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**INHIBITION OF THE SODIUM-IODIDE SYMPORTER BY PERCHLORATE:  
AN EVALUATION OF LIFE STAGE SENSITIVITY USING  
PHYSIOLOGICALLY BASED PHARMACOKINETIC (PBPK) MODELING**

U.S. Environmental Protection Agency  
Office of Research and Development

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## LIST OF ACRONYMS

<b>AAP</b>	American Academy of Pediatrics
<b>AFRL</b>	Air Force Research Laboratory
<b>BW</b>	body weight
<b>EPA</b>	U.S. Environmental Protection Agency
<b>FDA</b>	U.S. Food and Drug Administration
<b>GFR</b>	glomerular filtration rate
<b>GI</b>	gastrointestinal
<b>GW</b>	gestation week
<b>HA</b>	health advisory
<b>HRL</b>	health reference level
<b>IO</b>	Immediate Office
<b>IRIS</b>	Integrated Risk Information System
<b>i.v.</b>	intravenous
<b>LOD</b>	limit of detection
<b>MCLG</b>	maximum contaminant level goals
<b>NCCT</b>	National Center for Computational Toxicology
<b>NCEA</b>	National Center for Environmental Assessment
<b>NHEERL</b>	National Health and Environmental Effects Research Laboratory
<b>NIS</b>	sodium-iodide symporter
<b>NOEL</b>	no-observed-effect level
<b>NRC</b>	National Research Council
<b>ORD</b>	Office of Research and Development
<b>OSP</b>	Office of Science Policy
<b>PA</b>	permeability-area cross product
<b>PBPK</b>	physiologically based pharmacokinetic
<b>PD</b>	pharmacodynamic
<b>PK</b>	pharmacokinetic
<b>PND</b>	postnatal day
<b>POD</b>	point of departure
<b>RAIU</b>	radioactive iodide uptake
<b>RfD</b>	reference dose
<b>SD</b>	standard deviation
<b>SDWA</b>	Safe Drinking Water Act
<b>SEM</b>	standard error of the mean
<b>TDS</b>	Total Diet Study

## EXECUTIVE SUMMARY

Perchlorate competitively inhibits uptake of iodide by the sodium-iodide symporter (NIS) in laboratory animals and humans. NIS is found in many tissues but is primarily responsible for sequestering iodide from the bloodstream into the thyroid, enabling biosynthesis of thyroid hormones. The National Research Council (2005) advised that the perchlorate reference dose be based on the inhibition of iodide uptake by the thyroid. In this analysis, the physiologically based pharmacokinetic (PBPK) models of perchlorate and radioiodide, which were developed to describe thyroidal radioactive iodide uptake (RAIU) inhibition by perchlorate for the “average” adult (Merrill et al., 2005), pregnant woman and fetus, lactating woman and neonate, and young child (Clewell et al., 2007), were evaluated based on their ability to provide additional information about this critical effect for potentially sensitive subgroups.

The U.S. Environmental Protection Agency (EPA) evaluated the PBPK model code provided by the model authors and found minor errors in mathematical equations and computer code, as well as some inconsistencies among model code files. Office of Research and Development (ORD) scientists made corrections to the code, with agreement from model authors that the corrections should be made, in order to harmonize the models and more adequately reflect the biology.

EPA determined that model parameters describing urinary excretion of perchlorate and iodide were particularly important in prediction of RAIU inhibition in all subgroups; therefore, a range of biologically plausible values available in peer-reviewed literature was evaluated in depth by using the PBPK models. EPA also determined that exposure rates were critical for estimation of RAIU inhibition by the models and thus evaluated exposure rates further.

EPA’s analysis identified the near-term fetus (only gestation week 40 fetus could be adequately modeled) as the most sensitive subgroup with respect to percent RAIU inhibition at a perchlorate dose equal to the point of departure (7  $\mu\text{g}/\text{kg}\text{-day}$ ) that was used for the derivation of the reference dose (RfD). Specifically, at a perchlorate dose of 7  $\mu\text{g}/\text{kg}\text{-day}$ , the percent RAIU inhibition predicted by the model for the near-term fetus is 6.7-fold greater than that for the average adult. After correcting the model for reduced urinary clearance in infants, the same analysis predicts percent RAIU inhibition approximately two- to threefold higher for the breast-fed and bottle-fed infant (7–60 days) than for the average adult (differing from Clewell et al. [2007]) and predicts percent RAIU inhibition for the 1- to 2-year-old child about equal to the average adult. Clewell et al. (2007) predicted percent RAIU inhibition in the older child to be about one-half that of the adult; ORD’s results are closer to, but still less than, those for the adult.

Overall, detailed examination of Clewell et al. (2007) and Merrill et al. (2005) reflected that the model structures were appropriate for predicting percent inhibition of RAIU by perchlorate in most life stages. The lack of biological information and data that might be used to



calibrate key model parameters (in humans of specific life stages) and validate model predictions, particularly for early fetal development, limits EPA's confidence on predictions for fetal endpoints. Therefore, EPA simply chose not to use model predictions for the early- or mid-term fetus. However, because many of the physiological and iodide- and perchlorate-specific parameters in the late-term fetus are expected to be quite close to those of the newborn and there are many more data available for validation of the model in the newborn, the higher confidence in model predictions for the newborn is then partially extended to the late-term fetus (although there is still lower confidence in the late-term fetal predictions than in those for the newborn). Quantitative outputs of the PBPK models as updated by EPA differ by up to threefold from published values, though many of the outputs are within 20% of the published values. Nevertheless, the EPA evaluation determined that, with those modifications as described herein, the Clewell et al. (2007) and Merrill et al. (2005) models are acceptable to calculate the life stage differences in the degree of thyroidal NIS RAIU inhibition at a given level of perchlorate exposure.

## 1. INTRODUCTION

The sodium-iodide symporter (NIS) transports iodide from blood into the thyroid gland, enabling biosynthesis of thyroid hormones. Perchlorate is a potent competitive inhibitor of the NIS. Perchlorate has been shown to cause thyroid-related pathologies and neurodevelopmental effects in rodents by disrupting the thyroid axis homeostasis (Gilbert and Sui, 2008; National Research Council [NRC], 2005; York et al., 2005 a, b). NRC (2005) evaluated the human health implications of perchlorate and stated that inhibition of iodide uptake has been unequivocally demonstrated in humans exposed to perchlorate, and it is the key event that precedes all thyroid-mediated effects of perchlorate exposure. NRC advised that the perchlorate reference dose (RfD) be based on the inhibition of iodide uptake by the thyroid. NRC further advised that, while they did not deem iodide uptake inhibition by itself to be adverse, this effect was a precursor to adverse health effects of perchlorate exposure. The lowest dose (7 µg/kg-day) administered in the Greer et al. (2002) study of radioactive iodide uptake (RAIU) inhibition in a group of healthy adult volunteers was considered a no-observed-effect level (NOEL). NRC recommended that the RfD be derived by using this dose as the point of departure (POD), applying an intraspecies uncertainty factor of 10 to account for variability in the human population; fetuses of pregnant women who might have hypothyroidism or iodine deficiency were thought to be the population subgroup of particular concern. The U.S. Environmental Protection Agency's (EPA) Integrated Risk Information System (IRIS) adopted NRC's recommendations (U.S. EPA, 2005).

Merrill et al. (2005) described a deterministic, physiologically based pharmacokinetic (PBPK) model for radioiodide and perchlorate and the competitive interaction of perchlorate and radioiodide at the NIS in adult humans. Clewell et al. (2007) extended this work and previous life stage models in the rodent (Clewell et al., 2003a, b) to predict inhibition of the NIS for pregnant and lactating women, nursing infants, and the subsequent stages of childhood.

The following report provides a quantitative analysis of perchlorate-mediated inhibition of the NIS in humans by using the Merrill et al. (2005) and Clewell et al. (2007) PBPK models, focusing on the variability in the degree of NIS inhibition as a function of life stage. This set of models, largely completed after the NRC (2005) report, provides new information that may be useful to EPA for addressing differences in human responses to perchlorate across life stages.

The evaluation of the PBPK models was conducted in two stages. First, the scientific credibility of the models was evaluated based on the information presented in Merrill et al. (2005) and Clewell et al. (2007), limited inspection of the computer codes of the models, and limited execution of the computer model codes as supplied by the model authors. In concluding this first stage of model evaluation, EPA decided that the PBPK models were potentially suitable to inform regulatory decision making by the Agency, but a more detailed and thorough

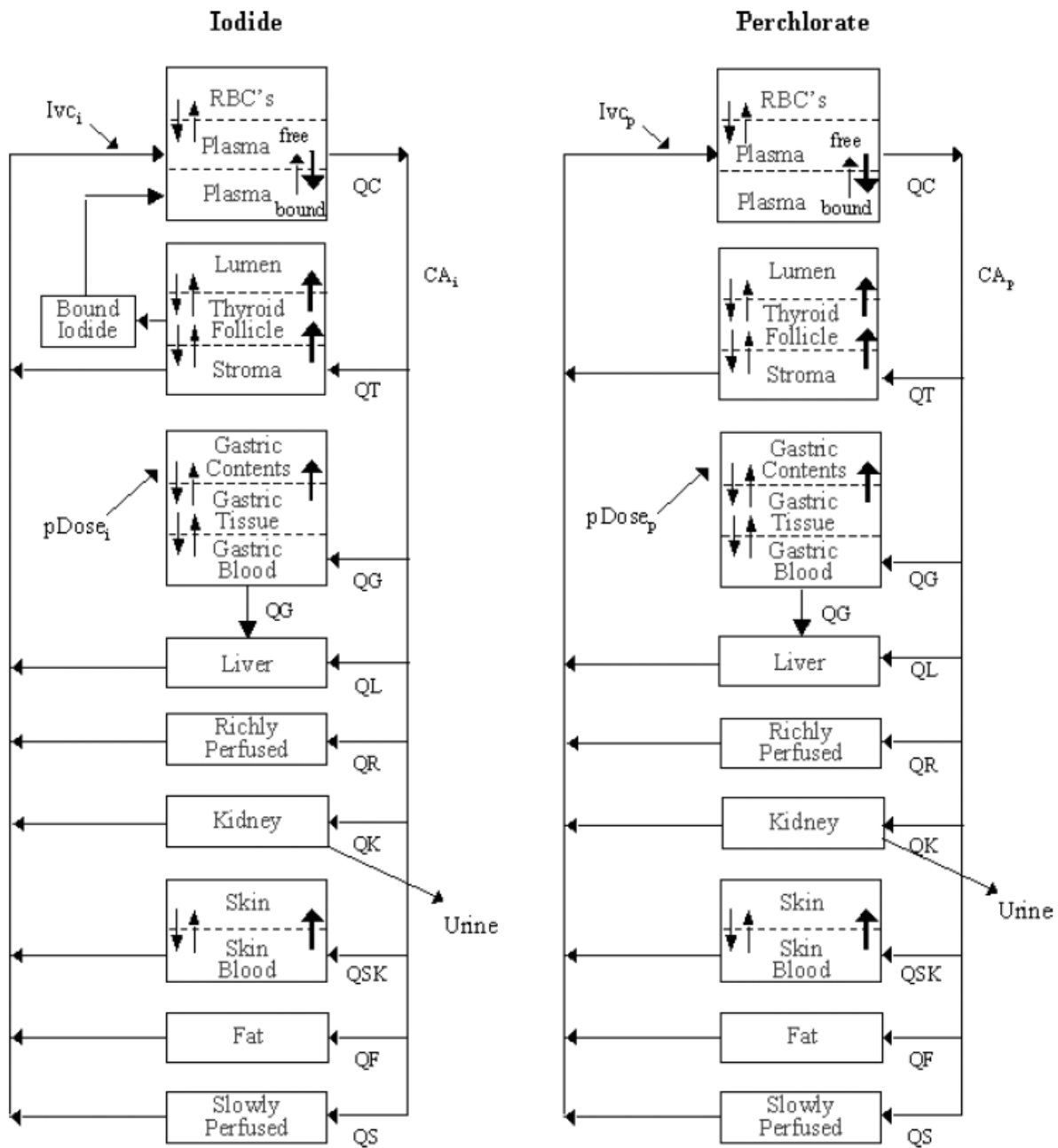
evaluation of the models was necessary. Thus, the second stage of model evaluation involved a more complete inspection of the computer codes and examination of the technical approach used to develop the model structures and parameter values. In addition, the published models were modified by EPA to fix errors and incorporate new data, particularly data on life stage variability in the urinary clearance of perchlorate, to which NIS inhibition is sensitive. The overall approach taken for both the first and the second stages of this evaluation followed the recommendations for evaluation of PBPK models provided by Clark et al. (2004) and Chiu et al. (2007).

PBPK model-predicted inhibition of thyroidal NIS radioiodide uptake by perchlorate was evaluated for several life stages. The PBPK models were developed to evaluate the effect of perchlorate on RAIU inhibition. This was also the dose metric suggested by NRC (2005) as a precursor to the critical effect; thus, the 24-hour RAIU inhibition predicted by the PBPK models was used in this analysis. The life stages evaluated in this modeling analysis included the pregnant woman, fetus, lactating woman, breast-fed infant, bottle-fed infant, 1-year-old and 2-year-old child, “average” adult, and nonpregnant woman of childbearing age. Clewell et al. (2007) developed separate PBPK model codes for the pregnant woman/fetus and for the lactating woman/breast-fed infant. These model codes were provided to EPA by the authors of Clewell et al. (2007). EPA obtained results for the bottle-fed neonate by altering the dose specification in the model for the breast-fed infant. Simulation results for bottle-fed infants were compared to information contained in a consultative letter transmitted from the U.S. Air Force Research Laboratory (AFRL) to EPA (Mattie, 2006). The PBPK model code for the average adult was obtained from the authors of Merrill et al. (2005), while the code for the nonpregnant woman of childbearing age was modified by EPA from the pregnant woman’s code by removing the placental and fetal compartments but retaining the mammary compartment.

The PBPK model for each life stage is presumed to represent a typical or average individual from that life stage. Thus, in evaluating specific PBPK parameter choices, especially for urinary clearance, EPA’s objective was to ensure that the values appropriately represented an average or central value for a given life stage and age. However, in some cases there is considerable uncertainty on what that average is or should be, and alternative means of predicting the average were considered. In particular, sensitivity analyses were developed by EPA for alternate values of urinary clearance. In the case where more than one alternative clearance value appeared to be equally likely (i.e., had a similar quality and amount of supporting data), then the more “sensitive” option was selected (i.e., the option that leads to predictions of the highest sensitivity to perchlorate-induced RAIU). However, the overall analysis did not seek to select parameters associated with highest predicted effect and is not intended to represent an upper-bound estimate of the average effect of perchlorate in these populations. Again, modeling choices are assumed to represent the best or most likely (central) estimate for an average individual within the population. There is uncertainty about the average, and EPA has selected from within the range of uncertainty, but there is still expected to be

population variability around the model predictions based on that choice, with the model prediction being near the center of the distribution.

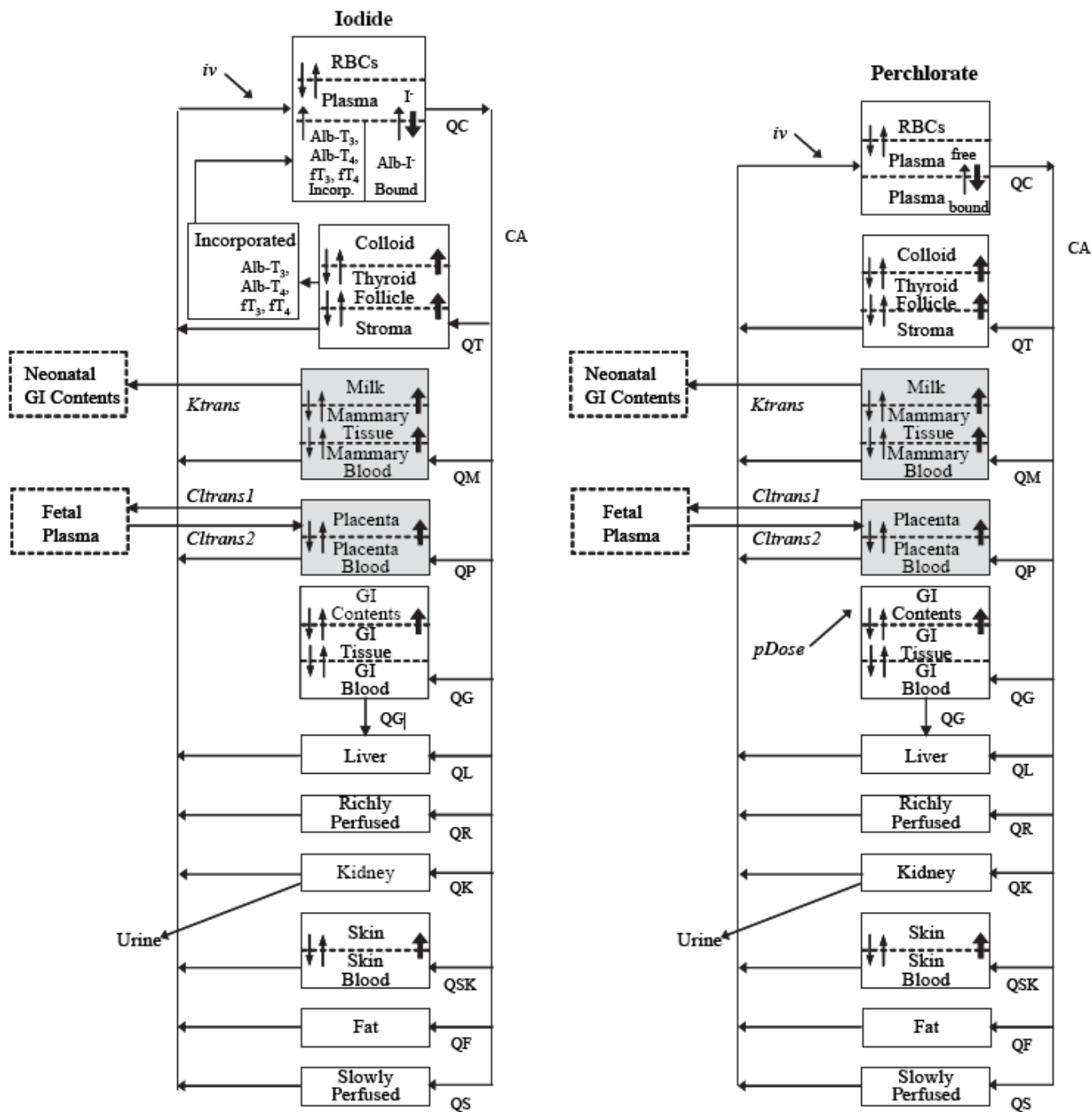
The PBPK model structures as depicted by Merrill et al. (2005) and Clewell et al. (2007) are shown in Figures 1-1 and 1-2, respectively. EPA's evaluation of model code and modifications did not result in changes to the overall model structure as shown in these figures.



**Figure 1-1. PBPK model structure for radioiodide (left) and perchlorate (right) for the average adult.**

Bold arrows represent active transport (some compartments; plasma binding also indicated with a bold arrow), double arrows represent passive diffusion, and thin single arrows represent first-order rates.

Source: Merrill et al. (2005). Used by permission. Copyright *Toxicological Sciences*.



**Figure 1-2. PBPK model structure for radioiodide (left) and perchlorate (right) in the pregnant and lactating woman.**

The shaded compartments indicate where the model structure differs for infants and neonates. Bold arrows represent active transport (some compartments; plasma binding also indicated with a bold arrow), double arrows represent passive diffusion, and thin single arrows designate first order rates.

Source: Clewell et al. (2007). Used by permission. Copyright *Journal of Toxicology and Environmental Health Part A*.

## 2. EXAMINATION OF PBPK MODEL COMPUTER CODE

The PBPK model computer code was provided to EPA by the authors (Clewell et al., 2007; Merrill et al., 2005). Several inconsistencies among the various code files and between those files and the published papers were identified. As a result, EPA made changes to the model code that have impacts of varying sizes on predictions of RAIU inhibition. Modeling changes having significant effects are discussed in the text here and/or are shown in Appendix A, Section A.4. Except as noted, correction of errors or inconsistencies resulted in only minor changes in model outputs. The model codes, as modified by EPA, were still able to reproduce human data sets shown in Clewell et al. (2007).

An example of a coding error relates to NIS inhibition in tissues other than the thyroid. Inhibition of NIS radioiodide transport by perchlorate was described for the thyroid in all model codes, but other NIS-containing tissues (e.g., gastrointestinal [GI] tract, skin, mammary gland, placenta, and breast (tissue-milk transport)) were found to inconsistently include perchlorate inhibition of radioiodide transport across model codes. Discussion with the Clewell et al. (2007) model authors concluded that the competitive inhibition of NIS radioiodide transport by perchlorate should have been described for all NIS-containing tissues. Thus, EPA added inhibition of radioiodide transport by perchlorate when it was absent in the model codes obtained from the authors.

The model code obtained from the authors of Clewell et al. (2007) for human pregnancy included inhibition of NIS radioiodide transport by perchlorate in the skin and GI tract, but this inhibition was not described mathematically in the model code for the lactating woman, breast-fed neonate, or young child. As noted in Appendix A, section A.4, addition of iodide transport inhibition in the skin had some effect, reducing estimated RAIU inhibition in the average adult model. However, addition of inhibition of NIS radioiodide transport by perchlorate in the skin and GI tract into the code for the pregnant woman did not significantly impact the kinetics or predictions of percent inhibition of thyroidal uptake of radioiodide.

In contrast, the radioiodide excretion into breast milk by NIS in the lactating woman code did not account for inhibition by perchlorate, and inclusion of this inhibition markedly increased the predicted percent inhibition of thyroidal radioiodide uptake in the breast-fed infant. For example, at a maternal dose equal to the POD (i.e., when the mother's ingestion is 7  $\mu\text{g}/\text{kg}\text{-day}$ ), if all model corrections are made *except* that inhibition of iodide transport into breast tissue and breast milk is *not* included, then the radioiodide transport to breast milk is predicted to be *increased* by about 0.3% vs. control due to the inhibition of iodide transport into other maternal tissues. The model then also predicts that thyroid radioiodide uptake by the 60-day-old breast-fed infant (of the mother receiving this perchlorate dose and if the radioiodide exposure also occurs via breast feeding) is decreased by 7.1%, because the inhibition in the infant thyroid from

the perchlorate he or she ingests more than offsets this small increase in radioiodide ingestion. However, when inhibition of iodide transport in breast tissue and milk is appropriately included in the model, this maternal perchlorate dose is predicted to *decrease* iodide transport into milk by 3.1%, and net predicted inhibition of radioiodide uptake by her 60-day-old infant is predicted to be 10.3%. Thus, correction of this coding error decreased the predicted breast milk transport of iodide, with a nearly additive increase in the net inhibition of radioiodide uptake by the 60-day-old infant thyroid: an impact of 1.5-fold on the effect in the infant. However, these changes also led to a prediction of decreased inhibition in the lactating woman herself, since inhibition of iodide transport into breast milk results in more iodide in maternal circulation and hence is available for maternal thyroid uptake. These and other model code modifications made by EPA are described in detail in Appendix A.

EPA has not identified any coding errors that invalidate the use of the overall model structure of Clewell et al. (2007) for quantitative prediction of perchlorate-mediated competitive inhibition of thyroidal NIS uptake of radioiodide, although EPA predictions with the corrected code differ to some extent from those described in Clewell et al. (2007) as a result of those corrections. EPA has conducted a complete audit of model codes for potential errors. This effort was in support of EPA's in-house analysis of the model code, and a report of the analysis is attached as Appendix C. A PDF document of the model code modified by EPA and used in this analysis is available at <http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=199347>.



### **3. EVALUATION OF PBPK MODEL TECHNICAL APPROACH—DEVELOPMENT AND MODEL PARAMETERIZATION**

Because the PBPK models for pregnancy, lactation, and infant/child growth integrate exposure over periods of time when various physiological parameters are also changing, it is helpful to have simple, algebraic equations that describe parameter dependence on gestation week (GW), age, and/or body weight (BW). In several cases algebraic functions were fit to data to evaluate model alternatives (some of which were then used). These curve fits were conducted by using curve-fitting tools available in Microsoft Excel, so the results are reproducible and expected to represent or approximate population-average behavior. However, in most cases the data used were measurement averages or the average together with points at  $\pm 1$  standard deviation (SD), and thus results would not be identical to what would be obtained if all the original measurement values were available and modeled. However, EPA believes that differences in the quantitative PBPK model predictions between using these curve fits and what might be obtained by more extensive analyses would be small. A strength of PBPK modeling is that model predictions are usually not overly dependent on the precise value of any one parameter: small changes in a parameter or function will not lead to large changes in model predictions. As modifications were evaluated during this exercise, it was indeed found to be the case that slight changes in these curve fits made little difference in model predictions, compared for example to the larger impact of using the “low” vs. “high” urinary clearance options (discussed in detail below).

Good modeling and statistical practice is to use as few parameters as possible to represent a given data set, while capturing the overall  $x$ - $y$  relationship observed in the data. Therefore, model forms with only two or three fitted parameters but capable of matching the shape of the observed data were preferred. For example, a quadratic model form was used to represent changes in urinary clearance during pregnancy (as observed by Aboul-Khair et al. [1964]) because it was simple, had only two adjustable parameters (with the intercept set to zero), and could be estimated by fitting to data in a Microsoft Excel chart (plot).

EPA’s approach was to generate algebraic functions through curve fitting, which would provide good approximations of time- or BW-dependent changes in parameter behavior, using a relatively small number of parameters and simple analytic tools (curve fitting in Microsoft Excel) that could be quickly applied. Advanced statistical methods could be applied but are unlikely to substantially change the resulting model predictions.

#### **3.1. URINARY CLEARANCE**

The urinary clearance values for perchlorate and iodide across all life stages were determined to be sensitive parameters for prediction of NIS thyroidal iodide uptake inhibition by

perchlorate. Thus, urinary clearance was examined further to determine if the approach used in Clewell et al. (2007) appropriately represents the available peer-reviewed literature data on urinary clearance. Details of this evaluation are found in Appendix B, and a brief summary follows.

For parameters based on human data, the following issues were identified. Clewell et al. (2007) scaled urinary clearance of perchlorate and iodide by BW as a function of overall metabolism and clearance ( $BW^{0.75}$ ). This scaling causes urinary clearance per unit of BW to increase as BW decreases. EPA determined, however, that this relationship does not accurately describe the reported rate of urinary clearance in neonates. In fact, several indices of renal function indicate that urinary clearance of perchlorate and iodide in neonates is considerably slower than is indicated by  $BW^{0.75}$ . For example, glomerular filtration rate (GFR) (normalized to body surface area) in 1-week-old neonates is  $11.0 \pm 5.4$  mL/minute/ $1.73$  m<sup>2</sup>, while in infants aged 9–12 months, GFR is  $86.9 \pm 8.4$  mL/minute/ $1.73$  m<sup>2</sup> (Gomez and Norwood, 2005). (Note: A convention in literature reporting these data is to calculate GFR per unit of body surface area [m<sup>2</sup>] for the tested individuals but to express the results normalized to a standard adult surface area [ $1.73$  m<sup>2</sup>] regardless of the tested individuals' ages.) Data on urinary elimination of a number of compounds, including drugs and drug metabolites, also indicate that renal clearance is slower per unit of BW in neonates (Dorne et al., 2004; Clewell et al., 2002). Modification of the PBPK models to describe slower clearance of perchlorate and iodide in neonates (approximately 50% of adult values when normalized to BW) vs. that described in Clewell et al. (2007) (approximately 200% of adult values when normalized to BW) resulted in an increase in predicted levels of perchlorate-induced NIS inhibition in infants. For example, at a perchlorate dose rate of 7 µg/kg-day (amount ingested by the infant equal to the POD for the RfD), the 7-day-old bottle-fed infant model RAIU inhibition predictions increased from 1.5% (after other corrections were made) to 4.4% (Table 4-2) as urinary clearance decreased.

An analysis of urinary excretion data in children (2–12 years) showed that the default scaling used by Clewell et al. (2007) fell within the range of the data for cimetidine, whose primary clearance mechanism is renal excretion (Lloyd et al., 1985), but the average for those data was better described as scaling by  $BW^{1.0}$ , resulting in a somewhat lower average predicted clearance (see Appendix B for details). Therefore, EPA chose to estimate perchlorate-induced inhibition by using scaling of urinary clearance proportional to BW for children at 1 year of age and older, which results in somewhat higher estimates of iodide uptake inhibition than reported by Clewell et al. (2007) though still slightly less than predicted for the average adult exposed at the same dose (see Appendix B, including Figure B-4, for additional details). EPA's estimates of urinary clearance in infants and children are lower than those used in Clewell et al. (2007) but are values that EPA judges to be best scientific estimates, not bounds. In particular, the GFR values used for infants were obtained based on the best estimates of statistical fits to experimental data (DeWoskin and Thompson, 2008; Guignard et al., 1975). The clearance

estimates on which EPA relied were the averaged values presented in DeWoskin and Thompson (2008). The compiled data include those of Guignard et al. (1975); however, considered as a group, the clearance estimates in Guignard et al. (1975) would support lower clearance estimates than the averaged values used here. Thus, it is important to note that the clearance estimates used here are intended to be expected central estimates, and lower (or higher) mean clearance estimates could also be made.

Data were also identified indicating that urinary clearance of iodide by the mother during pregnancy and the first few postpartum months may be as much as two times higher than in the nonpregnant woman (Aboul-Khair et al., 1964). These data include measurements of radioiodide uptake during pregnancy and lactation. Details of the PBPK model analyses with data from Aboul-Khair et al. (1964) are discussed in Appendix B. Model fits using these data also required the assumption of higher maternal NIS levels over this period, which may be consistent with the information indicating higher thyroid activity during pregnancy (Fantz et al., 1999). Some caution is needed in evaluating these data, since the comparisons for pregnancy and nursing were relative to measured adult clearances that were substantially below what is used in the perchlorate PBPK modeling. The reasons for this are not known.

Delange (2004) reviewed data pertaining to urinary clearance of iodide during pregnancy and found studies suggesting both increases and decreases in renal clearance of iodide; thus, urinary clearance changes during pregnancy are inconclusive. In this analysis by EPA and in general pharmacokinetics, clearance is defined as the ratio of the elimination rate (amount/time) to the blood concentration (amount/volume) with units of volume/time and is considered to be the volume of blood “cleared” of the compound per unit of time. Thus, a true evaluation of urinary clearance requires that *both* the urinary excretion rate and the blood concentration be determined, and clearance cannot be determined from only observing urine excretion rates or concentrations. Since it is common and often relatively simple to measure urinary levels of a compound but much less common and more difficult to (simultaneously) measure blood concentrations, it is at least possible that many of the data referred to by Delange (2004) are excretion data rather than clearance data. Since iodide is not metabolized, its excretion would on average equal its ingestion rate in iodide-sufficient conditions; so, even if clearance did change, its excretion would remain the same as long as dietary exposure remained about constant.

Finally, the existing model of Clewell et al. (2007) applies maternal urinary clearance constants for iodide and perchlorate that are about half of the average adult, based on pregnant vs. nonpregnant comparisons in rats, which is the opposite of what the data in Aboul-Khair et al. (1964) suggest and is inconsistent with conclusions by Delange (2004, Tables 1 and 2). While EPA’s general preference is to use observations (data) taken directly from humans instead of extrapolating from rats, the fact that baseline adult clearances in this study differ from the model’s assumed baseline and that the specific iodide urinary clearance observations of Aboul-

Khair et al. (1964) differ from the statements of Delange (2004) creates uncertainty around the human data.

Because of these differences in urinary clearance values reported in the literature, EPA considered the following three alternatives for urinary clearance during pregnancy as being equally likely:

1. urinary clearance values reported by Clewell et al. (2007) equal to about half of the average adult
2. increased urinary clearance based on the pregnant:nonpregnant ratio reported by Aboul-Khair et al. (1964)
3. urinary clearance constants assumed to be unchanged in pregnancy from the average adult based on observations of Delange (2004) (see Table 4-2)

To evaluate the impact of this uncertainty, for each case the urinary clearances of perchlorate and iodide were assumed to vary proportionately (i.e., the ratio of perchlorate:iodide clearance constants as estimated in average adults is maintained) (from Merrill et al. [2005]). Unfortunately, perchlorate clearance has not been measured in other human life stages, so there are no human data with which to validate this assumed constant proportionality. The result was that, with adjustments in the fitted parameter representing the thyroid NIS levels, all three assumptions led to equally good fits to the existing data. Therefore, it appears that all three possible cases are equally likely.

Since there are no conclusive human pregnancy data to distinguish which of these alternatives is more likely, EPA selected option 1, the lower clearance values reported in the peer-reviewed paper by Clewell et al. (2007) for relative response estimation (life stage sensitivity analysis), since this value leads to the most sensitive predictions. These lower clearance values were used in producing Tables 4-1, 4-3, and 4-5 below. While this analysis uses the lowest urinary clearance value among the alternatives evaluated, it does not provide an overall upper-bound effect estimate because the impact of uncertainty and variability in parameters other than those examined here (e.g., uncertainty in thyroid NIS parameters and interindividual variability in urinary excretion) was not evaluated.

For lactation, Clewell et al. (2007) used a clearance rate for iodide equal to the average adult but a clearance rate for perchlorate of about 40% of the average adult value, again based on life stage comparisons in rats. However, these values are inconsistent with the values fit to data in lactating rats (Clewell et al., 2003b) and male rats (Merrill et al., 2003) for which the clearance values were almost identical. Further, the data of Aboul-Khair et al. (1964) show an iodide clearance rate that is close to the late pregnancy value immediately following birth but that falls to within control range at postnatal week 12. Thus, EPA again considered three possibilities in a sensitivity analysis:

1. clearance parameters as used by Clewell et al. (2007)

2. clearance (for both iodide and perchlorate) higher than the average adult, based on Aboul-Khair et al. (1964)
3. clearance equal to nonpregnant average values

In this case option 1 was considered to be less likely than options 2 or 3, since it does not appear to be actually supported by observations (in rats) and it seems biologically unrealistic. There is no known biological basis for perchlorate clearance to be reduced while iodine clearance is not reduced. Simulation results with all three options are shown below in Table 4-2. EPA decided not to use the lower perchlorate clearance of Clewell et al. (2007) (option 1) because of its lack of consistency with available data and because this option was judged to be less plausible. Given the biological uncertainty between setting both clearance values equal to the average adult (option 3) and the higher clearance indicated by Aboul-Khair et al. (1964) (option 2), both options appear to be equally likely, so EPA chose to base the model predictions on option 2, since that leads to the most sensitive predictions. The resulting estimates of perchlorate effects are not upper-bound values, since the options considered were by no means representative of percentiles in a population distribution and this analysis did not address a range of other uncertainties in the modeling, such as uncertainty thyroid NIS parameters and interindividual variability in urinary clearance.

### **3.2. PARAMETER SCALING**

For parameters scaled up from rodents, Clewell et al. (2007) scaled permeability-area cross products (PAs) by  $BW^{0.75}$ . The use of such scaling is common practice for PBPK model parameters describing metabolic clearance and has been tested through applications with a number of chemicals, although substantial chemical-specific differences from these general scaling assumptions may occur. EPA tested the impact of scaling PA values by  $BW^{0.5}$  or  $BW^{1.0}$  for most tissues and found that this variation had little impact on predictions of NIS inhibition.

During pregnancy and lactation in the rodent, the placenta and mammary gland tissues undergo size changes that are disproportionate to overall BW. In particular, the placenta volume increases hundreds of times in size over the full course of pregnancy and 16-fold from the end of the first trimester to the end of pregnancy, while total BW increases only about 10%. So even if the scaling power of 0.75 is accepted as being correct, the assumption that the transport parameters in these tissues change with total BW rather than tissue weight appears biologically inappropriate because this assumption leads to the prediction that total NIS levels in the placenta remain approximately constant as the size of the placenta increases hundreds of times.

Therefore, EPA tested the effect of alternate scaling of the chemical-specific placental constants. Specifically, EPA tested scaling of the chemical-specific placental constants by tissue weight rather than total BW, but this scaling approach was found to only result in minimal quantitative changes. However, with the model code as obtained from the authors of Clewell et

al. (2007), high-frequency oscillations in fetal levels of perchlorate were predicted at the time when the fetal thyroid begins to develop, which was presumed to be biologically unrealistic. Changing the scaling for these constants to depend on tissue weight rather than total BW removed these oscillations. Thus the oscillations appear to result from a numerical instability in the model. Nevertheless, in keeping with EPA's intent to change only those model components that are either clear coding errors or have significant quantitative impact, EPA left these quantities as described in the original code for its reported simulations.

In addition, while the adult woman PBPK code was adjusted by the model authors to reflect changes in body fat and mammary gland size during lactation, the equation used to adjust blood flow to the mammary gland in proportion to its size appeared to be in error, since the flow rate set by it at birth only reflected the mammary gland volume change (increase) during pregnancy rather than the initial (pre-pregnancy) volume plus the increase (i.e., if  $V_{M,init}$  is the initial volume and  $V_{M,preg}$  is the increase during pregnancy, such that the total volume during pregnancy is  $V_{M,total} = V_{M,init} + V_{M,preg}$ , the flow rate was set to provide only the flow expected for  $V_{M,preg}$  rather than  $V_{M,total}$ ). The result is that the mammary gland blood flow at birth was less than the pre-birth blood flow, even though the tissue volume was greater. The equation was corrected to reflect the total tissue volume at the end of pregnancy/birth.

Finally, the equation and scaling used for binding of perchlorate and iodide to blood proteins are believed to not appropriately reflect the biology. In particular, if the concentration of the binding protein ( $C_{bp}$ ) is constant across age, as stated by Clewell et al. (2007) and the blood volume ( $V_B$ ) is a constant fraction of BW (i.e.,  $V_B = VC_B \times BW$ ), as is assumed in the model, then the total amount of binding protein should scale as blood (i.e.,  $A_{bp} = C_{pb} \times V_B = C_{pb} \times VC_B \times BW^{1.0}$ ). Since the maximum rate of blood binding would be expected to be proportional to the total amount of protein, it then follows that this maximal rate should scale as  $BW^{1.0}$  not  $BW^{0.75}$  as currently modeled. However, the impact of changing the scaling coefficient from 0.75 to 1.0 was found to be minimal, so the scaling was left in the original form for EPA's subsequent analysis.

Blood binding of perchlorate and iodide is described by using a Michaelis-Menten equation:  $r_{bind} = V_{max,b} \times C / (K_{m,b} + C)$ , where  $C$  is the blood concentration of perchlorate or iodide. In the case where  $C$  is approximately constant over a long period of time, as is expected for dietary iodide, this equation would result in a constant rate of binding. However, as the amount of bound material increases, assuming that the *total* concentration of binding protein is constant, the amount of *free* binding protein available would be expected to decrease, which in turn would cause a decrease in the rate of binding. The Michaelis-Menten equation used in the model is qualitatively inconsistent with that mechanistic expectation. Since changing the scaling of this rate had minimal effect on EPA's predictions for tracer radioiodide uptake (where blood concentration is not constant and declines to near-zero levels over a few days), this matter was not pursued further and the model was considered adequate for the evaluation of such tracer

kinetics. However, if the models were to describe dietary iodide rather than the current trace amounts of radioiodide, describing blood binding by using a Michaelis-Menten equation would not allow for an appropriately variable rate of binding, thus limiting EPA's confidence in using these models for predictions of various intake rates of dietary iodide.

### 3.3. POSTNATAL PBPK MODELING

Procedurally, to account for exposure to perchlorate throughout pregnancy and hence that the newborn and mother would carry a level of perchlorate from the moment of birth, simulations for both the mother and fetus should first be run by using the pregnancy model to estimate body levels at birth. However, EPA found that, by postnatal day (PND) 7, the predicted fetal and maternal levels had almost no dependence on pre-birth levels vs. exposures that began after birth. Rapid changes in infant thyroid function in the first few days immediately following birth also make the model parameters, and hence model predictions of RAIU inhibition, quite uncertain for those days. Therefore, EPA chose to simulate postnatal exposure, beginning at birth, and to use PND 7 as EPA's earliest prediction.

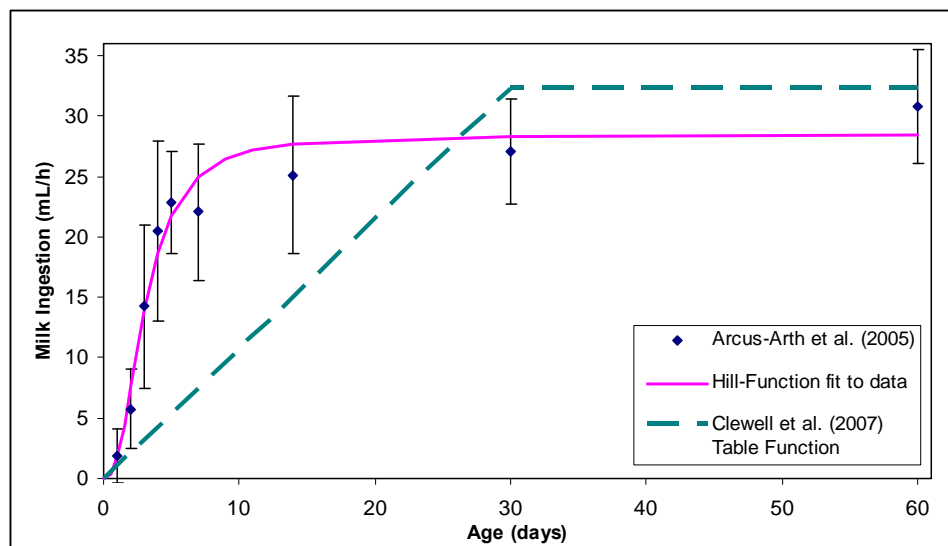
Key parameters of postnatal exposure are the suckling rates for breast-fed and bottle-fed infants. EPA selected the 90<sup>th</sup> percentile drinking water intake value for many of the subpopulations evaluated in this report in order to characterize the effects of high-end drinking water exposure to perchlorate. This approach is consistent with the *Guidelines for Exposure Assessment* (U.S. EPA, 1992) and with EPA policies and procedures for deriving health-based drinking water values, including maximum contaminant level goals (MCLGs), health advisory (HA) values, and health reference levels (HRLs). However, for the breast-fed infant, the model explicitly accounts for ingestion by the mother and the child's suckling rate. Therefore, EPA chose to use the 90<sup>th</sup> percentile ingestion rate for the breast-feeding mother, in which case the perchlorate concentration predicted in her breast milk would represent the high-end consumer but an *average* suckling rate for the breast-feeding infant, since the concentration in the breast milk is reflective of the mother's 90<sup>th</sup> percentile ingestion rate. Assuming that infant suckling rate and mother's ingestion rate vary independently and using 90<sup>th</sup> percentile rates for both the infant and the mother would effectively result in an ingestion rate greater than the 90<sup>th</sup> percentile for the breast-fed infant. This would be inconsistent with the Agency guidelines, policies, and procedures as well as the other life stage evaluations, including bottle-fed infants.

### 3.3.1. Breast-Fed Infant Suckling Rate

The suckling rate (breast milk ingestion rate) used by Clewell et al. (2007) was determined to be an inadequate description based on data currently available in the peer-reviewed literature. The original model used a table function to describe the baby's suckling rate, which is a volumetric transport rate (L/h) between the breast milk and baby's stomach, as the route of exposure for perchlorate. Fairly recent data on breast milk ingestion rates (Arcus-Arth et al., 2005) indicate that, in the first couple weeks of life, suckling rates are higher than those set by the table function implemented in Clewell et al. (2007) but are slightly below the table function between 2.5 weeks and several months of age (Figure 3-1). Therefore, to improve PBPK model predictions for the first 2 months after birth, the suckling rate was altered from the original approach used by the authors. A smooth Hill function of infant age (in days) was found to best fit to the mean ingestion rate data from Arcus-Arth et al. (2005) ( $r^2 = 0.98$ ) and was implemented in the description of infant breast milk ingestion:

$$\text{Milk Ingestion Rate (mL/h)} = \text{KTRANS} = \frac{28.4 \times \text{Age\_Days}^{2.4}}{3.06^{2.4} + \text{Age\_Days}^{2.4}}$$

This equation is not used to describe the breast milk ingestion beyond the first two months of age, the point at which the ingestion rate begins to decrease.



**Figure 3-1. Breast milk ingestion values used by EPA.**

Prior to the plotting of data from Arcus-Arth et al. (2005), the data were converted from g/kg-day to mL/h by using model-derived BWs at the given ages (BW were not provided in Arcus-Arth et al. [2005] for all life stages).

Data source: Arcus-Arth et al. (2005).



In addition, while iodide uptake inhibition in the infant was estimated by Clewell et al. (2007) using a simulated radioiodide injection directly to the infant, that approach would not account for the effect of maternal perchlorate exposure on iodide ingestion by the infant in breast milk. The model code already described iodide transport to breast milk; however, EPA added perchlorate inhibition of that transport, as noted above. Additionally, the model was extended to allow for transfer of the breast milk iodide to the breast-fed neonate's stomach (using the same suckling rate as for perchlorate) and estimated total infant thyroid iodide at 24 hours after simulated intravenous (i.v.) injection in the mother as the measure of effect. No change was needed for the bottle-fed infant, since the amount of iodide in the formula was presumed to be unaffected by the presence of perchlorate. Predicted radioiodide kinetics in infant blood was much different under this scenario, with a slower rise and fall and a peak around 12 hours after maternal injection. Thus, some of the dissimilarity between bottle-fed and breast-fed infant predictions can be attributed to this difference in radioiodide kinetics.

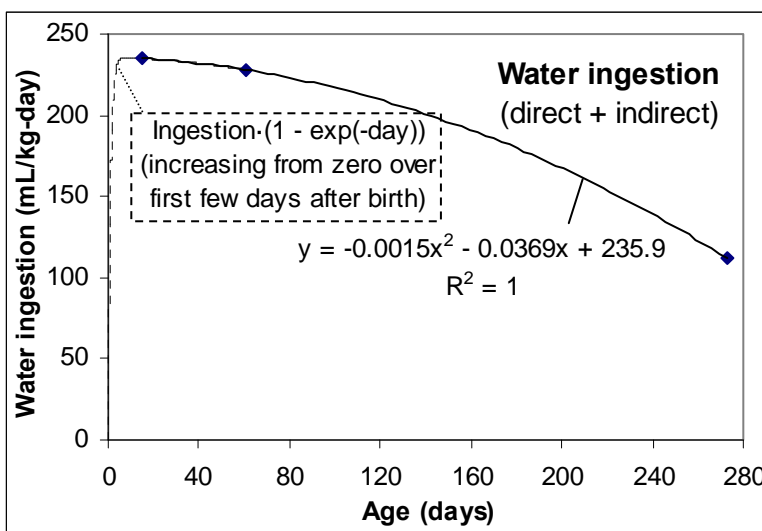
It should also be noted that, for breast-fed infant simulations under the RAIU inhibition scenarios being evaluated, the i.v. dose of radioiodide was treated as being given to the mother, a portion of which passed to the infant through breast milk. The portion passing to the infant in the absence of perchlorate served as the control value. So, in addition to the inhibition of iodide uptake by the infant's thyroid predicted by the model, the ingestion of perchlorate by the mother inhibited iodide transport into the breast milk, thus accounting for a perchlorate-induced alteration in nutritional iodide. The reduction of iodide transport in milk was small in the sense that the predicted reduction was only 0.7–1.0% when the infant was receiving 7  $\mu\text{g}/\text{kg}\text{-day}$  of perchlorate (for infants between 7 and 60 days old, with maternal perchlorate clearance at the average adult value). But this reduction had a near additive effect on the predicted reduction of iodide uptake by the infant's thyroid at this dose rate, which was 2.5% in the 60-day-old bottle-fed infant (see below) but 3.5 % for the breast-fed infant of the same age (both obtained by using the lower infant clearance, based on glomerular filtration but adult-average maternal clearance).

### **3.3.2. Bottle-Fed Infant Model Simulation Approach**

The model code for the breast-fed infant was modified to allow for exposure to perchlorate via ingestion of a water-based formula rather than breast milk. This provided for direct comparison of a bottle-fed infant with a breast-fed infant by using the same PBPK model structure and parameter set but with an alternate exposure scenario. Briefly, the lactating mother's perchlorate dose rate was set equal to zero, which allowed the infant's perchlorate exposure to be controlled independent of the mother's. The model was coded such that a fixed dose of perchlorate could be administered to the infant or a water concentration could be multiplied by a formula ingestion rate. Also, a direct i.v. dose of radioiodide to the bottle-fed infant was used in the determination of perchlorate inhibition of iodide uptake. The presence of

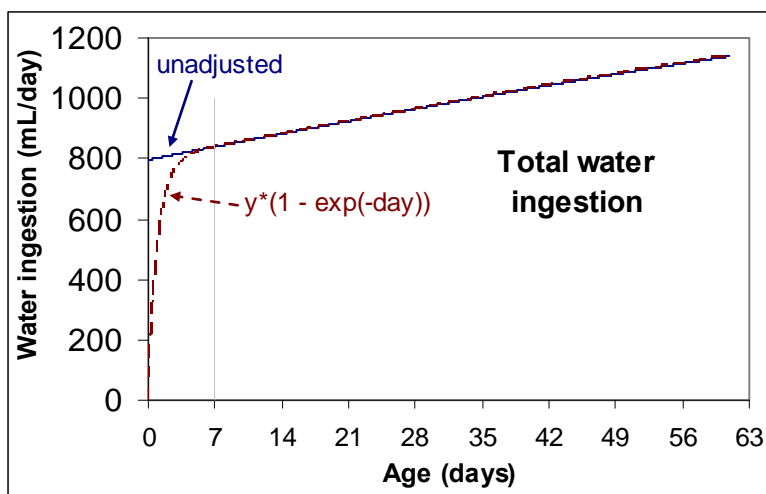
perchlorate in formula is assumed not to decrease the iodide available to the infant in the formula. More details of this approach are included in Appendix A.

To account for the fact that water ingestion varies with age and BW in the bottle-fed infant, a smooth function of age was fit to the results of Kahn and Stralka (2009), which had been plotted against the midpoint for each age range, as shown in Figure 3-2A. However, to account for the minimal ingestion occurring in the first couple days of life, the equation was multiplied by a rising exponential function:  $1 - e^{-\text{day}}$ . Note that the function describes the BW-specific ingestion rate (L/kg-day), so it is multiplied by BW to obtain the total ingestion (L/day), which is then a continuously increasing function of age, as shown in Figure 3-2B. (The function is only used for predictions up to 60 days of age.)



**Figure 3-2A. Ninetieth percentile water consumption values used by EPA for the bottle-fed infant.**

BW-specific function (mL/kg-day) shown as fit to 90<sup>th</sup> percentile water ingestion data from Kahn and Stralka (2009).



**Figure 3-2B. Ninetieth percentile water consumption values used by EPA for the bottle-fed infant.**

Plot of total ingestion (mL/day), calculated by multiplying age-specific ingestion rate (mL/kg-day) from Figure 3-2A by age-specific BW (kg).

A detailed description of issues with parameterization and coding errors in the PBPK models and the resolution of these issues is provided in Appendix A.

## 4. PBPK MODEL RESULTS AND LIFE STAGE ANALYSIS

### 4.1. PBPK MODEL RESULTS

Table 4-1 compares model predictions published by Clewell et al. (2007) with predictions for the model incorporating EPA’s corrections to equations and parameters—with the important exception that the urinary clearance rates in the published model are retained here. This analysis is intended to show the impact of the various other changes EPA made to the model. The two sets of values are generally close with the exception of the lactating mother, in which model predictions are approximately one-third of the values previously published in the lower dose range. This change is primarily due to the corrections in model code whereby inhibition of iodide transport into maternal skin and particularly breast tissue/milk by perchlorate was added to the code. Adding these terms leads to the prediction that, with perchlorate exposure, more iodide is kept in the circulating maternal blood rather than being transported to skin or transferred to the infant. Hence, more is available for uptake by the thyroid, reducing the impact of perchlorate inhibition of thyroid uptake.

**Table 4-1. Comparison of Clewell et al. (2007) published model predicted percent inhibition of thyroidal radioiodide uptake across life stages with EPA modified versions (but retaining the Clewell urinary clearance rates)\***

External dose (mg/kg-day)	Fetus <sup>a</sup> (% inhibition)		Breast-fed neonate <sup>b</sup> (% inhibition based on dose to mother)		Child <sup>c</sup> (% inhibition)		Pregnant woman <sup>a</sup> (% inhibition)		Lactating woman <sup>d</sup> (% inhibition)	
	Clewell	EPA	Clewell	EPA	Clewell	EPA	Clewell	EPA	Clewell	EPA
0.001	1.1	1.3	0.9	1.9	0.3	0.3	1	0.92	1.1	0.60
0.01	10	11	8	17	3	2.7	9	8.5	10	6.0
0.1	49	51	34	68	21	22	50	49	54	43
1	84	85	63	95	72	74	91	90	92	89

\*For this comparison, infant and maternal urinary clearance rates were set as in Clewell et al. (2007).

<sup>a</sup>Fetus and pregnant woman shown at GW 38 using clearance values as published in Clewell et al. (2007) that are equal to about half of the average adult value.

<sup>b</sup>Breast-fed neonate shown at postnatal month 1.5 (day 45). External dose is that ingested by the mother. Neonate ingestion (mg/kg-day) is 4.4, 4.1, 2.6, and 0.85 times maternal ingestion at external doses of 0.001, 0.01, 0.1, and 1 mg/kg-day, respectively, due to saturation of NIS-mediated transport of perchlorate into breast tissue and milk at higher doses.

<sup>c</sup>Child shown at 7 years of age with the EPA prediction using “medium” estimate for urinary clearance.

<sup>d</sup>Lactating woman shown at PND 7.

Table 4-2 compares the effects of alternate urinary clearance parameters on the EPA-modified PBPK model predictions of RAIU inhibition for the different life stages. A decrease in the PBPK model urinary clearance rate of iodide and perchlorate resulted in increases of RAIU inhibition predictions for all life stages. The largest effect was seen for the near-term fetus

(GW 40), such that the prediction of fetal RAIU inhibition increased from 3.4% inhibition at the highest rate of clearance to 11% inhibition at the lowest rate of clearance (Table 4-2). The detailed effects on RAIU inhibition resulting from the EPA model modifications, some of which are described in section 3, are provided in Appendix A.

**Table 4-2. Effect of urinary clearance on model predicted percent inhibition of thyroidal radioiodide uptake at the POD (7 µg/kg-day) for various life stages, using the EPA version of model code**

Urinary clearance rate	Gestation model <sup>b</sup>		Lactation model <sup>c</sup>						Older child <sup>f</sup>				
	Pregnant woman GW 40	Fetus GW 40	Lactating woman <sup>d</sup>			Breast-fed infant (based on dose to infant) <sup>e</sup>			Bottle-fed infant <sup>c,e</sup>			10 kg 0.97yr	14 kg 2yr
			7d	30d	60d	7d	30d	60d	7d	30d	60d		
High	1.6%	3.4%	1.3%	1.7%	1.9%	2.8%	3.0%	3.3%	1.4%	1.5%	1.5%	1.3%	1.3%
Medium <sup>a</sup>	2.9%	5.2%	2.1%	2.0%	2.0%	3.1%	2.8%	2.9%	1.8%	1.3%	1.2%	1.7%	1.7%
Low	6.1%	11%	4.4%	4.0%	4.0%	6.2%	4.4%	4.2%	4.3%	2.9%	2.5%	2.1%	2.1%

<sup>a</sup>Average adult value for urinary clearance (clearance constants  $CLUC_i = 0.11$  and  $CLUC_p = 0.125$ ) was used as a “medium” estimate for the pregnant and lactating woman and the older child.

<sup>b</sup>“High” value for urinary clearance was determined from Aboul-Khair et al. (1964) and  $cluc_i = (1 + 0.0703 \times GW - 0.0012 \times GW^2) \times 0.11$  and  $cluc_p = (1 + 0.0703 \times GW - 0.0012 \times GW^2) \times 0.125$ , where  $GW = 40$ . The  $V_{max}$  for thyroidal uptake of iodide and perchlorate were adjusted ( $V_{max}Tc \times 1.8631$ ) to fit Aboul-Khair et al. (1964) maternal thyroid uptake data (more detail in Appendix B). Fetus percent inhibition of RAIU was affected by maternal urinary clearance and thus included in the table; however, no fetal parameters were altered. Maternal NIS  $V_{max}$  values were not readjusted for the medium clearance. “Low” value for urinary clearance was used as published in Clewell et al. (2007) where  $CLUC_i = 0.06$  and  $CLUC_p = 0.05$ , determined from the parallelogram parameterization approach.

<sup>c</sup>The initial conditions used to estimate the percent inhibition of thyroidal radioiodide uptake for the lactating woman and breast- and bottle-fed infants were set at the final pregnant woman and fetus values by using the medium clearance rate.

<sup>d</sup>High clearance rate for the lactating woman was set equal to that of the average adult values from Merrill et al. (2005), but the  $V_{max}$  for thyroid perchlorate and iodide uptake was adjusted to fit literature data. Central estimate did not include  $V_{max}$  adjustment. The low estimate was as published by Clewell et al. (2007), where the clearance for iodide was equal to the average adult but the clearance rate for perchlorate was about half ( $CLUC_p = 0.05$ ).

<sup>e</sup>The dose to the lactating woman was adjusted so that the breast-fed infant received a total dose of 7 µg/kg-day perchlorate from maternal milk. Maternal dose rates lower than the POD were needed to provide 7 µg/kg-day to the infant, as follows in µg/kg-day: high maternal clearance at 7 days, 4.3; 30 days, 3.8; and 60 days, 3.9; medium maternal clearance at 7 days, 2.7; 30 days, 3.0; and 60 days, 3.6; and low maternal clearance at 7 days, 1.1; 30 days, 1.5; and 60 days, 1.8. The bottle-fed infant was simulated by using a constant 7 µg/kg-day perchlorate dose rate. High is estimated by using  $BW^{0.75}$  scaling as published in the Clewell et al. (2007) model. Medium assumes that clearance is equal to GFR (i.e.,  $CLUC_i = CLUC_p = [7.5 \text{ L/h}]/[70^{2/3}]$ , with a scaling coefficient of 2/3). Low assumes that clearance/GFR is the same as in the adult ( $\approx 40\%$ ).  $V_{max}$  for thyroid iodide uptake was adjusted for all clearance rates to fit literature data shown in Clewell et al. (2007, Figures 5 and 9).

<sup>f</sup>High value was estimated as published in Clewell et al. (2007) by using iodide and perchlorate urinary clearance constants equal to the average adult and the constants were scaled by  $BW^{0.75}$ . Medium used the same constants but scaled by  $BW^{1.0}$ ; this scaling described the average renal excretion of cimetidine (Lloyd et al., 1985) better. The low clearance was estimated by scaling by  $BW^{1.0}$  and multiplying by the ratio (0.76) of the lower 95% confidence bound to the mean for the Lloyd et al. (1985) data.

#### **4.1.1. PBPK Model Predictions Compared with Literature Data**

EPA-modified PBPK model predictions were compared with recent literature data as well as data previously simulated by Clewell et al. (2007) and Merrill et al. (2005). These comparisons of model data with literature data are detailed in the sections below.

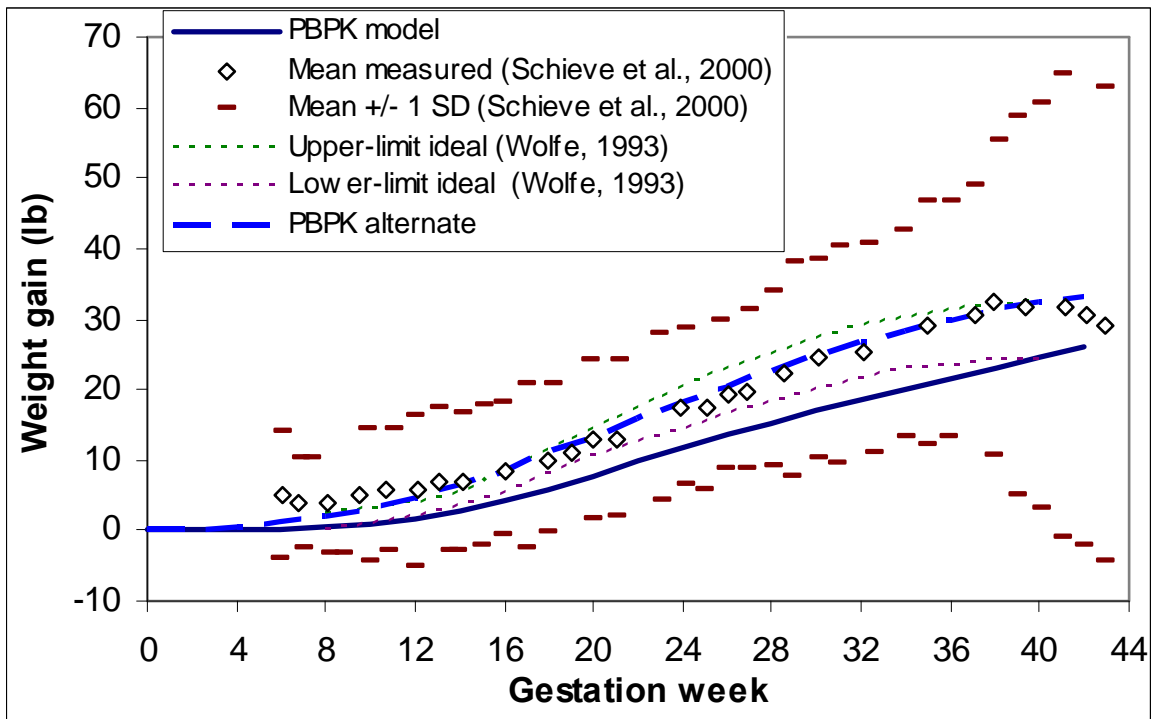
##### **4.1.1.1. BW Change During Pregnancy**

The Clewell et al. (2007) gestational model prediction of BW change during pregnancy was compared with literature data that were not used to develop the growth equations. While the Centers for Disease Control and Prevention collects data on maternal weight gain and other health indicators for pregnancy, these data appear to be reported as total weight gain (from conception to just before birth), reported in the form of percent of population falling into various categories (“underweight,” “normal weight,” etc.) and do not include information on specific growth. However, Schieve et al. (2000) reported BW gains during pregnancy from data for 3,511 mother-infant pairs from the 1988 National Maternal and Infant Health Survey, and Wolfe (1993) provided “upper-limit” and “lower-limit” values for ideal weight gain during pregnancy. These data were compared with the model-predicted BW gain (Figure 4-1).

Except for the apparent plateau in the data starting around GW 38, the shape of the model growth curve matches the observed growth curve quite well. While the curve falls somewhat below the observed mean (Schieve et al., 2000), it is well within one SD of the mean. Considering that these were data not used in specifying the model, the agreement appears to be quite good.

The data for the last few time points represent women who had not already given birth by that time (e.g., women who gave birth at GW 40 would not be included in the population represented at GW 42). So the apparent plateau should not be construed to mean that an individual woman’s BW would plateau or begin to decline in the last few GWs. Rather, it likely indicates that women who had the highest increase in BW by GW 38 (when the peak in the mean value occurs) gave birth in that week and so were not included in the population analyzed at later weeks. Since there is a tendency to induce labor before the child becomes too large to be safely delivered, the downward trend is then explained as a population shift, whereas the model growth curve represents the change in a particular individual.

Finally, to test the sensitivity to BW, an alternate model version with the growth curve shifted upward (Figure 4-1) was tested and compared to the original model. RAIU inhibition was evaluated at GW 40, given an exposure to 7  $\mu\text{g}/\text{kg}\text{-day}$  perchlorate (the POD). With the model growth curve as specified by the authors (Clewell et al. 2007), the RAIU inhibition predicted for the mother and fetus were 6.10 and 11.0%, respectively, and, with the BW increased to better match the observed mean, the model predicted 6.14 and 11.1%, respectively. Thus, the sensitivity of model predictions to changes in this range is negligible.



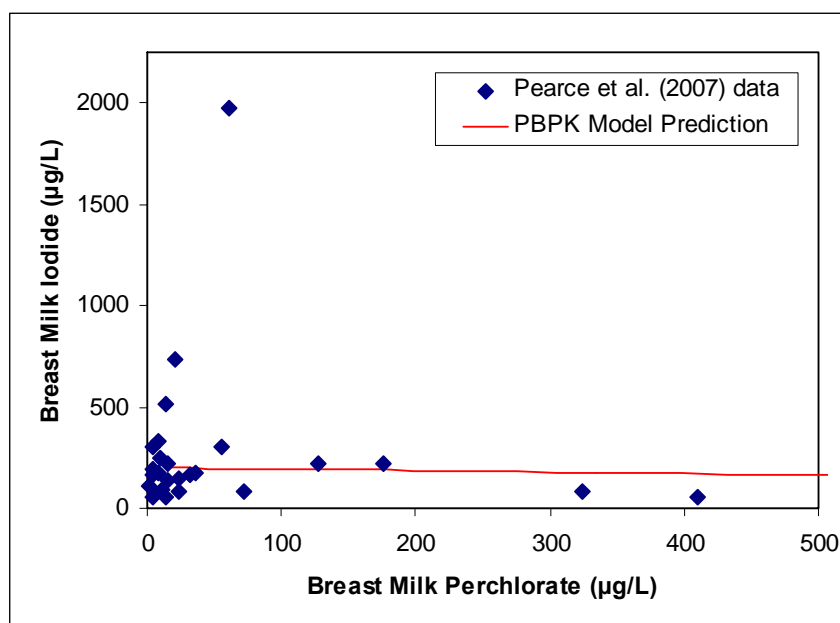
**Figure 4-1. Weight gain during gestation as predicted by the PBPk model equations implemented by Clewell et al. (2007) compared with recent literature data.**

The BW gain predicted by the PBPk model is within one SD of the mean data throughout gestation, with the best correlation at GWs 36 to 40.

#### 4.1.1.2. *Perchlorate and Iodine in Breast Milk*

For this exercise, the mean breast milk iodide for breast milk perchlorate concentrations less than 20 µg/L was considered to be “control” and estimated to be 199 µg/L by digitizing data from Pearce et al. (2007, Figure 1). Because the PBPk model does not simulate dietary iodide, the PBPk model-predicted 24-hour inhibition of radioiodide transfer into breast milk (%BM<sub>inh</sub>) was converted to a value relative to the Pearce et al. (2007) data by using the following calculation:  $(100 - \%BM_{inh}) \times 199 \mu\text{g/L} \div 100$ . For example, when the model predicted breast milk perchlorate to be 100 µg/L, a corresponding 3.3% decrease in 24-hour radioiodide in breast milk was observed:  $(100 - 3.3) \times 199 \mu\text{g/L} \div 100 = 192 \mu\text{g/L}$ . When the model predicted breast milk perchlorate concentration to be 334 µg/L, a corresponding 11% inhibition in breast milk 24-hour radioiodide was predicted. This value falls within the range of the Pearce et al. (2007) data, and thus the model predictions appear to be in line with the epidemiology data (Figure 4-2).





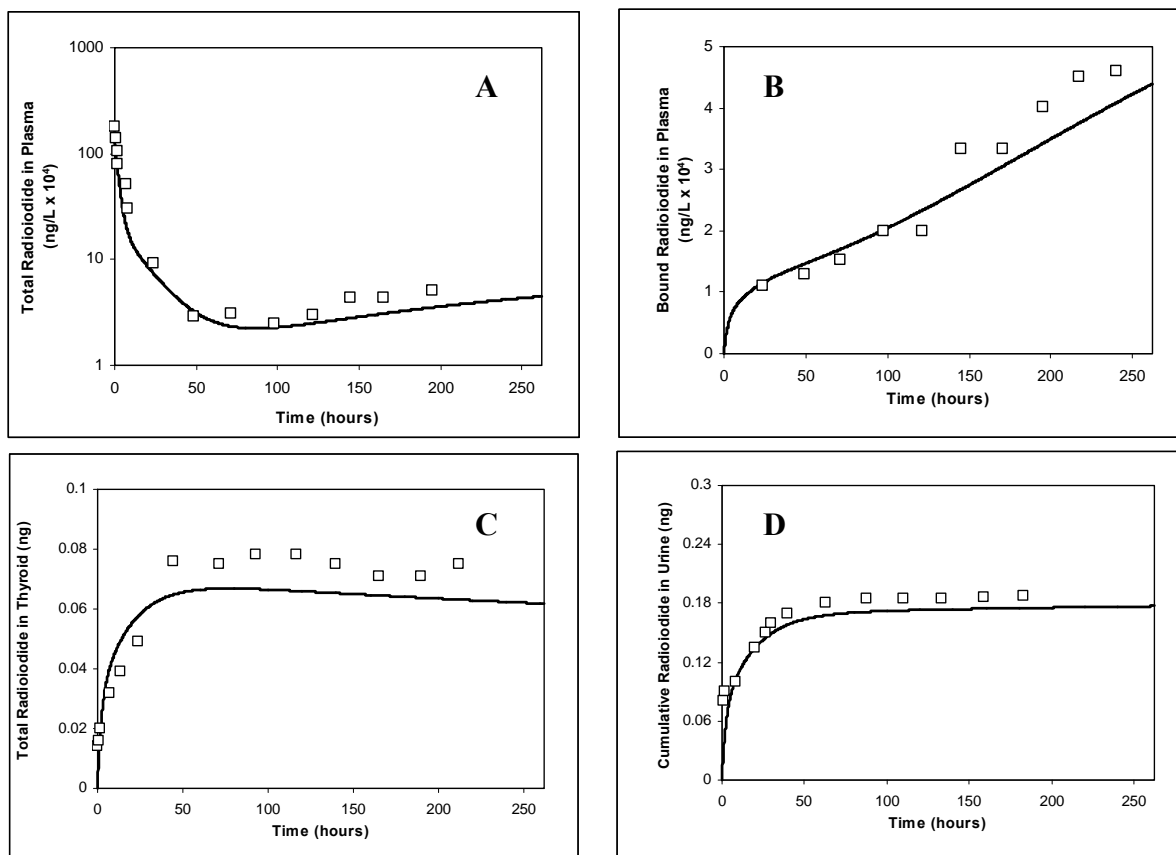
**Figure 4-2. PBPK model prediction of breast milk perchlorate and iodide compared with data from Pearce et al. (2007).**

The PBPK model was used to predict perchlorate concentration in breast milk and reduction in breast milk radioiodide concentration at 48 days post partum (median from Pearce et al. [2007]), using a range of perchlorate oral dose rates (1–50 µg/kg-day).

#### **4.1.1.3. Comparison with Data Previously Simulated by Merrill et al. (2005) and Clewell et al. (2007)**

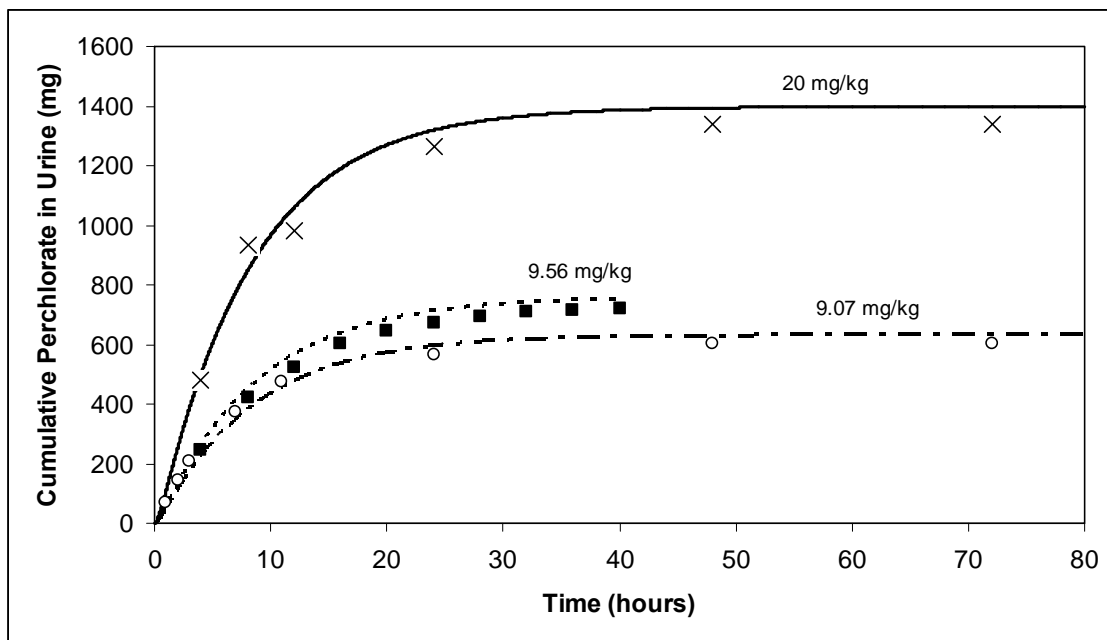
The following sections show comparisons of data with a few simulations, using the EPA-modified PBPK models for the average adult (Merrill et al., 2005) and gestation, lactation, and older child life stages (Clewell et al., 2007). Data in the figures below correspond to figures in the original model publications by Merrill et al. (2005) and Clewell et al. (2007). The modified PBPK model versions used by EPA in this evaluation remain in agreement with kinetic data shown in the original manuscripts and previously compared with the Merrill et al. (2005) and Clewell et al. (2007) PBPK models for radioiodide and perchlorate.

**4.1.1.3.1. Average adult.** The EPA-modified PBPK model for the average adult was tested for its ability to predict data shown in figures presented in Merrill et al. (2005). A few of the model simulations compared with observed data are shown below in Figures 4-3 and 4-4.



**Figure 4-3. EPA-modified PBPK model simulations for radioiodide in the average adult.**

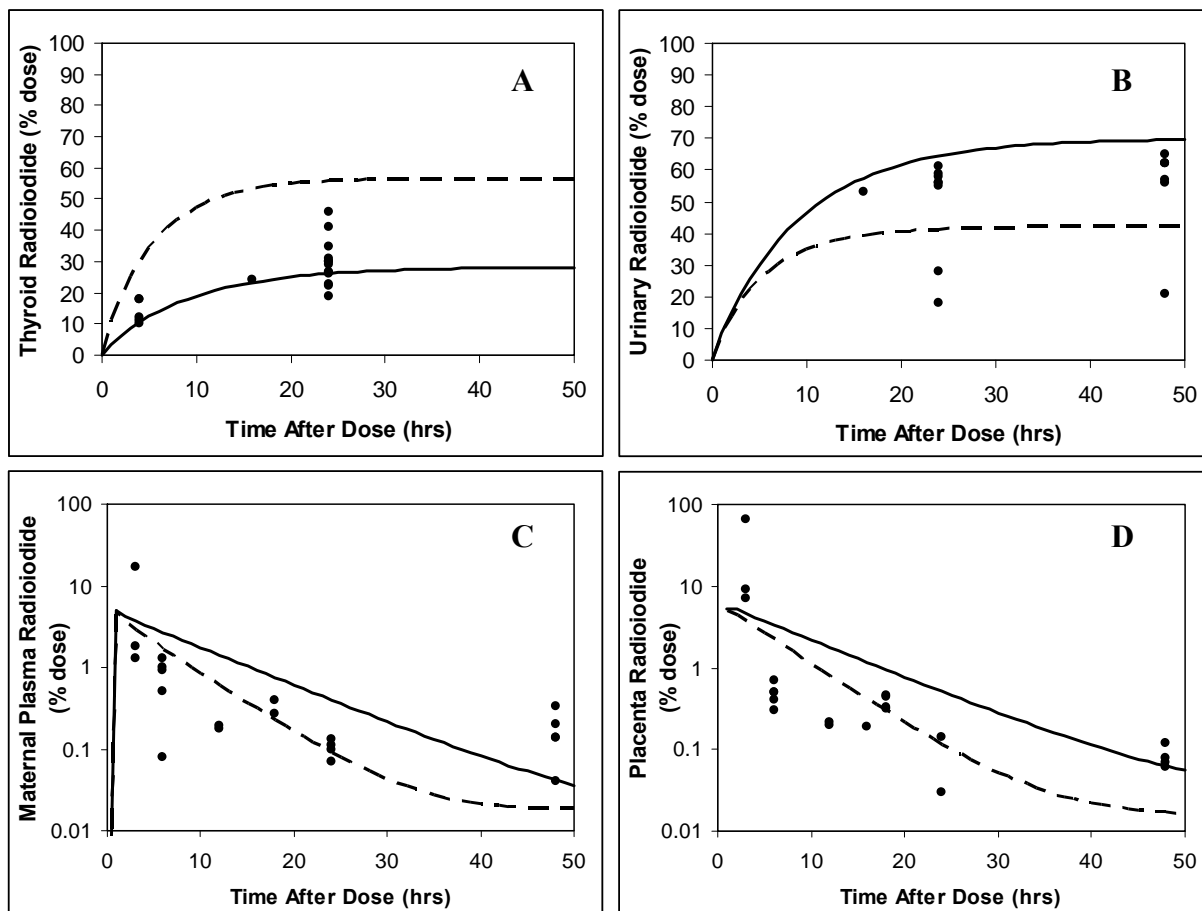
Model simulations (lines) shown with measured radioiodide (points) for (A) plasma, (B) protein-bound radioiodide in plasma, (C) total radioiodide in thyroid, and (D) cumulative radioiodide in urine after a tracer radioiodide i.v. dose at time zero. Similar simulations were originally shown in Merrill et al. (2005, Figure 3).



**Figure 4-4. EPA-modified PBPK model simulations for perchlorate excretion by the average adult.**

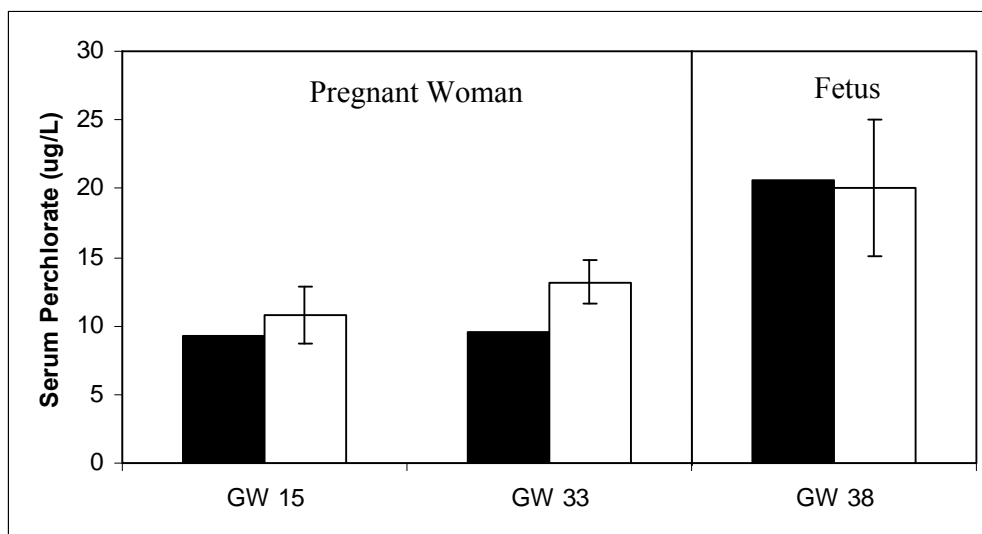
Model simulations (lines) shown with cumulative urinary perchlorate (points) measurements. Simulations correspond to figures originally shown in Merrill et al. (2005, Figures 8–10). Perchlorate oral bolus doses (9.07, 9.56, or 20 mg/kg) were administered to healthy male volunteers at time zero, and urinary perchlorate was measured at various times following dose.

**4.1.1.3.2. Pregnant woman and fetus.** The EPA-modified PBPK model for the pregnant woman and fetus was tested for its ability to predict data shown in figures presented in Clewell et al. (2007). A few of the model simulations compared with observed data are shown below in Figures 4-5 and 4-6.



**Figure 4-5. EPA-modified PBPK model simulations of radioiodide in the pregnant woman at GW 22.**

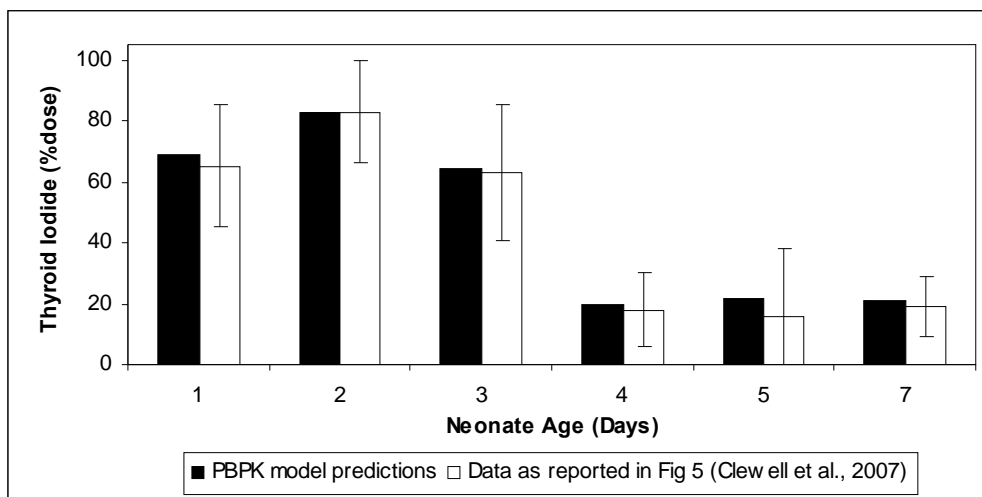
Model simulations (lines) shown with measured radioiodide (points) for (A) total radioiodide in thyroid, (B) cumulative excretion in urine, (C) maternal plasma, and (D) radioiodide in the placenta after a tracer radioiodide i.v. dose at time zero. Similar simulations were originally shown in Clewell et al. (2007, Figure 6). As in Clewell et al. (2007), the solid and broken lines indicate the model simulation using thyroid NIS iodide  $V_{max}$  mean ( $1.22 \times 10^5$  ng/hour) and maximum ( $6.52 \times 10^5$  ng/hour) values, respectively.



**Figure 4-6. EPA-modified PBPK model predictions of perchlorate in the pregnant woman at GWs 15 and 33 and in the fetus at birth (GW 38).**

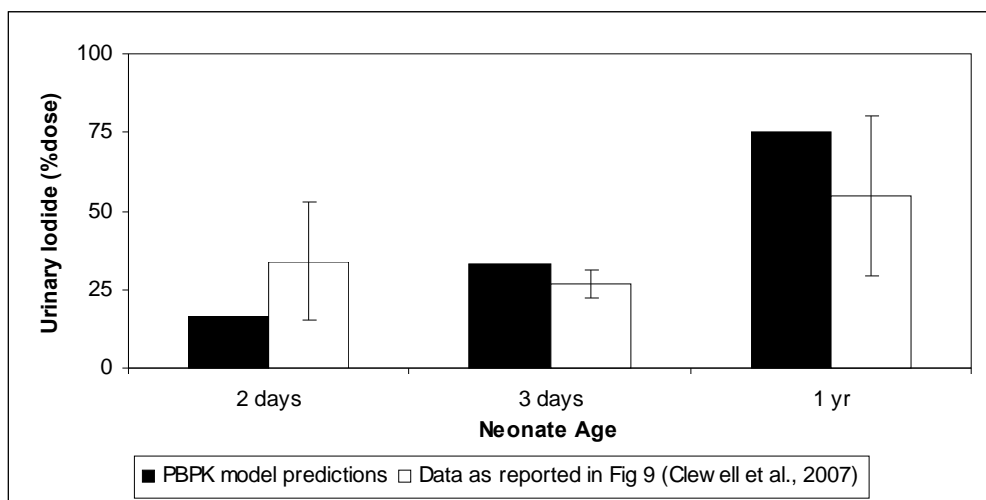
Model predictions (dark bars) shown with measured perchlorate (light bars  $\pm$  1 SD) following exposure to 114 ppm perchlorate in drinking water. Similar simulations were originally shown in Clewell et al. (2007, Figure 10).

**4.1.1.3.3. Lactating Woman and Neonate.** Model predictions obtained by using the PBPK models as modified by EPA were compared with published radioiodide and perchlorate data previously simulated by Clewell et al. (2007) and are shown below in Figures 4-7 and 4-8.



**Figure 4-7. EPA-modified PBPK model predictions of neonatal thyroid radioiodide uptake compared with data  $\pm$  SD as reported in Clewell et al. (2007, Figure 5).**

Twenty-four hour thyroid iodide uptake (as % dose) following i.v. dose to the neonate at 1, 2, 3, 4, 5, and 7 days following birth.



**Figure 4-8. EPA-modified PBPK model predictions of neonatal urinary radioiodide excretion compared with data  $\pm$  SD as reported in Clewell et al. (2007, Figure 9).**

Twenty-four hour urinary excretion (as % dose) following i.v. dose to the neonate at 2 days, 3 days, and 1 year following birth.

#### 4.2. LIFE STAGE RELATIVE RESPONSE ANALYSIS

For this document and analysis, *sensitivity* is defined as the predicted response in percent RAIU inhibition 24 hours after iodide i.v. injection for an average individual within a specific subgroup (e.g., bottle-fed infants) relative to the predicted response in percent RAIU inhibition for an average, nonpregnant adult, where response is the percent RAIU inhibition 24 hours after iodide i.v. injection.

The PBPK models published by Merrill et al. (2005) and Clewell et al. (2007) were modified as described above and in the appendices and used to estimate the predicted percent RAIU inhibition for the average adult and different subgroups, including potentially sensitive subgroups. These estimates were made assuming a dose equal to the POD of 7  $\mu\text{g}/\text{kg}\text{-day}$ , which was identified by NRC (2005) as a NOEL for the derivation of the RfD and adopted by EPA. The POD was the level at which no significant inhibition of RAIU was observed in a group of healthy adult volunteers. Table 4-3, column 3, shows the PBPK model predictions of percent inhibition of RAIU at the 7  $\mu\text{g}/\text{kg}\text{-day}$  dose rate. The relative sensitivity of different subgroups was determined by comparing the percent RAIU inhibition of each subgroup to the percent RAIU inhibition for an average adult at a dose equal to the POD (Table 4-3, column 4).

**Table 4-3. Model-predicted RAIU inhibition and relative sensitivity of different subgroups compared with the average adult at a dose equal to the POD based on EPA's modified PBPK models**

Population or life stage	BW (kg) <sup>a</sup>	RAIU inhibition at the POD (7 µg/kg-day)	Relative sensitivity vs. average adult at the POD
Average adult	70	1.6%	1
Woman (child-bearing age)	68	3.0% <sup>b</sup>	1.8
GW 13	Mother: 69	6.3% <sup>c</sup>	3.8
GW 20	Mother: 71	6.3% <sup>c</sup>	3.8
GW 40	Mother: 79	6.1% <sup>c</sup>	3.7
	Fetus: 3.5	11% <sup>c</sup>	6.7
Mother and breast-fed infant (7 days)	Mother: 74	2.1% <sup>d</sup>	1.3
	Infant: 3.6	5.4% <sup>d, e, f</sup>	3.3
Mother and breast-fed infant (30 days)	Mother: 73	2.0% <sup>d</sup>	1.2
	Infant: 4.2	4.4% <sup>d, e, f</sup>	2.7
Mother and breast-fed infant (60 days)	Mother: 72	2.0% <sup>d</sup>	1.2
	Infant: 5.0	4.2% <sup>d, e, f</sup>	2.5
Bottle-fed infant (7 days)	Infant: 3.6	4.3% <sup>e</sup>	2.6
Bottle-fed infant (30 days)	Infant: 4.2	2.9% <sup>e</sup>	1.8
Bottle-fed infant (60 days)	Infant: 5.0	2.5% <sup>e</sup>	1.5
Child (0.97 years) <sup>g</sup>	Child: 10	1.7% <sup>h</sup>	1.1
Child (2 years)	Child: 14	1.7% <sup>h</sup>	1.1

<sup>a</sup>The BW (70 kg) for the average adult is the default weight used by the Office of Water. All other BWs are generated by the model.

<sup>b</sup>Results were obtained by using modified code in which fetal and placental compartments were removed from the code for pregnancy. Maternal BW was held at the value defined at the start of pregnancy (BW = 67.77 kg), and the "average adult" urinary clearance values as published by Merrill et al. (2005) were used.

<sup>c</sup>Results are based on using the maternal urinary clearance as published in Clewell et al. (2007), which is equal to about half of the average adult clearance.

<sup>d</sup>Results are based on setting the maternal clearance rates of both perchlorate and iodide during lactation equal to the rate of the average adult. Clewell et al. (2007) used an iodide clearance rate equal to that of an average adult but a perchlorate rate only half that of the average adult.

<sup>e</sup>Percent RAIU inhibition given for the infant is provided based on a value of urinary clearance scaled from the adult by  $BW^{2/3}$  to approximate surface-area scaling and then multiplied by a rising fraction vs. age based on data (DeWoskin and Thompson, 2008) to reflect the reduction in GFRs (see section 3.1 and Appendix B.3 for further details). Clewell et al. (2007) scaled urinary clearance by  $BW^{0.75}$  rather than adjusting based on GFR.

<sup>f</sup>These percent RAIU inhibition values are based on an internal dose to the breast-fed infant of 7 µg/kg-day, the same as for the other subgroups. Maternal dose rates lower than the POD are needed to provide 7 µg/kg-day to the infant (see Table 4-2, notes), as follows: 7 days, 2.7 µg/kg-day; 30 days, 3.0 µg/kg-day; 60 days, 3.6 µg/kg-day. These doses differ due to changes in BWs and other pharmacokinetic factors with age.

<sup>g</sup>Because Office of Water typically uses a 10 kg child as a default assumption for its health advisories, the model was run for a child at 0.97 years, the age at which the model-simulated BW for a child is 10 kg.

<sup>h</sup>Results were obtained by setting urinary clearance constants for the older child equal to the average adult (Merrill et al., 2005) and scaling by  $BW^{1.0}$ .

EPA's model predictions may generally be considered central estimates for each subgroup (at the consumption levels modeled) that account for pharmacokinetic (PK) differences and do not take into account within-group variability in pharmacokinetics, uncertainty in model parameters and predictions, or population differences in pharmacodynamics (PD). Fetal simulations are only reported for the end of gestation (GW 40), since key fetal parameters are considered to be too uncertain for reliable use earlier in gestation and there are no data to support model predictions or parameters for earlier gestation. Note that results for the GW 40 fetus should not be assumed to also represent the sensitivity of the first or second trimester fetus, a life stage for which reliable estimates cannot be made. However, maternal parameters are considered to be more reliable, so maternal predictions are also shown for GWs 13 and 20.

In this analysis, urinary clearance was identified as a key parameter (i.e., model predictions were highly sensitive to the value used for this variable). Given the range of uncertainty about urinary clearance during pregnancy and early infancy, the most "sensitive" (lowest) value was selected from a range of potential values that were identified, while during lactation (breast-feeding woman), the middle option (2) was selected. See section 3.1, above, for details. However, a full population analysis of urinary clearance was *not* conducted, and given that variability in other PK parameters was not addressed, these estimates should not be considered a true upper confidence bound on RAIU inhibition.

*When compared to the average adult, the fetus was identified by EPA's analysis as the most sensitive subgroup with respect to percent RAIU inhibition at a dose equal to the POD.* This finding is consistent with prior PBPK modeling analyses by Clewell et al. (2007). The predicted percent RAIU inhibition is approximately 6.7-fold higher for the fetus at GW 40 than for the average adult. (Simulations at earlier GWs indicate that the fetus is more sensitive than the adult throughout pregnancy but are considered too quantitatively uncertain to assign exact relative sensitivities.) The same analysis shows that the predicted percent RAIU inhibition is approximately one- to threefold higher for the breast-fed and bottle-fed infant (7–60 days) than for the average adult and is approximately equal for the 1- to 2-year-old child compared to the average adult. While the difference estimated for fetuses is larger than for other groups, EPA's analyses indicate that, due to differences in exposure, fetuses whose mothers drink water containing perchlorate would be predicted to have somewhat lower predicted RAIU inhibition than would other sensitive subgroups.

#### **4.3. LIFE STAGE COMPARISON FOR THREE DRINKING WATER CONCENTRATIONS**

EPA evaluated the percent RAIU inhibition at various water concentrations, with and without perchlorate intake from food sources, for the different life stages. EPA-adjusted models based on Clewell et al. (2007), as described earlier, were used to simulate three drinking water concentrations (15, 20, 24.5 ppb) (Table 4-5). Available literature was used to estimate water



intake rates for the different life stages as well as the dietary contribution to the average daily dose of perchlorate, as described below.

The water intake rates used for the average adult, nonpregnant and pregnant woman are based on normalized 90<sup>th</sup> percentile values for total (direct and indirect) consumers-only water intake multiplied by the age- or GW-dependent BW. (Direct water consumption is that used for drinking; indirect consumption is that used for food preparation and therefore ingested with food.) EPA selected the 90<sup>th</sup> percentile drinking water intake value for most of the subpopulations evaluated in this report in order to explore the potential effects of high-end exposure to perchlorate from public water systems. The approach is consistent with the *Guidelines for Exposure Assessment* (U.S. EPA, 1992) and with EPA policies and procedures for deriving health-based drinking water values, including MCLGs, HA values, and HRLs. EPA acknowledges that modeling a complete distribution of water ingestion would be more informative; however, due to time and data constraints, EPA selected a high-end water intake estimate as the most appropriate means to explore the potential effects of drinking water exposure on subpopulations. EPA selected community water ingestion for the analysis to characterize the high-end exposure to perchlorate from public water systems (as opposed to bottled water or private well water, which is captured in the total water ingestion). For this assessment, in support of EPA's evaluation needs under SDWA, EPA's interest in the impact of perchlorate from direct and indirect community water consumption is considered. EPA further notes that less than 1% of public water systems reported detecting perchlorate at concentrations greater than 15 µg/L. Therefore, by evaluating populations consuming the 90<sup>th</sup> percentile of drinking water with concentrations of perchlorate at 15, 20, and 24.5 µg/L, EPA is providing a realistic high-end exposure estimate.

The water intake rates used to estimate daily perchlorate exposure from drinking water were 0.032 L/kg-day (Kahn and Stralka, 2009; U.S. EPA, 2004) for the average adult and nonpregnant woman and 0.033 L/kg-day for the pregnant woman (U.S. EPA, 2004). However, a constant water intake rate (2.96 L/day, 90<sup>th</sup> percentile, consumers only [U.S. EPA, 2004]) for the lactating mother was used since her BW is expected to decrease during the weeks following pregnancy, while demands of breast-feeding increase. For the 6- to 12-month-old and 1- to 2-year-old children, the water intake rates of 0.112 L/kg-day and 0.056 L/kg-day, respectively, were set based on 90<sup>th</sup> percentile values for direct and indirect water consumers-only intake (from Kahn and Stralka [2009, Table 4]). Additionally, to calculate L/day for these age groups, the corresponding age group mean BWs obtained from National Health and Nutrition Examination Survey 1999–2006 were used: 9.2 kg for 6- to 12-month-old children and 11.4 kg for 1- to 2-year-old children. Using the PBPK model-predicted BW from growth equations, this approach resulted in model predictions for a 9.6-month-old child and a 1.3-year-old child. A different approach was used to estimate the breast- and bottle-fed infant breast milk and formula intake rates, respectively (see sections 3.3.1 and 3.3.2 for details regarding these intake rates).

The dietary doses of perchlorate used correspond to the midpoint of the range of lower- and upper-bound average perchlorate dietary intakes for each subgroup, as identified from the U.S. Food and Drug Administration (FDA) Total Diet Study (TDS) (Murray et al., 2008), except for the breast- and bottle-fed infants. The breast-fed infants are assumed to have no direct exposure via food or water. The estimates for breast-fed infants in Table 4-5 result from the combined food and water dose to the mother providing breast milk to the infant.

The bottle-fed infant TDS was derived from the infant formula concentrations of perchlorate reported in the FDA TDS data available online (U.S. FDA, 2008a). EPA selected the FDA TDS data to represent the dietary intake of perchlorate because the TDS is the best available nationally representative estimate of dietary exposure to perchlorate for the majority of the sensitive subpopulations of concern, including infants and children. The data gathered for 2005–2006 resulted in detection of perchlorate in 8 of the 12 samples (soy- and milk-based formulas) with a detected concentration mean of 1.875 ppb (limit of detection [LOD] 1.0 ppb). Using 0.5 ppb (half the LOD) for the samples in which perchlorate was not detected, the average is 1.42 ppb. Each of the 12 values represents a composite sample, based on samples collected four times a year in four geographical locations for 5-week periods and in three cities in each region. The samples reported by the FDA are shown in Table 4-4, along with the market basket averages and the overall average calculated as described above.

**Table 4-4. The FDA TDS perchlorate data in infant formula for 2005–2006**

TDS food #	Food description	Perchlorate level (ppb)			
		Market basket	Market basket	Market basket	Market basket
		1	2	3	4
202	Infant formula, milk, high iron	ND	2.5	2	2
203	Infant formula, milk, low iron	1.2	ND	3.6	2.1
309	Infant formula, soy	ND	ND	0.8 <sup>a</sup>	0.8 <sup>a</sup>
	Market basket average <sup>b</sup>	0.73	1.17	2.13	1.63
	Overall average	1.42			

<sup>a</sup>Estimated value; result was below level of quantitation but above LOD.

<sup>b</sup>Non-detects (NDs) were averaged at one-half the LOD of 1 ppb.

Source: U.S. FDA (2008a)

In addition to the FDA TDS data, EPA also considered the results of a study by Pearce et al. (2007). Samples of 17 brands of prepared liquid formula analyzed by Pearce et al. (2007) averaged 1.45 ppb perchlorate, consistent with the FDA TDS information. In addition to calculating the bottle-fed infant percent RAIU inhibition for 1.42 ppb contribution from infant formula (Table 4-5), the bottle-fed infant model sensitivity to a change in TDS was tested by using 1 ppb and 3.6 ppb. A decrease from 1.42 to 1 ppb resulted in a change of –0.05 and –0.03% RAIU inhibition for the 7- and 60-day-old bottle-fed infants, respectively. Conversely,

an increase from 1.42 to 3.6 ppb resulted in a change of +0.28% and +0.17% RAIU inhibition for the 7- and 60-day old bottle-fed infants, respectively.

Assuming a 90<sup>th</sup> percentile water ingestion rate of 0.033 L/kg-day and perchlorate intake from food consumption of 0.1 µg/kg-day and using the Clewell et al. (2007) PBPK model-fitted BW, the pregnant woman's dose of perchlorate was estimated to not exceed the RfD if she consumed less than 15 µg/L of perchlorate in drinking water.

There are uncertainties associated with this modeling, as there are for any modeling effort. For example, this analysis does not take into account within-group variability in PK, uncertainty in model parameters and predictions, or population differences in PD. Also, the NRC identified fetuses of pregnant women that are hypothyroid or iodine deficient as the most sensitive subpopulation. These models were not designed to account for whether the pregnant woman is hypothyroid or iodine deficient. Model predictions of doses in the various subgroups apply to a subgroup average for typical, healthy individuals and effectively describe the RAIU inhibition relative to that same individual as his/her own control. Some members of a group would be expected to have RAIU inhibition greater than indicated in Table 4-5 for a particular perchlorate concentration, while others would have lesser inhibition. This would be expected for fetuses as well as for other subgroups. Likewise, the model does not allow for predictions of how RAIU inhibition, or the impact of that inhibition, might change with dietary iodide status (i.e., in an iodide deficient individual or one with more than sufficient dietary iodide).

There is also some uncertainty regarding the water intake rates, particularly for infants. EPA described water intake by infants as a smooth function fit to the 90<sup>th</sup> percentile community water-consumers' intake rate data (intake per unit BW) of Kahn and Stralka (2009), which is then multiplied by the age-dependent BW to account for the changes occurring over the first weeks of life. This resulted in an estimated 90<sup>th</sup> percentile water intake rate of 0.84 L/day for the 7-day-old bottle-fed infant and was used by EPA in PBPK model simulations. General information on water and formula intake for 7-day-old infants is also available in guidelines for healthy growth and nutrition of the American Academy of Pediatrics (AAP) (Hagan et al., 2008). The values estimated by using the guidelines from the AAP (0.126 L/kg-day, assuming 80% is the percent water used in preparation of formula) for 7-day-old infants are close to the mean consumers-only intake rate for the 1- to 30-day-old infants from the Kahn and Stralka (2009) study (0.137 L/kg-day; n = 40).

**Table 4-5. Predicted percent RAIU inhibition and corresponding perchlorate intake ( $\mu\text{g}/\text{kg}\text{-day}$ ) at three different water concentrations with and without food intake**

	Body weight (kg) <sup>a</sup>	90th Percentile water intake (L/day) <sup>b</sup>	% RAIU inhibition Perchlorate intake ( $\mu\text{g}/\text{kg}\text{-day}$ )			TDS food ( $\mu\text{g}/\text{kg}\text{-day}$ ) <sup>c</sup>	% RAIU inhibition Perchlorate intake ( $\mu\text{g}/\text{kg}\text{-day}$ )		
			Water only				Food + water		
			15 $\mu\text{g}/\text{L}$	20 $\mu\text{g}/\text{L}$	24.5 $\mu\text{g}/\text{L}$		Food + 15 $\mu\text{g}/\text{L}$	Food + 20 $\mu\text{g}/\text{L}$	Food + 24.5 $\mu\text{g}/\text{L}$
<b>Average adult</b> $\mu\text{g}/\text{kg}\text{-day}$	<b>70</b>	<b>2.24</b>	0.11 0.48	0.15 0.64	0.18 0.78	<b>0.1</b>	0.13 0.58	0.17 0.74	0.20 0.88
<b>Nonpregnant woman</b> $\mu\text{g}/\text{kg}\text{-day}$	<b>66</b>	<b>2.11</b>	0.21 0.48	0.27 0.64	0.34 0.78	<b>0.1</b>	0.25 0.58	0.32 0.74	0.38 0.88
<b>Pregnant woman</b>									
<b>Mother—GW 13</b> $\mu\text{g}/\text{kg}\text{-day}$	<b>69</b>	<b>2.18</b>	0.47 0.50	0.63 0.66	0.77 0.81	<b>0.1</b>	0.57 0.60	0.73 0.76	0.87 0.91
<b>Mother—GW 20</b> $\mu\text{g}/\text{kg}\text{-day}$	<b>71</b>	<b>2.34</b>	0.46 0.50	0.62 0.66	0.76 0.81	<b>0.1</b>	0.56 0.60	0.72 0.76	0.85 0.91
<b>Mother—GW 40</b> $\mu\text{g}/\text{kg}\text{-day}$	<b>78</b>	<b>2.57</b>	0.45 0.50	0.60 0.66	0.74 0.81	<b>0.1</b>	0.54 0.60	0.69 0.76	0.83 0.91
<b>Fetus—GW 40</b>	<b>3.5</b>	—	0.89 —	1.2 —	1.4 —		1.1 —	1.4 —	1.6 —
<b>Breast-fed infant</b>									
<b>Mother—7 d</b> $\mu\text{g}/\text{kg}\text{-day}$	<b>74</b>	<b>2.96</b>	0.18 0.60	0.24 0.80	0.30 0.98	<b>0.1</b>	0.21 0.70	0.27 0.90	0.33 1.1
<b>Infant—7 d</b> $\mu\text{g}/\text{kg}\text{-day}$	<b>3.6</b>	<b>0.60<sup>d</sup></b>	1.1 1.6	1.5 2.1	1.8 2.6	— <sup>d</sup>	1.3 1.8	1.7 2.4	2.0 2.8
<b>Mother—60 d</b> $\mu\text{g}/\text{kg}\text{-day}$	<b>72</b>	<b>2.96</b>	0.18 0.61	0.24 0.82	0.29 1.0	<b>0.1</b>	0.21 0.71	0.26 0.92	0.32 1.1
<b>Infant—60 d</b> $\mu\text{g}/\text{kg}\text{-day}$	<b>5</b>	<b>0.68<sup>d</sup></b>	0.75 1.2	1.0 1.6	1.2 2.0	— <sup>d</sup>	0.87 1.4	1.1 1.8	1.3 2.2
<b>Bottle-fed infant</b>									
<b>Infant—7 d</b> $\mu\text{g}/\text{kg}\text{-day}$	<b>3.6</b>	<b>0.84<sup>e</sup></b>	2.0 3.5	2.6 4.7	3.2 5.8	<b>1.42 <math>\mu\text{g}/\text{L}</math></b>	2.2 3.9	2.8 5.0	3.4 6.1
<b>Infant—60 d</b> $\mu\text{g}/\text{kg}\text{-day}$	<b>5</b>	<b>1.14<sup>e</sup></b>	1.2 3.4	1.6 4.6	2.0 5.6	<b>1.42 <math>\mu\text{g}/\text{L}</math></b>	1.3 3.7	1.7 4.9	2.1 5.9
<b>Older child</b>									
<b>6–12 mo<sup>f</sup></b> $\mu\text{g}/\text{kg}\text{-day}$	<b>9.2</b>	<b>1.03</b>	0.42 1.68	0.56 2.24	0.69 2.74	<b>0.275</b>	0.49 1.96	0.63 2.52	0.75 3.02
<b>1–2 yr<sup>f</sup></b> $\mu\text{g}/\text{kg}\text{-day}$	<b>11.4</b>	<b>0.64</b>	0.21 0.84	0.28 1.12	0.35 1.37	<b>0.37</b>	0.31 1.21	0.38 1.49	0.44 1.74

- <sup>a</sup>Calculations for a 70 kg average adult are shown, while the BW for the nonpregnant woman is from U.S. EPA (2004, based on CSFII 94-96, 98), and BWs for the child are mean values from Kahn and Stralka (2009). BWs in italics are predicted weights (functions of age or GW), using growth equations from Gentry et al. (2002) as implemented in the PBPK models (Clewell et al., 2007) (nonpregnant value is BW at day 0 of gestation).
- <sup>b</sup>Water intake levels for adults other than the lactating mother are based on normalized 90<sup>th</sup> percentile values for total water intake (direct and indirect) multiplied by the age- or GW-dependent BW as follows: 32 mL/kg-day for average adult and nonpregnant woman; 33 mL/kg-day for the pregnant woman. A fixed ingestion rate was used for the lactating mother because, while her BW is expected to drop during the weeks following the end of pregnancy, the demands of breast-feeding will be increasing. Values are from Kahn and Stralka (2009), with the exception of the values for women, which come from U.S. EPA (2004).
- <sup>c</sup>The dietary values used correspond to the midpoint of the range of lower- and upper-bound average perchlorate levels for each subgroup, as identified from the FDA TDS in Murray et al. (2008), except for the bottle-fed infant. EPA used 1.42 µg/L as the concentration of perchlorate in infant formula. This is based on the average of available FDA TDS data (U.S. FDA, 2008a), with ½ LOD included in the average for the samples in which perchlorate was not detected.
- <sup>d</sup>The breast-fed infants are assumed to have no direct exposure via food or water. The prediction for breast-fed infants in this table results from the dose from both food and water to the mother providing breast milk to the infant. Breast-fed infant “water intake” is the breast milk ingestion rate obtained from the age-dependent Hill function fit to the breast milk ingestion data (L/kg-day) from Arcus-Arth et al. (2005), as shown in Figure 3-1. Urinary clearance rates for the lactating woman equal to that of the average adult were used, consistent with data presented in Delange (2004).
- <sup>e</sup>For the bottle-fed infant, normalized total water intake (direct and indirect, L/kg-day) was described as a smooth function of infant age, fit to the results from Kahn and Stralka (2009) and multiplied by BW (age). The FDA has suggested an alternate approach, using the caloric intake requirement of a 7-day-old infant as the basis for calculating consumption (U.S. FDA, 2008b). This would likely yield a lower estimate of intake than the 0.84 L/day that EPA used in the model.
- <sup>f</sup>For the 6- to 12-month-old and 1- to 2-year-old children, EPA set the water ingestion based on published exposure tables and selected the age at which the model-predicted BW matched the exposure-table mean. This approach resulted in model predictions for 0.796-year-olds (to represent 6- to 12-month-old children) and 1.285-year-olds (to represent 1- to 2-year-old children).

## **5. OTHER SUSCEPTIBLE SUBGROUPS, MODEL UNCERTAINTY, AND DATA NEEDS**

### **5.1. OTHER SUSCEPTIBLE SUBGROUPS**

This analysis focused on the inhibition of thyroid radioiodide uptake by perchlorate on the average adult, nonpregnant woman, lactating woman, breast-fed neonate, bottle-fed neonate, and older child. The individuals in each of these categories were considered to be average, euthyroid individuals. Iodine deficiency, hypothyroidism, and other thyroid diseases were not considered. Additionally, effects of perchlorate in individuals also exposed to other iodide uptake inhibitors (e.g., thiocyanate, nitrate) were not evaluated.

The PBPK models developed by Merrill et al. (2005) and Clewell et al. (2007) were not developed with the goal of providing simulations of dietary iodide. The models accurately simulate trace amounts of radioiodide and inhibition of radioiodide uptake into the thyroid; however, the models are unable to handle the large mass flux of dietary iodide compared to radioiodide. Thus, in order to simulate iodine deficiency using these models, only qualitative estimates could be provided. In iodine deficiency or the hypothyroid state, the thyroid NIS is up-regulated (or stimulated) by thyroid-stimulating hormone. Thus, an arbitrary increase in  $V_{\max}$  of NIS transport of iodide and perchlorate could give some qualitative indication of how the system might be affected (e.g., the degree to which NIS transport would change as well as the secretion, or loss of iodide, rate constant currently used in the model), but too many uncertainties exist for this information to be useful in this assessment. Thus, we do not have modeling predictions available for effects of perchlorate on iodide-deficient individuals.

### **5.2. MODEL UNCERTAINTIES AND LIMITATIONS**

#### **5.2.1. Model Uncertainties**

Lack of data in various human life stages for model parameterization and validation is the cause for uncertainties associated with the use of the PBPK models developed by Merrill et al. (2005) and Clewell et al. (2007). In humans, the only data available for validation of thyroid percent RAIU inhibition model predictions are for the average adult human and were used in Merrill et al. (2005).

In the absence of thyroid percent RAIU inhibition data, other data sets have been used to demonstrate the ability of the models to predict radioiodide and perchlorate kinetics in various life stages. For the evaluation of their model, Clewell et al. (2007) used such data, the majority of which were collected in the 1950s and 1960s. These “validation” exercises are valuable in that they provide confidence that the models are predicting perchlorate and radioiodide distribution and elimination. In the absence of PBPK models, it would be necessary to rely on external dose as the estimate of maternal, fetal, and neonatal exposure. However, since

perchlorate kinetics are governed by active transport as well as passive diffusion, the use of external dose does not provide a reliable estimate of internal dose.

The PBPK model parameters utilized by Clewell et al. (2007) were primarily derived by using a parallelogram approach, such that the relationship between model parameters for various life stages in the rat is similar in humans. The data available for validation of these parameters were minimal, especially in the developing fetus and neonate. Thus, confidence in these model predictions is less than in the average adult. But given the fact that these models are able to predict some data on the distribution of radioiodide in the fetus (see Clewell et al. [2007], Figure 7 and Table 3), serum perchlorate in the fetus/neonate at birth (see Clewell et al. [2007], Figure 10), and urinary iodide in the neonate (see Clewell et al. [2007], Figure 9), this provides a “medium” level of confidence in these model predictions.

Some model parameters are more certain than others. For instance, the NIS affinity constant for iodide and perchlorate has more substantial laboratory data than the estimates for urinary clearance and is known to be highly conserved (has little genetic variation) among humans. For this reason, a range of urinary clearance estimates were tested by EPA. Data needs that would help reduce model uncertainties are discussed further in section 5.3.

### **5.2.2. Model Limitations**

Several limitations of the PBPK models are evident for evaluation of health effects of perchlorate. First, the PBPK models developed by Clewell et al. (2007) and Merrill et al. (2005) were not developed with the goal of describing dietary intake of iodide nor are they capable of such simulations because the parameters are not calibrated to handle such a large mass flux of iodide. Furthermore, these models were developed to predict percent inhibition of thyroid radioiodide uptake (percent RAIU inhibition) and were not elaborated on to include thyroid hormones and thyroid axis regulation. Thus, a qualitative evaluation of what effect perchlorate may have on RAIU inhibition in a hypothyroid (up-regulated NIS, increased NIS  $V_{max}$ ) may be tested, but this has little importance because it cannot be directly compared with data. There are likely many other changes in iodide kinetics and thyroidal iodide processing in hypothyroid and other thyroid disease states that give a qualitative analysis little relevance.

The models are also not formulated to predict the combined inhibition of radioiodide uptake jointly when an individual is exposed to a mixture of perchlorate and other iodide uptake inhibitors (e.g., thiocyanate and nitrate). It should be noted, however, that perchlorate's potency as an inhibitor of radioiodide uptake at the NIS is 15 and 240 times greater than that of thiocyanate and nitrate, respectively (Tonacchera et al., 2004). It is the combination of potency and the relative blood concentration of these anions that ultimately determines the impact of total exposure on iodide uptake.

### 5.3. DATA NEEDS

There are significant research needs that would contribute to the understanding of physiological processes pertaining to perchlorate and iodine and thereby reduce model uncertainty. Additional research could also provide the needed data for expansion of the PBPK models to include interaction with other iodide uptake inhibitors.

First, a detailed evaluation of the mechanisms of perchlorate renal clearance, including the effect of perchlorate on the clearance of NIS substrates, and perchlorate-induced perturbations in the thyroid axis and perchlorate inhibition of NIS are needed. Additional data on renal/urinary clearance of anions and environmental pollutants in various life stages, especially during pregnancy, post partum, and in neonates, are also needed. Furthermore, additional information to characterize perchlorate exposure to neonates is desired (i.e., measurements of perchlorate in non-composited baby formula samples and samples of powdered formula reconstituted with perchlorate-free water).

To further the understanding of effects of perchlorate in individuals exposed to other iodide uptake inhibitors, such as thiocyanate and nitrate, investigations of the toxicity of these compounds in the presence and absence of perchlorate, including effects on iodide uptake and the thyroid axis, would be useful. Biomonitoring of perchlorate, iodide, thiocyanate, and thyroid hormones during and after pregnancy and during lactation in smoking and nonsmoking women could provide information to better characterize co-exposure effects. In these instances blood levels and urine levels of compounds should be collected simultaneously, so that the relationship of blood levels and urinary excretion can be directly evaluated.



## 6. SUMMARY AND CONCLUSIONS

Detailed examination of Clewell et al. (2007) determined that the model structure is appropriate for predicting percent inhibition of thyroidal RAIU following perchlorate exposure. While some coding errors were found, correction of these led to only minor changes in the NIS inhibition prediction in most cases. Beyond the issue of coding errors, a number of concerns and questions were raised regarding choices of parameter values in the models for each life stage. However, discussions with the authors of Clewell et al. (2007) about the technical basis for some of the parameter values clarified most such issues. Several issues with model choices made by the authors have also been noted and tested but found to not have marked impacts on model predictions (e.g., the equation and scaling used for binding of perchlorate and iodide to blood proteins, as described above). In cases that represent differences in scientific judgment rather than coding errors (some noted in Appendix A) and that result in minimal changes to the results, the model code has been left as is. The existing model structure has previously been peer reviewed; thus, it was concluded that the most expedient course was to not make these changes because of their minimal impact.

A few adjustments to the model components and procedures were made if it was determined that they could have more substantive impacts on the results. For example, the arrangement of terms in the equations describing transport in blood (mixing of venous blood streams and arterial blood compartment) was giving rise to numerical instabilities in the computer implementation. While the software used to solve the model appeared to be robust enough to handle this instability, such that there were minimal changes in model predictions when the equations were adjusted to remove them, it was deemed appropriate and better to use the modified code without instabilities.

Use of more accurate values for the rate of urinary clearance in neonates led to the greatest changes in predicted levels of NIS inhibition relative to those provided by Clewell et al. (2007). A few adjustments were also made to the lactation/breast-feeding model components and procedures, including the neonate's rate of milk ingestion. Overall, however, while the quantitative outputs of the PBPK model as modified by EPA differ from those published in Clewell et al. (2007) (see Table 4-1), EPA evaluation determined that, with modifications as described herein, Clewell et al. (2007) is acceptable to calculate the life stage differences in the degree of NIS inhibition of thyroidal radioiodide uptake at a given level of perchlorate exposure.

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## APPENDIX A: DESCRIPTION OF MODEL CODE ISSUES AND RESOLUTION

Computer code (acsl language, .csl and .cmd files) were provided by the authors (R.A. Clewell and E.A. Merrill) for the average adult (human10.csl [Merrill et al., 2005]), pregnant woman and fetus (HPregF.csl [Clewell et al., 2007]), lactating woman and breast-fed infant (HLactF.csl [Clewell et al., 2007]), and older child (HKidF.csl [Clewell et al., 2007]). Descriptions of specific issues and discrepancies identified in the code or among the code and model descriptions in the published papers follow, along with the resolutions. At the end of this appendix is a brief “impact of changes” listing that shows the impact of each change or set of changes on model predictions of inhibition of radioiodide uptake given exposure at the POD (7 µg/kg-day) and illustrates the quantitative results of these corrections.

### A.1. Perchlorate Inhibition of Iodide Transport

During the review of the acsl CSL code provided by the authors for the average adult (human10.csl [Merrill et al., 2005]), pregnant woman and fetus (HPregF.csl [Clewell et al., 2007]), lactating woman and breast-fed infant (HLactF.csl [Clewell et al., 2007]), and older child (HKidF.csl [Clewell et al., 2007]), several apparent discrepancies between the manuscript and the code were noted that were related to NIS and pendrin inhibition of iodide transport by perchlorate. Understanding of the biology of NIS, along with the statement “[i]nhibition of iodide uptake was included in the maternal, neonatal, and fetal thyroid follicle and colloid, GI contents, and skin, as well as the maternal placenta, mammary gland, and milk, based on various literature sources showing inhibition in these tissues in laboratory animals and humans” in Clewell et al. (2007), led to the following concerns and actions:

1. Transport of iodide via NIS into GI contents was described with perchlorate inhibition in the human10.csl and HPregF.csl code for the average adult and pregnant woman and fetus but was not included in the code for lactating woman/infant and older child.

*Action:* GI inhibition of NIS iodide transport by perchlorate was added to the model code for the older child and lactating woman/infant.

2. Transport of iodide via NIS into skin was described with perchlorate inhibition in the model code for the pregnant woman and fetus but was not included in the model code for the other life stages.

*Action:* Skin inhibition of NIS iodide transport was added to model code for the average adult, older child, and lactating woman/infant.

(a) Perchlorate inhibition of mammary iodide active transport was not included in the model code for both the pregnant woman and lactating woman. Also, iodide transport

from mammary tissue to milk was not inhibited by perchlorate in the lactating woman model code.

(b) Of particular concern is that, qualitatively, a dual impact would be expected on the breast-fed infant, due to reductions in iodide received in breast milk, which the model code obtained from the authors did not predict due to the lack of an inhibition in the lactation/milk compartment.

*Action:* (a) Inhibition of iodide transport by perchlorate into the mammary tissue was added to the model code for the pregnant woman and lactating woman, and inhibition of iodide transport to milk was added in the lactating woman model code.

(b) The value of  $Km\_Mkp$  of  $10^6$  ng/L in breast milk for the lactating woman was used. Iodide transfer to the infant was then simulated as the iodide concentration in breast milk times the suckling rate—a clearance term in the existing maternal model, by adding the term to the infant gastric juice compartment. This revised code was used to simulate  $^{125}I$  levels in the infant thyroid at 24 hours after maternal  $^{125}I$ -dosing (along with lactational transfer of perchlorate) to obtain the percent inhibition in the breast-fed infant.

## **A.2. Tissue and Blood Flow During Pregnancy/Lactation**

While the adult woman PBPK code was adjusted by the model authors to reflect changes in body fat and mammary size during lactation, the equation used to adjust blood flow to the mammary gland in proportion to its size appeared to be in error, since the flow rate set by the equation at birth only reflected the portion of mammary volume resulting from the change (increase) during pregnancy, and not the initial (pre-pregnancy) volume to which that increase is added. The result is that the maternal mammary blood flow just after giving birth was less than the pre-pregnancy blood flow, even though the tissue volume was greater. The equation was corrected to reflect the total tissue volume at the end of pregnancy/birth.

## **A.3. Residual Milk Volume**

Residual milk volume is the volume of milk available to but not consumed by the infant (Dewey et al., 1991) is the volume of milk that is retained in the mammary gland between feedings and was used in the PBPK models to determine the concentration of radioiodide and perchlorate in breast milk. It is unclear what reference was used by Clewell et al. (2007) to derive the residual milk volume ( $VMk = 0.632$  L). The reference cited in the acsl computer code was Gentry et al. (2001); however, this reference was not included in the Clewell et al. (2007) manuscript. Furthermore, a search of PubMed and Google did not find a Gentry reference from 2001 or with the  $VMk$  value.

We evaluated Dewey et al. (1991), as suggested by an external reviewer, and agreed that it provided a value (0.109 L) for  $VMk$  that is appropriate for use in the lactation PBPK model.

The VMk parameter was changed from 0.632 to 0.109 L, and the model sensitivity was tested. Model sensitivity to VMk was tested, and an 83% decrease from 0.632 to 0.109 L for VMk resulted in a less than 3% change in model calculated percent inhibition of radioiodide uptake. Thus, the model is not considered sensitive to this parameter, and the value for VMk was changed from 0.632 to 0.109 L.

#### **A.4. Modification to Lactating Woman/Breast-Fed Infant Model to Provide for Bottle-Fed Infant Simulations**

The model code for the lactating woman/breast-fed infant was modified slightly to provide model simulations of the bottle-fed infant. Specifically, for bottle-fed infant simulations, the perchlorate dose to the mother was set equal to zero, then either the existing direct-dosing rate (PDOSE\_N) was set > 0 for fixed  $\mu\text{g}/\text{kg}\text{-day}$  rates and/or a fixed concentration was multiplied by the suckling rate (KTRANS in revised code) for fixed water concentrations. In those simulations the maternal code still ran but contributed nothing since the maternal dose was set to zero. An i.v. dose of iodide to the infant was simulated in order to calculate percent of thyroidal radioiodide uptake (percent RAIU) inhibition in the bottle-fed infant in contrast to the iodide dose being received via breast milk for the calculation of percent RAIU inhibition in the breast-fed infant.

#### **A.5. Blood Flow vs. Plasma Flow**

The models were constructed by using arterial plasma concentration coupled with whole blood (plasma plus red blood cells) flow rates to describe the amounts of iodide and perchlorate delivered to the tissues. Thus, the mass flow of free perchlorate (in plasma), to and from the tissue compartments, is nearly double what physiology indicates. Either the influx in all mass balance equations should correspond to the effective concentration in whole blood (i.e.,  $[\text{plasma\_concentration}] \times [\text{volume\_of\_plasma}] / [\text{volume\_of\_plasma} + \text{volume\_of\_red\_blood\_cells}]$ ) or the flow rate should correspond to plasma flows, since the red blood cell:plasma partition coefficient (PRBC\_p) is not always equal to 1, and the chemical movement between plasma and red blood cells is diffusion limited.

The effect of using plasma flow vs. blood flow on the predictions of percent RAIU inhibition was found to be very small; however, the model was corrected to reflect appropriate physiology. Cardiac output (total blood flow) was adjusted to reflect plasma flow by multiplying by the plasma:whole blood volume ratio ( $\approx 0.557$ ), thereby decreasing the flow to the tissues. This had a small impact on model output although within the experimental error for the average adult (at  $7 \mu\text{g}/\text{kg}\text{-day}$ , it changed from 2.1% RAIU inhibition to 1.6%).

## A.6. Model Code Errors, Little to No Quantitative Impact on Model Predictions

In the binding equation for iodide in the blood for the pregnant woman, lactating woman, and older child models, the term for concentration of iodide in the arterial blood ( $Ca_i$  or  $Ca_{ni}$ ) appeared twice in the denominator. This effectively reduced the  $K_m$  and  $V_{max}$  for blood binding by half, but had minimal effect on model predictions at the concentrations tested (within linear range of blood binding). The extra  $Ca_i$  term was removed in these models.

The perchlorate mass balance equation, used as a flag for computational errors, was corrected for perchlorate by adding the amount of perchlorate in the “deep” thyroid,  $ADT_p$ , to the total mass in tissue ( $TM_{1p}$ ) equation. The iodide mass balance equation ( $BAL_i$ ) was corrected by adding the amount bound in blood ( $ABND_i$ ) to the total mass in blood ( $TM_{2i}$ ) equation and changing  $QS$  to  $QF$  in the equation for the rate of change in fat tissue ( $RAF_i$ ).

In the model code equation for the concentration of iodide in venous blood ( $CV_i$ ) in the average adult model, blood binding of iodide had an addition sign (+) instead of multiplication (\*) between the  $K_m$  and the perchlorate inhibition term; i.e.,

$$V_{max\_Bi} * CA_i / [CA_i + K_{m\_Bi} + (1 + Ca_p / K_{m\_Bp})]$$

instead of

$$V_{max\_Bi} * CA_i / [CA_i + K_{m\_Bi} \times (1 + Ca_p / K_{m\_Bp})]$$

EPA changed the + to a  $\times$ ; this had minimal impact on model predictions. The blood binding equation ( $RAbnd_i$ ) had the correct operator.

Additionally, blood binding of iodide and perchlorate are described slightly differently in the average adult model code (human 10.csl) and in the maternal and neonatal code (HlactF.csl). For perchlorate the rate of change of bound perchlorate is subtracted from the equation for the rate of change in the arterial plasma ( $RPLAS_p$ ), which determines the concentration in arterial blood ( $Ca_p$ ), and  $Ca_p$  is subsequently used in the Michaelis-Menten binding equation; however, for iodide, the rate of change of bound iodide is subtracted from the *venous* blood concentration ( $CV_i$ ) equation and  $Ca_i$  is still used in the Michaelis-Menten binding equation. EPA notes that  $Ca_i$  and  $Cv_i$  are typically very close if not equal to one another. The term for iodide binding was therefore moved from the venous to the arterial blood equation. As was expected, this change had minimal impact on model predictions; however, making this change did seem to improve computation speed and stability.

The change in uterine weight was not included in the total maternal BW calculation in the original code used in Clewell et al. (2007). Including the increase in uterine weight in the maternal BW calculation increased maternal BW by approximately 3%, but this correction did not affect prediction of percent RAIU inhibition in the pregnant woman or fetus for the time points examined.



## A.7. Impacts of Various Changes on Model Predictions

Described below are differences between the percent RAIU inhibition predictions of the PBPK models as originally published/described by the authors and the percent RAIU inhibition predictions now obtained with the models. The differences are illustrated with model predictions at the POD of 7 µg/kg-day. The predictions of the models originally published/described are in the first sub-bullet (>) in each category, and the effects of EPA's changes are noted in the subsequent sub-bullets. In most cases, only those adjustments that resulted in relatively large changes were noted. For each of these life stages/populations, other technical corrections that are not described here and had only minimal effects were also made.

### Changes in percent RAIU inhibition predicted by the model with changes in code and parameters

- Average (nonpregnant) adult—original model predictions assumed perchlorate in four equal doses at 4-hour intervals, corresponding to the Greer et al. (2002) study protocol:
  - > 3.3%—original value from Table 2 (1<sup>st</sup> one, page # 8 at bottom), Mattie (2006)
  - > 3.1%—assuming continuous/steady-state perchlorate exposure
  - > 2.1%—adding inhibition of iodide transport in skin (correction)
  - > 1.6%—flow to tissues adjusted from blood flow to plasma flow (correction)
- Pregnant woman:
  - > 6.4%—original value from Table 2 (Mattie, 2006), GW 38
  - > 6.3%—multiple small corrections (e.g., + inhibition in mammary gland), continuous exposure
  - > 6.1%—maternal and fetal flow to tissues adjusted from blood flow to plasma flow (correction), GW 38—using original Clewell et al. (2007) clearance values
- Fetus (exposure is to mother, per total maternal BW):
  - > 8.6%—original value from Table 2 (Mattie, 2006), GW 38
  - > 7.6%—multiple small corrections, as above, GW 38
  - > 9.9%—inhibition at GW 40
  - > 11.0%—maternal and fetal flow to tissues adjusted from blood flow to plasma flow (correction) at GW 40—using original Clewell et al. (2007) clearance values
- Lactating woman—original model used four doses at 4-hour intervals:
  - > 6.9%—original value, PND 30
  - > 3.8%—adding inhibition of iodide transport in skin and breast milk (corrections)
  - > 4.1%—assume continuous dosing/ingestion; other small changes (e.g., blood binding)
  - > 2.0%—revised urinary clearance (increased from original), KTRANS equation changed to Hill function (revised lactation expression), PND 30
  - > 1.8%—flow to tissues adjusted from blood flow to plasma flow (correction), PND 30
  - > 2.0%—VMk constant changed from 0.632 to 0.109 L, PND 30
- Breast-fed neonate (exposure is to mother, 7.0 µg/kg maternal BW):
  - > ≈7%—original value (interpolated from Table 1, Mattie [2006]), age 1 month
  - > 11.6%—including inhibition of iodide transfer in breast milk and maternal skin; other fixes

- > 18.5%—revised (reduced) urinary clearance to scale as GFR vs. adult (Appendix B)
  - > 12.1%—age 7 days, KTRANS equation changed to Hill function (revised lactation expression)
  - > 12.1%—age 7 days, flow to tissues adjusted from blood flow to plasma flow (correction)
  - > 12.5%—age 7 days, VMk changed from 0.632 to 0.109 L
- Bottle-fed neonate (since dose-rate fixed, milk ingestion rate does not impact these):
    - > 1.3%—original value (interpolated from Table 1, Mattie [2006]), 1-month-old
    - > 1.2%—small corrections
    - > 3.0%—revised (reduced) urinary clearance to scale as GFR vs. adult (Appendix B)
    - > 4.1%—age 7 days
    - > 4.3%—age 7 days, flow to tissues adjusted from blood flow to plasma flow (correction),
    - > 2.9%—age 30 days, flow to tissues adjusted from blood flow to plasma flow (correction),
- Older child:
    - > 2.1%—original value Table 5 (Clewell et al., 2007); 7-year-old
    - > 1.7%—with inhibition in GI tract and skin, steady-state exposure simulation
    - > 2.0%—revised clearance for perchlorate (scale as  $BW^{1.0}$ , Appendix B), 7-year-old
    - > 1.9%—revised clearance, 2-year-old
    - > 1.9%—revised clearance, 1-year-old
    - > 1.7%—flow to tissues adjusted from blood flow to plasma flow (correction), 2-year-old
    - > 1.7%—flow to tissues adjusted from blood flow to plasma flow (correction), 1-year-old

## APPENDIX B: EVALUATION OF URINARY CLEARANCE PARAMETERS

### B.1. Overview

In the PBPK models of Merrill et al. (2005) and Clewell et al. (2007), the urinary clearance of perchlorate and iodide are described by using a common form of allometric scaling by BW raised to the  $\frac{3}{4}$  power. The actual urinary clearance constant for an individual of a given BW is given as follows:

$$CLU_k = CLUC_k \times BW^{0.75} \quad (\text{Eq. B1})$$

where  $CLU_k$  has units of L/hour and “ $k$ ” is either “P” for perchlorate or “I” for iodide.

Note 1: The tables in the papers identify the units of the CLUCs as L/hour-kg, but clearly this should be L/hour-kg<sup>0.75</sup> to be consistent with this mathematical formulation, which is how the CLU values are calculated in the computer code. This form of allometric scaling is commonly used in PBPK models and is generally considered appropriate for estimating changes with individual BWs. Also, the tables list  $CLUC_P = 0.13 (\pm 0.05$  in Merrill et al. [2005]) and  $CLUC_I = 0.11$  (Merrill et al., 2005) or 0.1 (Clewell et al., 2007). The values as set in the computational command (.cmd) file were 0.125 and 0.11 for perchlorate and iodide, respectively, so these values will be used below.

These values for  $CLUC_k$  were determined from PK data in adult humans, so henceforth they will be identified as the “adult” values.

Note 2: The similar values of  $CLUC_k$  for perchlorate and iodide suggest that these are handled similarly by the kidney, as would be expected given their similar charge and ionic/molecular diameter. As will be discussed below, there is evidence for re-uptake activity by the pendrin transporter for iodide in the kidney, though only significant at lower concentrations, and for perchlorate-iodide interactions in renal clearance. While this transporter appears to operate on both iodide and perchlorate, the  $V_{max}$  for iodide is significantly higher than for perchlorate, and other tissues where it is explicitly described in the models capture this differential activity. This difference between iodide and perchlorate transport, and the fact that it appears to have small impact at higher (test) iodide concentrations corresponds nicely with the small difference in adult  $CLUC_k$  values: if  $\approx 10\%$  of iodide is actively resorbed, but a much smaller fraction of perchlorate, such a difference would be predicted.

The current model does not include active renal transport per se but describes renal excretion as follows:

$$r\text{CLU}_k = \text{CLUC}_k \times \text{CVK}_k \quad (\text{Eq. B2})$$

where  $\text{CVK}_k$  is the concentration of  $k$  in the venous blood exiting the kidney. Inclusion of active (saturable) transport would lead to a nonlinear formulation. The error from not including the active transport is considered to be within the realm of PK uncertainty and variability that is *not* included in the current model applications. So, a revision of the model to include it is not proposed. But in application of these results, the fact should be considered that not all of the interindividual variability and uncertainty in the perchlorate and iodide PK has been quantified.

Given that this linear formulation is accepted and the implicit suggestion that renal clearance is largely controlled by glomerular filtration and nonspecific fluid resorption, the expectation is that the relative clearance for iodide and perchlorate (i.e.,  $\text{CLU}_I/\text{CLU}_P$ ) should be constant across ages, BWs, and life stages. In EPA's evaluation for the child and average (nonpregnant, non-lactating) adult, this proportionality has been maintained.

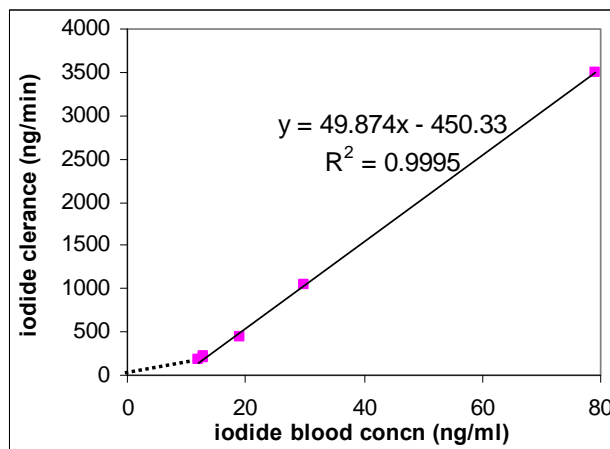
Note 3: In Clewell et al. (2007) it is stated "The maternal urinary clearance value ( $\text{Cluc}_i$ ) was set at 60% of the value in the non-pregnant human based on observed difference in the pregnant and male rat models (Clewell et al., 2003b; Merrill et al., 2003)." In fact both  $\text{CLUC}_I$  and  $\text{CLUC}_P$  were set to 0.05 in the pregnant woman (both reduced by about the same proportion), but, in the lactating woman, only  $\text{CLUC}_P$  was so reduced while  $\text{CLUC}_I$  was not. These maternal lactation values go against the argument given above that the proportionality should be maintained, but EPA chose to use the maternal values as set. It is likely worthwhile to evaluate these maternal values in light of the generally higher urinary excretion seen in pregnant/lactating women, but alteration of these clearance constants would require refitting of other parameters, and so EPA chose not to conduct that specific evaluation.

## **B.2. Urinary Clearance in Adults (Outside of Pregnancy/Lactation)**

With the use of the calculations as indicated above, the clearance rate from the Merrill et al. (2005) model for average adults is  $\text{CLU}_P = \text{CLUC}_P \times 70^{0.75} = 3.025 \text{ L/hour} = 50.4 \text{ mL/minute}$ . The average GFR in adults is  $125 \text{ mL/minute} = 7.5 \text{ L/hour}$ , so  $\text{CLU}_P/\text{GFR} = 40\%$ . For iodide the values are  $2.662 \text{ L/hour}$  or  $44.3 \text{ mL/minute}$ , 35% of GFR.

For comparison, Gardner et al. (1988) examined the effects of iodide supplementation in men, and a plot and regression of their data (urinary clearance vs. blood concentrations) is shown in Figure B-1. The slope of the regression line,  $49.9 \text{ mL/minute}$ , is quite close to the clearance value used by Merrill et al. (2005), and, if the intercept is forced to zero, the slope reduces to  $40.5 \text{ mL/minute}$ , bracketing that value. However, as indicated by the dashed line, drawn for illustration, the clearance must be considerably reduced at lower concentrations, assuming that

clearance does not become zero until the blood concentration also drops to zero. (The slope of the dashed line is about 10 mL/minute.)



**Figure B-1. Iodide clearance vs. blood levels in men, from Gardner et al. (1988).**

What these data and regression indicates is that there is a nonlinearity in clearance at low levels, which could well be due to active reabsorption that becomes saturated at higher concentrations. The presence of the pendrin transporter in the kidney is noted in the review of Soleimani and Xu (2006).

While EPA does not propose changing the model to explicitly include active transport in the kidney, thereby describing this nonlinearity in excretion, it is noted here as a source of uncertainty or variability in model predictions: clearance values obtained at high concentrations (clinical experiments) might not be completely predictive of values at lower concentrations.

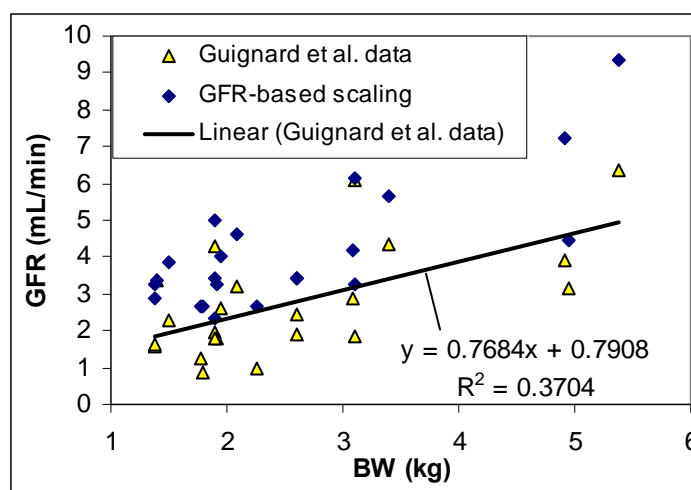
The presence of a transporter (more) active towards iodide, could explain the slightly lower clearance of iodide vs. perchlorate in the adult. It is assumed that this transporter acts at a similar proportion of activity in all life stages and hence that the ratio of perchlorate:iodide transport is approximately constant. Likewise, it is assumed (as one means of estimation) that the clearance rates remain at about 40 and 35% of GFR for perchlorate and iodide, respectively.

### **B.3. Urinary Clearance in the Neonate**

Note 4: The analysis here focuses on clearance of perchlorate, but, as indicated above, iodide clearance was always changed in parallel to maintain the ratio of 0.11/0.125. Furthermore, for each alternate value of  $CLU_k$  evaluated, the  $V_{max}$  for NIS-mediated uptake of both iodide and perchlorate in the follicle (from blood to follicle tissue) was adjusted to maintain model fits to RAIU data (available for infants and other life stages in the absence of perchlorate).

Data from Guignard et al. (1975) on GFR in infants (age 1–25 days) with a linear regression are shown in Figure B-2. As a basis for comparison, EPA will consider the clearance

of a 3.6 kg child, the (average) weight predicted by the model to occur at 7 days of age. Based on the regression shown below, GFR at that age/BW is 3.557 mL/minute or 0.21 L/hour. As implemented in Clewell et al. (2007), the clearance of perchlorate is predicted to be  $CLU_P = 0.125 \times 3.6^{0.75} = 0.33$  L/hour. Clearly this value of  $CLU_P$  does not fit with the assumptions on clearance/GFR stated above; the only way in which clearance could be higher than GFR is if there is active excretion of iodide with no or substantially reduced resorption. EPA is aware of no data on renal transporters during infancy to suggest the level and pattern of expression changes required to bring about such an effect. If, instead, an assumption is made that perchlorate clearance was 40% of GFR, as it is in the adult, the value obtained at 3.6 kg BW would be 0.085 L/hour, almost four times lower than the default extrapolation.



**Figure B-2. GFR vs. BW in infants (Guignard et al., 1975).**

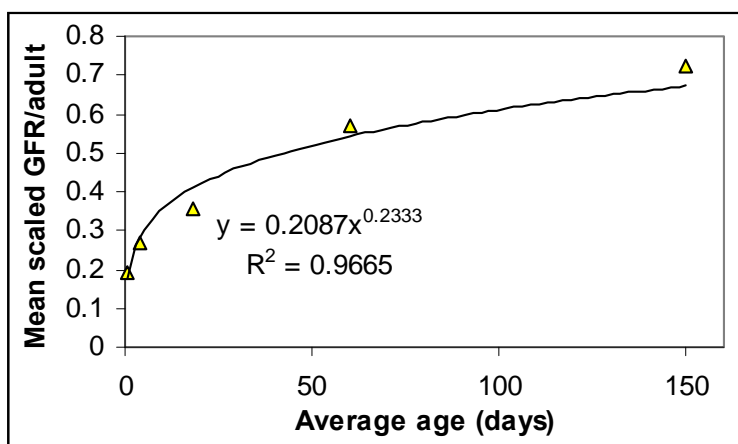
GFR-based scaling uses equation B3 (with total adult GFR of 125 mL/minute vs. 3.025 L/hour perchlorate clearance) with individual age (day) and BW values from Guignard et al. (1975).

Since the intent is to account for BW changes in a convenient way, it should be noted that GFR is typically normalized to body surface area, since this scaling has been found to explain much of interindividual variability. Even with such normalization GFR is below adult levels near birth, rising toward adult values over the first few months (DeWoskin and Thompson, 2008). Therefore, consideration was given to scaling of renal excretion for infants by  $BW^{2/3}$  as an approximation of surface area normalization and using the average values compiled by DeWoskin and Thompson (2008) for different age ranges. Values for the ratio of normalized GFR in infants vs. adults (ratio of mean infant value to adult, plotted vs. midpoint of each age range) are shown in Figure B-3, along with a simple power-function curve fit,  $SGR = 0.2087 \times \text{day}^{0.23333}$ , where day is the child's age in days. This function was used (a

correction factor), together with  $BW^{2/3}$  scaling (default surface area-based scaling), to estimate urinary clearance for perchlorate in the infant, based on GFR as follows:

$$\begin{aligned} CLUp_{\text{child}} &= SGR \times CLUp_{\text{adult}} \times \left( \frac{BW_{\text{child}}}{BW_{\text{adult}}} \right)^{2/3} \\ &= 0.2087 \times \text{day}^{0.23333} \times 3.025 \times \left( \frac{BW_{\text{child}}}{70} \right)^{2/3} \end{aligned} \quad (\text{Eq. B3})$$

Clearance for iodide is similarly calculated from the adult clearance value.



**Figure B-3. Ratio of surface area-normalized GFR (mL/minute/surface-area) in infants vs. adults as a function of age.**

Data from DeWoskin and Thompson (2008).

The result of applying equation B3 to the individual data of Guignard et al. (1975), but using the total adult GFR of 125 mL/minute (7.5 L/hour) instead of the iodide clearance of 3.025 L/hour, is shown in Figure B-2. The result is not a smooth function of BW because of the variation in BW vs. age in that data set. However, one can see that the resulting predictions are generally higher than the observations in that particular data set. This is not surprising since the average clearance values of Guignard et al. (1975) are lower than many of the other results included in the calculation of geometric means for each age range by DeWoskin and Thompson (2008), on which the multiplicative function shown in Figure B-3 and used in equation B3 is based. Likewise, applying equation B3 to a 7-day-old 3.6-kg infant results as follows:

$$CLU_{\text{child}} = 0.2087 \times 7^{0.23333} \times 3.025 \times \left( \frac{3.6}{70} \right)^{2/3} = 0.14 \text{ L/hour} \quad (\text{Eq. B4})$$

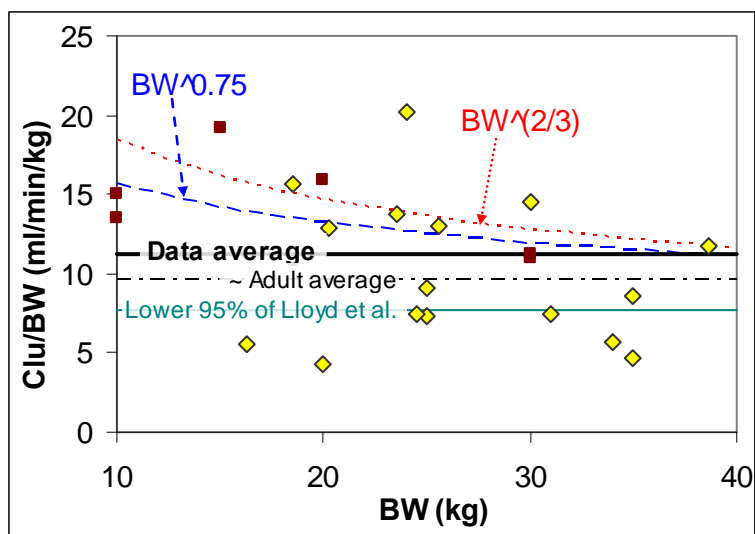
Compared to the GFR of 0.21 L/hour, this seems reasonable, although it is 67% of the GFR rather than 40%. At 60 days, when an average child is 5 kg, equation B3 yields

0.28 L/hour or 4.7 mL/minute, which also appears reasonable in comparison to the data in Figure B-1, again noting that this is iodide clearance rather than total GFR. While the data shown above are for children below 25 days, EPA extends its extrapolation to a 60-day-old 5-kg child, though with greater uncertainty at that age (since renal clearance does rise rapidly during that time). However, the estimates obtained up until 30 days are expected to be fairly sound.

#### **B.4. Urinary Clearance in the Older Child**

For older children, consideration was given to the data of Chin et al. (1982) and Lloyd et al. (1985) for cimetidine, which is primarily cleared by urinary excretion. The subjects were children being treated primarily for closed-head injuries (“secondary to motor vehicle accident”), and EPA restricted the Lloyd et al. (1985) data (larger data set) analysis to only that injury category (excluding a few cases of sepsis, for example) and to ages less than 12 years (youngest was 4.1 years). The Chin et al. (1982) data are included because they include children as young as 1 year, though there were fewer subjects. Unlike in neonates, from these data it appears that either direct BW scaling, normalization, or scaling by  $BW^{0.75}$  may be appropriate. A plot of clearance/BW (y-axis) vs. BW is shown in Figure B-4, with lines indicating  $BW^{1.0}$  (constant, dash-dot horizontal line with this normalization),  $BW^{0.75}$ , and  $BW^{2/3}$  scaling from adult values.





**Figure B-4. Cimetidine clearance data (Chin et al. [1982] [maroon squares] and Lloyd et al. [1985] [yellow diamonds]) and possible scaling relationships.**

$BW^{0.75}$  and  $BW^{2/3}$  curves are normalized to BW after applying this scaling. The data average line is likewise determined by combining the normalized Chin et al. (1982) and Lloyd et al. (1985) data, irrespective of BW, calculating the average of those values, and plotting a horizontal line with that value. The lower 95% of Lloyd et al. line is likewise plotted at the lower 95% confidence limit on the mean of the Lloyd et al. data, determined from considering the normalized values as a set of samples independent of BW.

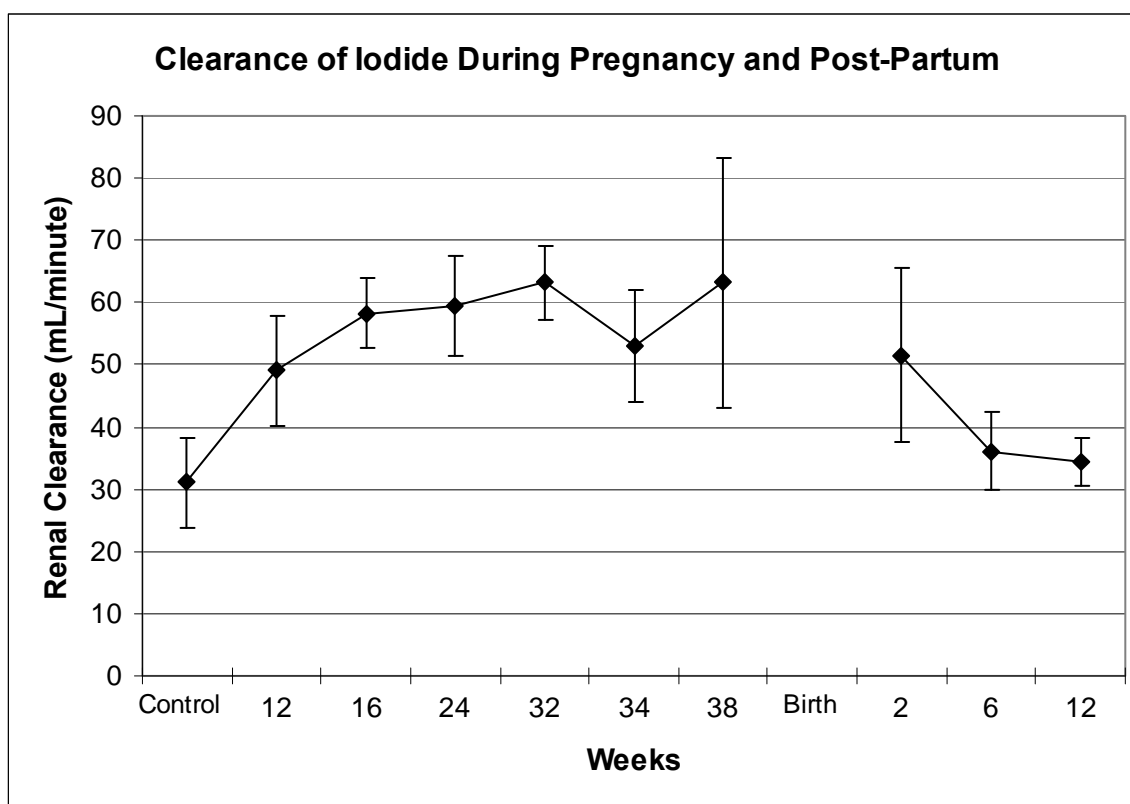
Lloyd et al. (1985) stated that the range of clearance rates in adults is 9–10.3 mL/minute-kg, and the average of these two values is shown for comparison (with no trend vs. BW) as well as the average of the data shown and the results of using  $BW^{0.75}$  and  $BW^{2/3}$  to scale from that approximate adult average. It can be seen that the data of Lloyd et al. (1985) (normalized to  $BW^{1.0}$ ) show little residual trend vs. BW, although the more limited data of Chin et al. (1982) show a downward trend similar to the results of scaling by  $BW^{0.75}$  and  $BW^{2/3}$ . (Regression of the Lloyd et al. [1985] data yields a slope that is negative but not significantly different from zero.)

While the allometrically scaled relationships are clearly within the range of the data and may be considered a reasonable estimation, the closeness of the normalized data average to that for adults and the fact that the  $BW^{0.75}$  scaling falls above the data average suggest that simple scaling of Clu by  $BW^{1.0}$  better describes the data over much of the range. In the face of the variability shown by these data and lack of clear fit by any of these functions, EPA chose to represent the average clearance in older ( $\geq 1$  year of age) children by scaling adult clearance values by  $BW^{1.0}$ , although this relationship may be low for younger children. The results of using  $BW^{0.75}$  scaling, as in the original publication of Clewell et al. (2007), are also shown in Table 4-2 as representing high clearance values, and the results of scaling by  $BW^{1.0}$  but

multiplying by the ratio (0.76) of the lower 95% confidence bound to the mean for the Lloyd et al. (1985) data are shown for the low clearance values.

### B.5. Urinary Clearance in Pregnancy and Lactation

Clewell et al. (2007) estimated clearance of perchlorate and iodide during pregnancy and lactation based on parallel changes in the rat (vs. average adult), obtaining clearance for both compounds of about half the average adult values during pregnancy. During lactation this approach led to half of the average adult clearance for perchlorate but a value for iodide equal to that in adults. The data for iodide clearance in humans of Aboul-Khair et al. (1964) during pregnancy and early postnatal times, shown in Figure B-5, were also considered.



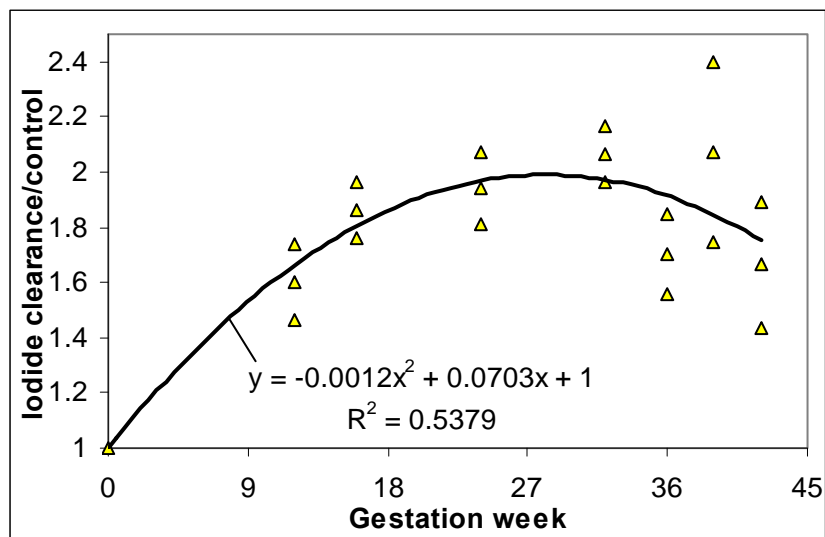
**Figure B-5. Renal clearance of iodine (mean ± 2 SEM) in pregnancy and the postpartum period compared with nonpregnant, control values.**

Data source: Aboul-Khair et al. (1964).

These data show that renal (urinary) clearance for iodide is elevated to as much as two times control (nonpregnant) values during pregnancy, and, while clearance then declines fairly rapidly towards control after birth, it is still elevated in the first couple of months, where EPA's analysis of neonatal clearance has focused attention. In keeping with the assumed proportionality between perchlorate and iodide and based on these data, the same relationship

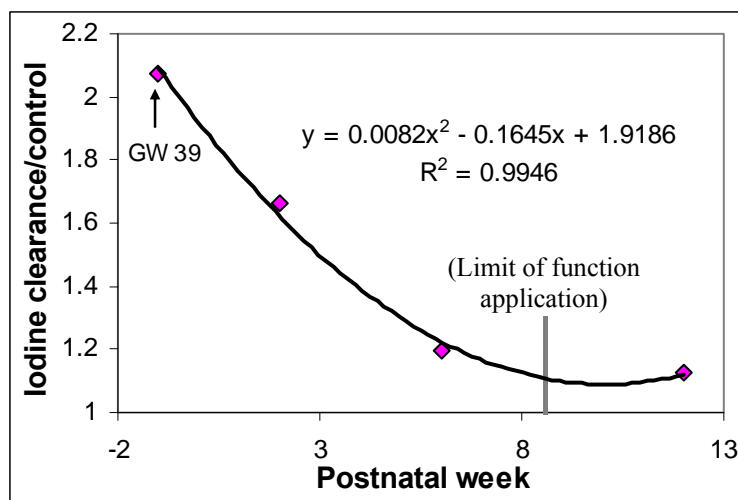
would be expected to hold as higher rather than reduced clearance. A dilemma occurs in considering the data of Aboul-Khair et al. (1964), however, in that the control iodine clearance as measured by the authors is  $31.05 \pm 3.66$  mL/minute (mean  $\pm$  standard error of the mean [SEM]), while the value determined by Merrill et al. (2005) for nonpregnant adults is 44.3 mL/minute. Likewise Aboul-Khair et al. (1964) reported thyroid iodide uptake at 2.5 hours postinjection as  $21.4 \pm 1.4\%$  of the administered dose, but the amount predicted by the Merrill et al. (2005) model (in the absence of perchlorate) is 7.6%. Therefore, the data of Aboul-Khair et al. (1964) were normalized to their own controls for both urinary clearance and iodide uptake, and then that relative change was used as a model input (for clearance, multiplying the nonpregnant clearance rate constant by the pregnant:control ratio from Aboul-Khair et al. [1964]) or in estimating changes in thyroid NIS (to fit relative increases in thyroid uptake).

The urinary iodine clearance data from Aboul-Khair et al. (1964) for pregnancy and at 1 week post partum, with a quadratic interpolation function, are shown in Figure B-6. A quadratic function was likewise fit to the data for the early postnatal period (along with the last gestational data point) as shown in Figure B-7. The latter function was only used up to 60 days (8.6 weeks) of infant age, since the data indicate a decline toward control values after that point.



**Figure B-6. Relative pregnant:nonpregnant iodide clearance values from Aboul-Khair et al. (1964), with quadratic interpolation function.**

Points are mean  $\pm$  SEM.



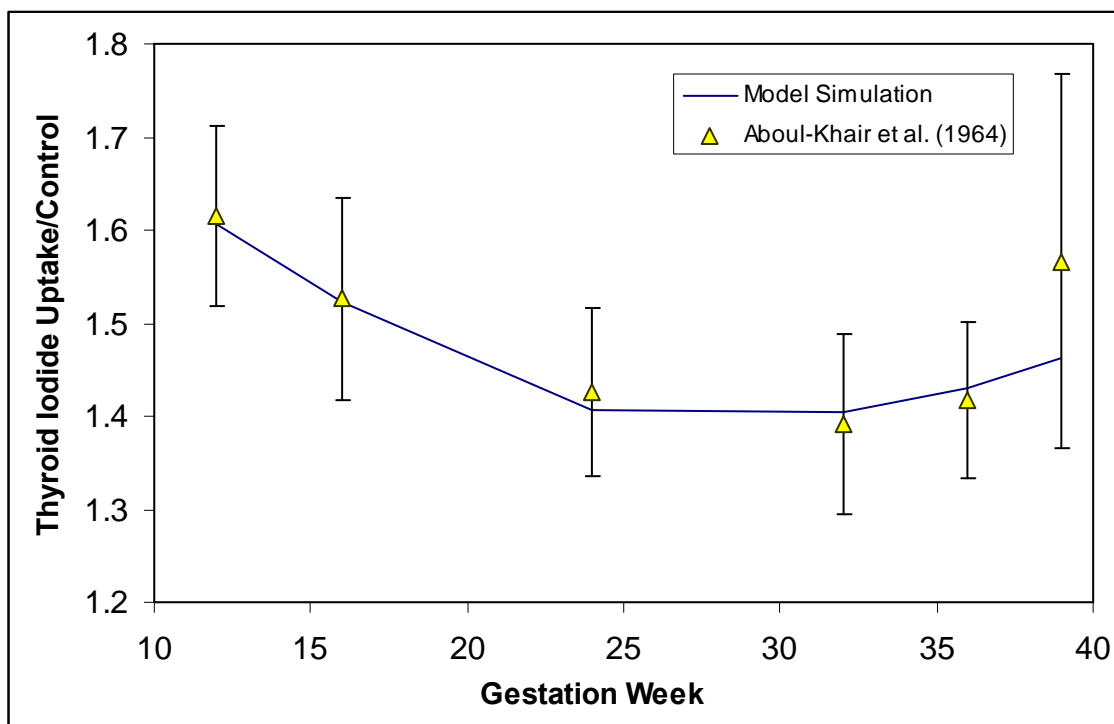
**Figure B-7. Maternal iodine urinary clearance and approximation function for early postnatal period.**

Data source: Aboul-Khair et al. (1964).

For pregnancy, the  $V_{\max}$  values for maternal thyroid NIS-mediated uptake of perchlorate and iodide were adjusted specifically to fit iodide uptake data of Aboul-Khair et al. (1964) collected at the same pregnancy time points as the urinary clearance data. A multiplier function

$$RV_{\max}(\text{pregnancy}) = 0.0009 \times GW^2 - 0.054 \times GW + 2.6 \quad (\text{Eq. B5})$$

was used to adjust both the perchlorate and iodide values. The fit to these iodide uptake data, given the increased urinary clearance as shown in Figure B-6 and the fitted quadratic for increased maternal NIS, is shown in Figure B-8.



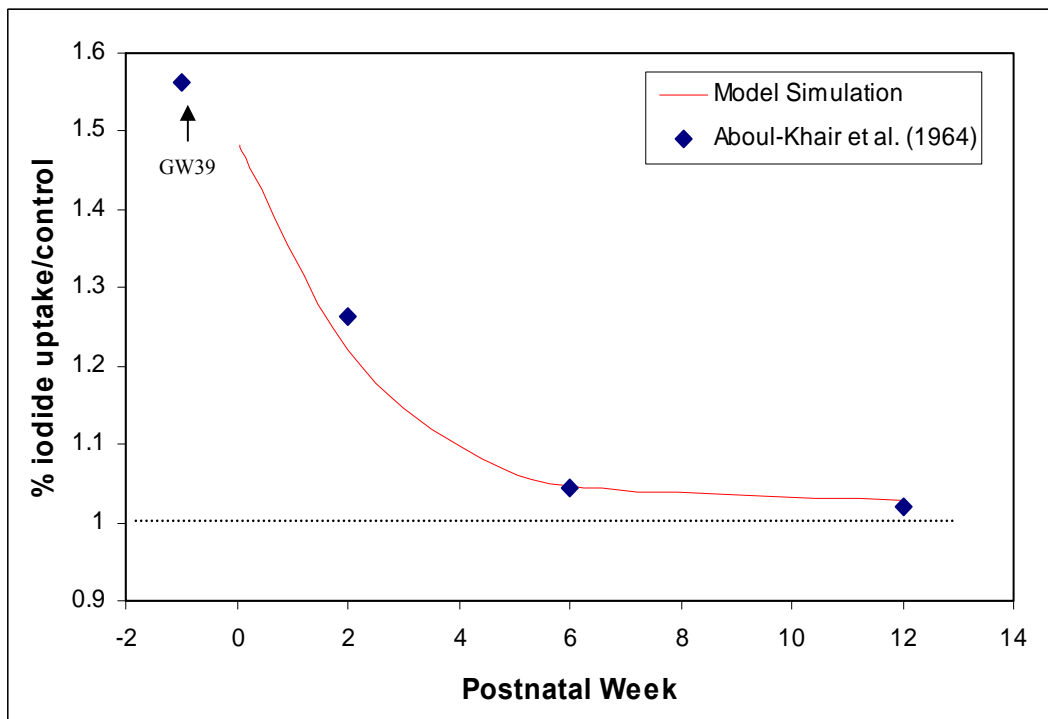
**Figure B-8. Thyroid iodide uptake at 2.5 hours post i.v. injection relative to control.**

Data (points) are from Aboul-Khair et al. (1964) (mean ± SEM). The line represents the model simulation. Note that, while values drop from GW 12 to 32, they are consistently greater than one.

During the postpartum period the urinary clearance and iodide uptake data of Aboul-Khair et al. (1964) are both falling toward control values, but there is the situation that the control uptake measured, 21.4% of the i.v. dose at 2.5 hours postinjection, is well above the value estimated by the PBPK model for an average adult: 7.6%. Furthermore, a number of the physiological parameters differ in the lactating woman model vs. the average adult, and are expected to change over time. Therefore, EPA first ran the lactating woman model, using the average adult clearance constants (scaled by  $BW^{0.75}$ ) at postnatal week 50 to estimate the model-control maternal uptake at 2.5 hours post iodide injection: 6.4%. Then, to evaluate the impact of using these observations, the NIS levels during this period were adjusted by assuming that, as clearance falls from about two times control values at GW 39 (1 week prior to birth) towards control, the NIS  $V_{max}$  values follow suit, dropping to the values of Merrill et al. (2005) for the adult. In particular, if RCLF is the fold increase of urinary clearance over control, then the NIS  $V_{max}$  was multiplied by the following:

$$RV_{max}(\text{lactation}) = (\text{RCLF} - 1)^2 + 1 \quad (\text{Eq. B6})$$

Thus, for  $RCLF = 2.09$  (from the equation in Figure B-7) at GW 39 (postnatal week “-1”),  $RV_{max}$  equals 2, and, as  $RCLF$  falls towards 1.0 (i.e., clearance approaches control values),  $RV_{max}$  also falls to 1.0, so  $V_{max}$  values will approach controls. A plot of the RAIU uptake measured by Aboul-Khair et al. (1964) and the simulated values resulting from use of this function are shown in Figure B-9, where both have been normalized by their respective control values.



**Figure B-9. Radioiodide uptake in late pregnancy and early postnatal period.**

Data are from Aboul-Khair et al. (1964). PBPK simulations are with adjusted lactation model (see text above).



## Overview

Battelle received PBPK models from EPA that were revised from Clewell et al. (based on the 2007 manuscript *Perchlorate and Radioiodide Kinetics Across Life Stages in the Human: Using PBPK Models to Predict Dosimetry and Thyroid Inhibition and Sensitive Subpopulations Based on Developmental Stage*) and performed the following tasks:

- Evaluated model code for internal consistency
- Digitized figures from published manuscripts
- Compared manuscript figures to current ACSL model outputs

## Results

The check of the model code found no outstanding coding discrepancies beyond those corrected by EPA staff (as noted in the code/comments of the model files). Additionally, the EPA staff corrections (as identified by comments in the code) all appear to appropriately result in code equations which now reflect the model as described in the manuscript.

Model Checked:

Lactational Model     **HLactFrev.csl**

Pregnancy Model     **HPregF\_Y\_pms2.csl**

The model code has been significantly revised by EPA staff to correct mistakes (typos) in equations, harmonize model code with statements in the manuscript, clean model code for readability, and reduce model run irregularities (i.e. long simulation times). Extensive model checking by Battelle was conducted on prior versions of the csl files. The current csl files were also checked to verify that corrections/additions were properly implemented. The m-files for producing the figures are attached in the Appendix.

From the 2007 Clewell paper, the following figures were analyzed:

Figure 5 – Thyroid of newborns;

Figure 6 – Maternal Concentrations;

Figure 7 – Total fetal burden;

Figure 8 – Lactating women; and

Figure 9 – Neonatal urine.

EPA staff also provided additional parameter values and some adjusted m-files for some of the simulations as noted in the discussion of each figure.



In addition to the output of the current model, simulation lines presented in the original manuscript were digitized (using DigitizeIt, share-it! - Digital River, Eden Prairie, MN) and are presented in most of the figures below.

In general, the model simulations were similar to, but occasionally not identical to, the published model results. This may be due to modifications made by Clewell et al. (2007) after publication or EPA's corrections to the submitted model.

Figure 5 – Thyroid of newborns:

- Produced using the **pregnancy** model and the **Fig5\_GFR.m** file.
  - The figure presents model-simulated thyroid radioiodide uptake 24 h postdosing in the newborn infant.
  - The 3D nature of the manuscript figure makes it difficult to determine the exact values of the points.
  - Since the experimental data exists only at 24 hour after dosing (for each dose group), only this time point was simulated.
  - EPA staff provided additional constants not documented in the original model code (see attached Fig5\_GFR.m file).
  - The simulation closely represents the published simulations and the experimental data.

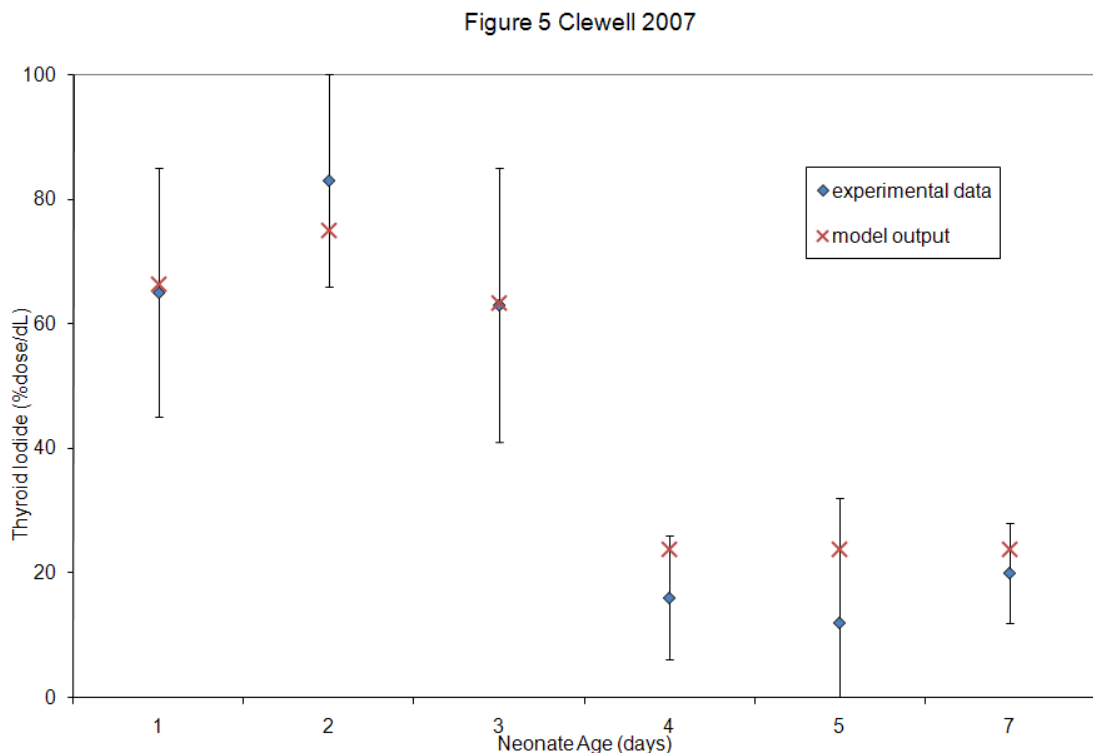


Figure 6 – Maternal Concentrations

- Produced using the **pregnancy** model and the **Fig6.m** file.
  - The figure presents predicted radioiodide concentration in maternal (A) thyroid, (B) urine, (C) whole blood, and (D) placenta.
  - The experimental data in the m files matches that presented in the manuscript with a few minor differences for both the mean and max Vmax values.

Figure 6a Clewell 2007

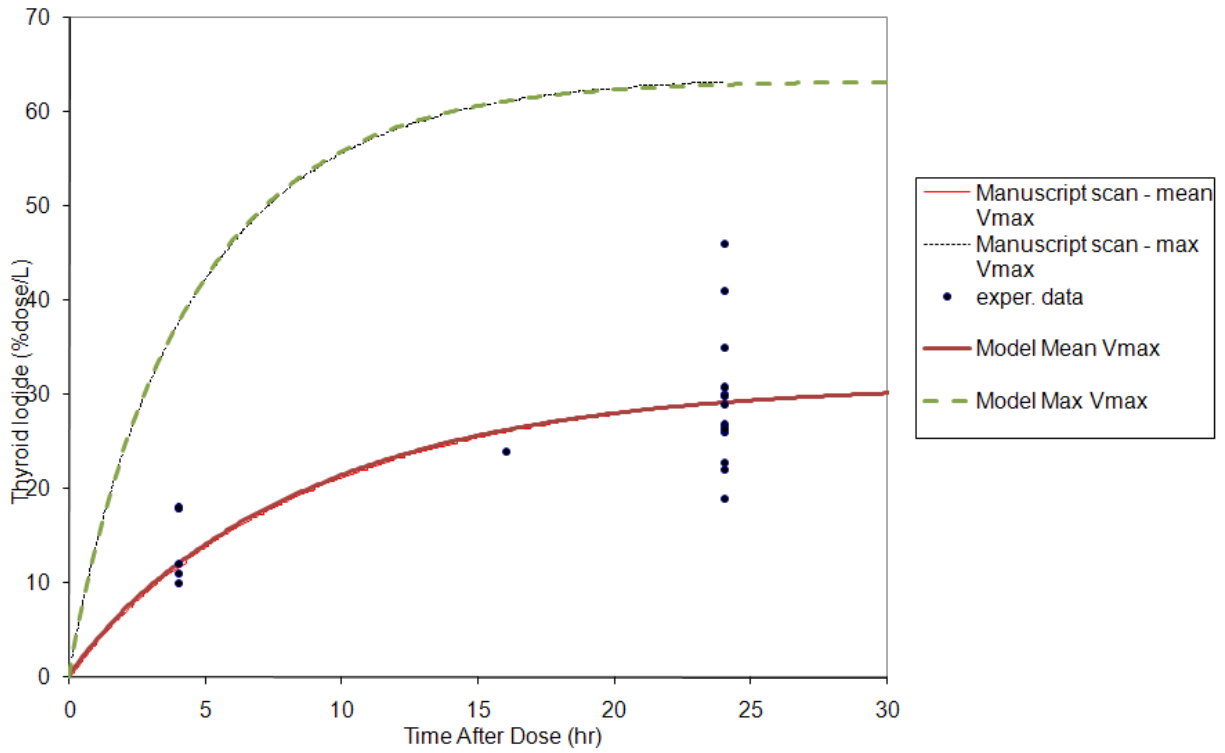


Figure 6b Clewell 2007

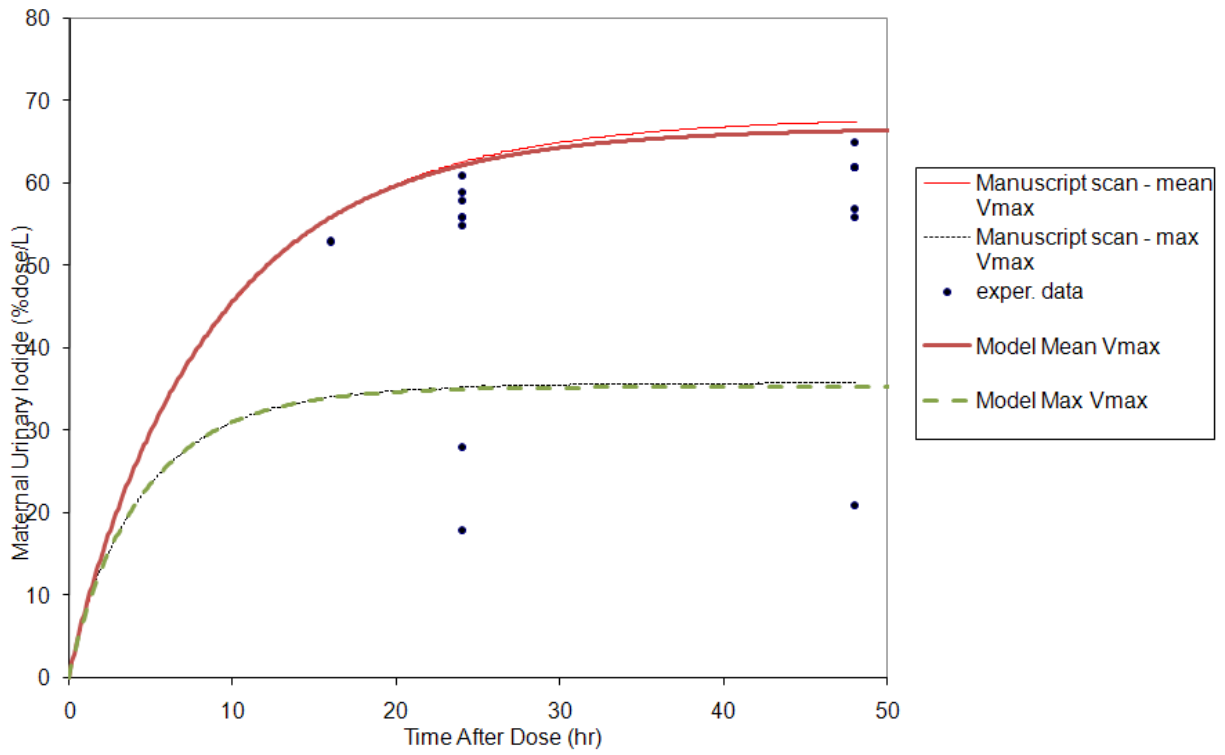


Figure 6c Clewell 2007

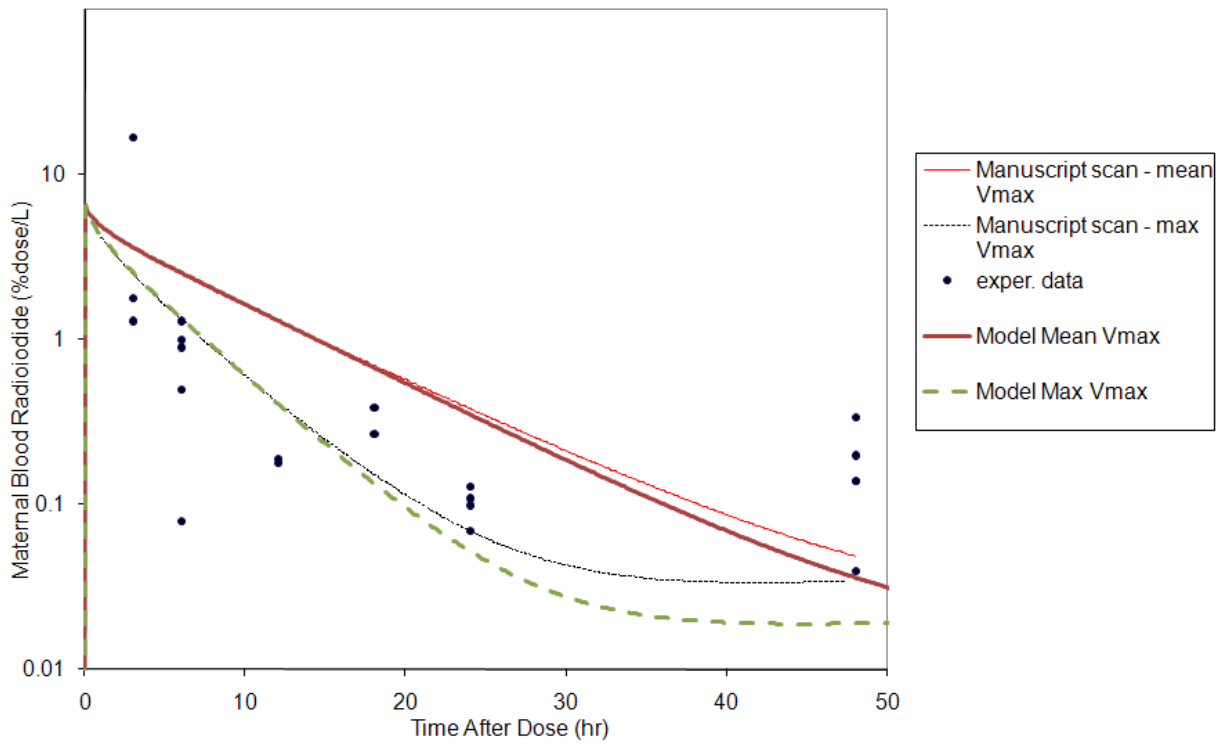
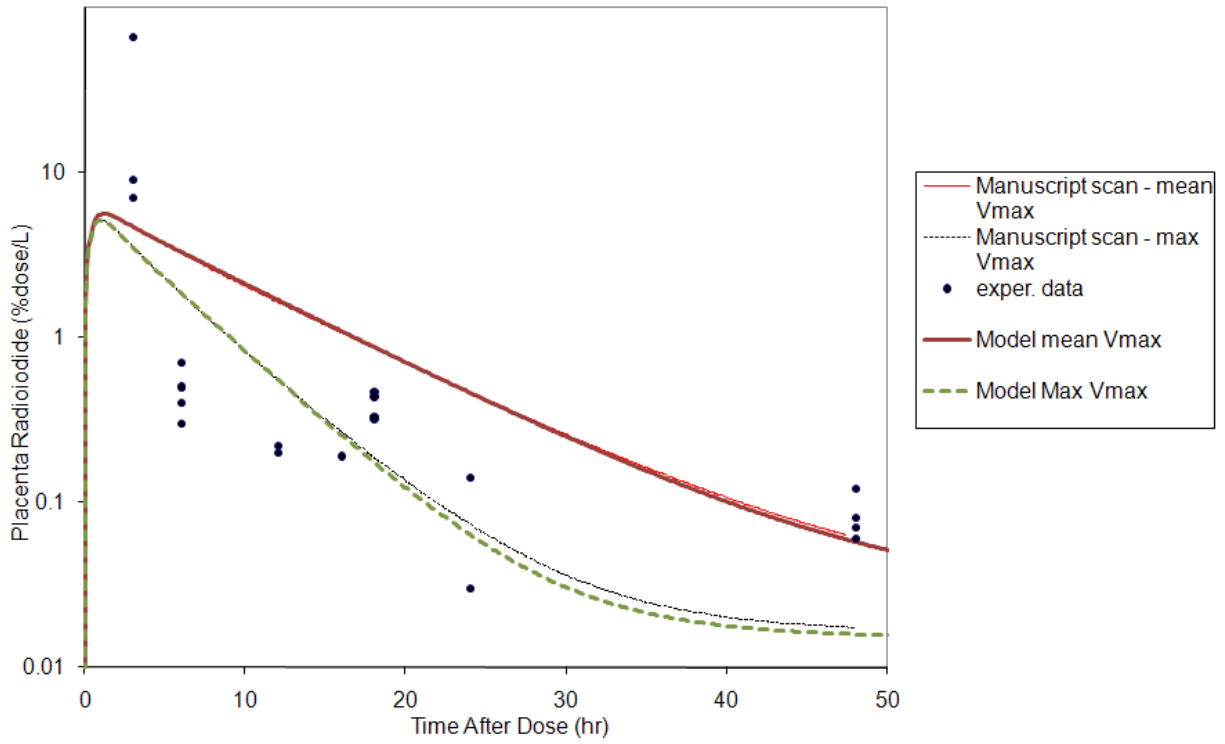


Figure 6d Clewell 2007



## Figure 7 – Total fetal burden

- Produced using the **pregnancy** model and the **Fig7.m** file.
  - The figure presents Total fetal  $^{131}\text{I}^-$  burden.
  - Experimental data appears to match (except for an anomalous value in the manuscript day 15 panel, at 20 hours and 90+ percent iodide in the fetus).
  - The simulation and publication differ slightly in the rate of elimination with the acsIXtreme code showing slightly faster elimination.

Figure 7 - GW 13, Clewell 2007

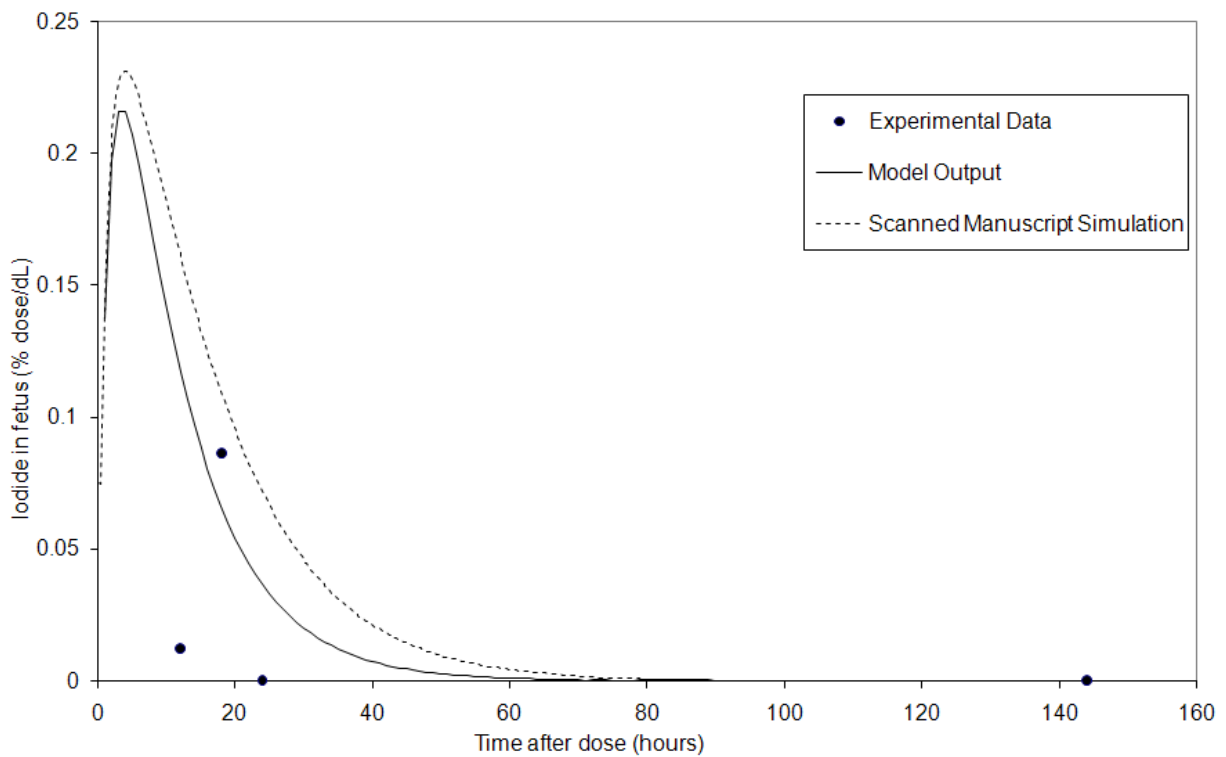


Figure 7 - GW 14, Clewell 2007

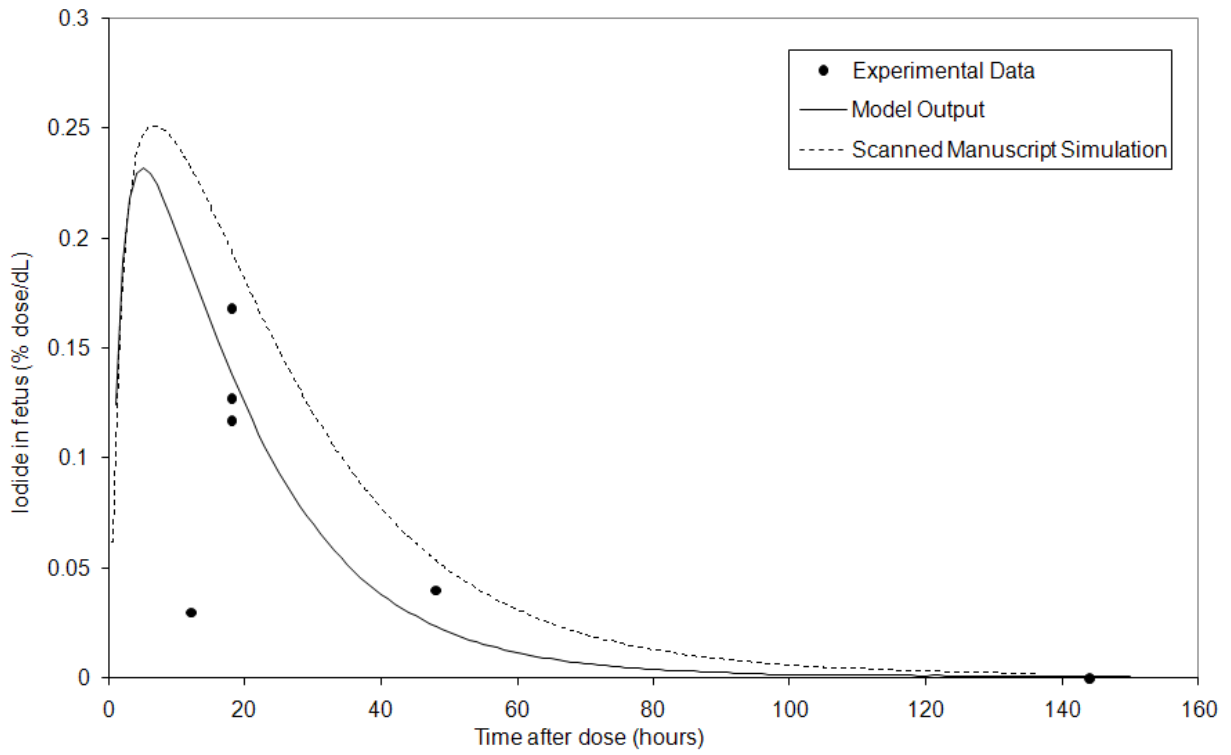


Figure 7 - GW 15, Clewell 2007

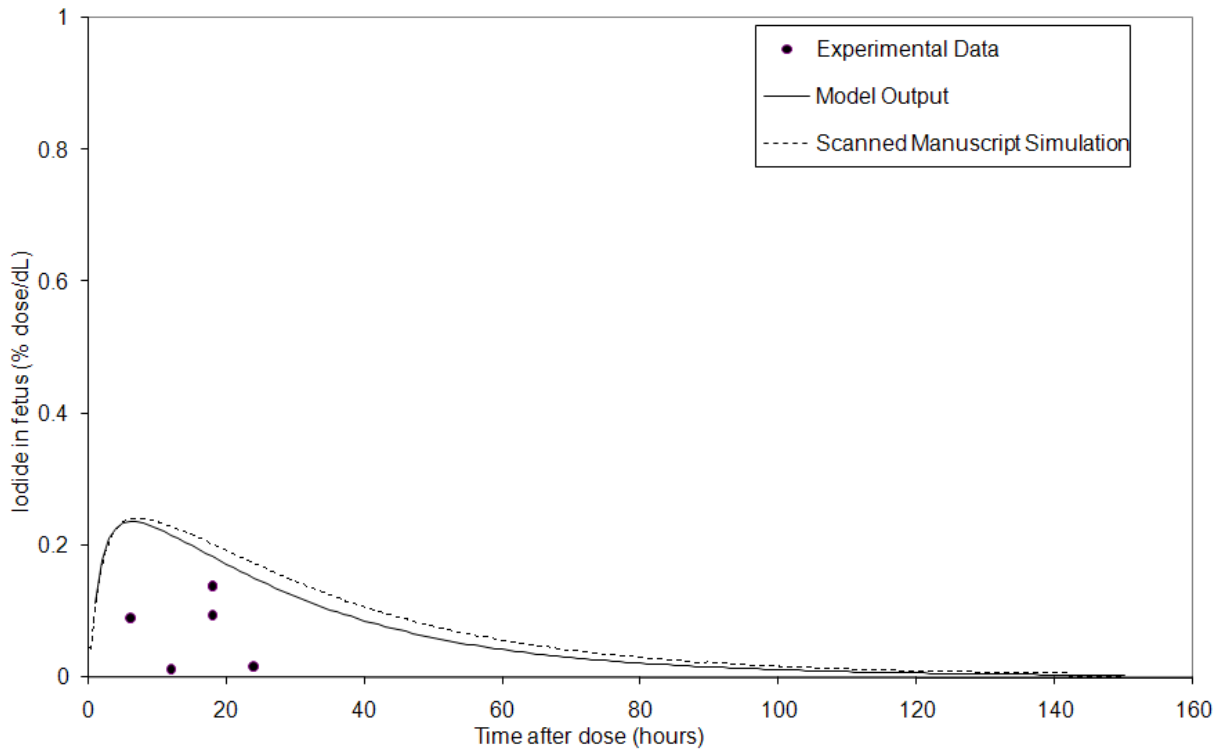


Figure 8 – Lactating women

- Produced using the **lactational** model and the **Fig7.m** file.
  - The figure presents predicted radioiodide concentration in the (A) thyroid, (B) urine, and (C) breast milk of lactating women.
  - The experimental data in the m files matches that presented in the manuscript (although there are data at longer post-dosing times in the m file not presented in the paper). EPA staff also supplied a modified m-file containing additional parameters.
  - The model simulations are close but it appears that some parameters have slightly changed.

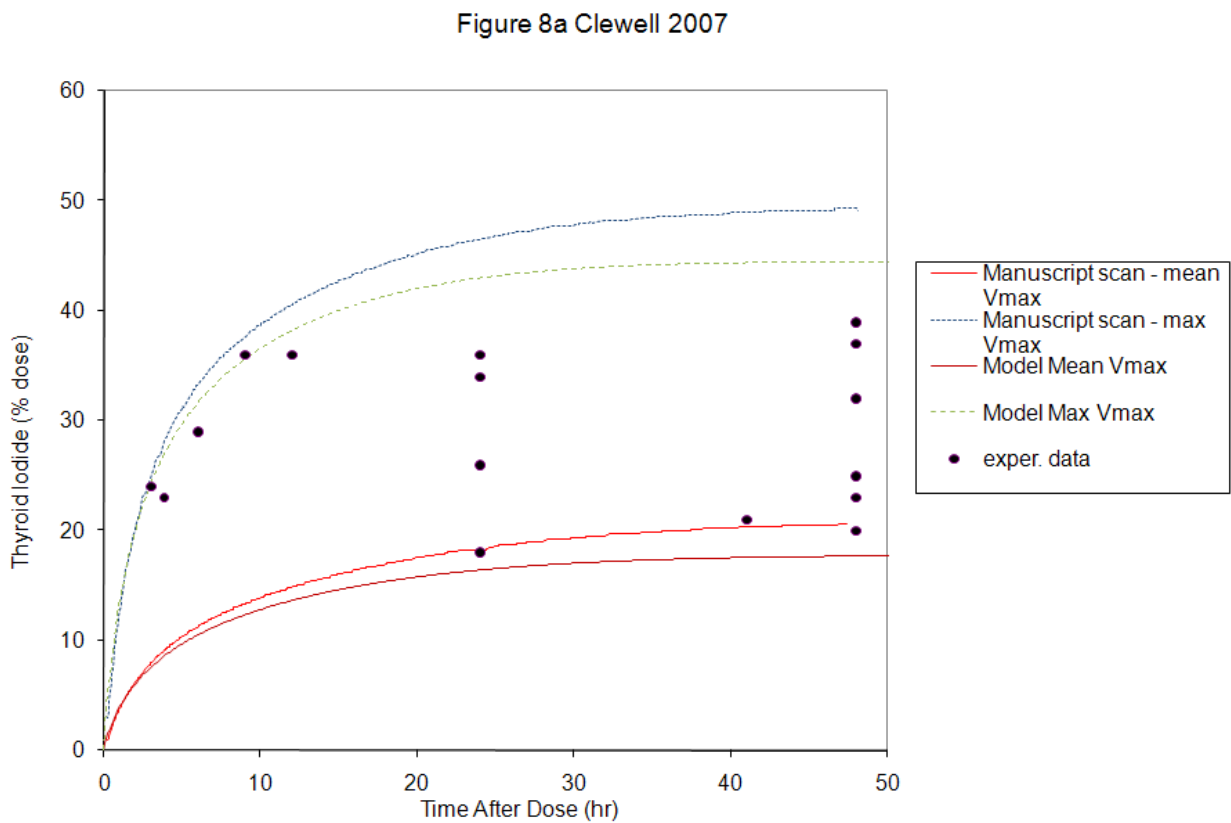


Figure 8b Clewell 2007

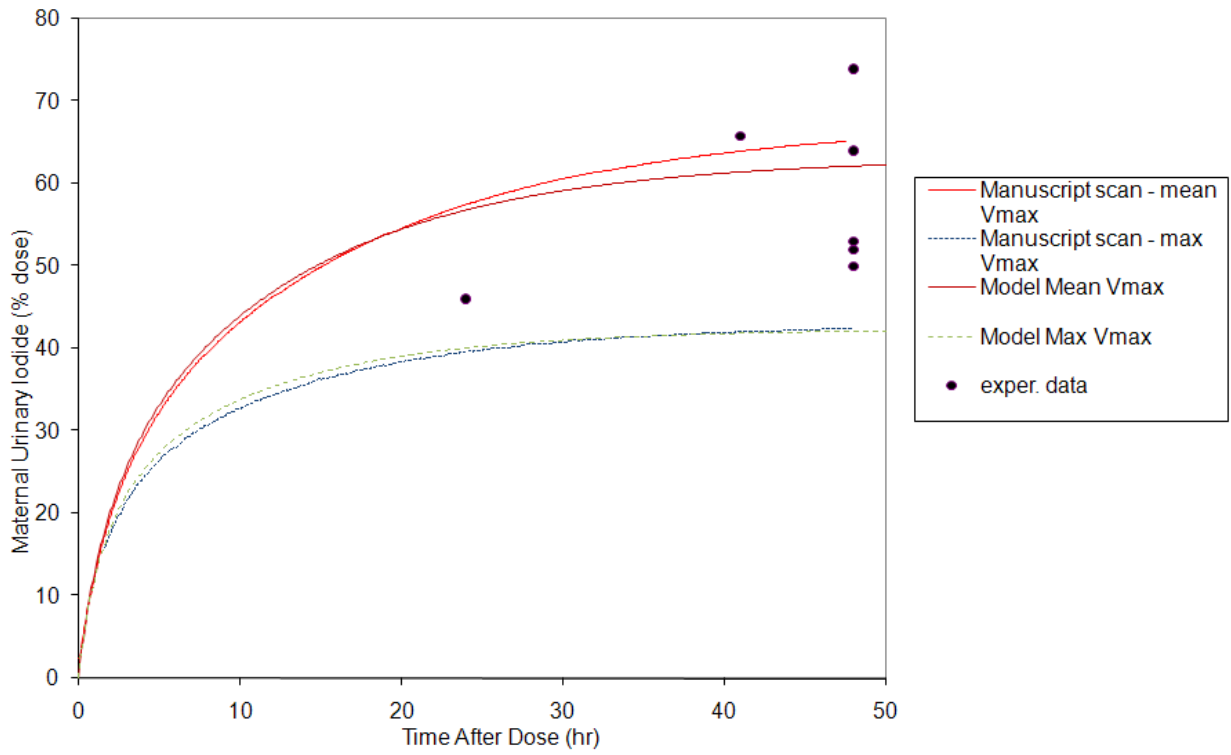
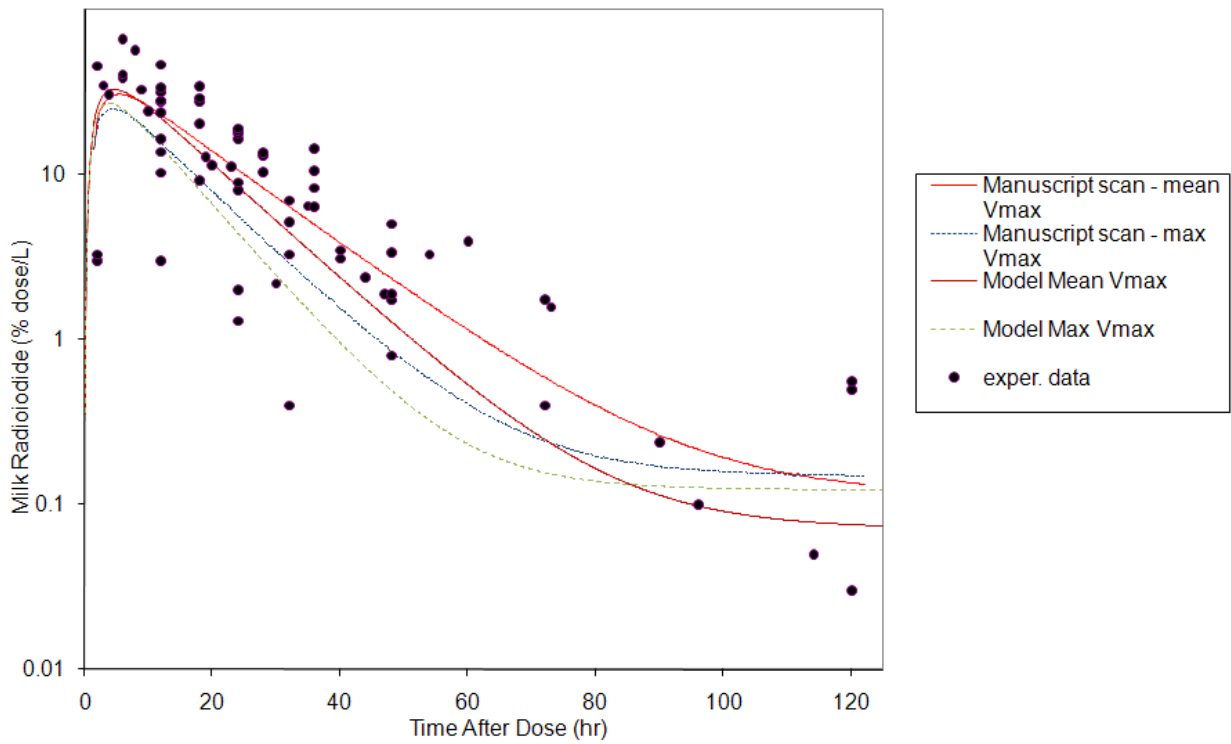


Figure 8c Clewell 2007

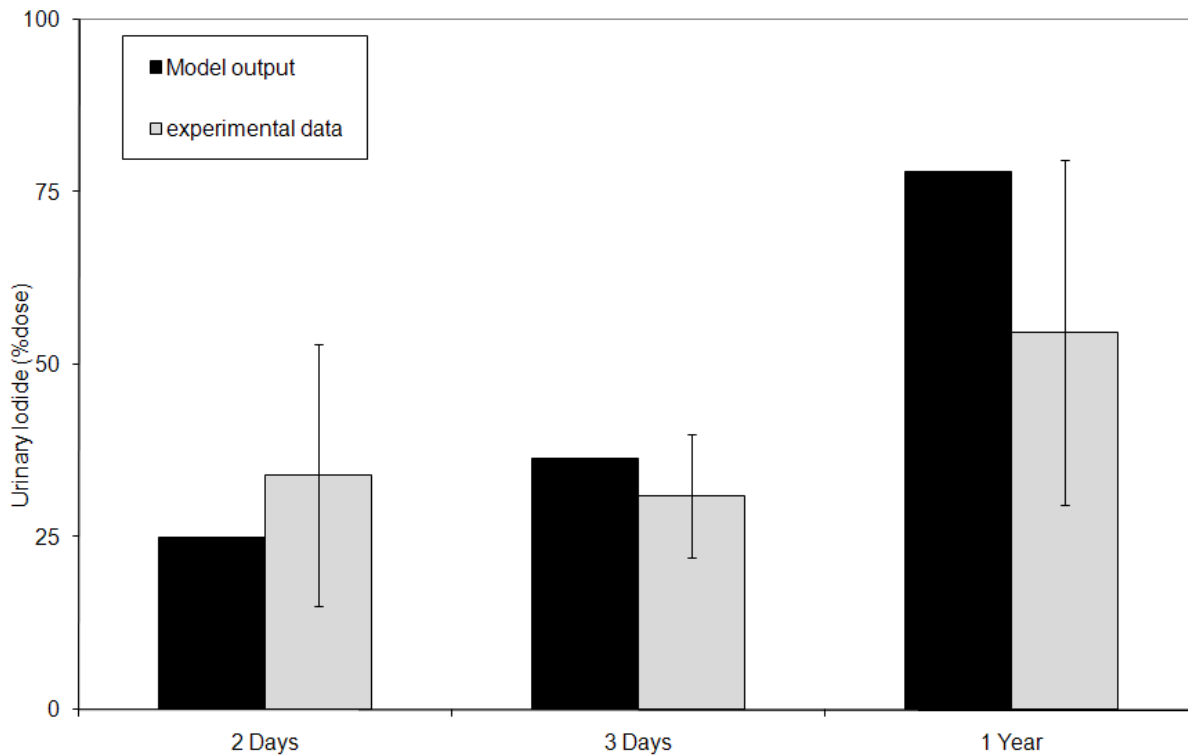




## Figure 9 – Neonatal urine

- Produced using the **lactational** model and the **Fig9\_GFR.m** file.
  - The figure presents predicted radioiodide in neonatal urine after a direct oral  $^{131}\text{I}^-$  dose.
  - The experimental data in the m files matches that presented in the manuscript.
  - The model simulations closely match the simulations presented in the manuscript.

Figure 9 Clewell 2007



## Additional Items

- Originally the pregnancy model had to run its init routine twice to get acceptable values. EPA staff fixed this issue by correcting the order of setting constant values in the m-files. The model currently does not use the init routine supplied by the original authors; rather constants are set in the csl and m-files.
- The m-files should be fully converted away from the old command language for consistency.
- Many of the figures are now easily produced by running m-files provided by the EPA staff.
- Given the evolution of the model, simulation of figures from the author's older papers on this model was attempted but the number of changes made this a difficult comparison.

---

## Appendix: m-files for figures

---

```
% Fig5_GFR.m
WESITG=0; WEDITG=0;
output @clear
prepare @clear DAYS CA_NI CTTOT_NI
nio_upt=[]; CINT=.1;
ACLU=0.75; RU=1.0; VCHNG=0.0; AKT=1; NDRNK=1;
cluc_i=0.11; cluc_p=0.125; %adult values
    CLUC_NI=cluc_i*RU*(70^(0.75 - ACLU));
    CLUC_NP=cluc_p*RU*(70^(0.75 - ACLU));
PDOSE=0; IVDOSE_I=0; IVDOSE_NI=50; PPB=0; PDOSE_N=0; CONC=0; DOSE_RI=0;
for day=[1:5,7]
    IVSTART_I=(day-1)*24; TSTOP=IVSTART_I+24; IVSTRT_NI=IVSTART_I;
    start @nocallback
    nio_upt = [nio_upt;[day, BW, BW_N, 100*ATTOT_NI/IVDOSE_NI]]
end
```

---

```
%maternal iodide vs. combined literature data
%corresponds to Figure 6 in JTEH, 2005
%DATA Miodide (t, au_i, attot_i, catot_i, cpl_i)
MiodideD=[3699      NaN      NaN      16.8      9
3699      NaN      NaN      1.8      66
3699      NaN      NaN      1.3      7
3700      NaN      11      NaN      NaN
3700      NaN      12      NaN      NaN
3700      NaN      18      NaN      NaN
3700      NaN      10      NaN      NaN
3700      NaN      18      NaN      NaN
3702      NaN      NaN      0.9      0.4
3702      NaN      NaN      .08      0.5
3702      NaN      NaN      1.3      0.7
3702      NaN      NaN      0.5      0.3
3702      NaN      NaN      1.0      0.5
3708      NaN      NaN      0.18      0.2
3708      NaN      NaN      0.19      0.22
3712      53      24      NaN      0.19
3714      NaN      NaN      0.27      0.32
3714      NaN      NaN      0.39      0.328
3714      NaN      NaN      NaN      0.468
3714      NaN      NaN      NaN      0.436
3720      58      46      0.1      0
3720      28      30      0.13      0.14
3720      61      22      0.11      0
3720      55      22.8      0.07      0.03
3720      59      26      0.0      NaN
3720      56      26.8      NaN      NaN
3720      18      29      NaN      NaN
3720      56      30      NaN      NaN
3720      NaN      26.4      NaN      NaN
3720      NaN      30.8      NaN      NaN
3720      NaN      29.8      NaN      NaN
3720      NaN      35      NaN      NaN
3720      NaN      41      NaN      NaN
3720      NaN      19      NaN      NaN
3744      65      NaN      0.2      0.08
3744      62      NaN      0.14      0.06
3744      56      NaN      0.04      0.12
3744      62      NaN      0.34      0.07
3744      57      NaN      0.14      0
3744      21      NaN      NaN      0
3744      62      NaN      NaN      0
3792      NaN      NaN      0.2      NaN
3792      NaN      NaN      0.22      NaN
3840      NaN      NaN      0.54      NaN
3840      NaN      NaN      0.03      NaN];
```

```
%Fig6.m
%Plot Figure 6
DOSE_I=100; TSTART=3000; IVSTART_I=696; TSTOP=800;
VMAXC_TI=1.22e5;
%VCHNG=1.7; SPLA=0;

prepare ATTOT_I AU_I CATOT_I CPL_I T
```

---

---

```

start@nocallback
v1a=_attot_i, v2a=_au_i, v3a=_catot_i, v4a=_cpl_i, t1=_t
VMAXC_TI=6.52e5; %maximum vmax used in merrill et al.
start@nocallback;
v1b=_attot_i, v2b=_au_i, v3b=_catot_i, v4b=_cpl_i, t2=_t

plot (t1,v1a,t2,v1b,Miodided(:,1),Miodided(:,3),'o','fig6a.aps')
plot (t1,v2a,t2,v2b,Miodided(:,1),Miodided(:,2),'o','fig6b.aps')
plot (t1,v3a,t2,v3b,Miodided(:,1),Miodided(:,4),'o','fig6c.aps')
plot (t1,v4a,t2,v4b,Miodided(:,1),Miodided(:,5),'o','fig6d.aps')

```

---

```

%init_preg.m -- initialization file for pregnancy
WESITG=0;WEDITG=0;
%dam clo4 (Ln 45-52), fetus Cl04 (Ln 54-60), dam I125 (Ln 62-69), fetus I125 (Ln 71-76)

!!s ps_p=0.31, pr_p=0.56, pf_p=0.05, pk_p=0.99, pl_p=0.56, pg_p=1.29, pgj_p=1.76
!!s pt_p=0.13, pdt_p=7.0, psk_p=1.32, prbc_p=0.8, ppl_p=0.56, pmam_p=0.66
!!s vmaxc_tp=6e3, vmaxc_dtp=1.67e4, vmaxc_sp=1.2e6, vmaxc_gp=3.2e7
!!s vmaxc_pp=6e4, vmaxc_mp=2.2e4, Km_Tp=1.6e5, Km_DTp=1.0e8, Km_Gp=2.0e5
!!s Km_Sp=2.0e5, km_pp=2.0e5, km_mp=2.0e5, pagc_p=0.6, pagjc_p=1.0
!!s paskc_p=1.25, patc_p=1.0e-4, padtc_p=0.01, parbcc_p=10.0, papc_p=0.1
!!s pamc_p=0.04, cluc_p=0.05
!!s vmaxc_bp=588, km_bp=1.64e4, kunbc_p=0.03

!!s ktrans2c=0.12, ktrans1c=0.12
!!s vmxc_dtfp=1.67e4
!!s padtc_fp=0.01, patc_fp=0.01
!!s Km_TFp=1.6e5, Km_GFp=2.0e5, Km_SFp=2.0e5
!!s VmxC_SFp=8.0e5, paskc_fp=1.25
!!s Vmxc_GFp=4.0e6, pagjc_fp=1.0, pagc_fp=0.66
!!s vmxc_bfp=500, km_bfp=1.8e4, kunbc_fp=0.03

!!s ps_i=0.21, pr_i=0.4, pf_i=0.05, pk_i=1.09, pl_i=0.44, pg_i=1.0, pgj_i=2.0
!!s pt_i=0.15, pdt_i=7.0, psk_i=0.7, prbc_i=1.0, ppl_i=0.4, pmam_i=0.66
!!s vmaxc_ti=1.22e5, vmaxc_dti=1.0e8, vmaxc_si=8.4e4, vmaxc_gi=4.5e5
!!s vmaxc_pi=5e4, vmaxc_mi=4.0e4, Km_Ti=4.0e6, Km_DTi=1.0e9, Km_Gi=4.0e6
!!s Km_Si=4.0e6, km_pi=4.0e6, km_mi=4.0e6, pagc_i=0.16, pagjc_i=12.0
!!s paskc_i=0.06, patc_i=1.0e-4, padtc_i=1.5e-5, parbcc_i=10.0, papc_i=0.005
!!s pamc_i=0.01, cluc_i=0.06, khormc_i=0.03, ksecrc_i=3.1e-7
!!s vmaxc_bi=300, km_bi=7.8e5, kdeiodc_i=0.021

!!s ktrans1c_i=0.12, ktrans2c_i=0.12
!!s vmxc_dtfi=6.0e7
!!s padtc_fi=1.0e-4, patc_fi=0.01
!!s Km_TFi=4.0e6, Km_GFi=4.0e6, Km_SFi=4.0e6
!!s VmxC_SFi=3.0e5, paskc_fi=0.02
!!s Vmxc_GFi=2.0e5, pagjc_fi=0.3, pagc_fi=0.1
TSTOP=1;CINT=1;
start @NoCallBack

```

---

```

%Fig7.m
%Figure 7 plots
Data_HPregF
init_preg
prepare @clear TIME ATTOT_I CFET_I CTOT_FI
DOSE_I=10.0; TSTART=2100; IVSTART_I=84; TSTOP=IVSTART_I+150;
SPLA=0; start @nocallback
plot(_time-IVSTART_I,_cfet_i, ...
     ALL13d(:,1)-TSTART-IVSTART_I, ALL13d(:,3),'o','Fig7_GW13.aps')
TSTART=2300; IVSTART_I=52; TSTOP=IVSTART_I+150;
SPLA=1; start @nocallback
plot(_time-IVSTART_I,_cfet_i, ...
     ALL14d(:,1)-TSTART-IVSTART_I, ALL14d(:,3),'o','Fig7_GW14.aps')
ALL15d=[2526    190    0.09
2532    190    0.013
2532    64    0.012
2538    31.4  0.139
2538    35.2  0.0948
2538    140.3 0.0948
2538    225.2 0.139
2544    80    0.017
2544    221.  NaN

```

---

```

2544 73.2 NaN
2544 173.9 NaN
2544 88.9 NaN
2544 43.1 NaN
2544 55.2 NaN];

TSTART=2400; IVSTART_I=120; TSTOP=IVSTART_I+150;
ALL15d(:,1)=ALL15d(:,1)-TSTART-IVSTART_I;
start @nocallback
t=_time-IVSTART_I;pt=t>0;
plot(t(pt),_cfet_i(pt), ALL15d(:,1),ALL15d(:,3),'o','Fig7_GW15.aps')

```

---

%Figure 8 - RClewell et al, JTEH 2006

```

%Maternal iodide
%t, cmk_i, attot_i, au_i
MATID=[3242 45.80 NaN NaN
3242 3.00 NaN NaN
3242 3.30 NaN NaN
3243 35.00 24 NaN
3243 NaN NaN NaN NaN
3243 NaN NaN NaN NaN
3243 NaN NaN NaN NaN
3243.84 30.81 23 NaN
3246 40.72 29 NaN
3246 39.00 NaN NaN
3246 67.10 NaN NaN
3246 NaN NaN NaN NaN
3246 NaN NaN NaN NaN
3248 57.21 NaN NaN
3249 33.00 36 NaN
3250 24.50 NaN NaN
3252 31.74 36 NaN
3252 28.17 NaN NaN
3252 28.00 NaN NaN
3252 23.92 NaN NaN
3252 46.70 NaN NaN
3252 10.30 NaN NaN
3252 34.10 NaN NaN
3252 13.80 NaN NaN
3252 3.00 NaN NaN
3252 16.50 NaN NaN
3258 27.92 NaN NaN
3258 29.30 NaN NaN
3258 9.30 NaN NaN
3258 34.50 NaN NaN
3258 20.60 NaN NaN
3259 12.90 NaN NaN
3260 11.51 NaN NaN
3263 11.20 NaN NaN
3264 8.14 36 46
3264 19.22 18 NaN
3264 2.00 34 NaN
3264 1.30 26 NaN
3264 9.00 NaN NaN
3264 16.50 NaN NaN
3264 17.90 NaN NaN
3264 18.70 NaN NaN
3268 10.40 NaN NaN
3268 13.20 NaN NaN
3268 13.60 NaN NaN
3270 2.19 NaN NaN
3272 3.30 NaN NaN
3272 5.20 NaN NaN
3272 7.00 NaN NaN
3272 0.40 NaN NaN
3275 6.50 NaN NaN
3276 10.66 NaN NaN
3276 8.36 NaN NaN
3276 6.40 NaN NaN
3276 14.40 NaN NaN
3280 3.50 NaN NaN
3280 3.10 NaN NaN
3281 NaN 21 65.8

```

```

3284 2.40 NaN NaN
3287 1.90 NaN NaN
3288 1.74 32 50
3288 5.05 20 74
3288 0.80 25 64
3288 1.90 23 53
3288 3.40 37 52
3288 NaN 39 NaN
3288 NaN NaN NaN
3288 NaN NaN NaN
3288 NaN NaN NaN
3288 NaN NaN NaN
3294 3.29 NaN NaN
3300 3.95 NaN NaN
3312 1.76 29 NaN
3312 0.40 24 NaN
3313 1.58 NaN NaN
3330 0.24 NaN NaN
3336 0.10 26 NaN
3336 NaN 23 NaN
3354 0.05 NaN NaN
3360 0.56 24 NaN
3360 0.50 21 NaN
3360 0.03 NaN NaN
3378 0.01 NaN NaN
3384 0.01 20 NaN
3408 0.43 20 NaN
3408 NaN 21 NaN
3432 0.51 NaN NaN
3480 0.49 15 NaN
3504 0.44 NaN NaN
3528 0.38 NaN NaN
3552 0.33 NaN NaN
3672 0.18 NaN NaN
3720 0.13 NaN NaN
3792 0.13 NaN NaN
3960 0.07 NaN NaN
4152 0.01 NaN NaN ];

```

```

%Fig8.m
%Figure 8 - Rclewell et al, JTEH 2006
WESITG=0; WEDITG=0;
output @clear
prepare T CMK_I ATTOT_I AU_I
ACLU=0.75; RU=1.0; VCHNG=0.0; AKT=0.0; NDRNK=1;
cluc_i=0.11; cluc_p=0.125; %adult values
    CLUC_NI=cluc_i*RU*(70^(0.75 - ACLU));
    CLUC_NP=cluc_p*RU*(70^(0.75 - ACLU));
PDOSE=0; IVDOSE_I=100; IVDOSE_NI=0; PPB=0; PDOSE_N=0; CONC=0; DOSE_RI=0; IVDOSE_P=0;
IVSTART_I=3240; TSTOP=3365;
!! set TIME0=3240.0
VMAXC_TI = 1.39e5;
start@nocallback
cmk1=_cmk_i; attot1=_attot_i; au1=_au_i;

VMAXC_TI=7.4e5;
start@nocallback
cmk2=_cmk_i; attot2=_attot_i; au2=_au_i;
VMAXC_TI = 1.39e5;
!! set TIME0=0
plot (_t-TIME0,attot1,_t-TIME0,attot2, MATID(:,1)-TIME0,MATID(:,3),'o','Fig8a.aps')
plot (_t-TIME0,au1,_t-TIME0,au2,MATID(:,1)-TIME0,MATID(:,4),'o','Fig8b.aps')
plot (_t-TIME0,cmk1,_t-TIME0,cmk2, MATID(:,1)-TIME0,MATID(:,2),'o','Fig8c.aps')

```

```

% Fig9_GFR.m
WESITG=0; WEDITG=0;
output @clear
prepare @clear DAYS CA_NI CTTOT_NI
nio_urine=[]; CINT=.1;
ACLU=0.75; RU=1.0; VCHNG=0.0; AKT=1; NDRNK=1;
cluc_i=0.11; cluc_p=0.125; %adult values
    CLUC_NI=cluc_i*RU*(70^(0.75 - ACLU));
    CLUC_NP=cluc_p*RU*(70^(0.75 - ACLU));
PDOSE=0; IVDOSE_I=0; IVDOSE_NI=100; PPB=0; PDOSE_N=0; CONC=0; DOSE_RI=0;
!! set TIME0=0
for day=[2 3 366]
    IVSTART_I=(day-1)*24; TSTOP=IVSTART_I+24; IVSTRT_NI=IVSTART_I;
    if day>364
        !! set TIME0=8760
        TSTOP=IVSTART_I+48      % in .cmd file, TSTOP @ 48 hr after injection for 'Proced
year1'
    end
    start @nocallback
    nio_urine = [nio_urine;[day, BW, BW_N, AU_NI]]
end
!! set TIME0=0

```