, and total thyroid iodide during administration of LIDs. The model predicted the temporal response for decreases in serum T₄ and increases in serum TSH resulting from the lack of available iodide for thyroid hormone production in an acceptable manner with some exceptions. Across all studies, the predictions of serum T₃ may be less consistent with the experimental data compared to other predicted endpoints. Interestingly, the percent of daily T₃ production in the thyroid increases significantly under iodide deficient conditions (Abrams and Larsen, 1973 and Greer et al., 1968), which is in agreement with our model. The percent of overall T₃ production in the thyroid is predicted to increase from 15% (iodide sufficient 20 μg I/day intake) to 25% as iodide intake rate decreases to 1 μg/day and 45% at an iodide intake rate of 0.35 μg/day.

Model predictions during steady-state iodide deficiency of 1 μg I/day suggest that the percent of daily iodide intake excreted in urine decreases to about 65% and only 0.67 μg of iodide is utilized in daily thyroid hormone production. Thyroid iodide stores are severely depleted to about 20% (2.8 μg) of euthyroid, iodide sufficient values, resulting in a decrease of over 50% in serum T₄ concentrations.

Ultimately, the BBDR-HPT axis model was used to generate a dose-response plot for iodide intake and resulting serum T₄ and TSH concentrations (Figure 4.11). Using this model we confirm a sharp decline in serum T₄ such that the TSH stimulation of thyroid axis is unable to compensate for the lack of available iodide for thyroid hormone production. Others have
reported that laboratory rats require an iodide intake greater than 2 μg I/day to maintain euthyroid status (Pedraza et al., 2006).

**Challenges and Limitations**

As with every mathematical model, the utility and limitations of this BBDR-HPT axis model require discussion. Discovering details that pertain to how the HPT axis works is an active area of research. For example, our model does not describe events at the molecular level, or all metabolites of thyroid hormones (reverse T₃, T₂, T₁, or thyroid hormone conjugates other than T₄-glucuronide). Attempting to describe detailed biological process in a modeling framework would require focused laboratory studies and, in our opinion, widespread use of improved analytical tools for measuring thyroid hormones and TSH. Our model does not include physiological changes that occur during a long-term iodide deficient condition, resulting in a hypothyroid disease state. Structural changes in the thyroid (Colzani et al., 1999), increases in thyroid blood flow (Michalkiewicz et al., 1989), and altered biological activity of thyroid hormone metabolizing enzymes (Janssen et al., 1994; Pedraza et al., 2006; Obregon, et al., 2005) are examples of HPT axis alterations that are not accounted for in this model and may have affected the ability of our model to reproduce serum T₃ data.

The reported literature for the adult rat and HPT axis function varies dramatically. For example, reported TSH values for adult male Sprague-Dawley rats range from 4.6 ± 0.49 ng/mL to 8.73 ± 0.81 ng/mL (McLanahan et al., 2007), approximately 15 to 20 ng/mL (Siglin et al., 2000), 327 ± 174 ng/mL (Okamura et al., 1981a), to a high of 440 ± 220 ng/mL (Lemarchand-Beraud and Berthier, 1981). Several factors may contribute to this variability including, time of sampling, weight of animal, and radioimmunoassay (RIA) analytical method and standards.
employed. Thus, in reporting our model results we reported TSH as fold change to normalize and compare model simulations with more datasets.

Most of the ID studies occurred prior to 1990 and many methods for analysis of thyroid hormones, TSH, and iodide have evolved since their publication. However, the biggest concern when analyzing data from ID studies is the actual iodine concentration in the diet and the amount that the rat consumes. This amount can vary significantly between batches of rodent chow and can produce varying results as demonstrated by Naeije et al. (1978). Unfortunately the actual iodine concentration in rodent chow is not always measured by laboratories conducting ID studies, but rather the value reported by the manufacturer is included in the manuscript.

Other challenges were encountered during model development, including the inability of several parameters, which were optimized in the radiotracer sub-models to fit data points following iv doses of each compound, to reproduce steady-state, euthyroid iodide sufficient datasets. When the model parameters were re-optimized in the dietary iodide BBDR-HPT axis model, five parameters ($Cl_{uc,i}$, $V_{maxTc,i}$, $V_{maxBc,i}$, $V_{maxc^{UGT}T4}$, and $V_{maxc^{LU}T4}$) differed from radiotracer parameters by more than one fold. This suggests that radiotracer kinetics for the HPT axis may not adequately represent mass transfer kinetics of the endogenous substances.

**Future Directions**

The development of this model was initiated with the ultimate goal of integrating it with physiologically based pharmacokinetic (PBPK) models for thyroid toxicants. Thyroid toxicants are defined as compounds which alter serum thyroid hormone and TSH concentrations (Zoeller and Tan, 2007); and the BBDR-HPT axis model presented here is able to predict serum changes under iodine deficient conditions. In order to better understand the thyroid axis and temporal responses mathematically, it was necessary to first test the model under these iodine deficient
conditions. Some environmentally relevant compounds, such as perchlorate and thiocyanate, inhibit NIS thyroid iodide uptake and may result in conditions that ‘mimic’ iodide deficiency, a decline in available iodide for thyroid hormone synthesis (Wolff, 1998). As the field evolves and more data become available, this first-generation BBDR-HPT axis model can be expanded to contain other tissues of interest (e.g. brain and heart) and other thyroid axis compensatory mechanisms (e.g. changes in 5’-deiodinase activity). This model can also be expanded to relate dose-response and HPT axis status to frank toxicity or neurodevelopmental effects. However, the dietary iodide BBDR-HPT axis model presented here, which integrates a variety of physiologic processes, will be used to predict complex, non-linear dose responses resulting from exposure to thyroid toxic chemicals (e.g. PCB126 and perchlorate from McLanahan et al. (2007)), alone and in combination.

Funding

United States Environmental Protection Agency Science to Achieve Results research grant (RD83213401-0); United States Environmental Protection Agency Science to Achieve Results Fellowship (FP-91679301-0 to E.D.M).

Acknowledgments

The authors extend special thanks to Dr. Kyung O. Yu for providing experimental datasets for use in radioiodide model development. Sincere thanks to Dr. Jerry L. Campbell, Jr. for model review. The views expressed in this manuscript are those of the authors and do not represent official opinions of the United States Environmental Protection Agency. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.
References


Table 4.1. Model Boundaries for Parameter Estimation

<table>
<thead>
<tr>
<th>Boundary Condition</th>
<th>Literature Values</th>
<th>Radiotracer Model Predicted</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₄ metabolized to T₃ (%)</td>
<td>14-27%&lt;sup&gt;a&lt;/sup&gt;</td>
<td>40%</td>
</tr>
<tr>
<td>T₄ excreted in feces (T₄-G) (%)</td>
<td>10-38%&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>24%</td>
</tr>
<tr>
<td>T₃ excreted in feces (%)</td>
<td>4.9-54.9%&lt;sup&gt;c,d&lt;/sup&gt;</td>
<td>26%</td>
</tr>
</tbody>
</table>

<sup>a</sup> DiStefano <i>et al.</i>, 1982  
<sup>b</sup> Nguyen <i>et al.</i>, 1993  
<sup>c</sup> DiStefano <i>et al.</i>, 1987  
<sup>d</sup> DiStefano <i>et al.</i>, 1993
Table 4.2. Physiological Parameters for the Adult Rat

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Source</th>
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</thead>
<tbody>
<tr>
<td><strong>Tissue volumes</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body weight, BW (kg)</td>
<td>0.350</td>
<td>McLanahan et al., 2007</td>
</tr>
<tr>
<td>Liver, VLc (% BW)</td>
<td>3.66</td>
<td>Brown et al., 1997</td>
</tr>
<tr>
<td>Liver blood, VLBc (% VL)</td>
<td>21</td>
<td>Brown et al., 1997</td>
</tr>
<tr>
<td>Thyroid, VTc (% BW)</td>
<td>0.005</td>
<td>McLanahan et al., 2007</td>
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<tr>
<td>Thyroid blood, VTBc (% VT)</td>
<td>15.7</td>
<td>Malendowicz and Bednarek, 1986</td>
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<td><strong>Blood flows</strong></td>
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<td>Cardiac output, QCc (L/hr/kg^{0.075})</td>
<td>14.0</td>
<td>Brown et al., 1997</td>
</tr>
<tr>
<td>Liver, QLc (% QC)</td>
<td>17.4</td>
<td>Brown et al., 1997</td>
</tr>
<tr>
<td>Thyroid, QTc (% QC)</td>
<td>1.6 (^a)</td>
<td>Brown et al., 1997</td>
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</table>

\(^a\) Human value.
Table 4.3. Compound-Specific Parameters

<table>
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<tr>
<th>Parameter</th>
<th>Value</th>
<th>Source</th>
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</thead>
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<tr>
<td><strong>Volume of distribution (% BW)</strong></td>
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<tr>
<td>Iodide, $V_{dc_i}$</td>
<td>50-VT</td>
<td>Visual Fit</td>
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<td>TSH, $V_{dc_{TSH}}$</td>
<td>5.54</td>
<td>Connors et al., 1984</td>
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<tr>
<td>$T_4$, $V_{dc_{T4}}$</td>
<td>15.6-VL</td>
<td>Kohn et al., 1996</td>
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<tr>
<td>$T_3$, $V_{dc_{T3}}$</td>
<td>18.6-VL</td>
<td>DiStefano, 1986</td>
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<td><strong>Partition coefficients (unitless)</strong></td>
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<td>$T_4$ – Liver:blood, $PL_{T4}$</td>
<td>1.27</td>
<td>Escobar-Morreale et al., 1996</td>
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<tr>
<td>$T_3$ – Liver:blood, $PL_{T3}$</td>
<td>4.47</td>
<td>Escobar-Morreale et al., 1996</td>
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<td><strong>Permeability area cross-products ($L/hr/kg^{0.75}$)</strong></td>
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<td></td>
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<td>$T_4$ – Liver blood to liver tissue, $PAL_{T4}$</td>
<td>0.0423</td>
<td>Optimized</td>
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<tr>
<td>$T_3$ – Liver blood to liver tissue, $PAL_{T3}$</td>
<td>0.1699</td>
<td>Optimized</td>
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<tr>
<td>Iodide – Thyroid blood to thyroid tissue, $PAT_{T}$</td>
<td>0.0001</td>
<td>Merrill et al., 2003</td>
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<td><strong>Affinity constants (nmol/L)</strong></td>
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<td>Iodide – Thyroid NIS, $K_{m_{i}}$</td>
<td>31519</td>
<td>Merrill et al., 2003; Gluzman and Niepomniszcze, 1983</td>
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<td>TSH – Thyroid NIS, $K_{m_{TSH}}^{TSH}$</td>
<td>0.949</td>
<td>Optimized</td>
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<td>Iodide – Iodide organification in thyroid, $K_{b_{i}}$</td>
<td>244.59</td>
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<td>TSH – Iodide organification in thyroid, $K_{b_{TSH}}$</td>
<td>733.98</td>
<td>Optimized</td>
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<td>$T_4$ – Liver Type 1 $5'$-deiodinase, $K_{m_{DI_{T4}}}$</td>
<td>2300</td>
<td>Leonard and Visser, 1986</td>
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<td>$T_4$ – Liver glucuronidation, $K_{m_{UG_{T4}}}$</td>
<td>$1 \times 10^5$</td>
<td>Visser et al., 1993</td>
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<tr>
<td>$T_4$ – Liver uptake, $K_{m_{LU_{T4}}}$</td>
<td>650</td>
<td>Blondeau et al., 1988</td>
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<td><strong>Maximum velocities (nmol/hr/kg^{0.75})</strong></td>
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<tr>
<td>Iodide – Thyroid NIS, $V_{max_{Tc_{i}}}$</td>
<td>5738.267</td>
<td>Optimized</td>
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<tr>
<td>Iodide – Iodide organification in thyroid, $V_{max_{Bc_{i}}}$</td>
<td>1005.9$a$</td>
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<td>$T_4$ – Liver Type 1 $5'$-deiodinase, $V_{max_{DI_{T4}}}$</td>
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<tr>
<td>$T_4$ – Liver uptake, $V_{max_{LU_{T4}}}$</td>
<td>4384.73</td>
<td>Optimized</td>
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<td>$T_3$ – 1st order Liver uptake, $k_{LU_{T3}}$ (1/hr)</td>
<td>1.25</td>
<td>Optimized</td>
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<tr>
<td><strong>Clearance values</strong></td>
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<tr>
<td>Iodide – Urinary excretion, $Cl_{Uc_{i}}$ (L/hr/kg^{0.25})</td>
<td>0.0046</td>
<td>Optimized</td>
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<td>TSH – Vd clearance, $kel_{TSH}$ (L/hr/kg^{0.25})</td>
<td>1.8899</td>
<td>Lemarchand-Beraud and Berthier, 1981</td>
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<td>$T_4$ – Vd metabolism, $kel_{T4}$ (1/hr/kg^{0.25})</td>
<td>0.05$a$</td>
<td>Abrams and Larsen, 1973</td>
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<td>$T_3$ – Vd metabolism, $kel_{T3}$ (1/hr/kg^{0.25})</td>
<td>0.12$b$</td>
<td>Abrams and Larsen, 1973</td>
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<tr>
<td>$T_4$ – Liver metabolism, $kmet_{Lc_{T4}}$ (1/hr/kg^{0.25})</td>
<td>3.65</td>
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<td>$T_3$ – fraction of liver $T_3$ metabolism excreted in feces, $FT_{3} feces$ (unitless)</td>
<td>0.30</td>
<td>Visually Fit</td>
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<tr>
<td><strong>TSH / Thyroid hormone production parameters</strong></td>
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<tr>
<td>Thyroid hormone production constant, $k_{B_{TSH}}^{TSH}$ (L^2/nmol/hr)</td>
<td>$5 \times 10^{-7}$</td>
<td>Visually Fit</td>
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<tr>
<td>Maximum rate of TSH production in the absence of $T_4$, $k_0^{TSH}$ (nmol/hr/kg^{0.75})</td>
<td>6</td>
<td>Connors et al., 1984</td>
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<td>$T_4$ concentration for half-maximal TSH production, $K_{m_{T4}}^{TSH}$ (nmol/L)</td>
<td>0.2</td>
<td>Optimized</td>
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*a* Scaled by dividing by BW^{0.75}. See footnote in Materials and Methods.

*b* Calculated from serum half-life using $kel=\ln2/t_{1/2}$
**Figure 4.1.** Sub-model structure for radiotracer compounds used in model development and preliminary estimation of kinetic parameters. (A) $^{125}$Iodide sub-model for an iv dose to Vd, 1st order urinary elimination, and distribution to thyroid. Thyroid described with active uptake (bold arrow) and bidirectional diffusion. Bound $^{125}$I in thyroid is used in thyroid hormone production. (B) $^{125}$I-TSH sub-model described for an iv dose to Vd and 1st order elimination. (C) $^{125}$I-T$_3$ model described for an iv dose and 1st order clearance from Vd and distribution to the liver where it is transported via active uptake or diffusion. Liver clearance is described with one rate partitioned as 30% eliminated through feces and the remaining metabolized to free iodide. (D) $^{131}$I-T$_4$ sub-model for an iv dose to the Vd, 1st order clearance from Vd and distribution to the liver where it is also taken up via active transport or passive diffusion. T$_4$ in the liver is metabolized via Type I 5’-deiodnation and formation of T$_4$-glucuronide (T$_4$-G).
Figure 4.1.
**Figure 4.2.** BBDR-HPT axis model structure for the adult rat hypothalamic-pituitary-thyroid (HPT) axis, including sub-models (areas shaded in gray) for dietary iodide ($^{127}$I), TSH, T$_4$, and T$_3$. Solid arrows (→) represent blood flows, bold arrows (➡) within tissue compartments represent active uptake, solid arrows (➡) within tissue compartments represent diffusion limitation, dashed arrows between models (---) represent metabolic links, while the dashed and dotted arrow (---·➡) represents use of dietary iodide in thyroid hormone production, and the bold (➡➡) arrows connecting models processes controlled (stimulated or inhibited) by the compound. Specific details on the model links shown in the figure are as follows: 

1. Formation of free iodide from T$_3$ metabolism in the Vd and liver; 
2. Formation of free iodide from T$_4$ to T$_3$ metabolism in the Vd and liver; 
3. Loss of bound thyroidal iodide secreted as thyroid hormones; 
4. Metabolism of T$_3$ (30% into feces, 70% to free iodide); 
5. Deiodination of T$_4$ in the liver to T$_3$ and free iodide; 
6. Glucuronidation of T$_4$ (formation of T$_4$-glucuronide; T$_4$-G) and excretion into feces; 
7. TSH stimulation NIS iodide uptake; 
8. TSH stimulation of organification of iodide, forming thyroid hormone precursors; 
9. TSH stimulation of thyroid hormone production; 
10. T$_4$ negative feedback on TSH production.
Figure 4.2
**Figure 4.3.** Relationship between total amount of thyroidal iodide and fraction of total thyroid hormone production that is T3. This relationship was used to fractionate the thyroid production rate of thyroid hormones as detailed in *Methods*. Data points adapted from Pedraza *et al.* (2006) are shown with best fit exponential line. The equation for the line is adapted for use in the model as shown in Equation 18.
Figure 4.3
Figure 4.4. Model simulations (lines) compared with literature data (points) for iv doses of radiolabeled compounds used in HPT axis model development. (A) Serum $^{125}$Iodide ($^{125}$I, □) and cumulative $^{125}$I urinary excretion (■) following a tail vein iv dose of 33μg $^{125}$I/kg, data adapted from Yu et al. (2002) for serum (●) and urine (○); (B) Total (■) and bound (□) $^{125}$I in the thyroid after a tail vein iv dose of 33μg $^{125}$I/kg, data provided by Dr. Yu (personal communication) for total (■) and bound (□) thyroid $^{125}$I; (C) Serum (●) and liver (△) $^{125}$I-T$_3$ following iv injection of 0.83 ng $^{125}$I-T$_3$, data adapted from DiStefano et al. (1993) for serum (●) and liver (△); (D) Serum (●) and liver (△) $^{131}$I-T$_4$ following iv injection of 1.7 ng $^{131}$I-T$_4$, data adapted from Schroder van der Elst et al. (1997) for serum (●) and liver (△); and (E) Serum (●) $^{125}$I-TSH following iv dose of 5 ng $^{125}$I-TSH, serum data (●) adapted from Spira et al. (1979).
Figure 4.4C

Figure 4.4D
Figure 4.4E
Figure 4.5. Steady-state, iodide sufficient, model simulations (lines) shown with literature data (points) for HPT axis model calibration. (A) Total thyroid (__, μg) and free serum (__, μg/dL) iodide ($^{127}$I) model simulation, thyroid $^{127}$I data (○ ± SD) from McLanahan et al. (2007) and serum (● ± SD) from Eng et al. (1999); (B) Serum TSH (__, ng/mL) model simulation, data (● ± SD) for McLanahan et al. (2007); (C) Serum (__) and liver (●) T4 model simulations, serum data (● ± SD) from McLanahan et al. (2007) and liver (▽) adapted from Morreale de Escobar et al. (1994); and (D) Serum (__) and liver (●) T3 model simulations, serum data (● ± SD) unpublished data from McLanahan et al. (2007) study and liver (▽) from Morreale de Escobar et al. (1994).
Figure 4.5A

Figure 4.5B
Figure 4.5C

Figure 4.5D
Figure 4.6. Short term effects of LID (0.35 μg I/day) on serum thyroid hormones and total thyroid iodide of adult male Holtzman Sprague-Dawley rats. On Day 0, rats began a LID of approximately 0.35 μg I/day and continued for 26 days (Riesco et al., 1977). Model simulations are represented by lines for serum T₄ ( , ng/mL), T₃ ( , ng/mL), TSH ( , fold change), and total thyroid ¹²⁷I ( , μg). LID data for serum T₄ ( ▼ ± SD), T₃ ( ■ ± SD), TSH ( ○ ), and total thyroid ¹²⁷I ( ● ± SD) was adapted from Riesco et al. (1977).
Figure 4.6
**Figure 4.7.** Long term effects of LID (0.33 μg I/day) on serum thyroid hormones and total thyroid iodide of adult male (A) Simonsen Albino and (B) Holtzman Sprague-Dawley rats. On Day 0, rats began a LID of approximately 0.33 μg I/day and continued for 84 days (Okamura *et al.* 1981a). Model simulations are represented by lines for serum T₄ ( _____, ng/mL), T₃ ( — — — , ng/mL), TSH ( — — , fold change), and total thyroid ¹²⁷I ( ———— , μg). LID data for serum T₄ ( ▼ ± SD), T₃ ( ■ ± SD), TSH ( ◦ ), and total thyroid ¹²⁷I ( ● ± SD) was adapted from Okamura *et al.* (1981a).
Figure 4.7A

Figure 4.7B
**Figure 4.8.** Long term effects of LID (1.14 μg I/day) on serum thyroid hormones and total thyroid iodide of adult male Holtzman Sprague-Dawley rats. On Day 0, rats began a LID of 1.14 μg I/day and continued for 96 days (Okamura et al., 1981b). Model simulations are represented by lines for serum T4 ( , ng/mL), T3 ( , ng/mL), and total thyroid ¹²⁷I ( , μg). LID data for serum T₄ ( ▼ ± SD), T₃ ( ■ ± SD), and total thyroid ¹²⁷I ( ● ± SD) was adapted from Okamura et al. (1981b).
Figure 4.8
Figure 4.9. Recovery from ID in adult male Sprague-Dawley rats fed a LID for seven months.

After seven months on a LID (0.6μg I/day), rats were supplemented with iodide to provide total intake of approximately 2.6 μg I/day (black) or 8.6 μg I/day (dark grey) beginning on Day 0 and continuing for 9 days (Fukuda et al., 1975). Model simulations of serum T₄ (solid lines) and TSH (dashed lines) compared with recovery data modified from Fukuda et al. (1975) for serum T₄ (●, 2.6 μg I/day; ○, 8.6 μg I/day) and serum TSH (▲, 2.6 μg I/day; ▼, 8.6 μg I/day). Data expressed as percent of baseline recorded at Day 0.
Figure 4.9
Figure 4.10. Normalized sensitivity coefficient (NSC) graph for parameters that yielded a NSC of 0.90 or greater for at least one response (serum T4, T3, and TSH or total thyroid iodide) examined under iodide sufficient (20 μg/day) (left) and iodide deficient (1 μg/day) (right) steady-state conditions. Total amount of iodide in the thyroid is the most sensitive model response and followed by serum T4 and T3 concentrations, which were less sensitive than total thyroid iodide predictions to a one percent change in model parameters. Serum TSH was least sensitive to changes in model parameters.
Figure 4.10
**Figure 4.11.** Iodide dose-response plot for serum T₄ and TSH. BBDR-HPT axis model was used to determine steady-state serum T₄ and TSH concentrations over a wide range of iodide intakes, intakes ranged from insufficient (0-2 μg I/day) to sufficient (> 2 μg I/day).
Figure 4.11
Supplementary Data

“A Biologically Based Dose-Response Model for Dietary Iodide and the Hypothalamic-Pituitary-Thyroid Axis in the Adult Rat: Evaluation of Iodide Deficiency”

Eva D. McLanahan, Melvin E. Andersen, and Jeffrey W. Fisher

The supplementary data includes a table of model parameters optimized for radiotracer sub-models and used to make plots shown in Figure 4.4. These model parameters were re-optimized to euthyroid, steady-state iodide sufficient conditions in the dietary iodide BBDR-HPT axis combined model that often resulting in minor changes in the parameter.
<table>
<thead>
<tr>
<th>Parameter</th>
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<td><strong>Volume of distribution (% BW)</strong></td>
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<td>Merrill et al., 2003; Gluzman and</td>
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<td></td>
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<td>Niepomniscze, 1983</td>
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<td>1.8899</td>
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<td><strong>TSH / Thyroid hormone production parameters</strong></td>
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<td>Thyroid hormone production constant, $k_{IB_{TSH}}^{TSH}$ (L$^2$/nmol/hr)</td>
<td>$5 \times 10^{-7}$</td>
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$^a$ Scaled by dividing by BW$^{0.75}$. See footnote in *Materials and Methods*.

$^b$ Calculated from serum half-life using $kel=\frac{\ln(2)}{t_{1/2}}$. 

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128
CHAPTER 5

THE USE OF A BIOLOGICALLY BASED DOSE-RESPONSE MODEL OF THE
HYPOTHALAMIC-PITUITARY-THYROID (HPT) AXIS TO EVALUATE PERCHLORATE
INDUCED PERTURBATIONS OF THE HPT AXIS IN ADULT RATS

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To be submitted to Environmental Health Perspectives
Abstract

The perchlorate anion (ClO$_4^-$) is an environmental contaminant known to disrupt the thyroid axis of many terrestrial and aquatic species. It is well known that ClO$_4^-$ competitively inhibits iodide uptake into the thyroid at the sodium/iodide symporter (NIS), presumably leading to a decrease in available iodide for use in thyroid hormone production. A BBDR-HPT axis model for the adult male rat was combined with a PBPK model for ClO$_4^-$ to describe perturbations in the thyroid axis resulting from ClO$_4^-$ exposure in drinking water. First, the BBDR-HPT axis model was linked with a ClO$_4^-$ PBPK model via competitive inhibition of thyroidal iodide uptake by ClO$_4^-$.

However, model simulations were not able to predict the rapid decline in serum T$_4$ and rapid increase in serum TSH responses when laboratory rats were exposed to ClO$_4^-$ in drinking water. Thus, a hypothesis that thyroidal ClO$_4^-$ interferes with thyroid hormone synthesis was tested. When ClO$_4^-$ interference with thyroid hormone production was included in the model, it adequately simulated adult male rat thyroid axis perturbations in serum T$_4$ and TSH reported in literature from exposures to ClO$_4^-$ contaminated drinking water over a range of doses (1 mg ClO$_4^-$/kg-day to 15 mg ClO$_4^-$/kg-day). Perchlorate appears not only to affect iodide availability for thyroid hormone production, but also to interfere with production of thyroid hormones. TSH secretion in the presence of ClO$_4^-$ also appeared to be more sensitive than in iodide deficient conditions as evidenced by a more rapid increase in TSH in the presence of ClO$_4^-$ compared to changes under severe iodide deficiency. The integration of the ClO$_4^-$ PBPK and BBDR-HPT axis models provide further biological insights into mode of action of ClO$_4^-$ disruption of the thyroid axis and demonstrates the utility of a BBDR-HPT axis model.

Key Words: perchlorate, iodide, thyroid, rat, BBDR model, PBPK model, HPT axis
Introduction

The hypothalamic-pituitary-thyroid (HPT) axis is a dynamic system, composed of sophisticated feedback loops, often able to adapt to environmental and physiological insults that alter its function. The thyroid produces two major hormones, T4 and a smaller quantity of the biologically active hormone, T3. TSH is produced by the pituitary under normal conditions and synthesis increases in response to a decline in serum thyroid hormones. This is referred to as the T4/TSH negative feedback loop. TSH stimulates the thyroid by binding to extracellular TSH receptors on the basolateral plasma membrane of thyroid follicular cells, which results in an intracellular cascade of secondary messenger events (Ferreira et al., 2005; Riedel et al., 2001).

TSH upregulation of the thyroid can result in increased synthesis and activity of the NIS, increasing the rate of iodide sequestration by the thyroid, as well as events leading to increased rate of formation and secretion of thyroid hormones.

Environmental contaminants, such as perchlorate (ClO4\(^-\)), may affect thyroid axis homeostasis. Competitive inhibition of NIS iodide transport is the most well-known and well-defined mode of action for ClO4\(^-\) (Tonacchera et al., 2004; Yu et al., 2002; Wolff, 1998); although some have proposed that ClO4\(^-\) may also affect the formation and secretion of thyroid hormones (Hildebrandt and Halmi, 1981; Wolff, 1998; and Yu et al., 2002). The ClO4\(^-\) anion has recently been classified as a ubiquitous environmental contaminant throughout the United States, with detectable concentrations in many drinking water supplies (Motzer, 2001), food and beverage products (e.g. milk, lettuce, grains) (El Aribi et al., 2006), and also found as a contaminant in dietary supplements (Snyder et al., 2006). The occurrence of ClO4\(^-\) in the environment is most often attributed to anthropogenic uses of ClO4\(^-\) salts as oxidizers in solid
rocket propellants and application of Chilean nitrate fertilizers (Motzer, 2001); however, ClO$_4^-$ may also be formed naturally by atmospheric processes (Dasgupta et al., 2005).

The inhibition of iodide uptake into the thyroid by ClO$_4^-$ is thought to affect the thyroid axis by creating an iodide deficient (ID) condition within the thyroid, resulting in a decline in thyroid hormone production as reflected by decreased serum concentrations of thyroid hormones (Yu et al., 2002; Siglin et al., 2000). Under chronic exposures to ClO$_4^-$ in rodents the upregulation of the thyroid gland by TSH may not be able to compensate for the lack of available iodide in the thyroid, leading to hypothyroidism. Alterations in the HPT axis by ClO$_4^-$ have been observed in laboratory studies with rodents (inhibition of thyroidal uptake of radiolabeled iodide, decreased serum thyroid hormones and increased serum TSH) and clinical studies in humans (inhibition of thyroidal uptake of radiolabeled iodide). The human HPT axis is not as sensitive as rodents to ClO$_4^-$ induced perturbations and to date, only one human study suggests environmental levels of ClO$_4^-$ may disrupt the HPT axis in women with low intake of iodide (Blount et al., 2006). Thyroid hormones are essential for proper growth, development, reproduction, and metabolism. Transient changes in thyroid hormone economy during gestation and early development could result in life-long consequences, including irreversible neurological damage (Haddow et al., 1999).

Several ClO$_4^-$ physiologically based pharmacokinetic (PBPK) models have been developed for different life-stages of the rat and human (Clewell et al., 2003a, 2003b; Merrill et al., 2003, 2005). These models were combined with a radiolabeled iodide (¹²⁵I) PBPK model to predict inhibition of radiolabeled iodide uptake, which was determined to be the precursor to the critical effect used by the U.S. Environmental Protection Agency in development of the human RfD for ClO$_4^-$ (U.S. EPA, 2005). While these rodent and human models adequately predicted
the inhibition of radiolabeled iodide uptake, they did not include the downstream consequences of thyroidal iodide deficiency, such as alterations in serum thyroid hormone concentrations. The adult rat model presented herein integrates the BBDR-HPT axis model developed by McLanahan et al. (submitted) with a modified PBPK model for ClO$_4^-$ in the adult male rat (Merrill et al., 2003). This integration of models is an example of how the BBDR-HPT axis model can be combined with PBPK models for environmental contaminants, via specific modes of action, to predict perturbations in thyroid hormone homeostasis. Depending on the mode of action of the chemical, the BBDR-HPT axis model may need to be modified. In addition, combining PBPK models of thyroid disrupting chemicals with the BBDR-HPT axis model provides a means to test hypotheses concerning mode of action of these chemicals on the HPT axis.

**Materials and Methods**

To analyze the dose-response relationship between ClO$_4^-$ exposure and effects on the HPT axis, a physiologically-based pharmacokinetic (PBPK) model for ClO$_4^-$ was developed and integrated with the BBDR-HPT axis model previously reported (McLanahan et al., submitted) for the adult rat. Using these linked models provided a framework for evaluating the hypothesis that ClO$_4^-$ indirectly decreases serum T$_4$ concentrations by inhibiting thyroidal iodide uptake, causing a lack of available iodide for thyroid hormone synthesis.

**Model Structure**

The models were constructed in acslXtreme version 2.4.0.11 (AEgis Technologies, Huntsville, Alabama) and solved using the Gear algorithm for stiff systems. The BBDR-HPT axis model for dietary iodide and the thyroid axis was used as previously described (McLanahan et al., submitted). Briefly, the BBDR-HPT axis model includes four sub-models: dietary iodide, ...
thyroid stimulating hormone TSH, T₄, and T₃. The models combine to form a simplified representation of the thyroid axis in the adult rat that includes TSH stimulation of thyroidal iodide uptake at the NIS, incorporation of iodide into thyroid hormone precursors within the thyroid, and secretion of thyroid hormones (T₄ and T₃) into the bloodstream. The TSH/T₄ negative feedback loop is described, along with metabolism of thyroid hormones and recycling of iodide. Several additions were made to the BBDR-HPT axis model to incorporate the mode of action for ClO₄⁻ perturbation of the thyroid axis. These modifications are detailed in the following sections.

*Perchlorate PBPK Model Structure.* A simple model structure for ClO₄⁻ was constructed consisting of three compartments: plasma, thyroid, and rest of body. Perchlorate is rapidly absorbed after oral administration and is distributed throughout the body, but excreted virtually unchanged through the urine (Wolff, 1998). Urinary excretion of ClO₄⁻ was described as a first order clearance from the plasma.

The thyroid is described with a blood and tissue compartment using a diffusion limited equation to describe bidirectional passive diffusion of iodide between the thyroid gland and blood and a Michaelis-Menten equation to describe active uptake of iodide into the thyroid via the NIS protein. Several studies have shown that ClO₄⁻ and ³⁶ClO₄⁻ are transported into the thyroid via the NIS and an increased uptake of this anion has been observed in rats administered TSH and in conditions where TSH serum levels are increased in response to ClO₄⁻ exposure (Anbar *et al.*, 1959; Chow *et al.*, 1969; Chow and Woodbury, 1970; Goldman and Stanbury, 1973; and Yu *et al.*, 2002).

Datasets Used for Perchlorate PBPK Model Development. The PBPK model for ClO₄⁻ was developed and tested using iv dosing study kinetics of ³⁶ClO₄⁻ from Yu *et al.* (2002). Adult
male Sprague-Dawley rats were administered 3.3 mg $^{36}\text{ClO}_4^-$/kg bw and serum and thyroid concentrations determined 0.5, 6, 12, 24, 32, and 48 hrs post injection. Cumulative urinary excretion of $^{36}\text{ClO}_4^-$ was reported 12 and 24 hrs post injection. In addition, the ClO$_4^-$ serum and thyroid concentrations at 1, 5, and 14 days following administration of 1, 3, 10, and 30 mg ClO$_4^-$/kg bw in drinking water were simulated using the model (Yu et al., 2002).

**BBDR-HPT Axis and Perchlorate PBPK Model Integration.** The PBPK model for ClO$_4^-$ was integrated with the BBDR-HPT axis model for the adult male rat to test the hypothesis that ClO$_4^-$ disruption of HPT axis homeostasis is due to its ability to competitively inhibit NIS thyroidal iodide transport. To test this hypothesis, competitive inhibition of ClO$_4^-$ and iodide uptake at the thyroid NIS was described using the following equations:

\[
\frac{dTNIS_i}{dt} = \frac{V \max T_i^{TSH} \times Cvt_i}{Cvt_i + Km_i \times (1 + \frac{Cvt_p}{Ki_p})}
\]  \[1\]

\[
V \max T_i^{TSH} = \frac{V \max T_i \times Ca_{TSH}}{K_{TSH}^{NIS} + Ca_{TSH}}
\]  \[2\]

where Cvt$_i$ is the free concentration of iodide in thyroid blood (nmol/L), Km$_i$ is the affinity constant of iodide for the NIS (nmol/L), Ki$_p$ is inhibition constant of ClO$_4^-$ for iodide transport via the NIS (nmol/L), Cvt$_p$ is the concentration of ClO$_4^-$ in the thyroid blood (nmol/L), VmaxT$_i$ is the maximum rate of NIS iodide uptake (nmol/hr), Ca$_{TSH}$ is the serum concentration of TSH (nmol/L), and K$_{TSH}^{NIS}$ is the concentration of TSH that gives rise to half-maximal rate of NIS transport of iodide (nmol/L). A similar equation was used for the inhibition of iodide on the uptake of ClO$_4^-$ via the NIS; however, the inhibition of ClO$_4^-$ uptake is minimal because ClO$_4^-$ has a much greater affinity for the NIS compared to iodide (1500 nmol ClO$_4^-$/L vs. 31519 nmol I/L).
Model Parameters

When possible, model parameters were derived from the published literature. Default allometric scaling assumptions were used. Blood flows (Q), maximum velocities (Vmax), and permeability area cross products were multiplied by BW^{0.75} and clearance terms (Cl) were scaled by 1/BW^{0.25}.

Physiological Parameters. Physiological parameters including tissue volumes (V) and blood flows (Q) were obtained from literature (Brown et al. 1997; Everett et al., 1956; Malendowicz and Bednarek, 1986; McLanahan et al. 2007) and are shown in Table 1.

Chemical-Specific Parameters. All parameter values for the BBDR-HPT axis model were identical to those previously detailed in McLanahan et al. (submitted) for the initial evaluation of the hypothesis that ClO_4^- disturbed the HPT axis by inhibition of active uptake of thyroidal iodide by the NIS. However, the model was modified to test the hypothesis that ClO_4^- also affects thyroid hormone synthesis. The parameters for ClO_4^-, iodide parameters, and the BBDR-HPT axis model parameters for the second hypothesis are shown in Tables 5.2 and 5.3. The affinity constant, \( K_{mp} \), of ClO_4^- for the NIS was set equal to the inhibition constant (\( K_{ip} \)) for the inhibition of iodide uptake by ClO_4^-, determined to be 1.5 \( \mu M \) by an in vitro study in Chinese Hamster Ovary cells (CHO-4J) by Kosugi and colleagues (1996). The tissue: blood partition coefficient (\( PB_{Body}^{p} \), 0.416 unitless) for the rest of body compartment for ClO_4^- was first estimated by weighting the partition coefficients, based on tissue volume, used in the Merrill et al. (2003) adult male rat model. However to fit maximum serum ^{36}ClO_4^- concentrations following iv dose of 3.3 mg ^{36}ClO_4^-/kg (Yu et al., 2002), \( PB_{Body}^{p} \) was optimized to a value of 0.36.
Datasets used for Simulation of Perchlorate HPT Axis Perturbations

Two studies on the time-course of ClO$_4^-$ induced HPT axis perturbations were available. The first study conducted by Mannisto et al. (1979) examined the effect of a 15 mg ClO$_4^-$/kg-day drinking water exposure on adult male Sprague-Dawley (SD) rats. Serum T$_4$ and TSH concentrations were reported following 0, 2, 4, 6, 9, and 14 days of exposure. In the second study, Yu et al. (2002) administered 0, 1, 3, or 10 mg ClO$_4^-$/kg-day in drinking water to adult male SD rats. Serum total T$_4$, T$_3$, TSH, and ClO$_4^-$ concentrations were determined after exposure for 1, 5, and 14 days.

Another study reported HPT axis perturbations after administration of ClO$_4^-$ in drinking water for 14 days. Caldwell (1995) administered ClO$_4^-$ in drinking water to adult male and female SD rats at dose rates of 0, 0.1, 0.4, 1, 2, 4, 11, or 22 mg/kg-day for 14 days. Serum T$_4$ and TSH were reported by Caldwell (1995). This dataset did not include time course data; responses were only measured at the end of the 14 day exposure period.

Results

Perchlorate PBPK Model Parameterization

Parameters for the PBPK model for ClO$_4^-$ were derived from published literature when available. Several parameters were optimized (acslXtreme Parameter Estimation v 2.4.0.11) for the model as shown in Table 5.2. The $1^{st}$ order urinary clearance constant ($Cl_{uc,p}$, 0.018 L/hr-kg) was optimized to fit the cumulative urinary excretion data from Yu et al. (2002) of $^{36}$ClO$_4^-$ following an iv injection of 3.3 mg $^{36}$ClO$_4^-$/kg to adult male rats. Several parameters for the movement of ClO$_4^-$ into and out of the thyroid were optimized to the $^{36}$ClO$_4^-$ thyroid concentrations reported after the 3.3 mg $^{36}$ClO$_4^-$/kg bw iv dose reported by Yu et al. (2002).
These parameters included the maximum velocity for ClO₄⁻ NIS transport under TSH stimulation (V_{TSH}^{\text{maxTc}}, 177 nmol/hr-kg), concentration of serum TSH resulting in half-maximal rate of NIS transport (K_{TSH}^{\text{pNIS}}, 0.949 nmol/L), and the permeability area cross-product for bidirectional diffusion of ClO₄⁻ across the thyroid membrane (P_{c}^{\text{Tc}}, 2.8×10^{-4} L/hr-kg). The simple PBPK model developed for ClO₄⁻ adequately predicted serum and thyroid ³⁶ClO₄⁻ concentrations as well as cumulative urinary excretion following an iv dose of 3.3 mg ³⁶ClO₄⁻/kg (Yu et al., 2002) as seen in Figure 5.3.

**Model Parameterization of Integrated Perchlorate PBPK and BBDR-HPT axis Models**

After successfully simulating thyroid axis perturbations in iodide deficiency (McLanahan et al., submitted), and evaluating previously published ClO₄⁻ and radiolabeled iodide models (Clewell et al., 2003a, 2003b; Merrill et al., 2003, 2005), we tested the ability of the model to predict HPT axis perturbations resulting from ClO₄⁻ competitive inhibition of NIS thyroidal iodide transport, the universally recognized mode of action for HPT axis effects following exposure to ClO₄⁻. We then created model code to describe this mode of action for ClO₄⁻ (Equation 1) to predict ClO₄⁻ induced perturbations in the HPT axis. We speculated that the inhibition of thyroid iodide uptake by ClO₄⁻ would result in a lack of iodide available for thyroid hormone production, similar to decreased thyroid iodide seen after administration of low iodide diets to rodents.

Unexpectedly, using only the inhibition of iodide uptake via the NIS as the mode of action for ClO₄⁻ HPT axis perturbation (Equation 1), the model was unable to predict the rapid changes in serum T₄ and TSH following exposure to 1, 3, or 10 mg ClO₄⁻/kg-day (Figure 5.4). In the ID BBDR-HPT axis model, thyroidal iodide stores were closely related to perturbations in the HPT axis. With ClO₄⁻ it was apparent that the HPT axis was disturbed before the thyroidal
iodide stores were predicted to be depleted. For ID, slow depletion of thyroidal iodide pools was
governed by thyroidal iodide availability and the rate thyroid hormones were secreted and
metabolized. As seen in Figure 5.4C, the model generated serum T4 levels agreed favorably with
the time course data only on day 14 for the 10 mg ClO4⁻/kg-day exposure; however, other HPT
axis responses and time points were not predicted by the model.

To evaluate the role of the NIS in the discrepant behavior between prediction and
observation the model was configured to assume the ClO4⁻ caused complete inhibition of
thyroidal iodide uptake. Simulations predicted a slower decline in serum T4 concentrations than
observed after administration of ClO4⁻ in drinking water (data and simulation not shown). The
rodent thyroid stores 10-15 µg iodide (McLanahan et al., 2007), and uses about 1.4 µg/day in
thyroid hormone production. After 5 days of complete inhibition of thyroidal NIS iodide
transport, the model predicted a 30% decline in serum T4 concentrations compared to controls
and thyroid iodide stores were predicted to be about 8 µg (a decrease of 51%). With complete
inhibition of NIS iodide transport, passive diffusion of iodide into the thyroid provided 0.14 µg
I/day. However, after only one day of exposure to 10 mg ClO4⁻/kg-day in drinking water Yu et
al. (2002) reported a 25% decline in serum T4 concentrations relative to control animals.

Consequently, a new hypothesis was created which states that ClO4⁻ affects the
production and/or secretion of thyroid hormones in addition to the inhibition of NIS iodide
transport. This idea that ClO4⁻ may affect thyroid hormone synthesis has previously been
discussed in the literature as a possibility (Hildebrandt and Halmi, 1981; Wolff, 1998; and Yu et
al., 2002). Although the exact mechanism for the rapid decline in serum T4 due to ClO4⁻ has yet
to be elucidated; the use of a suppression constant, $K_p (1.4 \times 10^5 \text{ nmol ClO}_4^-/\text{L})$, for the effect of
ClO4⁻ on thyroid hormone production provided for a much better fit of literature data at the early
time points (Figure 5.5). Andersen et al. (1987) used inhibition constants to describe multiple mechanisms of substrate interaction for the metabolism of trichloroethylene and 1,1-dichloroethylene. A similar approach using suppression constants was employed by Vinegar et al. (1994), where a suppression constant was estimated for inhibition of the metabolism of HCFC-123 in rats.

A Hill coefficient ($n_2=2$) was also used in the equation to provide for a faster (steeper) slope for the rate of ClO$_4^-$ inhibition of thyroid hormone synthesis. Hill coefficients have been used previously for describing gene induction (Andersen et al., 1993, 1997 and Kohn et al., 1993) resulting from exposure to dioxin. Using modified Hill equations allows dose-response curves to take on variable shapes, sigmoidal or logarithmic. The modified equation used for thyroid hormone production is:

$$\frac{dT_{TH}^{pr}}{dt} = k_{TSH}^{IB} \times C_{a_{TSH}} \times C_{T_{iB}} \times \frac{(K_p)^{n_2}}{(C_{vt_p})^{n_2} + (K_p)^{n_2}}$$

where $k_{TSH}^{IB}$ is the rate constant for thyroid hormone production ($5 \times 10^{-7}$, L$^2$/nmol/hr), $C_{a_{TSH}}$ is the model predicted serum concentration of TSH (nmol/L), $C_{T_{iB}}$ (nmol/L) is the concentration of iodide bound as thyroid hormone precursors in the thyroid, and $C_{vt_p}$ (nmol/L) is the model predicted thyroid concentration of ClO$_4^-$.

Modifying the equation for thyroid hormone production resulted in better simulations for $T_4$, but still did not capture the rapid increase in serum TSH concentrations. Thus, the equation for TSH production used in the iodide deficiency BBDR-HPT axis model simulations was modified by adding a Hill coefficient ($n_1=0.94$) and the concentration of $T_4$ resulting in half-maximal TSH production ($K_{inh_{T4}}$) was increased to 0.3 nmol/L, compared to the 0.2 nmol/L used previously. The Hill coefficient for $n_1$ of 0.94 compared to 1 provided for a steeper dose
response for TSH production in low T₄ concentrations, less than $K^{inh}_{T₄}$. Thus, the modified T₄/TSH negative feedback equation describing the rate of TSH production:

$$\frac{dTSH_{pr}}{dt} = \frac{k_0^{TSH} \times \left(K^{inh}_{T₄}\right)^n}{\left(K^{inh}_{T₄}\right)^n + \left(Ca^{T₄}\right)^n}$$  \[4\]

where $k_0^{TSH}$ is the maximal rate of TSH production in the absence of T₄ (nmol/hr), $K^{inh}_{T₄}$ is the concentration of T₄ that results in half-maximal rate of TSH production (nmol/L), $Ca^{T₄}$ is the concentration of T₄ in the serum (nmol/L), and $n_1$ is a Hill coefficient (unitless).

The modified thyroid hormone (Equation 3) and TSH production (Equation 4) equations were used to visually fit the Hill coefficients ($n_1=0.94$ and $n_2=2$), serum T₄ control of TSH production ($K^{inh}_{T₄}=0.3$ nmol/L), and the suppression of thyroid hormone production by ClO₄⁻ ($K_p=1.4 \times 10^5$ nmol/L) to the time course of perturbations resulting from ClO₄⁻ reported by Yu et al. (2002). Using this approach, along with ClO₄⁻ competitive inhibition of thyroidal iodide uptake, the model was able to predict the rapid changes in serum T₄ and TSH seen that were observed after one day of exposure to 1, 3, and 10 mg ClO₄⁻/kg-day in drinking water (Figure 5.5). The model predicted only slight recovery over the 14 day period, but the predictions are in accordance with the literature reported data (Yu et al., 2002). Inhibition of synthesis over the 14 day period does not lessen, according to model predictions. However, TSH stimulation of thyroidal processes is an apparent compensatory mechanism to prevent serum T₄ levels from dropping below 50% at the highest dose administered (10 mg ClO₄⁻/kg-day). At this ClO₄⁻ dose rate, the model predicted TSH upregulation of thyroidal iodide uptake (2-fold), incorporation of the iodide in the thyroid into thyroid hormone precursors (2.5-fold), and the stimulation of hormone secretion (3-fold).
Simulated oscillations in $T_4$ and TSH concentrations result because the $\text{ClO}_4^-$ concentrations in the thyroid are used to describe the inhibition of thyroid hormone synthesis (Equation 3) and are periodic relative to $\text{ClO}_4^-$ consumption. Consumption of $\text{ClO}_4^-$ (and dietary iodide) takes place over a 12-hr period, during the dark hours, thus over the 12-hr period when $\text{ClO}_4^-$ is ingested serum and thyroid concentrations rise and are cleared during the period of non-ingestion (sleep). The periodicity of $\text{ClO}_4^-$ concentrations in the serum and thyroid as predicted by the model are shown in Figures 5.6A and 5.6B, respectively. The model adequately predicts serum concentrations following exposure to 1, 3, and 10 mg $\text{ClO}_4^-$/kg-day in drinking water for 1-14 days. However, $\text{ClO}_4^-$ thyroid concentrations are underpredicted by the model on day 5 of exposure to $\text{ClO}_4^-$ for the 3 and 10 mg/kg-day dose groups. One possible explanation for this discrepancy is that at these doses changes in the thyroid gland begin to occur, leading to hypertrophy and increased thyroid weight. Perchlorate thyroid concentration in rats given an iv dose of 3.3 mg $^{36}\text{ClO}_4^-$/kg-day were adequately predicted (Figure 5.4).

**Model Validation for the Adult Male Rat**

The BBDR-HPT axis model integrated with the $\text{ClO}_4^-$ PBPK model was tested for its ability to predict HPT axis disturbances observed in published literature $\text{ClO}_4^-$ datasets not used for model calibration. The Yu *et al.* (2002) data, physiological parameters (Table 5.1), and compound specific parameters (Table 5.2) either derived from literature, optimized, or estimated, were used to obtain a calibrated model. The calibrated model was then used to simulate the data from Mannisto *et al.* (1979) and Caldwell (1995). Mannisto *et al.* (1979) administered 15 mg $\text{ClO}_4^-$/kg-day to adult male Sprague-Dawley rats (10-20 µg I/day) and measured serum $T_4$ and TSH concentrations after 2, 4, 6, 9, and 14 days of exposure. The model simulations were able to predict the temporal increase in TSH over the study period as well as decrease in $T_4$ (Figure
The TSH upregulation of the thyroid prevented the serum T4 from decreasing more than 50% of control.

The model was tested over a wide range of concentrations to predict the adult rat T4 and TSH dose-response following a 14 day exposure period to ClO_4^- in drinking water. Caldwell (1995) administered 0, 0.1, 0.4, 1, 2, 4, 11, or 22 mg ClO_4^-/kg-day to male and female rats. The dose-response curve generated by the model for serum T4 and TSH is compared to the data reported by Caldwell (1995) in Figure 5.8. The model predicts the changes relative to control animals better for higher dose rates (>2 mg ClO_4^-/kg-day) of ClO_4^- in comparison to the under prediction of TSH and over prediction of T4 at low dose rates (< 2 mg ClO_4^-/kg-day).

**Discussion**

A dietary iodide biologically based dose-response (BBDR) model of dietary iodide the hypothalamic-pituitary-thyroid (HPT) axis (BBDR-HPT axis model) was integrated with a physiologically-based pharmacokinetic (PBPK) model for perchlorate (ClO_4^-). Alone, the BBDR-HPT axis model was shown capable of predicting HPT axis perturbations resulting from iodide deficiency (McLanahan *et al.*, submitted). For many decades, ClO_4^- perturbations of the HPT axis were thought to arise because of a lack of available iodide within the thyroid for thyroid hormone production (Wyngaarden *et al.*, 1952; Yu *et al.*, 2002). We first tested the hypothesis that competitive inhibition of thyroidal iodide transport by ClO_4^- at the sodium/iodide symporter (NIS), causes an iodide deficient condition within the thyroid and subsequent decrease in serum T4 and increase in serum TSH. The model failed to predict the rapid decline in serum T4 and rapid compensatory rise in serum TSH (Figure 5.4) observed in adult rats (Yu *et al.*, 2002). We believe this failure occurred because the model predicted iodide stores within the
thyroid are not depleted within 24 hours of cessation of iodide intake or complete inhibition of NIS iodide transport.

When the BBDR-HPT axis model linked with the ClO₄⁻ PBPK model via inhibition of iodide uptake failed to predict the rapid changes in thyroid indices reported in literature, we hypothesized an additional mode of action for ClO₄⁻ induced perturbation of the HPT axis. The second hypothesis tested was that thyroidal ClO₄⁻ inhibits the production and secretion of thyroid hormones. By modifying the description of thyroid hormone production (Equation 3) by the including ClO₄⁻ suppression of the rate of hormone synthesis and secretion, coupled with the competitive inhibition of thyroidal iodide transport, we were able to adequately describe many changes in serum T₄ observed in literature (Figures 5.5, 5.7 and 5.8). It was also necessary to include Hill coefficients in the equation for TSH production (Equation 4) to describe a more sensitive negative feedback effect of T₄ on TSH production. The Hill coefficient (n₁=0.94) along with a slight increase in the set-point for TSH secretion (K₄₄inh = 0.3 nmol/L) provided for a more rapid TSH increase seen when ClO₄⁻ is administered, compared to changes seen in iodide deficient conditions.

With these modifications, the model predicted literature reported perturbations following exposure to 1-22 mg ClO₄⁻/kg-day in drinking water (Caldwell, 1995; Mannisto et al., 1979; and Yu et al., 2002). However, the model was less successful at simulating low-dose exposures (<1 mg ClO₄⁻/kg-day). The reason for this is unknown, but model parameters could be adjusted to account for this difference in future iterations and applications of the model. Noteworthy, there are many difficulties surrounding determination of HPT axis perturbations resulting from low-dose exposures to thyroid active compounds (McLanahan et al., 2007). For example, the assay methods may not be sensitive enough to detect minor changes in the hormone levels; lack of
sensitivity of T4 at low doses may also be due to the cyclic and diurnal secretion of thyroid hormones (McNabb et al., 2004) and variability in time of sampling.

Several investigators have suggested that ClO$_4^-$ could be acting on the thyroid via modes of action yet to be elucidated. Hildebrandt and Halmi (1981) suggested that ClO$_4^-$ is capable of altering the processing and utilization of iodide within the thyroid as well as inhibiting NIS transport. In a review article, Wolff (1989) hypothesized that the anion effect, reduction in thyroid hormone secretion by anions themselves and not larger iodocompounds, resulting from excess iodide in the thyroid would be mimicked by similar anions that are related to iodide by hydration enthalpy, size, and accumulation in the thyroid. Iodide (I$^-$) and ClO$_4^-$ are similar in ionic size, charge, and hydration enthalpy. The ClO$_4^-$ anion has a hydration enthalpy of -238 and I$^-$ anion of -295 kJ/mol; thus it is plausible that the anion effect could be seen with ClO$_4^-$ resulting in a decrease in thyroid hormone production and secretion. Furthermore, due to the persistently low serum T$_4$ concentrations following ClO$_4^-$ exposure, Yu et al. (2002) suggested that in addition to inhibition of thyroidal iodide uptake, ClO$_4^-$ may also exert secondary effects on the thyroid. Although many researchers have suspected ClO$_4^-$ of exerting effects in addition to inhibition of thyroidal iodide uptake, additional modes of action have yet to be confirmed in the laboratory.

We used the BBDR-HPT axis model combined with a PBPK model for ClO$_4^-$ to test the hypothesis that ClO$_4^-$ inhibits thyroid hormone synthesis and secretion. In order to fit the rapid decrease in serum T$_4$, the model predicted a 25-70% decrease in overall thyroid hormone production for the range of ClO$_4^-$ doses examined (1-15 mg ClO$_4^-$/kg-day). The inhibition in thyroid hormone production does not appear to be alleviated by TSH, but does disappear when ClO$_4^-$ is excreted. In this empirical description of thyroid hormone production and ClO$_4^-$
suppression, TSH appears to compensate for the decrease in T₄ by stimulating the thyroid but the effect of ClO₄⁻ on inhibition of thyroid hormone persists over the 14 day period. Possibly, TSH is able to help compensate for the inhibition of iodide transport and thyroid hormone production, but is unable to compensate to overcome the effect of perchlorate.

In summary, the use of a BBDR-HPT axis model coupled with a ClO₄⁻ PBPK model provided a mathematical framework for exploration of ClO₄⁻ induced perturbations of the HPT axis in adult rats. A simple link between the models using inhibition of NIS thyroidal iodide transport by ClO₄⁻ was unable to predict the rapid changes in serum T₄ and TSH reported in literature. Previously, the BBDR-HPT axis model successfully simulated changes in the HPT axis from differing degrees of iodide deficiency (McLanahan et al., submitted); therefore, when the model failed to predict changes because of a lack of iodide in the thyroid due to ClO₄⁻ inhibition of uptake, we hypothesized that ClO₄⁻ also affected the synthesis and secretion of thyroid hormones. Modification of the thyroid hormone production rate with a suppression effect due to thyroidal ClO₄⁻ concentrations enabled the model to predict the ClO₄⁻ induced HPT axis perturbations reported in literature. Future laboratory experiments could confirm or reject this model generated hypothesis and more research is necessary to determine if this mode of action is applicable in humans, which exhibit less sensitivity to ClO₄⁻ perturbation than do rodents.
Acknowledgments

Funding provided by a U.S. EPA Science to Achieve Results research grant (RD83213401-0) and a U.S. EPA Science to Achieve Results Fellowship (FP-91679301-0 to EDM). Views expressed in this manuscript are those of the authors and do not represent official opinions of U.S. EPA.

References


Table 5.1. Physiological Parameters for the Adult Rat

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<tr>
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<td><strong>Tissue volumes</strong></td>
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<td>Body weight, BW (kg)</td>
<td>0.350</td>
<td>McLanahan et al., 2007</td>
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<tr>
<td>Plasma (% BW)</td>
<td>4.44</td>
<td>Brown et al., 1997; Everett et al., 1956</td>
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<td>Thyroid, VTc (% BW)</td>
<td>0.005</td>
<td>McLanahan et al., 2007</td>
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<tr>
<td>Thyroid blood, VTBc (% VT)</td>
<td>15.7</td>
<td>Malendowicz and Bednarek, 1986</td>
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<td>Rest of body, VBody</td>
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<tr>
<td><strong>Blood flows</strong></td>
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<td>Cardiac output, QCc (L/hr/kg^{0.075})</td>
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<td>Brown et al., 1997</td>
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<tr>
<td>Thyroid, QTc (% QC)</td>
<td>1.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Brown et al., 1997</td>
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<td>Rest of body, QBody</td>
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<sup>a</sup> Human value.
Table 5.2. Perchlorate and Iodide Parameters

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<td>Partition coefficients (unitless)</td>
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<td>Permeability area cross-product (L/hr/kg&lt;sup&gt;0.75&lt;/sup&gt;)</td>
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<td>Thyroid blood to thyroid tissue, PAT&lt;sub&gt;c&lt;/sub&gt;</td>
<td>1 × 10&lt;sup&gt;-4&lt;/sup&gt;</td>
<td>2.8 × 10&lt;sup&gt;-4&lt;/sup&gt;</td>
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<td>Affinity constants (nmol/L)</td>
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<tr>
<td>Thyroid NIS transport – Km</td>
<td>31519</td>
<td>1500</td>
<td>Gluzman and Niepomniszcze, 1983; Kosugi et al., 1996</td>
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<td>Thyroid NIS TSH stimulation, K&lt;sub&gt;NIS&lt;/sub&gt;/&lt;sub&gt;TSH&lt;/sub&gt;</td>
<td>0.949</td>
<td>0.949</td>
<td>Optimized</td>
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<tr>
<td>Maximum velocities (nmol/hr/kg&lt;sup&gt;0.75&lt;/sup&gt;)</td>
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<td>Iodide – Thyroid NIS, V&lt;sub&gt;max&lt;/sub&gt;T&lt;sub&gt;c&lt;/sub&gt;</td>
<td>4450</td>
<td>177</td>
<td>McLanahan et al., submitted; Optimized</td>
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<tr>
<td>Clearance values (L/hr/kg&lt;sup&gt;0.25&lt;/sup&gt;)</td>
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<td>Urinary excretion, CIU&lt;sub&gt;c&lt;/sub&gt;</td>
<td>0.0046</td>
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Table 5.3. Thyroid Hormone and TSH Production Parameters

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<td>nmol/L</td>
<td>Fitted</td>
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<td>Thyroid hormone production, k&lt;sup&gt;TSH&lt;/sup&gt;/&lt;sub&gt;T4&lt;/sub&gt;</td>
<td>5 × 10&lt;sup&gt;-7&lt;/sup&gt;</td>
<td>L&lt;sup&gt;2&lt;/sup&gt;/nmol/hr</td>
<td>McLanahan et al., submitted.</td>
</tr>
<tr>
<td>Suppression of hormone production, K&lt;sub&gt;p&lt;/sub&gt;</td>
<td>1.4 × 10&lt;sup&gt;5&lt;/sup&gt;</td>
<td>nmol/L</td>
<td>Fitted</td>
</tr>
<tr>
<td>Hill coefficients</td>
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<td></td>
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<tr>
<td>TSH production, n&lt;sub&gt;1&lt;/sub&gt;</td>
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<td>unitless</td>
<td>Fitted</td>
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<tr>
<td>Perchlorate suppression of hormone production, n&lt;sub&gt;2&lt;/sub&gt;</td>
<td>2</td>
<td>unitless</td>
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**Figure 5.1.** Perchlorate PBPK model structure for the adult rat. Perchlorate (iv or oral drinking water dose) enters the plasma where it is excreted in urine or distributed to the thyroid or rest of the body. Thyroid is modeled with bidirectional diffusion and active uptake (bold arrow) into the thyroid via the Na+/I- symporter (NIS). The “Rest of the Body” compartment is a flow-limited compartment and includes all other body tissues into which ClO₄⁻ may distribute.
Figure 5.1.
Figure 5.2. BBDR-HPT axis model structure as described previously (McLanahan et al., submitted). Briefly, the model includes sub-models (areas shaded in gray) for dietary iodide ($^{127}$I), TSH, T$_4$, and T$_3$. Solid arrows (→) represent blood flows, bold arrows (→) within tissue compartments represent active uptake, solid arrows (→) within tissue compartments represent diffusion limitation, dashed arrows between models (---) represent metabolic links, while the dashed and dotted arrow (---) represents use of dietary iodide in thyroid hormone production, and the bold (→) arrows connecting models processes controlled (stimulated or inhibited) by the compound. Specific details on the model links shown in the figure (1 - 10) are described in Chapter 4 (Figure 4.2; McLanahan et al., submitted).
Figure 5.2.
Figure 5.3. Model simulations (lines) of serum (---) and thyroid (---) concentrations following 3.3 mg $^{36}$ClO$_4$-/kg bw iv dose in adult male Sprague-Dawley rats compared with data (serum: ● ±SD and thyroid: ○ ± SD) from Yu et al. (2002). Cumulative urinary excretion model predictions of $^{36}$ClO$_4$ is shown (........) with data (▼ ±SD).
Figure 5.3.
Figure 5.4. BBDR-HPT axis and ClO$_4^-$ PBPK integrated model predictions with ClO$_4^-$ inhibition of NIS thyroidal iodide transport. Serum T$_4$ and TSH model simulations following administration of (A) 1, (B) 3, or (C) 10 mg ClO$_4^-$/kg-day. Serum T$_4$ (●) and TSH (○) are expressed as percent of control (100% represented by □). Linking the BBDR-HPT axis and ClO$_4^-$ PBPK models via ClO$_4^-$ inhibition of iodide uptake (Equation 1) fails to produce model simulations that adequately predict the temporal or degree of changes in serum T$_4$ (●) and TSH (○) reported by Yu et al. (2002) in adult rats following exposure to ClO$_4^-$ in drinking water.
Figure 5.4A.

Figure 5.4B.
Figure 5.4C.

- (C) 10 mg/kg-day
Figure 5.5. BBDR-HPT axis model predictions following exposure to ClO$_4^-$ in drinking water, including inhibition of thyroidal iodide uptake and suppression of thyroid hormone production by ClO$_4^-$. Serum T$_4$ and TSH model simulations following administration of (A) 1, (B) 3, or (C) 10 mg ClO$_4^-$/kg-day. Serum T$_4$ ( — ) and TSH ( . . ) are expressed as percent of control (100% represented by —). Testing the hypothesis that thyroidal ClO$_4^-$ also affects the synthesis and secretion of thyroid hormones provides for model simulations that adequately predict the rapid decrease in serum T$_4$ (●) and rise in TSH (○) reported by Yu et al. (2002) in adult rats following exposure to ClO$_4^-$ in drinking water.
Figure 5.5A.

Figure 5.5B.
Figure 5.5C.
Figure 5.6. Serum (A) and thyroid (B) concentrations of ClO$_4^-$ in adult rats following exposure to ClO$_4^-$ in drinking water. Model simulations (lines) are shown with data (points ± SD) from Yu et al. (2002). Perchlorate concentrations for 1 (----- and ●), 3 (///// and ▽), and 10 (--.-- and ■) mg ClO$_4^-$/kg-day.
Figure 5.6A.

Figure 5.6B.
Figure 5.7. HPT axis perturbations in serum T₄ (●) and TSH (○) following drinking water exposure to 15 mg ClO₄⁻/kg-day in adult rats. Data expressed as percent of control (100%, ▲▲▲) for serum T₄ (● ± SD) and TSH (○ ± SD) are adapted from Mannisto et al. (1979).
Figure 5.7.
**Figure 5.8.** Dose-response plot for serum T₄ and TSH after exposure to ClO₄⁻ in drinking water for 14 days. Model predictions of T₄ (solid line) and TSH (dashed line) are compared with adult rat data following exposure to 0, 0.1, 0.4, 1, 2, 4, 11, or 22 mg ClO₄⁻/kg-day for 14 days adult rats (Caldwell, 1995). Data expressed as percent of control (100%) for serum T₄ (●) and TSH (○).
Figure 5.8.
CHAPTER 6

CONCLUSIONS

The hypothalamic-pituitary-thyroid (HPT) axis is an example of a very complex endocrine system, which is able to maintain homeostasis through a multitude of feedback loops and autoregulatory controls. An understanding of thyroid axis regulation is necessary in order to interpret perturbations, such as those in serum thyroxine (T₄) and thyroid stimulating hormone (TSH) caused by environmental pollutants determined in laboratory studies evaluating HPT axis indices. The conclusions from the research are derived using statistical approaches for the binary mixture of 3,3’,4,4’,5-pentachlorobiphenyl (PCB126) and perchlorate (ClO₄⁻) and knowledge of HPT axis regulation and control mechanisms from published literature, as well as literature available on the mode of action for the thyroid active chemicals examined. Mathematical models are often used as tools to help us understand the kinetics of chemicals in the body, and this time we were able to couple our knowledge of the thyroid axis with existing information for ClO₄⁻ disturbances of the HPT and use the biologically based dose-response model of the HPT axis (BBDR-HPT model) as a dose-response analysis tool.

PCB126/Perchlorate Mixtures Study

Two large-scale laboratory experiments were conducted to determine the effects of ClO₄⁻ on the HPT axis of adult rats pretreated with PCB126. PCB126 has been shown to increase hepatic phase II metabolism of T₄ (increased rate in T₄-G formation), resulting in a decrease in serum T₄ concentrations and subsequent increase in TSH secretion and stimulation of the axis.
TSH stimulation of the axis results in a cascade of effects within the thyroid, one being the stimulation of sodium(Na\(^+\))/iodide(I\(^-\)) symporter (NIS) protein production and expression. This is significant, in interpreting results from our studies, because the most well-defined mode of action for ClO\(_4^-\) is competitive inhibition of thyroidal iodide uptake.

Results from our studies suggest that in an upregulated or TSH stimulated thyroid system, ClO\(_4^-\) has less of a pronounced effect on serum thyroid hormone status. The ability of ClO\(_4^-\) to affect the axis is diminished and an apparent shift to the right of the ClO\(_4^-\) dose response curve occurs. This suggests that a greater dose of ClO\(_4^-\) is necessary to cause a similar magnitude of effect on serum T\(_4\) and TSH. Our studies support this finding by a less than additive response in the HPT axis being observed when animals were pretreated with PCB126. Furthermore, our results did not indicate a synergistic response of the system by co-exposure to the compounds.

**BBDR-HPT Axis Iodide Deficiency Model**

The first generation dietary iodide BBDR-HPT axis model was constructed using simple model structure and empirical descriptions of the feedback loops. The simple model structures for the individual components were sufficient to describe radiolabeled tracer kinetic data for the individual components (\(^{125}\text{I}, \, ^{125}\text{I}-\text{TSH}, \, ^{131}\text{I}-\text{T}_4,\) and \(^{125}\text{I}-\text{T}_3\)) modeled. When linked via negative feedback, TSH stimulation, and hormone metabolism the model structures effectively described the iodide sufficient and iodide deficient rat. The availability of thyroidal dietary iodide alone drove the endogenous model and resulted in decreases in serum T\(_4\) and increases in serum TSH due to the lack of available iodide for thyroid hormone synthesis. Our model also was used to generate a dose-response plot for intake of dietary iodide and effects on serum T\(_4\) and TSH. This plot showed a region of critical intake rate of iodide to be less than 2 µg /day.
BBDR-HPT Axis Model of Perchlorate Perturbation

The BBDR-HPT axis model developed and presented first in Chapter 4, was combined with a PBPK model for ClO$_4^-$, an ubiquitous environmental contaminant. Perchlorate has been shown to disrupt the HPT axis by inhibition of iodide uptake into the thyroid; however, the models linked via ClO$_4^-$ competitive inhibition of thyroidal iodide uptake alone were unable to predict the rapid decrease in serum T$_4$ changes seen from ClO$_4^-$ exposure. This was surprising, because we hypothesized that the blocking of iodide uptake by ClO$_4^-$ resulted in an iodide deficient condition within the thyroid, and we expected the BBDR-HPT axis model to predict the changes in serum T$_4$ and TSH as it did under varying degrees of iodide deficiency. Furthermore, this led us to believe that ClO$_4^-$ might be acting via an additional mode of action that has not been fully elucidated in laboratory studies.

We hypothesized that ClO$_4^-$ also affected the synthesis and secretion of thyroid hormones and tested our hypothesis using the linked BBDR-HPT axis model and PBPK model for perchlorate. This hypothesis was tested and described using a suppression constant and Hill-type coefficients to inhibit thyroid hormone synthesis based upon the concentration of ClO$_4^-$ in the thyroid tissue. This description accounted for the rapid decrease in serum T$_4$, but did not result in the rapid rate or degree of increase in serum TSH seen in several studies. So additionally, the T$_4$/TSH negative feedback describing TSH production was modified using Hill coefficients and the set point for half-maximal negative feedback by T$_4$ was increased slightly. Using this modification, serum T$_4$ and TSH were predicted following exposure to 1-22 mg ClO$_4^-$/kg-day in drinking water, suggesting that the sensitivity of TSH is altered in the presence of perchlorate. The mode of action of ClO$_4^-$ should be further explored in the laboratory to confirm or reject the hypotheses suggested by this dose-response analysis using the mathematical models.
Future Applications of the BBDR-HPT Axis Model

The BBDR-HPT model developed can be linked to other PBPK models for thyroid active chemicals, via mode of action, to simulate and predict changes in thyroid hormones and TSH. For example, the BBDR-HPT axis model includes the phase II excretion of T₄ as T₄-glucornide (T₄-G) in the description of the liver compartment, and when combined with a PBPK model for PCB126, an increase in T₄-G formation is expected to result in the increased loss of T₄ from the body and subsequent rise in TSH to compensate. Linking the models together will provide for testing the hypothesis that PCB126 acts on the HPT axis via this single, well-defined mode of action. If the model is unable to predict changes in the HPT axis to the degree that is seen in experimental studies, then additional modes of action may be suggested and explored using the models (as demonstrated in Chapter 5). In addition, it is possible to link the BBDR-HPT axis model to multiple PBPK models to predict disturbances in the thyroid system from exposures to mixtures of compounds.

Finally, the development of this first-generation BBDR-HPT axis model will serve as a starting point for development of the maturing rat and human thyroid axis models. Other physiological compartments can be readily integrated into our model structure, such as the brain to correlate thyroid hormone levels in the brain to neurodevelopmental toxicity endpoints.
APPENDIX A

The acslXtreme (version 2.4.0.11) .csl file for BBDR-HPT axis radiolabeled model code (Chapter 4) is contained within this Appendix. Model code is structured as follows: First section contains physiological parameters and compound-specific constants. Second section includes scaled parameters and is followed by model code for the individual compounds, $^{125}$I-TSH, $^{125}$I, $^{131}$I-T$_4$, and $^{125}$I-T$_3$. 
PROGRAM: Male Rat HPT Axis Model, RadioTracer studies

!-----------------------------------------------------------------------
! File name: Labelnogut.csl
! Units in nmol, L, hr, kg
! Created 02/11/2007 by Eva McLanahan
! Radiolabeled I, T4, T3, and TSH
! Last Revised 07/09/07 by Eva McLanahan

INITIAL
  CONSTANT TSTOP=2   ! Length of experiment
  CONSTANT CINT=0.001 ! Communication interval

! ------------ Physiological Parameters------------------
CONSTANT BW = 0.350  ! kg - Animal body weight
CONSTANT QCC = 14.0  ! L/hr/kg - Total cardiac output [Brown 1997]
CONSTANT QLC = 0.174  ! %QC - Proportion cardiac output to the liver [Brown 1997]
CONSTANT QTC = 0.016  ! %QC - Proportion cardiac output to the thyroid - human value
  (Brown 1997 & Merrill 2003)
CONSTANT VLC = 0.0366 ! %BW liver tissue [Brown 1997 pg 416]
CONSTANT VLBC = 0.21  ! %VL as liver blood [Brown 1997]
CONSTANT VTC = 0.00005 ! %BW total thyroid tissue [My studies]
CONSTANT VTBC = 0.157  ! %VT as thyroid blood [Malendowicz and Bednarek, 1986]

! ------------ TSH Parameters------------------
CONSTANT MWTSH = 28000 ! g/mol - molecular weight TSH [chemfinder.com]
CONSTANT Vd_TSHC = 0.0554 ! L/kg - VdTSH - Connors et al 1984
CONSTANT KNIS_TSH = 1.8  ! nmol/L - Km TSH conc so Vmax of NIS I transport is 1/2 max
CONSTANT KbTSH = 1112.65 ! nmol/L - Km TSH conc so I binding in thyroid is 1/2 max
CONSTANT TSHc = 0.232  ! nmol/L-TSH concentration at steady-state in serum (calc from average of McLanahan et al 2007 studies 6.5ng/mL)
CONSTANT Kel_TSHC = 1.8899 ! 1/hr-kg - elimination rate constant for TSH from Vd
  (Lemarchand-Beraud and Berthier 1981)
### I (Iodide) Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Unit</th>
<th>Source/Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>MWI</td>
<td>126.90447</td>
<td>g/mol</td>
<td>Molecular weight of iodine [periodic table]</td>
</tr>
<tr>
<td>Vd_ic</td>
<td>0.5</td>
<td>L/kg</td>
<td>Volume of distribution of iodide</td>
</tr>
<tr>
<td>Km_i</td>
<td>31519</td>
<td>nmol/L</td>
<td>Affinity constant for NIS (Gluzman and Niepomniszcze, 1983 and Merrill 2003)</td>
</tr>
<tr>
<td>VmaxT_iC</td>
<td>1119.396</td>
<td>nmol/hr-kg</td>
<td>Maximal rate of NIS I uptake</td>
</tr>
<tr>
<td>PA_t_ic</td>
<td>0.0001</td>
<td>L/hr</td>
<td>PA term thyroid [Merrill 2003]</td>
</tr>
<tr>
<td>V0bindC</td>
<td>2243.598</td>
<td>nmol/hr-kg</td>
<td>Maximum rate of binding of iodide in thyroid [Merrill 2003]</td>
</tr>
<tr>
<td>Km_Ib</td>
<td>221.1</td>
<td>nmol/L</td>
<td>Km of iodide binding</td>
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<tr>
<td>ClU_iC</td>
<td>0.02</td>
<td>L/hr-kg</td>
<td>Urinary clearance of iodide</td>
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### T4 (Thyroxine) Parameters

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<th>Source/Details</th>
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<tbody>
<tr>
<td>MWT4</td>
<td>776.8742</td>
<td>g/mol</td>
<td>Molecular weight of T4 [calculated:C15H11I4NO4]</td>
</tr>
<tr>
<td>Vd_t4C</td>
<td>0.156</td>
<td>%Vd of BW</td>
<td>Distribution of T4 [Kohn 1996]</td>
</tr>
<tr>
<td>PL_t4</td>
<td>1.27</td>
<td>unitless</td>
<td>PC for T4 liver (EscobarMorreale 1996)</td>
</tr>
<tr>
<td>PA_t4</td>
<td>0.04875</td>
<td>L/hr-kg</td>
<td>PA term T4 liver</td>
</tr>
<tr>
<td>VMAXDIC</td>
<td>15.105</td>
<td>nmol/hr-kg</td>
<td>Vmax outer ring deiodinase</td>
</tr>
<tr>
<td>KMDI</td>
<td>2300</td>
<td>nmol/L</td>
<td>Km of outer ring DI in liver (Leonard &amp; Visser 1986)</td>
</tr>
<tr>
<td>KEL_t4C</td>
<td>0.05</td>
<td>1/hr-kg</td>
<td>Rate of elimination of T4 from body (vd)</td>
</tr>
<tr>
<td>KmUGT</td>
<td>100000</td>
<td>nmol/L</td>
<td>Km of UGT enzymes for T4 and T3 [Visser 1993]</td>
</tr>
<tr>
<td>VmaxT4GC</td>
<td>1080.32</td>
<td>nmol/hr</td>
<td>Vmax for T4-G formation in liver</td>
</tr>
<tr>
<td>VmaxT4luC</td>
<td>10552.8</td>
<td>nmol/hr-kg</td>
<td>Vmax for active uptake of T4 into liver</td>
</tr>
<tr>
<td>KmT4LU</td>
<td>650</td>
<td>nmol/L</td>
<td>Km for T4 active uptake into liver</td>
</tr>
<tr>
<td>FFT4</td>
<td>0.01</td>
<td>fraction free</td>
<td>Fraction free for T4 uptake into liver</td>
</tr>
</tbody>
</table>

### T3 (3,5,3'-Triiodothyronine) Parameters

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<th>Parameter</th>
<th>Value</th>
<th>Unit</th>
<th>Source/Details</th>
</tr>
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<tr>
<td>MWT3</td>
<td>650.97349</td>
<td>g/mol</td>
<td>Molecular weight of T3 [calculated:C15H12I3NO4]</td>
</tr>
<tr>
<td>Vd_T3C</td>
<td>0.186</td>
<td>L/kg</td>
<td>Vd_T3 per kg bw [thyroid hormone metab pg 67]</td>
</tr>
<tr>
<td>PL_t3</td>
<td>4.47</td>
<td>unitless</td>
<td>Liver T3 PC (EscobarMorreale1996)</td>
</tr>
<tr>
<td>PA_T3C</td>
<td>0.03</td>
<td>L/hr/kg</td>
<td>PA term liver T3</td>
</tr>
<tr>
<td>Kmetl_t3C</td>
<td>0.12</td>
<td>1/hr/kg</td>
<td>Rate of elimination of T3 from body</td>
</tr>
<tr>
<td>KLUT3</td>
<td>1.1</td>
<td>1/hr</td>
<td>Metabolism of T3 in the liver</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
!---Thyroid hormone production parameters
CONSTANT  ktshcib = 5e-7   !L2/nmol/hr- rate constant TH production
CONSTANT  ft3  = 0.20   !fraction of thyroid hormone production that is T3

!---T4 and T3 iodide equivalents
CONSTANT  I4CON  = 0.6534  !Fraction of t4 that is iodine
CONSTANT  I3CON  = 0.5848  !Fraction of T3 that is iodine

!IV dose parameters
CONSTANT  IVDOSEt4 = 0.0044   !131I-T4 IV Dose (ug)
CONSTANT  IVDOSEt3 = 0.00455   !125I-T3 IV Dose (ug)
CONSTANT  IVDOSEi = 33  !125I IV Dose (ug/kg)
CONSTANT  IVDOSEtsh = 5  !125I-TSH Dose (ng/rat)
CONSTANT  TINF  = .01    !Length of IV infusion (hr)
CONSTANT  TINF3  = 0.001  !Length of IV infusion for T3 (hr)

END ! INITIAL

DYNAMIC
ALGORITHM IALG = 2   !Gear method for stiff systems

DERIVATIVE

!------------- Scaled Parameters-------------
QC = QCC*BW**0.75   !L/hr - Cardiac output
QL = QLC*QC    !L/hr - blood flow to liver
QT = QTC*QC    !L/hr - blood flow to thyroid

VL1 = VLC*BW    !L - liver volume with blood
VLB = VLBC*VL1    !L - Volume of liver blood
VL = VL1-VLB    !L - Liver tissue volume without blood
VT1 = VTC*BW    !L - total thyroid volume
VTB = VTBC*VT1    !L - volume of thyroid blood
VT = VT1-VTB    !L - volume of thyroid without blood

!TSH Scaled Parameters
Vd_TSH = Vd_TSHC*BW   !L - Vd for TSH
Kel_TSH = Kel_TSHC/BW**0.25  !l/hr - TSH elimination rate
T4 scaled parameters:

- **VMAXDI** = VMAXDIC*BW**0.75  !nmol/hr – vmax T4 deiodination in liver
- **Vd_t4** = Vd_T4c*BW - VL1  !L – Vd of T4
- **PAL_t4** = PAL_t4c*BW**0.75  !L/hr – PA liver T4
- **Kel_t4** = KEL_t4c/BW**0.25  !1/hr – T4 metabolism in Vd
- **Vmaxt4g** = vmaxt4gc*BW**0.75 !nmol/hr – vmax T4-glucuronidation in liver
- **Vmaxt4lu** = vmaxt4luc*BW**0.75 !nmol/hr – vmax T4 liver uptake

T3 scaled parameters:

- **PAL_t3** = PAL_t3c*BW**0.75  !L/hr – PA liver T3
- **Vd_t3** = Vd_t3c*BW - VL1  !L – Vd of T3
- **Kel_t3** = Kel_t3c/BW**0.25  !1/hr – T3 metabolism in Vd
- **KmetL_t3** = Kmetl_t3c/BW**0.25 !1/hr – T3 metabolism in liver

Iodide scaled parameters:

- **Vd_i** = Vd_ic*BW - VT1  !L – volume of distribution of iodide
- **VmaxT_i** = VmaxT_iC*BW**0.75 !nmol/hr – maximum rate of iodide uptake by NIS is thyroid
- **ClU_i** = ClU_iC/BW**0.25  !L/hr – Urinary clearance of iodide
- **V0bind** = V0bindc/BW**0.75  !nmol/hr – vmax binding of i in thyroid
- **PAT_i** = PAT_ic*BW**0.75  !L/hr – pa term thyroid blood/tissue

MODEL CODE - TSH volume of distribution

IV = IV TSH dose

IVDOSE_tsh=(IVDOSETSH/MWTSH)


tsh_iv = pulse(0,tstop,tinf)
AIV_tsh=INTEG(Iv_tsh,tsh)

RC1TSH=Kel_TSH*AVdTSH
AC1TSH=INTEG(RClTSH,0.0)

RAVdTSH=IV_tsh-RC1TSH

TSHngml=(TSH/1000) * MWTSH  !ng/mL (same as ug/L)- TSH concentration in Vd

TSHRPDP=((Avdtsh/(AIV_tsh+1e-6))*100)/(Vd_tsh*1000)  !%dose/ml – Total rad %dose/ml SERUM
Iodide, IV dose w/ thyroid and Vd

**IV = Intravenous infusion rate (nmol/hr)**

\[
IVDOSE_i = (IVDOSE_i \times BW/MWI) \times 1000
\]

\[
iflag_i = \text{pulse}(0, tstop, tinf)
\]

\[
IV_i = \frac{IVDOSE_i}{TINF} \times iflag_i
\]

\[
AIV_i = \text{INTEG}(IV_i, 0.0)
\]

**Thyroid Iodide**

\[
ATIU = \text{amount of free iodide actively transported into thyroid by NIS}
\]

\[
RTNIS = \frac{(VmaxT_iTSH \times Cvt_i)}{(Km_i + Cvt_i)}
\]

\[
ATIU = \text{INTEG}(RTNIS, 0.0)
\]

\[
Atb_i = \text{amount of free iodide in thyroid blood}
\]

\[
RAtb_i = Qt \times (CA_i - Cvt_i) + PAt_i \times (Ctf_i - Cvt_i) - RTNIS
\]

\[
Cvt_i = \frac{Atb_i}{VTB}
\]

\[
At_i = \text{amount of FREE iodide in thyroid tissue}
\]

\[
RAt_iex1 = (PAt_i \times Ctf_i)
\]

\[
RAt_iex = (PAt_i \times Ctf_i) + RIB
\]

\[
RAt_iex2 = \text{RAt_iex1} + \text{Rth}
\]

\[
RAtf_i = \text{RTNIS} + PAt_i \times (Cvt_i - Ctf_i) - \text{RIB}
\]

\[
\text{dAtf}_i = \text{INTEG}(\text{RAtf}_i, 0.0)
\]

\[
At_i = \text{MAX}(\text{dAtf}_i, 0.0)
\]

\[
Ctf_i = \frac{At_i}{VT}
\]

\[
Ct_imgL = Ctf_i \times MWI / 10^{\times 6}
\]
\[
RIB = \frac{(V_{\text{maxbt}} \cdot C_{\text{tf}})}{(K_{\text{mb}} + C_{\text{tf}})} \quad \text{nmol/hr - rate of binding of iodide in thyroid}
\]

\[
V_{\text{maxbt}} = \frac{(V_{0\text{bind}} \cdot T_{\text{SHC}})}{(K_{bTSH} + T_{\text{SHC}})} \quad \text{nmol/hr – TSH stimulated Vmax of binding}
\]

\[
ARIB = \text{INTEG}(RIB, 0.0) \quad \text{nmol – amount of iodide bound in thyroid}
\]

\[
ARIB_{\text{ug}} = ARIB \cdot MWI / 1000 \quad \text{ug – amount of iodide bound in thyroid}
\]

\[
d_{\text{aribug}} = \frac{ARIB_{\text{ug}}}{(t+1e-6/24)} \quad \text{ug/d daily amt of iodide bound in thyroid}
\]

\[
RIB_i = RIB - \text{Rth} \quad \text{nmol/hr – rate of change of bound iodide}
\]

\[
d_{\text{AIB}} = \text{INTEG}(RIB_i, 0.0) \quad \text{nmol – amount of iodide bound in thyroid tissue}
\]

\[
AIB_i = \text{MIN}(d_{\text{AIB}}_i, 160) \quad \text{nmol/L – concentration of bound iodide in thyroid tissue}
\]

\[
CIB_i = AIB_i / VT \quad \text{mg/L – concentration of bound iodide in thyroid tissue}
\]

\[
\text{Approach used in endogenous dietary model}
\]

\[
RPR_{\text{th}} = k_{\text{ths}} cib \cdot T_{\text{SHC}} \cdot CIB_i \quad \text{nmol/hr – rate of thyroid hormone production}
\]

\[
RPR_{\text{t3}} = f_{\text{t3}} \cdot RPR_{\text{th}} \quad \text{nmol T3/hr – rate of T3 thyroidal production}
\]

\[
RPR_{\text{t4}} = RPR_{\text{th}} - RPR_{\text{t3}} \quad \text{nmol T4/hr – rate of T4 thyroidal production}
\]

\[
R_{\text{th}} = (RPR_{\text{t4}} \cdot I_{\text{4CON}}) + (RPR_{\text{t3}} \cdot I_{\text{3CON}}) \quad \text{nmol/hr – Rate of thyroid hormone production from bound}
\]

\[
\text{Ath} = \text{INTEG}(R_{\text{th}}, 0.0) \quad \text{nmol – amount of iodide used in thyroid hormone production}
\]

\[
\text{Ath}_{\text{ug}} = \text{Ath} \cdot MWI / 1000 \quad \text{ug – amount of iodide used in thyroid hormone production}
\]

\[
\text{TTI}_i = \text{Total Iodide in thyroid tissue (nmol)}
\]

\[
\text{TAT}_i = AIB_i + \text{Atf}_i \quad \text{nmol – total iodide in thyroid tissue}
\]

\[
\text{TAT}_{\text{mg}} = \text{TAT}_i \cdot MWI / 10**6 \quad \text{mg – total iodide in thyroid tissue}
\]

\[
\text{TCT}_{\text{mgL}} = \text{TAT}_{\text{mg}} / VT \quad \text{mg/L – total iodide in thyroid tissue}
\]

\[
\text{---------Volume of Distribution Iodide---------}
\]

\[
\text{RAP}_i = \text{IV}_i + QT \cdot C_{\text{vt}} - QT \cdot Ca_i - \text{RU}_i \quad \text{nmol/hr – rate of change of free iodide in Vd}
\]

\[
\text{AP}_i = \text{INTEG}(\text{RAP}_i, 0.0) \quad \text{nmol – amount of free iodide in Vd}
\]

\[
\text{CP}_i = \text{AP}_i / V_{\text{D}} \quad \text{nmol/L – concentration of iodide in Vd}
\]

\[
\text{Ca}_i = \text{CP}_i \quad \text{ng/mL – concentration of iodide in Vd}
\]

\[
\text{CP}_{\text{mgml}} = \text{CP}_i \cdot MWI \quad \text{ng/mL – concentration of iodide in Vd}
\]

\[
\text{CP}_{\text{mgL}} = (\text{CP}_{\text{mgml}} / 10**6) \quad \text{mg/L – concentration in Vd}
\]
RU_i=ClU_i*Ca_i  
AU_i=INTEG(RU_i,0.0)  
AU_iug=AU_i*MWI/1000

!---------MODEL CODE--- Total T4, IV dose with a Liver and Vd---------
!----T4 dosing--------iv dose radiolabeled-------------
!IV = Intravenous infusion rate(nmol/hr)
IVDOSE_t4=(IVDOSE_t4/MWT4)*1000
iflag_t4 = pulse(0,tstop,tinf)
IV_t4 = IVDOSE_t4/TINF*iflag_t4
AIV_t4 = INTEG(IV_t4,0.)

!--------------SERUM T4 (Volume of distribution)------------------
RAP_t4=IV_t4+(QL*CVL_t4)-QL*Ca_t4-RVdel_t4
AP_t4=INTEG(RAP_t4,0.0)
Ca_t4=AP_t4/Vd_t4
Ca_t4ugdl=Ca_t4*(0.0001)*MWT4
Ca_t4ngg=(Ca_t4*MWT4)/1000
RVdel_t4=Kel_t4*Ap_t4
AVdel_t4=INTEG(RVdel_t4,0.0)
T4RPDT4VD=(AP_t4/(AIV_t4+1e-6))*100
T4RPDT4P=((AP_t4/(AIV_t4+1e-6))*100)/(Vd_t4*1000)

!-------Liver T4-------------
RALb_t4=QL*(Ca_t4-Cvl_t4)+PAL_t4*(CL_t4-(Cvl_t4*FFT4))-RT4LU
ALb_t4=INTEG(RALb_t4,0.0)
Cvl_t4=ALb_t4/(VLB*PL_t4)
RAL_t4=(PAL_t4*((Cvl_t4*FFT4)-Cl_t4))-RAGL-RADIL+RT4LU
AL_t4=INTEG(RAL_t4,0.0)
CL_t4=AL_t4/VL
Cl_t4ngg=(CL_t4*MWT4/1000)/1.051

RU_i=ClU_i*Ca_i  
AU_i=INTEG(RU_i,0.0)  
AU_iug=AU_i*MWI/1000

!nmol/hr - rate of urinary clearance of iodide
!nmol - amount of iodide cleared in urine
!ug - amount of iodide cleared in urine

!nmol 125I-T4 - amt ivdose
!iflag = pulse dose at time 0
!nmol/hr - rate T4 iv infusion
!nmol 125I-T4 - Amount T4 iv dosed

!nmol/hr - rate of change of T4 in Vd
!nmol - amount of T4 in Vd
!nmol/L - concentration of T4 in Vd
!ug/dl - T4 in Vd
!ng/g - T4 in Vd
!nmol/hr - rate of T4 elimination from Vd
!nmol - amount of T4 eliminated from Vd

%!dose T4 rad in Vd
%!dose/ml - Total rad %dose/ml SERUM

!nmol/hr - rate of change of T4 in the liver blood
!nmol - amount of t4 in liver blood
!nmol/L - concentration of t4 in liver blood

%!nmol/hr - rate of change of T4 in liver tissue
!nmol - amount of T4 in liver tissue
!ng/g - concentration of T4 in liver tissue
(1.051=liver density, Obermoyer 1987)
RADIL=\left(\frac{VMAXDI\cdot CVL_t4}{CVL_t4+KMDI}\right) \quad \text{nmol/hr - rate of T4 deiodination in liver (D1)}

ADIL=\text{INTEG}(RADIL,0.0) \quad \text{nmol - Amount of T4 deiodinated (D1) in liver}

PC43L=(ADIL/(AIV_t4+1e-6))*100 \quad \% T4 converted by Type I 5'-Deiodinase in liver

RAGL=(VmaxT4G \cdot CVL_t4)/(KmUGT + CVL_t4) \quad \text{nmol T4/hr - rate of T4-glucuronidation in liver}

AGL=\text{INTEG}(RAGL,0.0) \quad \text{nmol T4 lost/used to make T4-G}

RAGLT4G=(RAGL*(MWT4G/MWT4)) \quad \text{nmol T4-G formed/hr}

RT4LU=(VmaxT4LU \cdot (CVL_t4+Ft4))/((KMT4LU+CVL_t4)) \quad \text{nmol/hr - rate T4 active uptake}

T4RPDL=(AL_t4/(AIV_t4+1e-6))*100 \quad \%dose T4 radioactivity in liver

T4RPDT4L=((AL_t4+ALb_T4)/(AIV_t4+1e-6))*100)/(VL*1000) \quad \%dose/ml - T4 rad %dose/ml liver

T4RPDGL=(AGL/(AIV_t4+1e-6))*100 \quad \%dose T4 glucuronidated in liver

PC43=((ADIL+AVdel_t4)/(AIV_t4+1e-6))*100 \quad \%dose T4 converted to T3 (whole body)

T4--Ratio amount in liver:blood in Wong paper should be around 1 up to 1hr post dose

LBAratio=AL_t4/(AP_t4+1e-6) \quad \text{Liver:blood amount ratio}

!----------MODEL CODE--- Total t3, IV dose with a Liver and Vd---------

IVDOSE_t3=(IVDOSET3/MWT3)*1000 \quad \text{nmol amt ivdose}

IV_t3 = IVDOSE_t3/TINF3*iflag_t3 \quad \text{rate t3 iv dosing(nmol/hr)}

AIV_t3 = \text{INTEG}(IV_t3,0.) \quad \text{Amount t3 iv dosed(nmol)}

!-------------SERUM t3 (Volume of distribution)--------------

RAP_t3=IV_t3+(QL\cdot CVL_t3)-QL\cdot Ca_t3-RVdel_t3 \quad \text{nmol/hr - rate of change of T3 in Vd}

AP_t3=\text{INTEG}(RAP_t3,0.0) \quad \text{nmol - amount of T3 in Vd}

Ca_t3=AP_t3/Vd_t3 \quad \text{nmol/L - concentration of T3 in Vd}

Ca_t3ugdl=Ca_t3*(0.0001)*MWT3 \quad \text{ug/dL - T3 in Vd}

Ca_t3ngg=(Ca_t3*MWT3)/1000 \quad \text{ng/g – T3 in Vd}

RVdel_t3=Kel_t3\cdot Ap_t3 \quad \text{nmol/h - rate of t3 elimination from Vd}

AVdel_t3=\text{INTEG}(RVdel_t3,0.0) \quad \text{nmol - amount of t3 eliminated from Vd}
t3RPDP=(AP_t3/(AIV_t3+1e-6))*100  
!%dose T3 rad in Vd

\[ \text{t3RPDP} = \left( \frac{\text{AP}_t3}{\text{AIV}_t3 + 1e-6} \right)\times100 \]

\[ \text{t3RPDP}_{3P} = \left( \frac{\left( \frac{\text{AP}_t3}{\text{AIV}_t3 + 1e-6} \right)\times100}{\text{Vd}_t3}\times1000 \right) \]

!%dose/ml - Total rad %dose/ml Vd

!-------Liver t3-------------

RALb_t3=QL*(CA_t3-Cvl_t3)+PAL_t3*(CL_t3-Cvl_t3)-RLt3U

\[ \text{RAL}_{t3} = \text{QL}\times(\text{CA}_{t3} - \text{Cvl}_{t3}) + \text{PAL}_{t3}\times(\text{CL}_{t3} - \text{Cvl}_{t3}) - \text{RLt3U} \]

\[ \text{AL}_{t3} = \text{INTEG}(\text{RAL}_{t3}, 0.0) \]

\[ \text{Cvl}_{t3} = \frac{\text{AL}_{t3}}{(\text{VL}\times\text{PL}_{t3})} \]

RAL_t3=(PAL_t3*(Cvl_t3-Cl_t3))-RLt3U

\[ \text{RAL}_{t3} = \left( \frac{\text{PAL}_{t3}\times(\text{Cvl}_{t3} - \text{Cl}_{t3})}{\text{RLt3U}} \right) - \text{RLt3U} \]

\[ \text{AL}_{t3} = \text{INTEG}(\text{RAL}_{t3}, 0.0) \]

\[ \text{CL}_{t3} = \frac{\text{AL}_{t3}}{\text{VL}} \]

\[ \text{CL}_{t3ngg} = \left( \frac{\text{cl}_{t3}\times\text{MWT3}}{1000} \right) \]

RLT3U=KLUT3*cvl_t3

\[ \text{RLT3U} = \text{KLUT3}\times\text{cvl}_{t3} \]

RAMLt3=AL_t3*KmetL_t3

\[ \text{RAML}_{t3} = \text{AL}_{t3}\times\text{KmetL}_{t3} \]

AMLt3=INTEG(RAMLt3, 0.0)

\[ \text{AML}_{t3} = \text{INTEG}(\text{RAML}_{t3}, 0.0) \]

RAT3feces=RAMLt3*0.30

\[ \text{RAT3feces} = \text{RAML}_{t3}\times0.30 \]

AT3_feces=INTEG(RAT3feces, 0.0)

\[ \text{AT3}_{feces} = \text{INTEG}(\text{RAT3}_{feces}, 0.0) \]

\[ \text{t3RPDL} = \left( \frac{(\text{AL}_{t3} + \text{ALb}_{t3})}{\text{AIV}_t3 + 1e-6} \right)\times100 \]

\[ \text{t3RPDL} = \left( \frac{(\text{AL}_{t3} + \text{ALb}_{t3})}{(\text{AIV}_t3 + 1e-6)} \right)\times100 \]

\[ \text{t3RPD}_{3L} = \left( \frac{(\text{AL}_{t3} + \text{ALb}_{t3})}{(\text{AIV}_t3 + 1e-6)} \right)\times100 \]

\[ \text{t3RPD}_{3L} = \left( \frac{(\text{AL}_{t3} + \text{ALb}_{t3})}{(\text{AIV}_t3 + 1e-6)} \right)\times100 \]

!Blood flow limited liver - removed

!RAL_t3=QL*(Ca_t3-Cvl_t3)-RAMLt3

!AL_t3=INTEG(RAL_t3,0:0)

!CL_t3=AL_t3/VL

!Cvl_t3=AL_t3/(VL*PL_t3)

!-------Mass balance T4-------------

TMASSt4=AP_t4+AL_t4+ADIL+Avdel_t4+AGL+Alb_t4

BALANCEt4=AIV_t4-TMASSt4
!----------Mass balance t3--------------
TMASSt3=AP_t3+AL_t3+Avdel_t3+AMLt3+Alb_t3
BALANCEt3=AIV_t3-TMASSt3

!----------Mass balance Iodide---------
TMASSi=Atb_i+Atf_i+Ath+AIB_i+AP_i+AU_i
BALANCEi=AIV_i-TMASSi

!----------Mass balance of TSH-----------
TMASStsh=Avdtsh+Acltsh
BALANCEtsh=AIV_tsh-TMASStsh

END!DERIVATIVE
TERMT (T.GE.TSTOP, 'checked on communication interval: REACHED TSTOP')
END!DYNAMIC
TERMINAL
     END!TERMINAL
END!PROGRAM
APPENDIX B

The acslXtreme (version 2.4.0.11) .m file for BBDR-HPT axis radiolabeled model simulations (Chapter 4) is contained within this Appendix. The .m file was used to generate model simulations shown in Figure 4.4 for the radiotracer sub-models. The sequences used to generate all plots in Figure 4.4 are ordered in the .m file as follows: $^{125}$I, $^{125}$I-T$_3$, $^{131}$I-T$_4$, and $^{125}$I-TSH.
%M file to plot 125I in serum and urine
%Figure 4.4A – Data from Yu et al 2002

%time(hrs), urine (ug)
RIIV33U = [ 24 9 ];

%time (hrs), serum (ng/mL)
RIIV33SF2 = [ 0.083  41.41
              0.25  54.03
              0.5  49.82
              1  44.63
              2  40.32
              6  24.97
              9  8.49
             24 1.95
             30 0.86
             48 0.52
             72 0.26
             96 0.19 ];

%Simulation commands
!!s ivdosei=33, tstop=96, cint=0.1, bw=0.300
!!prepare /All
!!start/nc

%Plotting Commands
plot(_t,_cp_ingml,RIIV33SF2(:,1),RIIV33SF2(:,2),_t,_au_iug,RIIV33U(:,1),RIIV33U(:,2),'urineplasma.aps');
RIIV33TT1 = [0.25 2.84
1 14.68
2 20.31
4 34.89 ];

RIIV33TB1 = [0.25 2.01
1 9.87
2 15.36
4 27.55 ];

RIIV33TT2 = [0.5 7.85
2 19.73
6 36.26 ];

RIIV33TB2 = [0.5 7.03
2 18.50
6 34.71 ];

RIIV33TT3 = [0.083 0.714
0.25 4.05
0.5 5.157
1 9.674
2 26.277
6 48.948
9 58.685
24 51.965
30 50.315
48 53.118
72 37.654
96 27.476 ];
RIIV33TB3 = [ 0.083 0.36
               0.25 3.202
               0.5  4.06
               1   8.505
               2   24.371
               6   46.482
               9   54.947
              24  49.834
              30  48.012
              48  50.095
              72  35.252
             96  24.611 ];

%Simulation commands
!!s ivdosei=33, tstop=96, cint=0.1, bw=0.300
!!prepare tct_img1, cib_img1, t, ct_img1
!!start/nc

%Plotting Commands
plot(_t, _tct_img1, RIIV33TT1(:,1), RIIV33TT1(:,2), RIIV33TT2(:,1), RIIV33TT2(:,2), RIIV33TT3(:,1), RIIV33TT3(:,2), _t, _cib_img1, RIIV33TB1(:,1), RIIV33TB1(:,2), RIIV33TB2(:,1), RIIV33TB2(:,2), RIIV33TB3(:,1), RIIV33TB3(:,2), 'thyroidbandt.aps');
%[125-I]-T3 data DiStefano1993
%Key for datasets in ThyroidDatasets.xls
%Units (%dose)
%Created 03/01/07
%Data from DiStefano et al 1993
%time (hrs), T3 (%dose)
RT3IVDP2 = [ 0.066666667 42.42424815
  0.116666667 8.333342858
  0.333333333 7.196966378
  0.666666667 6.439436908
  0.7 4.924302516 ];

RT3IVDL = [ 0.01 6.060606562
  0.116666667 46.59091824
  1.2 20.83338672 ];

%Simulation commands
!!s ivdoset3=0.00083, tstop=2, cint=0.001, bw=0.375
!!prepare /All
!!start/nc

%Plotting Commands
plot(_t, _t3rdp, _t, _t3rdl, RT3IVDP2(:,1), RT3IVDP2(:,2), RT3IVDL(:,1), RT3IVDL(:,2), 't3plasmaliver_Di
stefano93.aps');
%Schroder-vanderElst 1997 131I-T4 distribution in vehicle control rats
%Created 07/09/07 by Eva McLanahan
%iv dose of 0.0017ug 131I-T4 to female wistar rats
%Figure 4.4C - T4 from Schroder van der Elst 1997

%Time (hrs), Blood (%dose), Liver (%dose)
RT4IVSVEB = [ 0.25 42 29 0.5 31 30 1 34 23 2 25 19 3 27 16 4 21 15 6 21 14 ];

%Simulation commands
!!s ivdose t4=0.0017, bw=0.180, tstop=6, cint=0.001
!!prepare t, t4rpdt4vd, t4rpdl
!!start /nc

%Plotting Commands
plot(_t, _t4rpdt4vd, RT4IVSVEB(:,1), RT4IVSVEB(:,2), _t, _t4rpdl, RT4IVSVEB(:,1), RT4IVSVEB(:,3),'svet4.a ps')
%IV dose of TSH
%Created 07/22/07 by Eva McLanahan
%Last edited 07/22/07 by Eva McLanahan
%Spira 1979 iv dose male rats with 5ngTSH/rat via tail vein
%Male Hebrew University rats 80-100g
%Data expressed as %Dose TSH/ml plasma/100g bw - assuming 100grat

%Time (hrs), 125I-TSH (%dose/ml)
SpiraTSHiv = [ 0.01949119  16.70926392
0.050148872  14.16756717
0.081021331  13.04201494
0.101155515  10.39869552
0.121987729  10.83116423
0.173316539  8.993441313
0.234900371  7.165413202
0.266041305  7.310211755
0.296323115  5.367511656
0.420296204  4.637900996
0.482041119  3.930251676
0.75038995  2.592929802
1.039517304  1.745539131
1.349906438  1.442738244
1.681396214  1.37651532
1.991516786  1.026596369 ];

%Simulation Commands
!!s bw=0.100, ivdosetsh=5, tstop=2
!!prepare t, tshrdp
!!start/nc

%Plotting Commands
    plot (_t, _tshrdp, SpiraTSHiv(:,1), SpiraTSHiv(:,2), 'TSHiv.aps')
APPENDIX C

The acslXtreme (version 2.4.0.11) .csl file for BBDR-HPT axis iodide deficiency model code (Chapter 4) is contained within this Appendix. Model code with oral intake of iodide is structured as follows: First section contains physiological parameters and compound-specific constants. Second section includes scaled parameters and is followed by model code for TSH, dietary iodide, thyroxine (T₄), 3,5,3’-triiodothyronine (T₃), and ClO₄⁻. Finally the mass balance equations for each chemical conclude the model code.
PROGRAM: Male Rat HPT Axis Model
!-----------------------------------------------------------------------
!File name:EHPTnogut.csl created 03/01/07 from EHPT_Imod_021407.csl
!Units in nmol, L, hr, kg
!Endogenous I, T4, T3, and TSH
!Blood binding of T4 99% bound - taken into account for liver uptake (0.01*cvl)
!Last Revised 08/08/07 by Eva McLanahan

INITIAL
CONSTANT  TSTOP=1416  !Length of experiment (1416=59 days)
CONSTANT  CINT=0.5  !communication interval

! ------------ Physiological Parameters------------------
CONSTANT  QCC = 14.0  !L/hr/kg - Total cardiac output [Brown 1997]
CONSTANT  QLC = 0.174  !%QC - Proportion cardiac output to the liver [Brown 1997 p438]
CONSTANT  QTC = 0.016  !%QC - Proportion cardiac output to the thyroid - human value (Brown 1997 & Merrill 2003)
CONSTANT  VLC = 0.0366 !%BW liver tissue [Brown 1997 pg 416]
CONSTANT  VLBC = 0.21  !%VL as liver blood [Brown 1997]
CONSTANT  VTC = 0.00005 !%BW total thyroid tissue [McLanahan 2007]
CONSTANT  VTBC = 0.157  !%VT as thyroid blood [Merrill et al 2003]

!------GROWTH EQUATION PARAMETERS---------
CONSTANT  BWC = 0.320  !kg - body weight (a constant body weight)
CONSTANT  BWGon = 0  !if BWGon=1 then BW growth equation on, else uses BWC
CONSTANT  BWs = 170000  !mg - BW for start of study (must be given in lit.)
CONSTANT  BWt0 = 7314.70  !mg - initial BW at birth (Mirfazaelian 2007)
CONSTANT  KBW = 63.21  !days - age at inflection point (Mirfazaelian 2007)
CONSTANT  BWtmax = 521026.13  !mg - maximum body weight (Mirfazaelian 2007)
CONSTANT  gammaBW = 2.01  !unitless - hill coeff for BW growth (Mirfazaelian 2007)

!---------- TSH Parameters--------------------
CONSTANT  MWTSH  = 28000  !g/mol - molecular weight TSH [chemfinder.com]
CONSTANT  Vd_TSHC = 0.0554  !L/kg - VdTSH - (Connors et al 1984)
CONSTANT  K0TSHmaxC = 6  !nmol/hr - Max prod of TSH (absence of T4) (Connors 1984)
CONSTANT  Kinh_T4 = 0.2  !nmol/L - Km of T4 such that prod of TSH is 1/2 maximal
CONSTANT  KNIS_TSH  =  0.949  !nmol/L - TSH conc so Vmax of NIS I transport is 1/2 max
CONSTANT  KbTSH  =  733.98 !nmol/L - Km TSH conc such that I binding/organification in thyroid is 1/2 maximal
CONSTANT  Kel_TSHC =  1.8899 !1/hr-kg - elimination rate constant for TSH from Vd (Lemarchand-Béraud and Bertheir 1981)
CONSTANT TSHb =  5.08373 !ng/ml - TSH baseline to calc fold change. Set for each BW

!------------ I (Iodide) Parameters----------------------
CONSTANT  MWI  =  126.9047 !g/mol - molecular weight I [periodic table]
CONSTANT  Vd_iC  =  0.5  !L/kg - volume of distribution of iodide
CONSTANT  Km_i  =  31519  !nmol/L - affinity constant I for NIS (Gluzman and Niepomnisszcze 1983 and Merrill 2003)
CONSTANT  VmaxT_iC =  5738.267 !nmol/hr/kg - maximal rate of uptake
CONSTANT  PAt_iC =  0.0001 !L/hr - PA term thyroid [Merrill 2003]
CONSTANT  V0bindC =  1005.9 !nmol/hr-kg - maximum rate of binding of iodide in thyroid
CONSTANT  Kmb_i  =  244.59 !nmol/L - Km of iodide binding
CONSTANT  ClU_iC =  0.0046 !L/hr-kg - urinary clearance of iodide

!-------Thyroid hormone production parameters--------------
CONSTANT  ktshcib =  5e-7  !L2/nmol-hr - rate constant for thyroid hormone production

!---------- T4 (Thyroxine) Parameters----------------------
CONSTANT  MWT4 =  776.8742 !g/mol - molecular weight T4 [calculated:C15H11I4NO4]
CONSTANT  MWT4G =  952 !g/mol - molecular weight T4-Glucuronide [calculated: T4(776)+GA(194)-H2O(18)=952]
CONSTANT  Vd_t4c =  0.156  !L/kg bw - vd t4 (Kohn 1996)
CONSTANT  PL_t4  =  1.27  !Partition coefficient for T4 liver (EscobarMorreale 1996)
CONSTANT  PAL_t4C =  0.0423 !nmol/hr/kg - from tracer - PA term for T4 liver
CONSTANT  VMAXDIC =  19.89 !nmol/hr/kg - Vmax outer ring deiodinase
CONSTANT  KMDI  =  2300  !nmol/L - Km outer ring deiodinase in liver (Leonard and Visser 1986)
CONSTANT  KEL_t4C =  0.05  !1/hr-kg - rate of elimination of T4 from body (Vd) (Abrams & Larsen 1973 t1/2 used for calculation)
CONSTANT  KmUGT =  100000 !nmol/L - (Km of UGT enzymes for T4 and T3) (Visser 1993)
CONSTANT  VmaxT4GC =  3435.89 !nmol/hr - max rate of T4-G formation in liver
CONSTANT  VMAXT4LUC =  4384.73 !nmol/hr - Vmax for active uptake of T4 into liver
CONSTANT  KmT4LU =  650 !nmol/L - Km for uptake in hepatocytes (Blondeau 1988)
CONSTANT FFT4  =  0.01  !fraction of free t4 available for uptake to liver
CONSTANT t4b  =  48.8846  !t4 baseline - to calc % control - diff for each BW

!------------ T3 (3,5,3'-Triiodothyronine) Parameters------------------
CONSTANT  MWT3  =  650.97349 !g/mol - molecular weight T3 [calculated:C15H12I3NO4]
CONSTANT  Vd_T3C  =  0.186  !L/kg - Vd_T3 per kg BW [thyroid hormone metab pg 67]
CONSTANT  PL_t3  =  4.47  !Partition coefficient for T3 liver (EscobarMorreale 1996)
CONSTANT  PAL_t3C  =  0.1699  !L/hr/kg - from tracer - PA term liver T3
CONSTANT  Ke_l_t3C  =  0.12  !1/hr-kg- rate of T3 elimination from body
(CONSTANTS Kel_t3C = 0.12 Abrams and Larsen 1973 t1/2 used for calculation)
CONSTANT  KmetL_t3C  =  3.65  !1/hr-kg - fractional removal rate from liver
CONSTANT  KLUT3  =  1.25  !L/hr - 1st order liver uptake rate of T3
CONSTANT t3b  =  0.556647  !t3 baseline - to calc % control - diff for each BW

!---T4 and T3 iodide equivalents
CONSTANT  I4CON  =  0.6534  !Fraction of t4 as iodine (4 Im.w./T4m.w.) T4 iodide equiv
CONSTANT  I3CON  =  0.5848  !Fraction of T3 as iodine (3 Im.w./T m.w.) T3 iodide equiv
CONSTANT  T43CON = 0.8379  !T3/T4 molar equivalents (T3 m.w. / T4 m.w.)
CONSTANT  IFT4M  =  0.16335  !One I freed in T4 Metabolism (I mw./T4 m.w.)

!---IODIDE dosing parameters
!CONSTANT  pdose_i  =  20  !ug - oral dose of iodide (diet I intake for McLanahan 2007 calculated to be 20ug/day)
|pdose1 and pdose2 used instead of pdose_i for changing iodide intake during studies
CONSTANT  pdose1  =  20  !ug - 1st half of study iodide intake
CONSTANT  pdose2  =  20  !ug - 2nd half of study iodide intake

!Compartment initial amounts from running EHPT model to steady state 2000hrs (1/27/07)
CONSTANT  initAvdTSH  =  0.  !nmol TSH - initial amt of TSH in Vd
CONSTANT  initAP_t4  =  0.  !nmol T4 - initial amt of T4 in Vd (blood)
CONSTANT  initAlb_t4  =  0.  !nmol T4 - initial amt of T4 in liver blood
CONSTANT  initAl_t4  =  0.  !nmol T4 - initial amt of T4 in liver tissue
CONSTANT  initAlb_t3  =  0.  !nmol T3 - initial amt of T3 in liver blood
CONSTANT  initAl_t3  =  0.  !nmol T3 - initial amt of T3 in liver tissue
CONSTANT  initAP_t3  =  0.  !nmol T3 - initial amt of T3 in Vd (blood)
CONSTANT  initAP_i  =  0.  !nmol I - initial amt of iodide in Vd (blood)
CONSTANT  initAtb_i  =  0.  !nmol I - initial amt of iodide in thyroid blood
CONSTANT  initdAT_i = 0.  !nmol I - initial amt of free iodide in thyroid tissue
CONSTANT  initdAIB_i = 0.  !nmol I - initial amt of bound iodide in thyroid tissue

END ! INITIAL

DYNAMIC
ALGORITHM IALG = 2   !Gear method for stiff systems

DERIVATIVE
if (BWGon.eq.1) then
  BW=BWG else BW=BWC
end if

!------Growth equations------
BWG=(((BWt0*(KBW**gammaBW))+(BWtmax*(Age**gammaBW)))/((KBW**gammaBW)+(Age**gammaBW)))/10**6
  !BW (kg) at a given Age (days)
Age=Age0+days        !Age (days)
Age0=KBW*(((BWs-BWt0)/(BWtmax-BWs))**(1/(gammaBW))) !Age (days) at start of study if only
  !initial BWs (mg) is given

!--------------- Scaled Parameters--------------
QC = QCC*BW**0.75 !L/hr - Cardiac output
QL = QLC*QC  !L/hr - blood flow to liver
QT = QTC*QC  !L/hr - blood flow to thyroid
VL1 = VLC*BW  !L - liver volume with blood
VLB = VLBC*VL1  !L - Volume of liver blood
VL = VL1-VLB  !L - Liver tissue volume without blood
VT1 = VTC*BW  !L - total thyroid volume
VTB = VTBC*VT1  !L - volume of thyroid blood
VT = VT1-VTB  !L - volume of thyroid without blood

!--TSH scaled parameters---
Vd_TSH = Vd_TSHC*BW  !L - Vd for TSH
Kel_TSH = Kel_TSHC/BW**0.25 !l/hr - TSH elim rate
K0TSHmax = K0TSHmaxC*BW**0.75 !nmol/hr - max rate of TSH secretion (no T4)
!--T4 scaled parameters----

\[ V_d_{T4} = V_{d_{T4c}} \cdot BW - V_L1 \]  \hspace{1cm} !L - Vd for T4

\[ V_{MAXD_{I}} = V_{MAXD_{DIC}} \cdot BW^{0.75} \]  \hspace{1cm} !nmol/hr - Vmax for type 1 5'd in liver for t4

\[ PAL_{T4} = P_{AL_{T4C}} \cdot BW^{0.75} \]  \hspace{1cm} !L/hr - PA term for liver t4

\[ K_e_{l_{T4}} = K_{EL_{T4C}} / BW^{0.25} \]  \hspace{1cm} !1/hr - T4 elimination rate from body

\[ V_{maxT_{4G}} = V_{maxT_{4GC}} \cdot BW^{0.75} \]  \hspace{1cm} !nmol/hr - Vmax for t4 glucuronidation in liver

\[ V_{maxT_{4LU}} = V_{maxT_{4LUC}} \cdot BW^{0.75} \]  \hspace{1cm} !nmol/hr - Vmax for liver uptake of t4

!--T3 scaled parameters--

\[ V_d_{T3} = V_{d_{T3C}} \cdot BW - V_L1 \]  \hspace{1cm} !L - Vd for T3

\[ PAL_{T3} = P_{AL_{T3C}} \cdot BW^{0.75} \]  \hspace{1cm} !L/hr - PA term for T3 in liver

\[ K_e_{l_{T3}} = K_{EL_{T3C}} / (BW^{0.25}) \]  \hspace{1cm} !1/hr - T3 elimination rate from body

\[ K_{metL_{T3}} = K_{metL_{T3C}} / (BW^{0.25}) \]  \hspace{1cm} !1/hr - nonspecific T3 metabolism in liver

!--Iodide scaled parameters--

\[ V_d_{i} = V_{d_{ic}} \cdot BW - V_T1 \]  \hspace{1cm} !L - volume of distribution of iodide

\[ V_{maxT_{i}} = V_{maxT_{ic}} \cdot BW^{0.75} \]  \hspace{1cm} !nmol/hr - max rate of iodide uptake by NIS in thyroid

\[ C_{LU_{i}} = C_{LU_{ic}} / (BW^{0.25}) \]  \hspace{1cm} !L/hr - Urinary clearance of iodide

!Changed scaling from \( V_{0bindc} \cdot BW^{0.75} \) to below on 7/18/07 based on initialconditions.xls showing total thyroid iodide in thyroid not dropping below 7 for 120-180g rats and not above 20 for 400g rat

\[ V_{0bind} = V_{0bindC} / (BW^{0.75}) \]  \hspace{1cm} !nmol/hr - max rate of binding of iodide in thyroid

\[ P_{AT_{i}} = P_{AT_{ic}} \cdot BW^{0.75} \]  \hspace{1cm} !L/hr - PA term for thyroidal iodide

!----MODEL CODE----TSH volume of distribution with feedback T4-----------------

\[ R_{THSHPR} = (K_{0_{THSHmax}} \cdot K_{inh_{T4}}) / (K_{inh_{T4}} + C_{a_{t4}}) \]  \hspace{1cm} !nmol/hr - Rate of TSH production

\[ A_{THSHPR} = \text{INTEG} (R_{THSHPR}, 0.0) \]  \hspace{1cm} !nmol - Amount of TSH produced

\[ A_{THSHPRug} = A_{THSHPR} \cdot M_{WTSH} / 1000 \]  \hspace{1cm} !ug - amount of TSH produced

\[ d_{THSHPRugd} = (AT_{SHPRug} / (((t + 1e-6) / 24))) \]  \hspace{1cm} !ug/d - TSH production

\[ R_{CILS}_{TSH} = K_{el_{TSH}} \cdot A_{vd_{TSH}} \]  \hspace{1cm} !nmol/hr - clearance of TSH from Vd

\[ A_{CILS}_{TSH} = \text{INTEG} (R_{CILS}_{TSH}, 0.0) \]  \hspace{1cm} !nmol - amt of TSH cleared from Vd

\[ R_{AVd_{TSH}} = R_{THSHPR} - R_{CILS}_{TSH} \]  \hspace{1cm} !nmol/hr - rate of change of TSH in Vd

\[ A_{AVd_{TSH}} = \text{INTEG} (R_{AVd_{TSH}}, \text{initAVd_{TSH}}) \]  \hspace{1cm} !nmol - amt of TSH in Vd

\[ S_{TSH} = A_{vd_{TSH}} / V_d_{TSH} \]  \hspace{1cm} !nmol/L - Concentration TSH

\[ TSH_{ng/ml} = (TSH / 1000) \times M_{WTSH} \]  \hspace{1cm} !ng/mL (same as ug/L) - TSH concentration in Vd

!fold change tsh
TSHFOLD = (TSHng/ml/TShb) ! fold change TSH
TSHpercon = (TSHng/ml/TShb) * 100 ! TSH as % control

--- MODEL CODE ---
Iodide, IV dose with thyroid and Vd

--- Iodide dosing ---
oral dose

Change in iodide diet 1X - t = 5040 for Fukuda Refeeding data
pdose1 and pdose2 set in m file

if (t > 5040)
    pdose_i = pdose2
else
    pdose_i = pdose1
end if

Normal Oral Dosing parameters for I
dose_i = (pdose_i * 10^3) / MWi ! dietary intake amount (nmol)
Rdose_i = dose_i / 12 ! dose rate for eating period (hrs) per day

Food Consumption for a 12 hr period (light-dark cycle in rat)
pflag=pulse(0.0,24.0,12) ! for one 12 hr eating period per day
RMR_i = (Rdose_i * pflag) ! nmol/hr - dose rate for oral dose iodide

AST_i = INTEG(RMR_i,0.0) ! nmol - amount of iodide received orally entering stomach
d_AST_i = AST_i / (((t + 1e-6) / 24)) ! nmol - daily amount of iodide received orally in stomach

d_AST_iug = (d_AST_i * MWi) / (10^3) ! ug - daily amount of iodide received orally in stomach

--- Volume of Distribution of Free Iodide ---

RAP_i = RMR_i + QT * Cvt_i - QT * Ca_i + RAIFL_t4 + RAIFL_t3 + RAIFVd_t4 + RAIFVd_t3 - RU_i ! nmol/hr - rate of change of free iodide in serum

AP_i = INTEG(RAP_i, initAP_i) ! nmol - amount of free iodide in serum
CP_i = AP_i / Vd_i ! nmol/L - concentration of free iodide in serum
Ca_i = CP_i ! ug/dL - concentration of free iodide in serum
CP_ingml = (CP_i * MWi) / 1000 ! ng/ml - concentration of free iodide in serum
Ca_bi = (Ca_t4 * I4CON) + (Ca_t3 * I3CON) ! nmol/L - concentration of bound iodide in serum
Ca_bingml = (Ca_bi * MWi) / 1000 ! ng/ml - concentration of bound iodide in serum
Ca_ti = Ca_i + Ca_bi ! nmol/L - concentration of total iodide (bound + free) in serum
Ca_tingml = (Ca_ti * MWi) / 1000 ! ng/ml - concentration of total iodide

RU_i = ClU_i * Ca_i ! nmol/hr - rate of urinary clearance of iodide

--- Model Code ---
Iodide, IV dose with thyroid and Vd

change in iodide dose IX - t = 540 for Fukuda Refeeding data

--- Model Code ---
Iodide, IV dose with thyroid and Vd

TSHpercon = (TSHng/ml/TShb) * 100
TSHfold = (TSHng/ml/TShb) * 100

TSHFOLD = (TSHng/ml/TShb) ! fold change TSH
TSHpercon = (TSHng/ml/TShb) * 100
TSHfold = (TSHng/ml/TShb) * 100
AU_i=INTEG(RU_i,0,0) \quad \text{!nmol} \quad \text{amount of iodide cleared in urine}

d_{au_i}=AU_i/((t+1e-6)/24) \quad \text{!nmol/d} \quad \text{iodide cleared in urine}

AU_iug=AU_i*MWI/1000 \quad \text{!ug} \quad \text{amount of iodide cleared in urine}

d_{AU_iug}=AU_iug/((t+1e-6)/24) \quad \text{!ug/d} \quad \text{iodide cleared in urine}

PINEX=(d_{AU_i}/(d_{AST_i}+1e-6))*100 \quad \% \quad \text{of daily intake of iodide excreted in urine}

---Rate of metabolism of TH in Vd -- freeing of iodide---

RAIFvd_t4=Rvdel_t4*IFT4M \quad \text{!nmol/hr} \quad \text{rate of I freed from T4->T3 metabolism in Vd}

AIFVd_t4=INTEG(RAIFvd_t4,0,0) \quad \text{!nmol} \quad \text{amount of I freed from T4->T3 metabolism in Vd}

RAIFvd_t3=(Rvdel_t3*I3CON) \quad \text{!nmol/hr} \quad \text{rate of I freed from T3 metabolism in Vd -- assume all goes to T3, that T3 metab is rate limiting step}

AIFVd_t3=INTEG(RAIFvd_t3,0,0) \quad \text{!nmol} \quad \text{amount of I freed from T3 metabolism in Vd}

--------Liver iodide metabolism of THs, added to Iodide Vd---------

RAIFL_t4=RADIL*IFT4M \quad \text{!nmol/hr} \quad \text{rate of I freed in liver from T4 metab to T3}

AIFL_t4=INTEG(RAIFL_t4,0,0) \quad \text{!nmol} \quad \text{amt of iodide released in liver from T4 metab to T3}

RAIFL_t3=RAML_t3*0.70*I3CON \quad \text{!nmol/hr} \quad \text{EST 70% of T3 metabolized into free I-}

AIFL_t3=INTEG(RAIFL_t3,0,0) \quad \text{!nmol} \quad \text{amount of I freed in liver from T3 metabolism}

--------THYROID IODIDE-------------------------------

Rate of active iodide uptake into thyroid by the NIS

RTNIS=(VmaxT_i*TSH*Cvt_i)/(Cvt_i+Km_i) \quad \text{!nmol/hr} \quad \text{rate of I active uptake (NIS)}

VmaxT_iTSH=((VmaxT_i*TSH)/(KNIS_TSH+TSH)) \quad \text{!nmol/hr} \quad \text{change in Vmax due to TSH stimulation}

ATIU=INTEG(RTNIS,0,0) \quad \text{!nmol} \quad \text{Amount of I uptake (active) into thyroid}

Rate of change of iodide in thyroid blood

RAtb_i=Qt*(Ct_i-Cvt_i)+PAt_i*(Ctf_i-Cvt_i)-RTNIS \quad \text{!nmol/hr} \quad \text{rate of change of I in thy blood}

Atb_i=INTEG(RAtb_i,initAtb_i) \quad \text{!nmol} \quad \text{amount of I in thyroid blood}

Cvt_i=Atb_i/VTB \quad \text{!nmol/L} \quad \text{conc of I in thyroid blood}

Rate of change of FREE IODIDE IN THYROID

RAtf_i=RTNIS+PAt_i*(Ct_i-Ctf_i)-RIB \quad \text{!nmol/hr} \quad \text{rate of change of FREE IODIDE IN THYROID}

d_{Atf_i}=INTEG(RAtf_i,initdAt_i) \quad \text{!nmol} \quad \text{amount of free iodide in thyroid lumen was}

Atf_i=MAX(d_{Atf_i},0) \quad \text{!ug} \quad \text{amount of free iodide in thyroid lumen}

Ctf_i=Atf_i/VT \quad \text{!nmol/L} \quad \text{conc of free iodide in thyroid tissue}

Ctf_imgl=Ctf_i*MWI/10**6 \quad \text{!mg/L} \quad \text{concentration of free I in thyroid tissue}
!Rate of incorporation (binding) of iodide in thyroid tissue
\[ \text{RIB} = \frac{(V_{\text{maxbt}_i} \cdot \text{Ctf}_i)}{(K_{\text{mb}_i} + \text{Ctf}_i)} \] !nmol/hr - rate of incorporation of iodide in thyroid
\[ V_{\text{maxbt}_i} = \frac{(V_0 \cdot \text{bind} \cdot \text{TSH})}{(K_b \cdot \text{TSH} + \text{TSH})} \] !nmol/hr - vmax of binding change (stimulated by TSH concentration in Vd
\[ \text{ARIB} = \text{INTEG}(\text{RIB}, 0.0) \] !nmol - amount of iodide incorporated in thyroid
\[ \text{ARIBug} = \frac{\text{ARIB} \cdot \text{MWI}}{1000} \] !ug - amount of iodide incorporated in thyroid
\[ d_{\text{aribug}} = \frac{\text{ARIBug}/((t + 1e-6)/24))}{24} \] !ug - daily amt of iodide incorporated in thyroid

!Rate of change of BOUND iodide in thyroid tissue
\[ \text{RIB}_i = \text{RIB} - \text{Rth} \] !nmol/hr - rate of change of bound iodide in thyroid (rate of binding - loss as secretion of thyroid hormone)
\[ d_{\text{AIB}_i} = \text{INTEG}(\text{RIB}_i, \text{initdAIB}_i) \] !ug - amt iodide bound in thyroid
\[ \text{CIB}_i = \frac{\text{AIB}_i \cdot \text{MWI}}{1000} \] !nmol/L - concentration of iodide bound in thyroid
\[ \text{CIB}_\text{imgl} = \frac{\text{CIB}_i \cdot \text{MWI}}{10^{12}} \] !mg/L - concentration of iodide bound in thyroid

!Set a maximum and minimum amt of iodide stores in thyroid - max is not really needed even for iodide up to 500ug/day
if (\text{dAIB}_i \cdot \text{GT.160}) then
\text{AIB}_i = 160
else if (\text{dAIB}_i \cdot \text{LT.0}) then
\text{AIB}_i = 0
else
\text{AIB}_i = \text{dAIB}_i
end if

!Rate of utilization of bound I secreted as TH (rate of production of T4 and T3 in iodide equiv.)
\[ \text{Rth} = (RPR_{\text{t4}} \cdot \text{I4CON}) + (RPR_{\text{t3}} \cdot \text{I3CON}) \] !nmol I/hr - rate of utilization of thyroid I in TH prod
\[ \text{Ath} = \text{INTEG}(\text{Rth}, 0.0) \] !nmol - amount of iodide used in TH prod
\[ d_{\text{ath}} = \frac{\text{Ath}/((t + 1e-6)/24)}{1000} \] !nmol/day - daily amt of iodide used in TH prod
\[ \text{Athug} = \frac{\text{Ath} \cdot \text{MWI}}{1000} \] !ug - amount of iodide used in TH prod
\[ d_{\text{athug}} = \frac{\text{Athug}/((t + 1e-6)/24)}{1000} \] !ug/day - daily amount of iodide used in TH prod
\[ \text{PINITHPR} = \left( \frac{\text{d}_{\text{ath}}}{(\text{d}_{\text{ast}_i} + 1e-6)} \right) \times 100 \] !% of daily intake of iodide used in TH prod

!Rate of change of FREE iodide in thyroid tissue - allows you to look at the free entering and leaving, also loss of free to binding
\[
R_{At_{ien}} = R_{TNIS (P_{At_i} C_{vt_i})}
\]

- \(R_{At_{ien}} = \text{INTEGRAL}(R_{At_{ien}}, 0.0)\)
- \(At_{ien} = \text{INTEGRAL}(R_{At_{ien}}, 0.0)\)
- \(R_{At_{iex1}} = (P_{At_i} C_{tf_i})\)
- \(At_{iex1} = \text{INTEGRAL}(R_{At_{iex1}}, 0.0)\)
- \(R_{At_{iex2}} = R_{At_{iex1} + R_{th}}\)
- \(At_{iex2} = \text{INTEGRAL}(R_{At_{iex2}}, 0.0)\)

- \(T_{At_i} = A_{IB_i} + A_{tf_i}\)
- \(T_{At_{img}} = (T_{At_i} M_{WI}) / 10^{**6}\)
- \(T_{Ct_{imgL}} = T_{At_{img}} / V_T\)
- \(T_{At_{iug}} = T_{At_i} M_{WI} / 1000\)

---MODEL CODE---Thyroid hormone production in the Thyroid---

- \(R_{PR_{th}} = k_{tshcib} TSH C_{IB_i}\)
- \(F_{T3calc} = 0.2652 ((TAT_{iug})^{-0.4684})\)
- \(R_{PR_{t3}} = F_{T3calc} R_{PR_{th}}\)
- \(A_{PR_{t3}} = \text{INTEGRAL}(R_{PR_{t3}}, 0.0)\)
- \(A_{PR_{t3}ug} = A_{PR_{t3}} M_{WT3} / 1000\)
- \(d_{PRT3Tug} = (A_{PR_{t3}Tug} / (((t+1e-6)/24)))\)
- \(R_{PR_{t4}} = R_{PR_{th}} - R_{PR_{t3}}\)
- \(A_{PR_{t4}} = \text{INTEGRAL}(R_{PR_{t4}}, 0.0)\)
- \(A_{PR_{t4}ug} = A_{PR_{t4}} M_{WT4} / 1000\)
- \(d_{PRT4Tug} = (A_{PR_{t4}Tug} / (((t+1e-6)/24)))\)

- \(T3/T4\) ratio
- \(MR34T = A_{PR_{t3}} / (A_{PR_{t4}} + 1e-6)\)
-----MODEL CODE---- Total T4 with a Liver and Vd-------

**SERUM T4 (Volume of distribution)**

\[
\begin{align*}
\text{RAP}_t4 &= \text{RPR}_t4 + (\text{QL} \times \text{CVL}_t4) - \text{QL} \times \text{Ca}_t4 - \text{RVdel}_t4 \\
\text{AP}_t4 &= \text{INTEG}(\text{RAP}_t4, \text{initAP}_t4) \\
\text{Ca}_t4 &= \frac{\text{AP}_t4}{\text{Vd}_t4} \\
\text{Ca}_t4_{ugdl} &= \frac{\text{Ca}_t4 \times \text{MWT}_4}{10000} \\
\text{Ca}_t4_{ngg} &= \frac{\text{Ca}_t4 \times \text{MWT}_4}{1000} \\
\text{t4percon} &= \frac{\text{Ca}_t4_{ngg}}{\text{t4b}} \times 100
\end{align*}
\]

\[
\begin{align*}
\text{AVdel}_t4 &= \text{amount of T4 cleared from Vd} - \text{assumed to go to T3 + free iodide} \\
\text{RVdel}_t4 &= \text{Kel}_t4 \times \text{Ap}_t4 \\
\text{AVdel}_t4 &= \text{INTEG}(\text{RVdel}_t4, 0.0) \\
\text{dAVdel}_t4 &= \frac{\text{AVdel}_t4}{\left(\frac{t+1e-6}{24}\right)}
\end{align*}
\]

**Liver T4**

\[
\begin{align*}
\text{RALb}_t4 &= \text{QL} \times (\text{Ca}_t4 - \text{CVL}_t4) + \text{PAL}_t4 \times (\text{CL}_t4 - (\text{CVL}_t4 \times \text{FFT}_4)) - \text{RLT4U} \\
\text{ALb}_t4 &= \text{INTEG}(\text{RALb}_t4, \text{initAlb}_t4) \\
\text{Cvl}_t4 &= \frac{\text{ALb}_t4}{\text{VLB} \times \text{PL}_t4} \\
\text{RAL}_t4 &= (\text{PAL}_t4 \times ((\text{CVL}_t4 \times \text{FFT}_4) - \text{CL}_t4)) - \text{RAGL} - \text{RADIL} + \text{RLT4U} \\
\text{AL}_t4 &= \text{INTEG}(\text{RAL}_t4, \text{initAl}_t4) \\
\text{CL}_t4 &= \frac{\text{AL}_t4}{\text{VL}} \\
\text{CL}_t4_{ngg} &= \frac{\text{CL}_t4 \times \text{MWT}_4 / 1000}{1.051} \\
\text{RLT4U} &= \frac{\text{VmaxT4LU} \times (\text{Cvl}_t4 \times \text{FFT}_4)}{\text{KmT4LU} + (\text{Cvl}_t4 \times \text{FFT}_4)} \\
\text{ALT4U} &= \text{INTEG}(\text{RLT4U}, 0.0)
\end{align*}
\]
! Metabolism of T4 in liver - via deiodination
RADIL=(((VMAXDI*Cvl_t4)/(Cvl_t4+KMDI)) !nmol/hr - rate of T4 deiodination in liver (D1)
ADIL=INTEG(RADIL,0.0) !(nmol) Amount of T4 deiodinated (D1) in liver
| d_ADIL_T4=(ADIL/(((t+1e-6)/24))) |

| PC43L=(ADIL/(APR_t4+1e-6))*100 |

! Metabolism of T4 in the liver - via glucuronidation
RAGL=(VmaxT4G * Cvl_t4)/(KmUGT + Cvl_t4) !nmol T4/hr - rate of T4-glucuronidation in liver
AGL=INTEG(RAGL,0.0) !nmol T4 lost/used to make T4-G
RAGLT4G=(RAGL*(MWT4G/MWT4)) !nmol T4-G formed/hr
AGLT4G=INTEG(RAGLT4G,0.0) !nmol T4-G formed
RGLT4Gpmolhr=RAGLT4G*1000 !pmol T4-G formed/hr/liver

| d_AT4_feces=(AGL/(((t+1e-6)/24))) |

PT4PRinfeces=(d_AT4_feces/(d_PRT4nmold+1e-6))*100 !% of T4 produced excreted in feces/day

! -- Overall T4 Metabolism --
| AWBT4Met=total amount of T4 metabolized (liver gluc + liver deiod + Vd metab) |
RWBT4Met=RVdel_t4+RAGL+RADIL !nmol/hr - whole body rate of T4 metabolism
| AWBT4Met=INTEG(RWBT4Met,0.0) |
| d_AWBT4Met=(AWBT4Met/(((t+1e-6)/24))) |

| AT4T3=Avdel_t4+Adil |
| FMWBT4=(AWBT4Met/(APR_t4+1e-6))*100 |
| FMvT4=(AVdel_t4/(APR_t4+1e-6))*100 |
| (FMGLT4=(AGL/(APR_t4+1e-6))*100 |
| FMDILT4=(ADIL/(APR_t4+1e-6))*100 |
| FMT4T3=FMvdT4+FMDILT4 |

%!% of produced T4 that is metab. Sum of all pathways
%!% of produced T4 that is metab in Vd
%!% of produced T4 that is metab to T4-G in liver
%!% of produced T4 that is metab to t3 in liver
%!% of T4 converted to T3
!---------MODEL CODE--- Total t3, with a Liver and Vd---------
!------------------SERUM t3 (Volume of distribution)-----------------
!----production of T3 in the Vd from T4 metabolism
!AT3FVd=amount of T3 formed in the Vd from T4 metabolism
RT3FVd=(RVdel_t4*T43CON) !nmol t3/hr - all T4 to T3+I
AT3FVd=INTEG(RT3FVd,0.0) !nmol - amt of T3 formed in Vd from T4 metabolism
AT3FVdug=AT3FVd*MWT3/1000 !ug - amount of T3 produced from T4 metabolism in Vd
RAP_t3=(QL*CVL_t3)-QL*Ca_t3-RVdel_t3+RPR_t3+RT3FVd !nmol/hr - rate of change of t3 in Vd
AP_t3=INTEG(RAP_t3,initAP_t3) !nmol - amount of t3 in Vd
Ca_t3=AP_t3/Vd_t3 !nmol/L - concentration of t3 in Vd
Ca_t3ugdl=Ca_t3*(0.0001)*MWT3 !ug/dL - t3 in Vd
t3percon=(ca_t3ngg/t3b)*100 !%control - Vd T3
RVdel_t3=Kel_t3*Ap_t3 !nmol/h - rate of t3 elim from Vd
assumed all metab to free I-
AVdel_t3=INTEG(RVdel_t3,0.0) !nmol - amount of t3 elim from Vd

!-------Liver t3----------
!----production of T3 in liver
RAT3FL=RADIL*T43CON !nmol/hr - rate of T3 formed in liver from T4 deiod. in T3 equiv.
AT3FL=INTEG(RAT3FL,0.0) !nmol - amount of T3 formed in liver
RALb_t3=QL*(CA_t3-Cvl_t3)+PAL_t3*(CL_t3-Cvl_t3)-RLt3U !nmol/hr - rate of change in liver blood (t3)
ALb_t3=INTEG(RALb_t3,initAlb_t3) !nmol - amount of t3 in liver blood
Cvl_t3=ALb_t3/(VLB*PL_t3) !nmol/L - concentration of t3 in liver blood
RAL_t3=(PAL_t3*(Cvl_t3-Cl_t3))+RAT3FL-RLAM_t3+RLt3U !nmol/hr - rate of change in liver tissue (t3)
AL_t3=INTEG(RAL_t3,initAl_t3) !nmol - amount of t3 in liver tissue
CL_t3=AL_t3/VL !nmol/L - concentration of t3 in liver tissue
Cl_t3ngg=(CL_t3*MWT3/1000)/1.051 !ng/g - concentration of T3 in liver tissue
(1.051=liver density, Obermoyer 1987)
RLT3U=Cvl_t3*KLUT3 !nmol/hr - 1st order rate of liver uptake of T3
ALT3U=INTEG(RLT3U,0.0) ! nmol - amt of T3 actively transported into liver

RAML_t3=AL_t3*KmetL_t3 ! nmol/hr - rate of T3 metabolism in liver (unspecified) - assume small portion excreted and rest forms free I-

AML_t3=INTEG(RAML_t3,0.0) ! nmol - amount of T3 metabolized in liver

RAT3feces=RAML_t3*0.30 ! nmol/hr - rate of T3 excreted in feces

AT3_feces=INTEG(RAT3feces,0.0) ! nmol - amt of T3 excreted in feces
d_AT3_feces=(AT3_feces/(((t+1e-6)/24))) ! nmol/d - amt of T3 excreted in feces/day

!----Total production of T3
TAPR_t3=AT3FVd+AT3FL+APR_t3 ! nmol - total amount of T3 produced
TAPR_t3ug=TAPR_t3*MWT3/1000 ! ug - total amt of T3 produced
d_PRT3ugd=TAPR_t3ug/((t+1e-6)/24) ! ug/d - whole body production of T3 per day
FAPR_t3Thy=(APR_t3Tug/(TAPR_t3ug+1e-6))*100 ! % of total T3 prod that occurs in the thyroid

!----T3 Metabolism contribution of pathways
RWBT3Met=RAML_t3+RVdel_t3 ! nmol/hr - Rate of overall T3 metabolism
AWBT3Met=INTEG(RWBT3Met,0.0) ! nmol - total amount of T3 metabolized
d_AWBT3Met=(AWBT3Met/(((t+1e-6)/24))) ! nmol - total daily amount of T3 metabolized

FMWBT3=(AWBT3Met/(TAPR_t3+1e-6))*100 ! % of produced T3 that is metab - sum of all pathways
FMVdT3=(AVdel_t3/(TAPR_t3+1e-6))*100 ! % of produced T3 that is metabolized in the Vd
FMLT3=(AML_t3/(TAPR_t3+1e-6))*100 ! % of produced T3 that is metabolize in the liver
FMFeT3=(AT3_feces/(TAPR_t3+1e-6))*100 ! % of produced T3 that is excreted in Feces

!-----END HPT AXIS MODEL-----------------------------------------------
!----------MASS BALANCES---------------------
!----------Mass balance TSH-------------------
TSHint=initAVdTSH !initial amts of TSH
TMASStsh=AClTSH+AvdTSH !total mass TSH
BALANCEtsh=TSHint+ATSHPR-TMASStsh !mass balance TSH (initial amt + amt produced - total mass)

!----------Mass balance T4---------------------
T4int=initAp_t4+initAlb_t4+initAl_t4 !initial amts of T4
tformt4=APR_t4 !total amt T4 produced in thyroid
TMASSt4=AP_t4+AL_t4+ADIL+Avdel_t4+AGL+Alb_t4 !total mass T4
BALANCEt4=T4int+tformt4-TMASSt4 !mass balance t4

!----------Mass balance T3---------------------
T3int=initAp_t3+initAlb_t3+initAl_t3 !initial amts of T3
tformt3=APR_t3+AT3FL+AT3FVd !total amount of T3 formed
TMASSt3=AP_t3+AL_t3+Avdel_t3+AML_t3 !total mass of T3
BALANCEt3=T3int+tformt3-TMASSt3 !mass balance t3

!----------Mass balance Iodide-------------------
Iint=initAP_i+initAtb_i+initdAt_i+initdAIB_i !initial amts of I
TMASSi=Atb_i+Atf_i+AIB_i+Ath+AP_i+AU_i !total mass of I
tformi=AST_i+AiFL_t4+AiFL_t3+AiFVd_t4+AiFVd_t3 !dose I & I freed from metabolism of T4 and T3
BALANCEi=Iint+tformi-TMASSi !mass balance iodide

!Days
days=((t+1e-6)/24) !days of model execution
END  !  DERIVATIVE

TERMT (T .GE. TSTOP, 'checked on communication interval: REACHED TSTOP')
END  !  DYNAMIC TERMINAL
T4END=ca_t4ngg
T3END=ca_t3ngg
TSHEND=tshngml
T4ENDPC=t4percon
TSHENDPC=tshpercon

END  !  TERMINAL

END  !  PROGRAM
APPENDIX D

The acslXtreme (version 2.4.0.11) .m file for BBDR-HPT axis iodide sufficient and deficient model simulations (Chapter 4) is contained within this Appendix. The .m file is organized by the order the figures appear in the Chapter 4. Code to simulate steady-state iodide sufficiency plots (iodide, T4, T3, and TSH) are followed by the sequences used to generate the iodide deficiency simulations compared to literature data. Finally, the .m file used to generate the iodide dose response plot for T4 and TSH is included.
% M file to plot Figures for BBDR-HPT axis LID model paper

%Figure 5A - Serum free iodide (ug/dL) and total amt in thyroid (ug)
%Male SD rats, 320g
%time(hrs), tat_iug(Yu2002-ug), and serum free iodide (Eng1999-ng/mL)
ISS = [  50 15 7.3
        50 12 10
        50 18  7
        50 NaN  7.6
        50 NaN 11.4
        50 NaN  8.6];

!!s bwgon=0, ivdosep=0, pdose_p=0, pdose1=20, tstop=100
!!BW320
!!prepare t, tat_iug, cp_iugdl, ca_bingml
!!start/nc
plot (_t,_tat_iug,ISS(:,1),ISS(:,2),_t,_cp_iugdl,ISS(:,1),ISS(:,3),'Fig5A_LIDpaper.aps');

%Figure 5B - Serum TSH (ng/mL)
%time(hrs), serum tsh (McLanahan2007-ng/mL)
TSHSS = [  50 6.5084
          50 9.0293
          50 3.9875];

!!s bwgon=0, pdose1=20, pdose_p=0, ivdosep=0, tstop=100
!!BW320
!!prepare t, tshngml
!!start/nc
plot (_t,_tshngml,TSHSS(:,1),TSHSS(:,2),'Fig5B_LIDpaper.aps');
Figure 5C - Serum T4 (ng/g) and Liver T4 (ng/g)
%time(hrs), serum T4 (McLanahan2007-ng/g), liver T4 (MorrealdeEscobar1994-ng/g)
T4SS = [ 50 40.6145991 25.51 50 29.3301941 23.22 50 51.89900409 18.7];
% Figure 5D - Serum T3 (ng/g) and Liver T3 (ng/g)
%time(hrs), serum T3 (McLanahan2007-ng/g), liver T3 (MorrealdeEscobar1994-ng/g)
T3SS = [ 50 0.461260479 4.91 50 0.358993714 5.71 50 0.563527244 3.72];

%%M file to plot FIGURE 6 for LID paper (LID plots)
%%Figure 6 - Riesco 1977 LID Diet

% 0.35ugI/day HSD rats
%time(days),total thyroid iodide(ug),serum T4(ng/g),serum T3(ng/g),serum TSH(fold change)

LIDR77A = [ 0 5.3632041 51.39660852 0.568138533 1
0 5.3632041 56.32452264 0.661482971 1
0 5.3632041 46.4686944 0.474794095 1
2 4.587266532 53.89146044 0.646783395 1.000001408
2 5.236509422 60.14695388 0.724539546 1.46666769
2 3.938023642 47.635967 0.569027244 0.533335126
4 3.414502333 51.46340794 0.703138094 1.733334979
4 4.485216854 61.299193794 0.823178757 2.400001024
4 2.343787812 41.62762194 0.583097431 1.066668934
6 2.275680693 36.50433073 0.51504231 1.266669585
6 3.005231253 43.66491697 0.635082974 1.733335764
6 1.546130133 29.34374449 0.395001646 0.866670614
8 1.83121078 30.95354164 0.589243891 1.600003094
8 2.645512921 39.9028406 0.686994446 2.333335574
8 1.016729235 21.99779922 0.491493336 0.866670614
11 1.189315295 20.817126 0.605017283 1.80000344
11 1.685409814 28.19651748 0.791706048 3.000001902
11 0.693220776 13.43782772 0.418328518 2.666669297
15 0.512031337 15.96106834 0.455503371 3.500001435
15 0.788775688 21.99780294 0.615310738 3.500001435
15 0.835268986 9.296333739 0.295696004 1.833337159
26 0.05 2.28122021 0.434472206 7.533332893
26 0.05 4.444817479 0.518893137 12.73331992
26 0.05 0.117622941 0.350051275 2.333336868];

!!s bwc=0.120, bwgon=0, pdose_p=0, tstop=630, pdose1=0.35
!!BW120
!!prepare days, tat_iug, ca_t4ngg, ca_t3ngg, tshfold
!!start/nc

plot(_days,_tat_iug,LIDR77A(:,1),LIDR77A(:,2),_days,_ca_t4ngg,LIDR77A(:,1),LIDR77A(:,3),_days,_ca_t3ngg,LIDR77A(:,1), LIDR77A(:,4),_days,_tshfold,LIDR77A(:,1),LIDR77A(:,5),'Fig6_LIDpaper.aps')
% Figure 7A - Okamura 1981 Simonsen Albino rats
% 0.45ugI/day (0.3-0.36?)
% time(days), total thyroid iodide(ug), serum T4(ng/g), serum T3(ng/g), serum TSH(fold change)

OK81SA = [  
0  10.82592  53  0.59 1  
0  13.68276  67  0.76 NaN  
0  7.96908  39  0.42 NaN  
14  1.925828  23  0.56 2.709677419  
14  3.426841  34  0.64 NaN  
14  0.424815  12  0.48 NaN  
28  0.642369  12  0.48 4.0609319  
28  0.98826  21  0.59 NaN  
28  0.296478  3  0.37 NaN  
56  0.4872  5.2  0.34 20.1827957  
56  0.5684  7.2  0.44 NaN  
56  0.4063.2  0.24 NaN  
84  0.478977  6.1  0.3 16.74910394  
84  0.623784  8.1  0.357 NaN  
84  0.33417  4.1  0.243 NaN ];

!!s bwgon=0, tstop=2016, pdose1=0.30
!!BW270
!!prepare days, tat_iug, ca_t4ngg, ca_t3ngg, tshfold
!!start/nc

plot(_days,_tat_iug,OK81SA(:,1),OK81SA(:,2),_days,_ca_t4ngg,OK81SA(:,1),OK81SA(:,3),_days,_ca_t3ngg,OK81SA(:,1),OK81SA(:,4),_days,_tshfold,OK81SA(:,1),OK81SA(:,5),'Fig7A_LIDpaper.aps')
%Figure 7B - Okamura 1981 Holtzman Sprague-Dawley rats
%0.45ug/day (0.3-0.36?)
%time(days),total thyroid iodide(ug),serum T4(ng/g),serum T3(ng/g),serum TSH(fold change)

OK81HSD = [ 0 9.68575 43 0.72 1
0 11.28105 52 0.789 NaN
0 8.09045 34 0.651 NaN
14 2.51784 34 0.72 1.272171254
14 3.67992 39 0.786 NaN
14 1.35576 29 0.654 NaN
28 2.854764 24 0.65 1.336391437
28 4.786776 39 0.76 NaN
28 0.922752 9 0.54 NaN
56 1.017093 12 0.58 3.972477064
56 2.082619 18 0.623 NaN
56 0 6 0.537 NaN
84 0.645216 7.8 0.44 11.82874618
84 0.852016 10.8 0.55 NaN
84 0.438416 4.8 0.33 NaN];

!!s bwgon=0, tstop=2016, pdose1=0.30
!!BW320
!!prepare days, tat_iug, ca_t4ngg, ca_t3ngg, tshfold
!!start/nc

plot(_days,_tat_iug,OK81HSD(:,1),OK81HSD(:,2),_days,_ca_t4ngg,OK81HSD(:,3),_days,_ca_t3ngg,OK81HSD(:,4),_days,_tshfold,OK81HSD(:,5),'Fig7B_LIDpaper.aps')
Figure 8 - Okamura 1981 Holtzman Sprague-Dawley rats
%1.14ugI/day, bw at end=0.391kg, at start=0.106kg
%time(days), total thyroid iodide (ug), serum T4 (ng/g), serum T3 (ng/g)

```
OK81BHSD = [ 19 3.6656 35 0.67
19 1.8676 30 0.62
19 5.4636 40 0.72
33 1.950676 28 0.65
33 1.573476 21 0.56
33 2.327876 35 0.74
63 3.4572 25 0.6
63 2.8452 23 0.52
63 4.0692 27 0.68
96 2.934455 18 0.51
96 2.444655 13 0.45
96 3.424255 23 0.57];
```

!!BW110
!!s bws=110000, bwgon=1, pdose1=1.14, tstop=2304
!!prepare days, tat_iug, ca_t4ngg, ca_t3ngg
!!start/nc

plot(_days,_tat_iug,OK81BHSD(:,1),OK81BHSD(:,2),_days,_ca_t4ngg,OK81BHSD(:,1),OK81BHSD(:,3),_days,
_ca_t3ngg,OK81BHSD(:,1),OK81BHSD(:,4),'Fig8_LIDpaper.aps');
%M file to plot FIGURE 9 for LID paper (LID plots)
%This data still has to be exported and percent baseline calculated to show in paper

Figure 9 - Fukuda 1975 I Refeeding study adult male Sprague-Dawley rats

Fed LID(0.6ug/day) for 7 months, then supplemented with iodide to provide total intake of 2.6ugI/day or 8.6ugI/day beginning on day 0 and continuing for 9 days

time(days), serum T4 (ng/g), serum TSH (ng/ml)

FRF2p6 = [ 5040 4.516133379 154.4905452
          5064 15.43012166 73.49038009
          5088 18.81722635 23.78036763
          5112 18.81723237 15.11962426
          5184 25.96777454 17.5055683
          5256 42 10.10610003 ];

FRF8p6 = [ 5040 4.892476996 140.8057019
          5064 23.3333464  37.32329471
          5088 31.61292306 12.0505026
          5112 27.84948729 13.16990279
          5184 36.88174628 9.685660734
          5256 60.59140593 7.173635116 ];

!!BW400
!!s tstop=5260, bwgon=1, bws=400000, pdose1=0.2, pdose2=2.6, pdose_p=0
!!prepare t, ca_t4ngg, tshngml
!!start/nc
t4FRF2p6= ca_t4ngg
tshFRF2p6=_tshngml

!!BW400
!!s tstop=5260, bwgon=1, bws=400000, pdose1=0.2, pdose2=8.6, pdose_p=0
!!prepare t, ca_t4ngg, tshngml
!!start/nc
t4FRF8p6= ca_t4ngg
tshFRF8p6=_tshngml

plot(_t,t4FRF2p6(:,1),FRF2p6(:,1),FRF2p6(:,2),_t,tshFRF2p6(:,1),FRF2p6(:,1),FRF2p6(:,3),_t,t4FRF8p6(:,1),FRF8p6(:,1),FRF8p6(:,2),_t,tshFRF8p6(:,1),FRF8p6(:,1),FRF8p6(:,3),'fFig9_LIDpaper.aps')
%%M file to generate dose response plot for iodide. This is case sensitive.

!!prepare t, t4end, tshend
output @Clear
global PDOSE1
!!BW320
!!s tstop=1000
!!s pdose_p=0.01
pdose1=[0.1:0.1:20]
for x=[1:200]
    PDOSE1=pdose1(x)
    start @NoCallback
    pdosei(x)=PDOSE1;
    t4final(x)=T4END;
    tshfinal(x)=TSHEND;
end
plot(pdosei,t4final,pdosei,tshfinal);
APPENDIX E

The procedure (PROCED) commands used in the BBDR-HPT axis model manuscript (Chapter 4) to set the initial values at each starting body weight are included in this Appendix. Initial values were determined by running the model to steady-state (2000hrs) under iodide sufficient (20 µg I/day) conditions. The PROCEDs were executed at the start of simulations for their corresponding body weight (see .m file code) to ensure steady-state conditions at the start of simulation.
!!PROCED BW500
s  bwc =  0.5
s  t4b =  28.2258
s  t3b =  0.413853
s  tshb =  8.7843
s  initavdtsh =  0.00869018
s  initap_t4 =  2.16906
s  initalb_t4 =  0.174259
s  inital_t4 =  0.304689
s  initalb_t3 =  0.00886592
s  inital_t3 =  0.074726
s  initap_t3 =  0.0474901
s  initap_i =  285.104
s  initatb_i =  0.00446223
s  initdat_i =  10.3138
s  initdaib_i =  149.254
END

PROCED BW450
s  bwc =  0.45
s  t4b =  30.5456
s  t3b =  0.42488
s  tshb =  8.12055
s  initavdtsh =  0.00723019
s  initap_t4 =  2.11259
s  initalb_t4 =  0.169723
s  inital_t4 =  0.296861
s  initalb_t3 =  0.00807036
s  inital_t3 =  0.0731042
s  initap_t3 =  0.0438799
s  initap_i =  250.898
s  initatb_i =  0.00392517
s  initdat_i =  8.52623
s  initdaib_i =  145.439
END

PROCED BW400
s  bwc =  0.4
s  t4b =  33.3644
s  t3b =  0.437739
s  tshb =  7.43768
s  initavdtsh =  0.00588639
s  initap_t4 =  2.05115
s  initalb_t4 =  0.164788
s  inital_t4 =  0.28835
s  initalb_t3 =  0.00725953
s  inital_t3 =  0.0713137
s  initap_t3 =  0.0401849
s  initap_i =  217.565
s  initatb_i =  0.00340184
s  initdat_i =  6.879
s  initdaib_i =  141.28
END

PROCED BW390
s  bwc =  0.39
s  t4b =  34.0033
s  t3b =  0.440585
s  tshb =  7.29855
s  initavdtsh =  0.00563188
s  initap_t4 =  2.03817
s  initalb_t4 =  0.163745
s  inital_t4 =  0.286553
s  initalb_t3 =  0.00709545
s  inital_t3 =  0.0709321
s  initap_t3 =  0.039435
s  initap_i =  210.992
s  initatb_i =  0.00329865
s  initdat_i =  6.56645
s  initdaib_i =  140.4
END

PROCED BW380
s  bwc =  0.38
s  t4b =  34.6715
s  t3b =  0.43537
s  tshb =  7.15852
s  initavdtsh =  0.00538218
s  initap_t4 =  2.02493
s  initalb_t4 =  0.162682
s  inital_t4 =  0.284721
s  initalb_t3 =  0.00693071
s  inital_t3 =  0.0705419
s  initap_t3 =  0.0386813
s  initap_i =  204.454
s  initatb_i =  0.00319601
s  initdat_i =  6.25976
s  initdaib_i =  139.503
END

PROCED BW370
s  bwc =  0.37
s  t4b =  35.3239
s  t3b =  0.430735
s  tshb =  7.02632
s  initavdtsh =  0.00513455
s  initap_t4 =  2.01171
s  initalb_t4 =  0.161623
s  inital_t4 =  0.280817
s  initalb_t3 =  0.00677912
s  inital_t3 =  0.0695767
s  initap_t3 =  0.0379389
s  initap_i =  200.467
s  initatb_i =  0.00299658
s  initdat_i =  5.95207
s  initdaib_i =  135.825
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<td>s bwc = 0.37</td>
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</tr>
<tr>
<td>s t3b = 0.446603</td>
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<td>s tshb = 7.01754</td>
<td>s tshb = 6.58861</td>
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<td>s initap_t4 = 1.9692</td>
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<tr>
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<td>s initalb_t4 = 0.158206</td>
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<td>s inital_t4 = 0.277009</td>
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<tr>
<td>s t3b = 0.449791</td>
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<tr>
<td>s tshb = 6.87558</td>
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<td>s initiap_t4 = 1.9545</td>
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<td>s initalb_t4 = 0.160491</td>
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</tr>
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<td>s initavdtsh = 0.00398709</td>
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<td>s inital_t4 = 0.272896</td>
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<td>s inital_t3 = 0.0679931</td>
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<td>s initiap_t3 = 0.034074</td>
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<td>s initiap_i = 165.964</td>
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<tr>
<td>s initatb_i = 0.00289148</td>
<td>s initatb_i = 0.00259105</td>
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<tr>
<td>s initdat_i = 5.37543</td>
<td>s initdat_i = 4.54528</td>
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<tr>
<td>s initdaib_i = 136.697</td>
<td>s initdaib_i = 133.699</td>
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<table>
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</table>
PROCED BW310
s  bwc  =  0.31
s  t4b  =  40.3832
s  t3b  =  0.467927
s  tshb  =  6.14992
s  initavdtsh  =  0.0037721
s  initap_t4  =  1.92405
s  initalb_t4  =  0.154579
s  inital_t4  =  0.270767
s  initap_i  =  159.672
s  initatb_i  =  0.002493
s  initdat_i  =  4.28098
s  initdaib_i  =  132.652
END

PROCED BW300
s  bwc  =  0.3
s  t4b  =  41.387
s  t3b  =  0.472085
s  tshb  =  6.00132
s  initavdtsh  =  0.00356221
s  initap_t4  =  1.90826
s  initalb_t4  =  0.153312
s  inital_t4  =  0.268586
s  initap_i  =  153.421
s  initatb_i  =  0.00239498
s  initdat_i  =  4.02313
s  initdaib_i  =  131.578
END

PROCED BW290
s  bwc  =  0.29
s  t4b  =  42.451
s  t3b  =  0.476457
s  tshb  =  5.85145
s  initavdtsh  =  0.003356221
s  initap_t4  =  1.89208
s  initalb_t4  =  0.152011
s  inital_t4  =  0.2670531
s  initap_i  =  147.207
s  initatb_i  =  0.00229735
s  initdat_i  =  3.77172
s  initdaib_i  =  130.476
END

PROCED BW280
s  bwc  =  0.28
s  t4b  =  43.581
s  t3b  =  0.481064
s  tshb  =  5.70026
s  initavdtsh  =  0.00315795
s  initap_t4  =  1.87547
s  initalb_t4  =  0.150679
s  inital_t4  =  0.264055
s  initap_i  =  141.04
s  initatb_i  =  0.00220056
s  initdat_i  =  3.52694
s  initdaib_i  =  129.345
END

PROCED BW270
s  bwc  =  0.27
s  t4b  =  44.7838
s  t3b  =  0.48931
s  tshb  =  5.54769
s  initavdtsh  =  0.00296366
s  initap_t4  =  1.8584
s  initalb_t4  =  0.149306
s  inital_t4  =  0.261699
s  initap_i  =  134.896
s  initatb_i  =  0.00210412
s  initdat_i  =  3.28835
s  initdaib_i  =  128.182
END

PROCED BW260
s  bwc  =  0.26
s  t4b  =  46.067
s  t3b  =  0.491084
s  tshb  =  5.39368
s  initavdtsh  =  0.00277466
s  initap_t4  =  1.84085
s  initalb_t4  =  0.147896
s  inital_t4  =  0.259277
s  initap_i  =  128.791
s  initatb_i  =  0.00200831
s  initdat_i  =  3.05644
s  initdaib_i  =  126.986
END
PROCED BW190
s  bwc  =  0.19
s  t4b  =  58.2524
s  t3b  =  0.538506
s  tshb  =  4.26842
s  initavdtsh  =  0.00160462
s  initap_t4  =  1.70107
s  initalb_t4  =  0.13667
s  inital_t4  =  0.240017
s  initalb_t3  =  0.00366091
s  inital_t3  =  0.0606396
s  initap_t3  =  0.0234818
s  initap_i  =  86.8389
s  initatb_i  =  0.00134997
s  initdat_i  =  1.62027
s  initdaib_i  =  117.436
END

PROCED BW180
s  bwc  =  0.18
s  t4b  =  60.6559
s  t3b  =  0.547625
s  tshb  =  3.89072
s  initavdtsh  =  0.00146009
s  initap_t4  =  1.67803
s  initalb_t4  =  0.134819
s  inital_t4  =  0.236849
s  initalb_t3  =  0.00348039
s  inital_t3  =  0.0599106
s  initap_t3  =  0.0226226
s  initap_i  =  80.9273
s  initatb_i  =  0.00125722
s  initdat_i  =  1.44274
s  initdaib_i  =  115.857
END

PROCED BW170
s  bwc  =  0.17
s  t4b  =  63.304
s  t3b  =  0.55761
s  tshb  =  3.92864
s  initavdtsh  =  0.00132143
s  initap_t4  =  1.65399
s  initalb_t4  =  0.132889
s  inital_t4  =  0.233545
s  initalb_t3  =  0.00329887
s  inital_t3  =  0.0599106
s  initap_t3  =  0.0217554
s  initap_i  =  75.0024
s  initatb_i  =  0.00125722
s  initdat_i  =  1.44274
s  initdaib_i  =  115.857
END

PROCED BW160
s  bwc  =  0.16
s  t4b  =  66.238
s  t3b  =  0.568605
s  tshb  =  3.75504
s  initavdtsh  =  0.00118874
s  initap_t4  =  1.62885
s  initalb_t4  =  0.130869
s  inital_t4  =  0.230092
s  initalb_t3  =  0.0031163
s  inital_t3  =  0.0583441
s  initap_t3  =  0.0208794
s  initap_i  =  69.1282
s  initatb_i  =  0.00107211
s  initdat_i  =  1.10938
s  initdaib_i  =  112.48
END

PROCED BW150
s  bwc  =  0.15
s  t4b  =  69.5089
s  t3b  =  0.58079
s  tshb  =  3.57873
s  initavdtsh  =  0.00106211
s  initap_t4  =  1.60245
s  initalb_t4  =  0.12875
s  inital_t4  =  0.226469
s  initalb_t3  =  0.00293264
s  inital_t3  =  0.0574976
s  initap_t3  =  0.0199939
s  initap_i  =  63.2203
s  initatb_i  =  0.00107211
s  initdat_i  =  0.953656
s  initdaib_i  =  110.662
END

PROCED BW140
s  bwc  =  0.14
s  t4b  =  73.1804
s  t3b  =  0.594391
s  tshb  =  3.39956
s  initavdtsh  =  0.00094178
s  initap_t4  =  1.57462
s  initalb_t4  =  0.126514
s  inital_t4  =  0.226469
s  initalb_t3  =  0.0027478
s  inital_t3  =  0.0566011
s  initap_t3  =  0.019098
s  initap_i  =  57.2967
s  initatb_i  =  0.00086515
s  initdat_i  =  0.805462
s  initdaib_i  =  108.74
END
PROCED BW130
s  bwc   =  0.13
s  t4b   =  77.3305
s  t3b   =  0.609676
s  tshb  =  3.21748
s  initavdtsh =0.000827583
s  initap_t4  =  1.54507
s  initialb_t4  =  0.124141
s  initial_t4  =  0.218601
s  initialb_t3  =  0.00256163
s  initial_t3  =  0.055645
s  initap_t3  =  0.0181899
s  initap_i  =  51.3558
s  initatb_i  =0.000793333
s  initdat_i  =  0.665139
s  initdaib_i  =  106.684
END
PROCED BW120
s  bwc   =  0.12
s  t4b   =  82.0362
s  t3b   =  0.626885
s  tshb  =  3.03328
s  initavdtsh =0.000720188
s  initap_t4  =  1.513
s  initialb_t4  =  0.121566
s  initial_t4  =  0.214206
s  initialb_t3  =  0.00237349
s  initial_t3  =  0.0546065
s  initap_t3  =  0.172646
s  initap_i  =  45.376
s  initatb_i  =0.000699542
s  initdat_i  =  0.532868
s  initdaib_i  =  104.406
END
PROCED BW110
s  bwc   =  0.11
s  t4b   =  87.0891
s  t3b   =  0.644923
s  tshb  =  2.85764
s  initavdtsh =0.000621944
s  initap_t4  =  1.47235
s  initialb_t4  =  0.1183
s  initial_t4  =  0.208594
s  initialb_t3  =  0.00217764
s  initial_t3  =  0.0533231
s  initap_t3  =  0.0162813
s  initap_i  =  39.3567
s  initatb_i  =0.000605082
s  initdat_i  =  0.410314
s  initdaib_i  =  101.124
END
!!
APPENDIX F

The acslXtreme (version 2.4.0.11) .csl file for BBDR-HPT axis and ClO₄⁻ PBPK model code (Chapter 5) is contained within this Appendix. Model code is structured as follows: First section contains physiological parameters and compound-specific constants. Second section includes scaled model parameters. Last section includes model code for the BBDR-HPT axis (TSH, iodide, T4, and T3) and is followed by the ClO₄⁻ PBPK model code.
PROGRAM: Male Rat HPT Axis Model
!-----------------------------------------------------------------------
!File name:EHPTnogut.csl created 03/01/07 from EHPT_Imod_021407.csl
!Units in nmol, L, hr, kg !Endogenous I, T4, T3, and TSH, and now combined with ClO4 PBPK model
!Blood binding of T4 99% bound - taken into account for liver uptake (0.01*cvl)
!Last Revised 10/12/07 by Eva McLanahan

INITIAL
CONSTANT  TSTOP=1416  !Length of experiment (1416=59 days)
CONSTANT  CINT=0.5  !communication interval

! ------------ Physiological Parameters------------------
CONSTANT  QCC = 14.0  !L/hr/kg - Total cardiac output [Brown 1997
CONSTANT  QLC = 0.174  !%QC - Proportion cardiac output to the liver [Brown 1997 p438]
CONSTANT  QTC = 0.016  !%QC - Proportion cardiac output to the thyroid- human value (Brown 1997 & Merrill 2003)
CONSTANT  VLC = 0.0366 !%BW liver tissue [Brown 1997 pg 416]
CONSTANT  VLBC = 0.21  !%VL as liver blood [Brown 1997]
CONSTANT  VTC = 0.00005 !%BW total thyroid tissue [McLanahan 2007]
CONSTANT  VTBC = 0.157 !%VT as thyroid blood [Merrill et al 2003]

!------GROWTH EQUATION PARAMETERS--------
CONSTANT BWC = 0.320   !kg - body weight (a constant body weight)
CONSTANT BWGon = 0   !if BWGon=1 then BW growth equation on, else uses BWC
CONSTANT BWs = 170000  !mg - BW for start of study (must be given in lit.)
CONSTANT BWt0 = 7314.70  !mg - initial BW at birth (Mirfazaelian 2007)
CONSTANT KBW = 63.21   !days - age at inflection point (Mirfazaelian 2007)
CONSTANT BWtmax = 521026.13 !mg - maximum body weight (Mirfazaelian 2007)
CONSTANT gammaBW = 2.01  !unitless - hill coeff for BW growth (Mirfazaelian 2007)

!--------- TSH Parameters-------------------------
CONSTANT MWTSH = 28000 !g/mol - molecular weight TSH [chemfinder.com]
CONSTANT Vd_TSHC = 0.0554 !L/kg - VdTSH - (Connors et al 1984)
CONSTANT K0TSHmaxC = 6  !nmol/hr - Max prod of TSH (absence of T4) (Connors 1984)
!CONSTANT Kinh_T4 = 0.2 !nmol/L - Km of T4 such that prod of TSH is 1/2 maximal
CONSTANT  Kinh_T4  =  0.3  !nmol/L - Km of T4 such that prod of TSH is 1/2 maximal  
(changed for Clo4 model)

CONSTANT  KNIS_TSH  =  0.949  !nmol/L - TSH conc so Vmax of NIS I transport is 1/2 max

CONSTANT  KbTSH  =  733.98  !nmol/L - Km TSH conc such that I binding/organification in 
thyroid is 1/2 maximal  

CONSTANT  Ke1_TSHC  =  1.8899  !1/hr-kg - elimination rate constant for TSH from Vd  
(Lemarchand-Beraud and Bertheir 1981)

CONSTANT  TSHb  =  5.08373  !ng/ml - TSH baseline to calc fold change. Set for each BW

CONSTANT  np  =  0.94  !unitless - Hill coefficient for production/feedback  

!------------ I (Iodide) Parameters----------------------

CONSTANT  MWI  =  126.90447  !g/mol - molecular weight I [periodic table]

CONSTANT  Vd_iC  =  0.5  !L/kg - volume of distribution of iodide

CONSTANT  Km_i  =  31519  !nmol/L - affinity constant I for NIS (Gluzman and  
Niepomniszcze 1983 and Merrill 2003)

CONSTANT  VmaxT_iC  =  5738.267  !nmol/hr/kg - maximal rate of uptake

CONSTANT  PA_iC  =  0.0001  !L/hr - PA term thyroid [Merrill 2003]

CONSTANT  V0bindC  =  1005.9  !nmol/hr-kg - maximum rate of binding of iodide in thyroid

CONSTANT  Kmb_i  =  244.59  !nmol/L - Km of iodide binding

CONSTANT  ClU_iC  =  0.0046  !L/hr-kg - urinary clearance of iodide

!-------Thyroid hormone production parameters--------------

CONSTANT  ktshcib  =  5e-7  !L2/nmol-hr - rate constant for thyroid hormone production

CONSTANT  np2  =  2  !unitless - Hill coefficient for TH production (clo4 supp.)

!------------ T4 (Thyroxine) Parameters---------------------

CONSTANT  MWT4  =  776.8742  !g/mol - molecular weight T4 [calculated:C15H11I4NO4]

CONSTANT  MWT4G  =  952  !g/mol - molecular weight T4-Glucuronide  
[calculated: T4(776)+GA(194)-H2O(18)=952]

CONSTANT  Vd_t4c  =  0.156  !L/kg bw - vd t4 (Kohn 1996)

CONSTANT  PL_t4  =  1.27  !Partition coefficient for T4 liver (EscobarMorreale 1996)

CONSTANT  PAL_t4C  =  0.0423  !nmol/hr/kg - from tracer - PA term for T4 liver

CONSTANT  VMAXDIC  =  19.89  !nmol/hr/kg - Vmax outer ring deiodinase

CONSTANT  KMDI  =  2300  !nmol/L - Km outer ring deiodinase in liver  
(Leonard and Visser 1986)

CONSTANT  KEL_t4C  =  0.05  !1/hr-kg - rate of elimination of T4 from body (Vd)  
(Abrams & Larsen 1973 t1/2 used for calculation)
CONSTANT KmUGT = 100000 \text{ nmol/L} - \text{(Km of UGT enzymes for T4 and T3)} \text{ (Visser 1993)}

CONSTANT VmaxT4GC = 3435.89 \text{ nmol/hr} - \text{max rate of T4-G formation in liver}

CONSTANT VMAXT4LUC = 4384.73 \text{ nmol/hr} - Vmax for active uptake of T4 into liver

CONSTANT KmT4LU = 650 \text{ nmol/L} - \text{Km for uptake in hepatocytes} \text{ (Blondeau 1988)}

CONSTANT FFT4 = 0.01 \text{ fraction of free t4 available for uptake to liver}

CONSTANT t4b = 48.8846 \text{ t4 baseline} - \text{to calc % control - diff for each BW}

!------------ T3 (3,5,3'-Triiodothyronine) Parameters------------------

CONSTANT MWT3 = 650.97349 \text{ g/mol} - \text{molecular weight T3 [calculated:C15H12I3NO4]}

CONSTANT Vd_T3C = 0.186 \text{ L/kg} - \text{Vd_T3 per kg BW [thyroid hormone metab pg 67]}

CONSTANT PL_t3 = 4.47 \text{ !Partition coefficient for T3 liver [EscobarMorreale 1996]}

CONSTANT PAL_t3C = 0.1699 \text{ !L/hr/kg} - \text{from tracer - PA term liver T3}

CONSTANT Kel_t3C = 0.12 \text{ !1/hr-kg} - \text{rate of T3 elimination from body}

CONSTANT KmetL_t3C = 3.65 \text{ !1/hr-kg} - \text{fractional removal rate from liver}

CONSTANT KLUT3 = 1.25 \text{ !L/hr} - \text{1st order liver uptake rate of T3}

CONSTANT t3b = 0.556647 \text{ t3 baseline} - \text{to calc % control - diff for each BW}

!---T4 and T3 iodide equivalents

CONSTANT I4CON = 0.6534 \text{ !Fraction of t4 as iodine (4 Im.w./T4m.w.) T4 iodide equiv}

CONSTANT I3CON = 0.5848 \text{ !Fraction of T3 as iodine (3 Im.w./T m.w.) T3 iodide equiv}

CONSTANT T43CON = 0.8379 \text{ !T3/T4 molar equivalents (T3 m.w. / T4 m.w.)}

CONSTANT IFT4M = 0.16335 \text{ !One I freed in T4 Metabolism (1 mw./T4 m.w.)}

!---IODIDE dosing parameters

!CONSTANT pdose_i = 20 \text{ !ug} - \text{oral dose of iodide (diet I intake for McLanahan 2007 calculated to be 20ug/day)}

!pdose1 and pdose2 used instead of pdose_i for changing iodide intake during studies

CONSTANT pdose1 = 20 \text{ !ug} - \text{1st half of study iodide intake}

CONSTANT pdose2 = 20 \text{ !ug} - \text{2nd half of study iodide intake}

!---PERCHLORATE MODEL PARAMETERS-------------------------------

CONSTANT MWP = 99.45 \text{ g/mol} - \text{molecular weight of clo4 (chemfinder.com)}

CONSTANT PT_p = 1 \text{ !unitless -}

CONSTANT PBody_p = 0.36 \text{ !unitless - should be weighted (0.416 from Merrill)}

CONSTANT VmaxT_pC = 177 \text{ !nmol/L/kg} - \text{opt - M.M Vmax for clo4 at thyroid NIS}
CONSTANT Km_p = 1500 !nmol/L - Michaelis-Menten Km for clo4 at thyroid NIS
(Kosugi et al 1996 - Ki value)
CONSTANT Ki_p = 1500 !nmol/L - Michaelis-Menten Km for clo4 at thyroid NIS
(Kosugi et al 1996 - Ki value)
CONSTANT PAT_pC = 0.00028 !L/hr/kg - OPT - thyroid permeation coef-area cross product
CONSTANT CLU_pC = 0.018 !L/hr-kg - OPT - urinary clearance of perchlorate 0.025
CONSTANT VmaxB_pC = 6000 !nmol/hr/kg - Vmax binding perchlorate to serum proteins
CONSTANT KmB_p = 225000 !nmol/L - Ka binding perchlorate to serum proteins
CONSTANT Clunb_pC = 0.032 !L/hr-kg - 1st order unbind of clo4 in serum (Merrill 2003)
CONSTANT KNIS_TSHp = 0.949 !nmol/L - OPT - TSH ½ max concentration for upreg of NIS
CONSTANT pat_ip = 0.0001 !just testing to see if diffusion out helps

!Compartment initial amounts from running EHPT model to steady state 2000hrs (1/27/07)
CONSTANT initAvdTSH = 0. !nmol TSH - initial amt of TSH in Vd
CONSTANT initAP_t4 = 0. !nmol T4 - initial amt of T4 in Vd (blood)
CONSTANT initAlb_t4 = 0. !nmol T4 - initial amt of T4 in liver blood
CONSTANT initAl_t4 = 0. !nmol T4 - initial amt of T4 in liver tissue
CONSTANT initAlb_t3 = 0. !nmol T3 - initial amt of T3 in liver blood
CONSTANT initAl_t3 = 0. !nmol T3 - initial amt of T3 in liver tissue
CONSTANT initAP_t3 = 0. !nmol T3 - initial amt of T3 in Vd (blood)
CONSTANT initAP_i = 0. !nmol I - initial amt of iodide in Vd (blood)
CONSTANT initAtb_i = 0. !nmol I - initial amt of iodide in thyroid blood
CONSTANT initdAT_i = 0. !nmol I - initial amt of free iodide in thyroid tissue
CONSTANT initdAIB_i = 0. !nmol I - initial amt of bound iodide in thyroid tissue

END ! INITIAL

DYNAMIC
ALGORITHM IALG = 2 !Gear method for stiff systems
DERIVATIVE
if (BWGon.eq.1) then
  BW=BWG
else
  BW=BWC
end if

!------Growth equations------
BWG=(((BWt0*(KBW**(gammaBW))+(BWtmax*(Age**(gammaBW))))/((KBW**(gammaBW))+(Age**(gammaBW))))/10**6
  !BW (kg) at a given Age (days)
Age=Age0+days
  !Age (days)
Age0=KBW*(((BWs-BWt0)/(BWtmax-BWs))**(1/(gammaBW)))
  !Age (days) at start of study if only initial BWs (mg) is given

!------------- Scaled Parameters-------------
QC = QCC*BW**0.75 !L/hr - Cardiac output
QL = QLC*QC !L/hr - blood flow to liver
QT = QTC*QC !L/hr - blood flow to thyroid
VL1 = VLC*BW !L - liver volume with blood
VLB = VLBC*VL1 !L - Volume of liver blood
VL = VL1-VLB !L - Liver tissue volume without blood
VT1 = VTC*BW !L - total thyroid volume
VTB = VTBC*VT1 !L - volume of thyroid blood
VT = VT1-VTB !L - volume of thyroid without blood

!---TSH scaled parameters---
Vd_TSH = Vd_TSHC*BW !L - Vd for TSH
Kel_TSH = Kel_TSHC/BW**0.25 !L/hr - TSH elim rate
K0TSHmax = K0TSHmaxC*BW**0.75 !nmol/hr - max rate of TSH secretion (no T4)
\textbf{!--T4 scaled parameters---}

\begin{itemize}
  \item \[ V_{d_{t4}} = V_{d_{t4}c} \cdot BW - VL1 \] (L - Vd for T4)
  \item \[ V_{\text{MAXDI}} = V_{\text{MAXDIC}} \cdot BW^{0.75} \] (nmol/hr - Vmax for type 1 5'd in liver for t4)
  \item \[ P_{\text{AL}_{t4}} = P_{\text{AL}_{t4}c} \cdot BW^{0.75} \] (L/hr - PA term for liver t4)
  \item \[ K_{\text{el}_{t4}} = K_{\text{EL}_{t4}c} / BW^{0.25} \] (1/hr - T4 elimination rate from body)
  \item \[ V_{\text{maxT4G}} = V_{\text{maxT4GC}} \cdot BW^{0.75} \] (nmol/hr - Vmax for t4 glucuronidation in liver)
  \item \[ V_{\text{maxT4LU}} = V_{\text{maxT4LUC}} \cdot BW^{0.75} \] (nmol/hr - Vmax for liver uptake of t4)
\end{itemize}

\textbf{!--T3 scaled parameters--}

\begin{itemize}
  \item \[ V_{d_{t3}} = V_{d_{t3}c} \cdot BW - VL1 \] (L - Vd for T3)
  \item \[ P_{\text{AL}_{t3}} = P_{\text{AL}_{t3}c} \cdot BW^{0.75} \] (L/hr - PA term for T3 in liver)
  \item \[ K_{\text{el}_{t3}} = K_{\text{EL}_{t3}c} / (BW^{0.25}) \] (1/hr - T3 elimination rate from body)
  \item \[ K_{\text{metL}_{t3}} = K_{\text{metL}_{t3}c} / (BW^{0.25}) \] (1/hr - nonspecific T3 metabolism in liver)
\end{itemize}

\textbf{!--Iodide scaled parameters--}

\begin{itemize}
  \item \[ V_{d_{i}} = V_{d_{ic}} \cdot BW - VT1 \] (L - volume of distribution of iodide)
  \item \[ V_{\text{maxT}_i} = V_{\text{maxT}_iC} \cdot BW^{0.75} \] (nmol/hr - max rate of iodide uptake by NIS in thyroid)
  \item \[ C_{\text{LU}_i} = C_{\text{LU}_ic} / (BW^{0.25}) \] (L/hr - Urinary clearance of iodide)
  \item \[ V_{0\text{bind}} = V_{0\text{bindc}} / (BW^{0.75}) \] (nmol/hr - max rate of binding of iodide in thyroid)
  \item \[ P_{\text{AT}_i} = P_{\text{AT}_ic} \cdot BW^{0.75} \] (L/hr - PA term for thyroidal iodide)
\end{itemize}

\textbf{!--Perchlorate scaled parameters--}

\begin{itemize}
  \item \[ V_{\text{Body}_{p}} = BW - VT - VTB - V_{\text{Plas}} \] (L - volume of "rest of body" for clo4 distribution)
  \item \[ V_{\text{Plas}} = V_{\text{PLC}} \cdot BW \] (L - volume of plasma)
  \item \[ Q_{\text{Body}_{p}} = QC - QT \] (L/hr - blood flow to rest of body for clo4 distribution)
  \item \[ C_{\text{LU}_{p}} = C_{\text{LU}_{pc}} / (BW^{0.25}) \] (L/hr - urinary clearance of clo4 (from rest of body))
  \item \[ V_{\text{maxT}_{p}} = V_{\text{maxT}_{pc}} \cdot BW^{0.75} \] (nmol/hr - vmax for perchlorate uptake into thyroid)
  \item \[ P_{\text{AT}_{p}} = P_{\text{AT}_{pc}} \cdot BW^{0.75} \] (L/hr - PAterm for clo4 diffusion into thyroid)
  \item \[ V_{\text{maxB}_{p}} = V_{\text{maxB}_{pc}} \cdot BW^{0.75} \] (nmol/hr - Vmax binding perchlorate to serum proteins)
  \item \[ C_{\text{unb}_{p}} = C_{\text{unb}_{pc}} / (BW^{0.25}) \] (L/hr - unbinding of clo4 to serum proteins)
\end{itemize}
!----MODEL CODE---TSH volume of distribution with feedback T4-----------------

RTSHPR=(K0TSHmax*Kinh_T4)/(Kinh_T4+Ca_t4) !nmol/hr - Rate of TSH production

RTSHPR=(K0TSHmax*Kinh_T4**np)/(Kinh_T4**np+Ca_t4**np))

ATSHPR=INTEG (RTSHPR,0.0) !nmol - Amount TSH produced

ATSHPRug=ATSHPR*MWTSH/1000 !ug - amount of TSH produced

d_TSHPRugd=(ATSHPRug/(((t+1e-6)/24)))) !ug/d - TSH production

RClTSH=Kel_TSH*AVdTSH !nmol/hr - clearance of TSH from Vd

AClTSH=INTEG (RClTSH,0.0) !nmol - amt of TSH cleared from Vd

RAvdTSH=RTSHPR-RClTSH !nmol/hr - rate of change of TSH in Vd

AVdTSH=INTEG (RAvdTSH,0.0) !nmol/hr - rate of change of TSH in Vd

TSH=AVdTSH/Vd_TSH !nmol/L - Concentration TSH

TSHngml=(TSH/1000) * MWTSH !ng/mL (same as ug/L)- TSH concentration in Vd

TSHFOLD=(TSHngml/TSHb) !fold change tsh

TSHpercon=(TSHngml/TSHb)*100 !TSH as % control

!----MODEL CODE---Iodide, IV dose w/thyroid and Vd -----------

Iodide dosing----------oral dose-----------------

!Change in iodide diet 1X - t=5040 for Fukuda Refeeding data
!pdose1 and pdose2 set in m file

if (t.GT.5040) then
    pdose_i=pdose2
else
    pdose_i=pdose1
end if

!Normal Oral Dosing parameters for I

dose_i = (pdose_i*10**3)/MWI !dietary intake amount (nmol)

Rdose_i = dose_i/12 !dose rate for eating period (hrs) per day

!Food Consumption for a 12 hr period (light-dark cycle in rat)

pflag=pulse(0.0,24.0,12) !for one 12 hr eating period per day

RMR_i = (Rdose_i * pflag) !nmol/hr - dose rate for oral dose iodide

AST_i = INTEG (RMR_i,0.0) !nmol - amt of iodide received orally entering stomach

d_AST_i = AST_i/(((t+1e-6)/24)) !nmol - daily amt of iodide received orally in stomach

d_AST_iug=(d_AST_i*MWI)/(10**3) !ug - daily amt of iodide received orally in stomach
---Volume of Distribution of Free Iodide-----

\[ \text{RAP}_i = \text{RMR}_i + QT \times \text{Cvt}_i - QT \times \text{Ca}_i + \text{RAIFL}_t4 + \text{RAIFL}_t3 + \text{RAIFvd}_t4 + \text{RAIFvd}_t3 - \text{RU}_i \]

\[ \text{AP}_i = \text{INTEG}(\text{RAP}_i, \text{initAP}_i) \]

\[ \text{CP}_i = \text{AP}_i / \text{Vd}_i \]

\[ \text{Ca}_i = \text{CP}_i \]

\[ \text{CP}_{iugdl} = (\text{CP}_i \times \text{MWI} / 10000) \]

\[ \text{Ca}_{bi} = (\text{Ca}_t4 \times \text{I4CON}) + (\text{Ca}_t3 \times \text{I3CON}) \]

\[ \text{Ca}_{bingml} = (\text{Ca}_{bi} \times \text{MWI}) / 1000 \]

\[ \text{Ca}_i + \text{Ca}_{bi} \]

\[ \text{RU}_i = \text{ClU}_i \times \text{Ca}_i \]

\[ \text{AP}_i = \text{INTEG}(\text{RU}_i, 0.0) \]

\[ d_{\text{AP}_i} = \text{AU}_i / ((t + 1e-6) / 24) \]

\[ \text{AU}_i = \text{INTEG}(\text{RU}_i, 0.0) \]

\[ \text{d}_{\text{AU}_i} = \text{AU}_i / ((t + 1e-6) / 24) \]

\[ \text{PINEX} = (d_{\text{AU}_i} / (d_{\text{AST}_i} + 1e-6)) \times 100 \]

---Rate of metabolism of TH in Vd -- freeing of iodide

\[ \text{RAIFvd}_t4 = \text{Rvdel}_t4 \times \text{IFT4M} \]

\[ \text{AIFVd}_t4 = \text{INTEG}(\text{RAIFvd}_t4, 0.0) \]

\[ \text{RAIFvd}_t3 = (\text{Rvdel}_t3 \times \text{I3CON}) \]

\[ \text{AIFVd}_t3 = \text{INTEG}(\text{RAIFvd}_t3, 0.0) \]

---Liver iodide metabolism of THs, added to Iodide Vd-------

\[ \text{RAIFL}_t4 = \text{RADIL} \times \text{IFT4M} \]

\[ \text{AIFL}_t4 = \text{INTEG}(\text{RAIFL}_t4, 0.0) \]

\[ \text{RAIFL}_t3 = \text{RAML}_t3 \times 0.70 \times \text{I3CON} \]

\[ \text{AIFL}_t3 = \text{INTEG}(\text{RAIFL}_t3, 0.0) \]

---THYROID IODIDE---------------------------

\[ \text{RTNIS} = (\text{VmaxT}_i \times \text{Cvt}_i) / (\text{Cvt}_i + \text{Km}_i) \]

\[ \text{RTNIS} = (\text{VmaxT}_i \times \text{Cvt}_i) / (\text{Cvt}_i + \text{Km}_i \times (1 + (\text{Cvt}_p / \text{Ki}_p))) \]
!nmol/hr - rate of active uptake (NIS) and inhibition by perchlorate

!very very low NIS to see effects

VmaxT_iTSH=((VmaxT_i*TSH)/(KNIS_TSH+TSH)) !nmol/hr - change in Vmax due to TSH stimulation

ATIU=INTEG(RTNIS,0.0) !nmol - Amount of I uptake (active) into thyroid

!Rate of change of iodide in thyroid blood

RAtb_i=Qt*(CA_i-Cvt_i)+PAt_i*(Ctf_i-Cvt_i)-RTNIS !nmol/hr - rate of change of I in thy blood

RAtf_i=RTNIS+PAt_i*(Cvt_i-Ctf_i)-RIB !nmol/hr - free iodide in thyroid

!Rate of change of FREE IODIDE IN THYROID

dAtf_i=INTEG(RAtf_i,initdAt_i) !nmol - amount of free iodide in thyroid lumen was

Atf_i=MAX(dAtf_i,0)

Atf_iug=Atf_i*MWI/1000 !ug - amount of free iodide in thyroid lumen

Ctf_i=Atf_i/VT !nmol/L - conc of free iodide in thyroid tissue

Ctf_imgl=Ctf_i*MWI/10**6 !mg/L - concentration of free I in thyroid tissue

!Rate of incorporation (binding) of iodide in thyroid tissue

RIB=(Vmaxbt_i*Ctf_i)/(Kmb_i+Ctf_i) !nmol/hr - rate of incorporation of iodide in thyroid

Vmaxbt_i=(V0bind*TSH)/(KbTSH+TSH) !nmol/hr - vmax of binding change (stimulated by TSH concentration in Vd

ARIB=INTEG(RIB,0.0) !nmol - amount of iodide incorporated in thyroid

ARIBug=ARIB*MWI/1000 !ug - amount of iodide incorporated in thyroid

d_aribug=(ARIBug/(((t+1e-6)/24))) !ug - daily amt of iodide incorporated in thyroid

!Rate of change of BOUND iodide in thyroid tissue

RIB_i=RIB-Rth !nmol/hr - rate of change of bound iodide in thyroid (rate of binding - loss as secretion of thyroid hormone)

ARIB_i=INTEG(RIB_i,initdAIB_i)

AIB_iug=(AIB_i*MWI)/1000 !ug - amt iodide bound in thyroid

CIB_i=AIB_i/VT !nmol/L - concentration of iodide bound in thyroid

CIB_imgl=CIB_i*MWI/10**6 !mg/L - concentration of iodide bound in thyroid

!Set a maximum and minimum amt of iodide stores in thyroid - max is not really needed even for iodide up to 500ug/day

if (dAIB_i.GT.160) then
AIB_i=160
else if (dAIB_i.LT.0) then
AIB_i=0
else
AIB_i=dAIB_i
end if

!Rate of utilization of bound I secreted as TH (rate of production of T4 and T3 in iodide equiv.)
Rth=(RPR_t4*I4CON)+(RPR_t3*I3CON) !nmol I/hr - rate of utilization of thyroid I in TH prod
Ath=INTEG(Rth,0.0) !nmol - amount of iodide used in TH prod
d_ath=Ath/((t+1e-6)/24) !nmol/day - daily amt of iodide used in TH prod
Athug=Ath*MWI/1000 !ug - amount of iodide used in TH prod
d_athug=Athug/((t+1e-6)/24) !ug/day - daily amount of iodide used in TH prod
PINITHPR=(d_ath/(d_ast_i+1e-6))*100 !% of daily intake of iodide used in TH prod

!Rate of change of FREE iodide in thyroid tissue - allows you to look at the free entering and leaving, also loss of free to binding
RAt_ien=RTNIS+(PAI_i*Cvt_i) !nmol/hr - rate of free iodide entering thyroid lumen (active uptake and diffusion)
At_i=INTEG(RAt_ien,0.0) !nmol - total amt of I entering thyroid (NIS and diff)
RAt_iex1=(PAI_i*Ctf_i) !nmol/hr - rate of free I diff out of thyroid tissue
At_iex1=INTEG(RAt_iex1,0.0) !nmol - amt of iodide diff out of thyroid (diffusion and secretion as thyroid hormone)
RAt_iex2=RAt_iex1+Rth !nmol/hr - total loss of iodide from thyroid (diffusion and secretion as thyroid hormone)
At_iex2=INTEG(RAt_iex2,0.0) !nmol - total amt of iodide loss from thyroid (diffusion and secretion as thyroid hormone)

!Total Iodide in thyroid tissue (nmol)
TAt_i=AIB_i+Atf_i !nmol - total amount of iodide in thyroid (free and bound)
TAt_img=(TAt_i*MWI)/10**6 !mg - total amount of iodide in thyroid (free and bound)
TCT_imgL=TAt_img/VT !mg/L - total concentration of iodide in thyroid (free and bound)
TAt_iug=TAt_i*MWI/1000 !ug (my data suggests should be between 10-18 ug)
---MODEL CODE---

**Thyroid hormone production in the Thyroid**

**Production of Total thyroid hormones** based on TSH and "bound" iodide pool:

\[
RPR\_th = k_tshcib \cdot TSH \cdot CIB_i \quad \text{nmol/hr - production rate of thyroid hormones (used in ID)}
\]

\[
RPR\_th1 = k_tshcib \cdot TSH \cdot CIB_i \quad \text{nmol/hr - production rate of thyroid hormones}
\]

\[
PTHS = \frac{(K_p\times np3)}{(K_p\times np3 + (C_t_p\times np3))} \quad \text{unitless - inhibition of thyroid production}
\]

\[
Rpr\_th = Rpr\_th1 \cdot PTHS \quad \text{nmol/hr – final rate of thyroid hormone production}
\]

\[
RPR\_th = 0.001 \quad \text{test shut off of thyroid hormone production}
\]

**Fractionation of thyroid hormone production**

\[
dFt3calc = 0.2652 \times ((TAT\_iug)^{-0.4684}) \quad \text{derived from Pedraza 2006}
\]

\[
RPR\_t3 = Ft3calc \cdot RPR\_th \quad \text{nmol/h - rate of T3 production from thyroid}
\]

\[
APR\_t3 = \text{INTEG}(RPR\_t3, 0.0) \quad \text{nmol - amt of T3 produced in thyroid}
\]

\[
d\_PRT3Tugd = (APR\_t3Tug / (((t+1e-6)/24))) \quad \text{ug/day - daily production rate of T3 in thyroid}
\]

\[
RPR\_t4 = RPR\_th - RPR\_t3 \quad \text{nmol/h - rate of T4 production from thyroid}
\]

\[
APR\_t4 = \text{INTEG}(RPR\_t4, 0.0) \quad \text{nmol - amt of T4 produced in thyroid}
\]

\[
d\_PRT4Tugd = (APR\_t4Tug / (((t+1e-6)/24))) \quad \text{ug/day - daily production rate of T4}
\]

\[
d\_PRT4nmold = (APR\_t4 / (((t+1e-6)/24))) \quad \text{nmol/day - daily production rate of T4}
\]

\[
T3/T4 ratio \quad MR34T = APR\_t3 / (APR\_t4 + 1e-6) \quad \text{T3/T4 production ratio}
\]

**Total T4 with a Liver and Vd**

**SERUM T4 (Volume of distribution)**

\[
AP\_t4 = \text{amount of total t4 in Vd} \quad !\text{nmol/hr - rate of change of T4 in serum}
\]

\[
RAP\_t4 = RPR\_t4 + (QL \times CVL\_t4) - QL \times Ca\_t4 - RVdel\_t4 \quad !\text{nmol - amount of T4 in SERUM}
\]

\[
AP\_t4 = \text{INTEG}(RAP\_t4, initAP\_t4) \quad !\text{nmol/L - concentration of T4 in SERUM}
\]

\[
Ca\_t4 = AP\_t4 / Vd\_t4 \quad !\text{ug/dL - T4 in SERUM}
\]

\[
Ca\_t4ugdl = (Ca\_t4 \times MWT4) / 10000 \quad !\text{ng/g or ng/mL - T4 in serum}
\]

\[
Ca\_t4ngg = (Ca\_t4 \times MWT4) / 1000 \quad !\text{%control - serum T4}
\]

\[
t4percon = (ca\_t4ngg / t4b) \times 100 \quad !\text{%control - serum T4}
\]
!AVdel_t4=amount of t4 cleared from vd - assumed to go to t3+free iodide
RVdel_t4=Kel_t4*Ap_t4 !nmol/h - rate of T4 elimination from Vd
AVdel_t4=INTEG(RVdel_t4,0.0) !nmol - amount of T4 eliminated from Vd
d_AVdel_t4=(AVdel_t4/(((t+1e-6)/24))) !nmol/d - daily amount of T4 eliminated from Vd

!-------Liver T4-------------
!Diffusion limited liver with active uptake of free serum T4 (added 3.20.07)
!Cvl*FFT4 assumes only a fraction of the total blood concentration is available for diffusion or
active uptake into the liver
RALb_t4=QL*(Ca_t4-Cvl_t4)+PAL_t4*(CL_t4-(Cvl_t4*FFT4))-RLT4U !nmol/hr - rate of change in liver blood (t4)
ALb_t4=INTEG(RALb_t4,initAlb_t4) !nmol - amount of t4 in liver blood
Cvl_t4=ALb_t4/(VLB*PL_t4) !nmol/L - concentration of t4 in liver blood

RAL_t4=(PAL_t4*((Cvl_t4*FFT4)-CL_t4))-RAGL-RADIL+RLT4U !nmol/hr - rate of change in liver tissue (t4)
AL_t4=INTEG(RAL_t4,initAl_t4) !nmol - amount of T4 in liver tissue
CL_t4=AL_t4/VL !nmol/L - concentration of T4 in liver tissue
CL_t4ngg=(CL_t4*MWT4/1000)/1.051 !ng/g - concentration of T4 in liver tissue
(1.051=liver density, Obermoyer 1987)

RLT4U=(VmaxT4LU*(Cvl_t4*FFT4))/(KmT4LU+(Cvl_t4*FFT4)) !nmol/hr - rate of liver T4 active
uptake (only FRACTION free available)
ALT4U=INTEG(RLT4U,0.0)

!Metabolism of T4 in liver - via deiodination
RADIL=((VMAXDI*Cvl_t4)/(Cvl_t4+KMDI)) !nmol/hr - rate of T4 deiodination in liver (D1)
ADIL=INTEG(RADIL,0.0) !(nmol) Amount of T4 deiodinated (D1) in liver
d_ADIL_T4=(ADIL/(((t+1e-6)/24))) !nmol/d - amount of T4 deiodinated in liver per day
PC43L=(ADIL/(APR_t4+1e-6))*100 !% T4 converted by Type I 5'-D in liver

!Metabolism of T4 in the liver - via glucuronidation
RAGL=(VmaxT4G * Cvl_t4)/(KmUGT + Cvl_t4) !nmol T4/hr - rate of T4-glucuronidation in liver
AGL=INTEG(RAGL,0.0) !nmol T4 lost/used to make T4-G
\[ RAGLT4G = (RAGL \times (MWT4G/MWT4)) \]  
\[ AGLT4G = \text{INTEG}(RAGLT4G, 0.0) \]  
\[ RGLT4Gpmolhr = RAGLT4G \times 1000 \]  
\[ d_{AT4\_feces} = (AGL/((t+1e-6)/24)) \]  
\[ PT4PR\_infeces = (d_{AT4\_feces}/(d_{PRT4nmold+1e-6}))*100 \]  

--- Overall T4 Metabolism ---  
\[ \text{AWBT4Met} = \text{total amount of T4 metabolized (liver gluc + liver deiod + Vd metab)} \]  
\[ RWBT4Met = RVdel\_t4+RAGL+RADIL \]  
\[ \text{AWBT4Met} = \text{INTEG}(RWBT4Met, 0.0) \]  
\[ d_{AWBT4Met} = (AWBT4Met/((t+1e-6)/24)) \]  

AT4T3 = Avdel\_t4+Adil  
FMWT4 = (AWBT4Met/(APR\_t4+1e-6))*100  
FMVdT4 = (AVdel\_t4/(APR\_t4+1e-6))*100  
(FMGLT4 = (AGL/(APR\_t4+1e-6))*100  
FMDILT4 = (ADIL/(APR\_t4+1e-6))*100  
FMT4T3 = FMvdT4+FMDILT4  

---------MODEL CODE--- Total t3, with a Liver and Vd--------  
--------production of T3 in the Vd from T4 metabolism--------  
\[ \text{RT3FVd} = \text{amount of T3 formed in the Vd from T4 metabolism} \]  
\[ RT3FVd = (RVdel\_t4*T4\_CON) \]  
\[ \text{RT3FVd} = \text{INTEG}(RT3FVd, 0.0) \]  
\[ \text{RT3FVdug} = \text{RT3FVd} \times \text{MWT3}/1000 \]  
\[ \text{RAP}\_t3 = (QL*CVL\_t3) - QL*Ca\_t3 - RVdel\_t3 + RPR\_t3 + RT3FVd \]  
\[ \text{AP}\_t3 = \text{INTEG}(\text{RAP}\_t3, \text{initAP}\_t3) \]  
\[ \text{Ca}\_t3 = \text{AP}\_t3/\text{Vd}\_t3 \]  
\[ \text{Ca}\_t3\_ug/dL = \text{Ca}\_t3 \times (0.0001) \times \text{MWT3} \]  
\[ \text{Ca}\_t3\_ng/g = (\text{Ca}\_t3 \times \text{MWT3})/1000 \]  
\[ \text{t3\_per\_con} = (\text{Ca}\_t3\_ng/g/\text{t3\_b}) \times 100 \]
RVdel_t3=Kel_t3*Ap_t3        !nmol/h - rate of t3 elim from Vd
AVdel_t3=INTEG(RVdel_t3,0.0)        !nmol - amount of t3 elim from Vd

!------Liver t3----------
!----production of T3 in liver

RAT3FL=RADIL*T43CON                !nmol/hr - rate of T3 formed in liver from T4 deiod. in T3 equiv.
AT3FL=INTEG(RAT3FL,0.0)  !nmol - amount of T3 formed in liver

!Diffusion limited liver
RALb_t3=QL*(CA_t3-Cvl_t3)+PAL_t3*(CL_t3-Cvl_t3)-RLt3U        !nmol/hr - rate of change in liver blood (t3)
ALb_t3=INTEG(RALb_t3,initAlb_t3)        !nmol - amount of t3 in liver blood
Cvl_t3=ALb_t3/(VLB*PL_t3)            !nmol/L - concentration of t3 in liver blood

RAL_t3=(PAL_t3*(Cvl_t3-CL_t3))+RAT3FL-RAML_t3+RLt3U        !nmol/hr - rate of change in liver tissue (t3)
AL_t3=INTEG(RAL_t3,initAl_t3)        !nmol - amount of t3 in liver tissue
CL_t3=AL_t3/VL        !nmol/L - concentration of t3 in liver tissue
Cl_t3ngg=(CL_t3*MWT3/1000)/1.051    !ng/g - concentration of T3 in liver tissue
(1.051=liver density, Obermoyer 1987)

RLT3U=Cvl_t3*KLUT3        !nmol/hr - 1st order rate of liver uptake of T3
ALT3U=INTEG(RLT3U,0.0)        !nmol - amt of T3 actively transported into liver

RAML_t3=AL_t3*KmetL_t3        !nmol/hr - rate of T3 metabolism in liver (unspecifed) - assume
AML_t3=INTEG(RAML_t3,0.0)  !nmol - amount of T3 metabolized in liver
RAT3feces=RAML_t3*0.30        !nmol/hr - rate of T3 excreted in feces
AT3_feces=INTEG(RAT3feces,0.0)        !nmol - amt of T3 excreted in feces
              d_AT3_feces=(AT3_feces/(((t+1e-6)/24)))        !nmol/d - amt of T3 excreted in feces/day

!----Total production of T3

TAPR_t3=AT3FVd+AT3FL+APR_t3        !nmol - total amount of T3 produced
TAPR_t3ug=TAPR_t3*MWT3/1000        !ug - total amt of T3 produced
d_FRT3ugd=TAPR_t3ug/((t+1e-6)/24)        !ug/d - whole body production of T3 per day
FAPR_t3Thy=(APR_t3Tug/(TAPR_t3ug+1e-6))&100    !% of total T3 prod that occurs in the thyroid
!---T3 Metabolism contribution of pathways

\[
RWBT3\text{Met}=RAML_{t3}+RVdel_{t3}
\]
\[
AWBT3\text{Met}=\text{INTEG}(RWBT3\text{Met},0.0)
\]
\[
d_{AWBT3\text{Met}}=(AWBT3\text{Met}/(((t+1e-6)/24)))
\]
\[
FMWBT3=(AWBT3\text{Met}/(TAPR_{t3}+1e-6))*100
\]
\[
FMVdT3=(AVdel_{t3}/(TAPR_{t3}+1e-6))*100
\]
\[
FMLT3=(AML_{t3}/(TAPR_{t3}+1e-6))*100
\]
\[
FMFeT3=(AT3_{feces}/(TAPR_{t3}+1e-6))*100
\]

!------END HPT AXIS MODEL---------------------------------------------------

!------BEGIN PBPK MODELS FOR CHEMICALS--------------------------------------

!-------ClO4 model-----iv and drinking water--------------------------------

!Oral Dosing (water consumption) parameters for perchlorate

\[
dose_p = (pdose_p1*BW*10**6)/MWP
\]
\[
Rdose_p=dose_p/12
\]
\[
pflag_p=pulse(0.0,24.0,12)
\]
\[
RMR_p=(Rdose_p*pflag_p)
\]
\[
AST_p=\text{INTEG}(RMR_p,0.0)
\]
\[
d_{AST_p}=AST_p/(((t+1e-6)/24))
\]

IV Dosing parameters for perchlorate

\[
\text{IVDOSE}_p=(\text{IVDOSE}_p*(10**6)*BW)/(MWP)
\]
\[
\text{iflag}_p=pulse(0,tstop,tinf)
\]
\[
RIV_p=(\text{IVDOSE}_p/TINF)*\text{iflag}_p
\]
\[
AIV_p=\text{INTEG}(RIV_p,0.)
\]

!----Plasma compartment for perchlorate-----

\[
Ca_p=Cv_p
\]
\[
RAb_p=RMR_p+RIV_p+(QT*Cvt_p)+(Qbody_p*Cvbody_p)-(QC*Ca_p)-RU_p-RBB_p
\]
\[
Ab_p=\text{INTEG}(RAb_p,0.0)
\]
\[
Cv_p=Ab_p/VPlas
\]

!Binding of perchlorate to serum proteins. Approach used by Merrill and Clewel. Determined not sensitive, so not used in this model.
!RBB_p=((VmaxB_p*Ca_p)/(KmB_p+Ca_p))-(Clunb_p*Cabnd_p)
  !nmol/hr - rate of change in amt of clo4 bound in plasma (binding rate - unbinding)
! Abbnd_p=INTEG(RBB_p,0.0)  !nmol - amt of clo4 bound in plasma
! Cabnd_p=Abbnd_p/Vplas    !nmol/L - concentration of clo4 bound in plasma
!PERB_p=Cabnd_p/(Catot_p+1e-6)  !Percent bound in blood

CaTot_p=Cv_p  !+Cabnd_p  !nmol - total clo4 in plasma (bound + free)
Ca_pugml=CaTot_p*MWP/10**6  !ug/mL - concentration of perchlorate in plasma

RU_p=ClU_p*Cv_p      !nmol/hr - rate of urinary clo4 excretion
AU_p=INTEG(RU_p,0.0)    !nmol - amt of clo4 excreted in urine
AU_pug=AU_p*MWP/1000    !ug - amt of clo4 excreted in urine

!------THYROID PERCHLORATE------
RTNIS_p=(VmaxT_pTSH*Cvt_p)/(Cvt_p+Km_p*(1+(Cvt_i/Km_i)))
  !nmol/hr - rate of NIS uptake of clo4 into thyroid (no iodide competition)
VmaxT_pTSH=((VmaxT_p*TSH)/(KNIS_TSHp+TSH))
  !nmol/hr - Vmax for clo4 uptake into thyroid stimulated by TSH
ATNIS=INTEG(RTNIS_p,0.0)    !nmol - amt of clo4 taken up into thyroid via NIS

RATB_p=QT*(Ca_p-Cvt_p)+PAt_p*(Ct_p-Cvt_p)-RTNIS_p
  !nmol/hr - rate of change in thyroid blood of clo4
Atb_p=INTEG(RATB_p,0.0)  !nmol - amt of clo4 in thyroid blood
Cvt_p=Atb_p/VTB    !nmol/L - conc of clo4 in thyroid blood

RAT_p=RTNIS_p+PAT_p*(Cvt_p-Ct_p)
  !nmol/hr - rate of change of clo4 in thyroid tissue
At_p=INTEG(RAT_p,0.0)  !nmol - amt of clo4 in thyroid tissue
Ct_p=At_p/(VT*PT_p)

Ct_pugg=(At_p/VT)*MWP/10**6  !ug/g - conc of clo4 in thyroid tissue

!To look at rates in and out of thyroid for perchlorate
RATp_in=RTNIS_p+(PAt_p*Cvt_p)
RATp_out=PAT_p*Ct_p
!-------Rest of Body Perchlorate-------
RABody_p=Qbody_p*(Ca_p-Cvbody_p)  !nmol/hr - rate of change of clo4 in 'rest of body'
ABody_p=INTEG(RABody_p,0.0)      !nmol - amt of clo4 in 'rest of body'
Cvbody_p=ABody_p/(VBbody_p*PBody_p) !nmol/L - clo4 in venous blood leaving 'rest of body'
Cbody_p=ABody_p/VBody_p          !nmol/L - concentration of clo4 in 'rest of body'

!----------MASS BALANCES------------------

!----------Mass balance TSH-------------
TSHint=initAVdTSH    !initial amts of TSH
TMASStsh=AClTSH+AvdTSH   !total mass TSH
BALANCEtsh=TSHint+ATSHPR-TMASStsh  !mass balance TSH (initial amt + amt produced - total mass)

!----------Mass balance T4--------------
T4int=initAp_t4+initAlb_t4+initAl_t4   !initial amts of t4
tformt4=APR_t4         !total amt T4 produced in thyroid
TMASSt4=AP_t4+AL_t4+ADIL+Avdel_t4+AGL+Alb_t4 !total mass T4
BALANCEt4=T4int+tformt4-TMASSt4     !mass balance t4

!----------Mass balance T3--------------
T3int=initAp_t3+initAlb_t3+initAl_t3   !initial amts of T3
tformt3=APR_t3+AT3FL+AT3FvD    !total amount of T3 formed
TMASSt3=AP_t3+AL_t3+Alb_t3+Avdel_t3+AML_t3 !total mass of T3
BALANCEt3=T3int+tformt3-TMASSt3     !mass balance t3

!----------Mass balance Iodide----------
Iint=initAP_i+initAtb_i+initdAt_i+initdAIB_i !initial amts of I
TMASSi=Atb_i+Atf_i+AIB_i+Ath+AP_i+AU_i   !total mass of I
tformi=AST_i+AIFL_t4+AIFL_t3+AIFvD_t4+AIFvD_t3 !dose I & I freed from metabolism of T4 and T3
BALANCEi=Iint+tformi-TMASSI         !mass balance iodide

!----------Mass Balance Perchlorate-----
TMASSp=Atb_p+AU_p+Ab_body_p+At_p    !total amount of perchlorate
TDosep=AST_p+AIV_p            !total dose of perchlorate
balancep=TDosep-TMASSp         !mass balance perchlorate

!Days
days=((t+1e-6)/24)            !days of model execution
END ! DERIVATIVE
    TERMT (T .GE. TSTOP, 'checked on communication interval: REACHED TSTOP')
END ! DYNAMIC
TERMINAL
    T4END=ca_t4ngg
    T3END=ca_t3ngg
    TSHEND=tshngml
    T4ENDPC=t4percon
    TSHENDPC=tshpercon
    END ! TERMINAL
END ! PROGRAM
APPENDIX G

The acslXtreme (version 2.4.0.11) .m files for BBDR-HPT axis and ClO$_4^-$ PBPK model simulations (Chapter 5) is contained within this Appendix. Each .m file includes the data plotted, simulation commands, and plotting commands.
%Serum and Thyroid Perchlorate concentrations in adult male SD rats (Yu et al 2002) following IV
dose of hot clo4 3.3mg/kg

File: HPERIVYU.m Created 04/13/07 by Eva McLanahan
File name change on 7/25/07 - Yu2002_iv_36ClO4.m
Last modified 07/25/07 by Eva McLanahan

M file for creating FIGURE 5.3

Data from Yu et al 2002 36-ClO4 tail vein IV injection (3.3mg/kg) - cl-36 data in male rat.xls

%time:serum clo4 (ug/g): thyroid clo4 (ug/g): urine clo4 (ug)

HPERIVYU = [ 0.5 8.62 19.55 NaN
0.5 8.16 17.66 NaN
0.5 9.07 21.45 NaN
6 2.09 15.83 NaN
6 1.87 12.85 NaN
6 2.31 18.81 NaN
12 0.47 9.19 885.502971
12 0.16 7.55 810.51
12 0.78 10.83 960.49
24 0.11 4.34 981.7950263
24 0.05 2.68 830.48
24 0.17 5.99 1133.12
32 0.03 1.43 NaN
32 0.01 0.55 NaN
32 0.05 2.30 NaN
48 0.01 0.45 NaN
48 0.00 0.18 NaN
48 0.01 0.72 NaN ];

%3.3 mg/kg of clo4 tail vein IV dose - Yu et al 2002)
!!s tstop=50, pdose_p=0, ivdosep=3.3, pdose1=20, cint=0.01, np=1
!!BW290
!!s bw=0.294, bwgon=0
!!prepare t, ca_pugml, ct_pugg, au_pug, ca_t4ngg, ca_t3ngg, tshngml, tat_iug
!!start/nc

Plot(_t, ca_pugml, HPERIVYU(:,1), HPERIVYU(:,2), _t, ct_pugg, HPERIVYU(:,1), HPERIVYU(:,3), _t, au_pug, HPERIVYU(:,1), HPERIVYU(:,4), 'HPERIVYU.aps')
%YuPerCON.m
%M file for plotting T4 and TSH from Yu 2002 clo4 drinking water study as percent of control (T4) and fold change (TSH)
%Created 07/20/2007 by Eva McLanahan
%M file for creating FIGURE 5.4 and 5.5
%Yu 2002 TSh Data as fold change (time, 0.1, 1, 3, 10 mg/kg-day clo4 dose)
YuTSH=[ 16 1.616182573 1.684647303 1.962655602 2.734439834
       112 1.347921225 1.971553611 2.704595186 3.146608315
       328 1.24009324  1.820512821 2.454545455 2.771561772 ];

%Yu 2002 T4 Data as percent of control change (time, 0.1, 1, 3, 10 mg/kg-day clo4 dose)
YuT4 = [ 16 94.47674419 88.6627907  88.37209302 76.1627907
        112 99.6969697  82.42424242 80.90909091 73.33333333
        328 97.25   95.5   86  84.25];

%Estimated parameters from visual fits
!!s np=0.94, np3=2, kp=140000, kinh_t4=0.3

%Thyroid Hormone Effects (THE) following 1 mg/kg of clo4 in drinking water 14 days - Yu et al 2002)
!!s tstop=336, ivdosep=0, pdose_p=1, pdose1=20, bwgon=0
!!BW330
!!prepare t, tshfold, t4percon, tat_iug
!!start/nc
yut4percon1=_t4percon
yutshfold1=_tshfold
yutat1=_tat_iug

%Thyroid Hormone Effects (THE) following 3 mg/kg of clo4 in drinking water 14 days - Yu et al 2002)
!!s tstop=336, ivdosep=0, pdose_p=3, pdose1=20, bwgon=0
!!BW330
!!prepare t, tshfold, t4percon, tat_iug
!!start/nc
yut4percon3=_t4percon
yutshfold3=_tshfold
yutat3=_tat_iug
%Thyroid Hormone Effects (THE) following 10 mg/kg of clo4 in drinking water 14 days – Yu et al 2002
!!s tstop=336, ivdosep=0, pdose_p=10, pdose1=20, bwgon=0
!!BW330
!!prepare t, tshfold, t4percon, tat_iug
!!start/nc
yut4percon10=_t4percon
yutshfold10=_tshfold
yutat10=_tat_iug

plot(_t,yut4percon1(:,1),_t,yut4percon3(:,1),_t,yut4percon10(:,1),YuT4(:,1),YuT4(:,3),YuT4(:,1),YuT4(:,4),YuT4(:,1),YuT4(:,5),'YuT4percon.aps')

plot(_t,yutshfold1(:,1),_t,yutshfold3(:,1),_t,yutshfold10(:,1),YuTSH(:,1),YuTSH(:,3),YuTSH(:,1),YuTSH(:,4),YuTSH(:,1),YuTSH(:,5),'YuTSHfold.aps')

plot (_t,yutat1(:,1),_t,yutat3(:,1),_t,yutat10(:,1),'YuTATstores.aps')
% Yu2002.m file
% Created 04/01/07 by Eva McLanahan
% Serum and Thyroid data from drinking water studies Yu et al 2002
% 04/02/07- Added 12 hrs to each time point to account for time of sacrifice compared to water intake (drink at night - sac in morning)
% Last Modified 04/02/07 by Eva McLanahan
%.M file for creating FIGURE 5.6
% Data plotted in Merrill et al 2003 from email from Jeff
% time (hrs), serum total perchlorate (ug/mL), thyroid perchlorate (ug/mL)

dw1 = [ 0 0 0
       328 0.000025 8.36
       328 0.3 10.44
       328 0.35 12.52 ];

dw3 = [ 0 0 0
       16 0.39 10.14
       16 0.45 14.43
       16 0.51 18.72
       112 1.22 48.65
       112 1.44 50.4
       112 1.66 52.15
       328 0.78 27.78
       328 0.91 30.98
       328 1.04 34.18 ];

dw10 = [ 0 0 0
       16 1.23 20
       16 1.92 23.68
       16 2.61 27.36
       112 4.05 166.29
       112 4.93 175.71
       112 5.81 185.13 ];
%Low dose (1mg/kg of ClO4 in drinking water 1, 4, 14 days - Yu et al 2002)
!!s tstop=350, ivdosep=0, pdose_p=1, pdose1=20, bwgon=0, cint=0.5
!!BW330
!!prepare t, ca_pugml, ct_pugg
!!start/nc
cap1=ca_pugml
ctp1=ct_pugg

%Mid dose (3mg/kg of ClO4 in drinking water 1, 4, 14 days - Yu et al 2002)
!!s tstop=350, ivdosep=0, pdose_p=3, pdose1=20, bwgon=0, cint=0.5
!!BW330
!!prepare t, ca_pugml, ct_pugg
!!start/nc
cap3=ca_pugml
ctp3=ct_pugg

%High dose (10mg/kg of ClO4 in drinking water 1, 4, 14 days - Yu et al 2002)
!!s tstop=350, ivdosep=0, pdose_p=10, pdose1=20, bwgon=0, cint=0.5
!!BW330
!!prepare t, ca_pugml, ct_pugg
!!start/nc
cap10=ca_pugml
ctp10=ct_pugg

plot(_t, ca_p1(:,1), _t, ca_p3(:,1), _t, ca_p10(:,1), dw10(:,1), dw10(:,2), dw3(:,1), dw3(:,2), dw1(:,1), dw1(:,2), 'YuDWCA.aps')
plot(_t, ct_p1(:,1), _t, ct_p3(:,1), _t, ct_p10(:,1), dw10(:,1), dw10(:,3), dw3(:,1), dw3(:,3), dw1(:,1), dw1(:,3), 'YuDWCT.aps')
Mannisto 1979 ClO4 in drinking water
15mg ClO4/kg-day, iodide intake 10-20ug/day (0.5-1.0mgI/kg chow), adult male SD 180-220g

Created 8/5/2007 by Eva McLanahan

.M file for creating FIGURE 5.7

Mannisto 1979 ClO4 in drinking water
15mg ClO4/kg-day, iodide intake 10-20ug/day, adult male SD 180-220g

MANN79_ClO4dw_percon = [ 0 100 100 100
0 113.0518234 105 118.0327869
0 86.94817658 95 81.96721311
48 78.3109405 105 118.0327869
48 84.68348952 112.6190476 140.2550091
48 71.93839148 97.38095238 95.81056466
96 91.17082534 80 183.6065574
96 96.85503586 86.875 192.5351288
96 85.48661481 73.125 174.6779859
144 87.14011516 80 179.5081967
144 92.64672309 83.75 192.2935848
144 81.63350723 76.25 166.7228086
336 NaN NaN 196.7213115
336 NaN NaN 209.2213115
336 NaN NaN 184.2213115 ];

Estimated parameters from visual fits to Yu 2002 data
np=0.94, np3=2, kp=140000, kinh_t4=0.3

Iodide intake tested 10-20 does not make a difference in model simulations
BW200

prepare t, t4percon, tshpercon

plot(_t,_t4percon,MANN79_ClO4dw_percon(:,1),MANN79_ClO4dw_percon(:,2),_t,_tshpercon,MANN79_ClO4dw_percon(:,1),MANN79_ClO4dw_percon(:,4),'Mannisto1979_clo4dw_percon.aps')
%Caldwell 1995 14-d Perchlorate Exposure (dose mg/kg-day)
%Male Rat Data. Digitized in Caldwell1995_CLO4.xls
%.M file for creating FIGURE 5.8
%Body weight and I diet not reported
%Caldwell 1995 14-d Perchlorate Exposure (dose mg/kg-day)
%Dose-Response plot data format
%clo4 dose (mg/kg-day), serum t3 (percent of control), serum t4 (% control), serum tsh (%control)
Cald95dr=[ 0.11  93.26088322 94.43432826 102.3669782
0.44  79.56524137 92.67576932 115.3847389
1.11  67.7717714  84.49084828 136.686509
2.28  57.11960464 82.69511045 207.6923824
4.32  53.31525974 80.90123157 213.6095383
11.44  49.89134977 66.92756484 233.7278661
22.16  49.51091586 59.33936349 256.213055 ];

%Estimated parameters from visual fits from Yu 2002 data
!!s np=0.94, np3=2, kp=140000, kinh_t4=0.3
!!prepare t, t4end, tshend, t3end, tat_iug output @Clear

output @Clear

global PDOSE_P
%Calculate and store T4, T3, and TSH for each dose then plot in one DR graph the final value at 14d
!!BW350
!!s bwgon=0, pdose1=20, tstop=336, pdose_p=0.11

pdose_p=[0:0.11:22.22]
for x=[1:202]
PDOSE_P=pdose_p(x)
start @NoCallback
pdosep(x)=PDOSE_P;
t4finalpc(x)=T4ENDPC;
tshfinalpc(x)=TSHENDPC;
tat_iug(x)=TAT_IUG;
end

plot(pdosep,t4finalpc, Cald95dr(:,1), Cald95dr(:,3),'caldwell95t4pc.aps')
plot(pdosep,tshfinalpc, Cald95dr(:,1), Cald95dr(:,4),'caldwell95tshpc.aps')