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

NOTICE

THIS DOCUMENT IS A PRELIMINARY DRAFT. It is undergoing peer review and public comment and should not at this stage be construed to represent any final Agency policy. It is being circulated for comment on its technical accuracy and policy implications.

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This document is intended to support EPA's preliminary regulatory determination for perchlorate; it is, however, still undergoing peer review under applicable information quality guidelines and public comment. As a result, this document may change. This document does not represent and should not be construed to represent any final Agency determination or policy. It is being circulated for review of its technical accuracy and science policy implications. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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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 (NRC, 2005) concluded that hypothyroidism is the first adverse effect in the continuum of effects that could result from perchlorate exposure. However, NRC advised that hypothyroidism not be used as the basis of the perchlorate RfD, recommending that the most health protective and scientifically valid approach was to base the perchlorate RfD 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 the young child (Clewell et al., 2007), were evaluated based on their ability to provide additional information about this critical effect for potentially sensitive subgroups.

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 between model code files. 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 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 μg/kg-day). Specifically, at a perchlorate dose of 7 μg/kg-day, the percent RAIU inhibition predicted by the model for the near-term fetus is 5-fold greater than the average adult. After correcting the model for reduced urinary clearance in infants, the same analysis predicts percent RAIU inhibition approximately 1- to 2-fold 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 slightly lower for the 1-2 year old child than for 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, 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 lifestages. Unfortunately, the lack of biological information and data that might be used to validate model predictions, particularly for early fetal development, limits EPA's confidence on predictions for fetal endpoints. Therefore the EPA simply chose not to use model predictions for the early- or mid-term fetus. However, because many of the physiological and iodide/perchlorate-specific parameters in the late-term fetus are expected to be quite close to those of the newborn, and there are much more data available for validation of the model in the newborn, our 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 the EPA differ by up to 3-fold 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 lifestage 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 (e.g., NRC, 2005; York et al., 2005 a, 2005b; Gilbert and Sui, 2008). The National Research Council (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 concluded that hypothyroidism is the first adverse effect in the continuum of effects that could result from perchlorate exposure. However, NRC advised that hypothyroidism not be used as the basis of the perchlorate RfD, recommending that the most health protective and scientifically valid approach was to base the perchlorate RfD on the inhibition of iodide uptake by the thyroid. NRC further concluded that iodide uptake inhibition, although not adverse, would precede any adverse health effects of perchlorate exposure. The lowest dose (7 μg/kg-day) administered in the Greer et al. (2002) study was considered a no-observed effect level (NOEL) because iodide uptake inhibition was considered to not be an adverse effect. The NRC also recommended that EPA use this dose as the point of departure and apply an intraspecies uncertainty factor of 10 to account for differences in sensitivity between the healthy adults in the Greer et al. (2002) study and the most sensitive population, fetuses of pregnant women who might have hypothyroidism or iodine deficiency. EPA's Integrated Risk Information System (IRIS) adopted the 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 lifestage models in the rodent (Clewell et al., 2003a, 2003b) to predict inhibition of the NIS for pregnant and lactating women, nursing infants, and for the subsequent stages of childhood.

The following report provides a quantitative analysis of perchlorate-mediated inhibition of the NIS in humans 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 lifestage. 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 lifestages. The analysis presented here was conducted to inform EPA's regulatory determination for perchlorate under the Safe Drinking Water Act. Specifically, the Office of Water requested that staff in the Office of Research and Development's (ORD) National Health and Environmental Effects Research Laboratory (NHEERL), National Center for Environmental Assessment (NCEA) and National Center for Computational Toxicology (NCCT) assist in a thorough evaluation of the Merrill et al. (2005) and Clewell et al. (2007) PBPK models to address their scientific soundness and to determine whether or not they are suitable to provide quantitative predictions to the Agency on the lifestage variability of perchlorate NIS inhibition of thyroidal iodide uptake.

The evaluation of the PBPK models was conducted in two stages. First, the scientific credibility of the models was evaluated based on (1) the information presented in Merrill et al. (2005) and Clewell et al. (2007), (2) limited inspection of the computer codes of the models, and (3) 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 for regulatory use 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 lifestage variability in the urinary clearance of perchlorate, to which NIS inhibition is sensitive.

The first-stage evaluation included staff[1] with expertise in PBPK modeling, developmental neurotoxicology, perchlorate toxicology, and risk assessment. The second-stage, more detailed evaluation was conducted by a subgroup[2] of those who conducted the preliminary

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[1] Staff involved in the 1st stage evaluation were:

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first-stage evaluation. The second-stage group consisted of NHEERL, NCEA and NCCT personnel with expertise in PBPK modeling and in the application of PBPK models to risk assessment, although expertise in developmental neurotoxicology and in risk assessment was retained. 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 lifestages. The lifestages evaluated by EPA included the pregnant woman, fetus, lactating woman, breast-fed infant, bottle-fed infant, 1 year old and 2 year old child, "average" adult, and non-pregnant woman of child-bearing 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 the 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 US Air Force Research Laboratory (AFRL) to the 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 non-pregnant woman of child-bearing age was modified by EPA from the pregnant woman code by removing the placental and fetal compartments, but retaining the mammary compartment.

2. EXAMINATION OF PBPK MODEL COMPUTER CODE

A number of coding errors were found in each model version/file provided to EPA by the authors and several inconsistencies between the various code files were identified. Except for those instances noted below and in Appendix A, correction of these errors resulted in only minor changes in model outputs, and the model codes were still able to reproduce human datasets shown in Clewell et al. (2007).

An example of a coding error with little quantitative impact 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 tract, skin, mammary gland, placenta, and excretion into breast-milk) were found

to inconsistently include perchlorate inhibition of radioiodide transport across model codes. 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 gastrointestinal tract, but this inhibition was not described mathematically in the model code for the lactating woman, breast-fed neonate, or young child. Addition by EPA staff of inhibition of NIS radioiodide transport by perchlorate in the skin and gastrointestinal 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 was not described in the lactating woman code as being inhibited by perchlorate, but inclusion of this inhibition markedly increased the predicted percent inhibition of thyroidal radioiodide uptake in the breast-fed infant (about 2-fold at lower perchlorate doses and less at higher doses of perchlorate). 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 staff added inhibition of radioiodide transport by perchlorate when it was absent in the model codes obtained from the authors. 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.

NOTE: A PDF document of the model code modified by EPA and used in this analysis is available upon request. Please contact Eva D. McLanahan at McLanahan.Eva@epa.gov or 919-541-1396 to request a copy of the code. Please include your name, affiliation, e-mail address, phone number, and reason for requesting the code.

3. EVALUATION OF PBPK MODEL TECHNICAL APPROACH - DEVELOPMENT AND MODEL PARAMETERIZATION

3.1. URINARY CLEARANCE

The urinary clearance values for perchlorate and iodide across all lifestages 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 body weight (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 surface area) in 1-week old neonates is 11.0 ± 5.4 mL/min/1.73 m^2 while in infants aged 9-12 months GFR is 86.9 ± 8.4 mL/min/1.73 m^2 (Gomez and Norwood, 2005). (*Note*: A convention in literature reporting these data is to calculate GFR per unit of body surface area (m^2) for the tested individuals, but to express the results normalized to a standard adult surface area (1.73 m^2) regardless of the tested individuals age.) Data on urinary elimination of a number of compounds including drugs and drug metabolites also indicate that renal clearance is slower per unit of body weight in neonates (Clewell et al., 2002; Dorne et al., 2004). Modification of the PBPK models to describe slower clearance of perchlorate and iodide in neonates (approximately 50% of adult values when normalized to BW) versus that described in Clewell et al. (2007) (approximately 200% of adult values when normalized to BW) resulted in an increase in predicted levels of NIS inhibition in infants at a perchlorate dose-rate of 7 μg/kg-day (amount ingested by the infant, equal to the point of departure for the RfD). For example, the 7-day-old bottle-fed infant model RAIU inhibition predictions increased from 1.5% (after other corrections were made) to 4.4% (Table 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 is renal excretion (Lloyd et al., 1985), but the average for those data was better described as scaling by BW^1, resulting in somewhat lower average predicted clearance (see Appendix B for details). Therefore, EPA chose to estimate perchlorate-induced inhibition 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 details.) EPA's estimates of urinary clearance in infants and children are lower than those used in Clewell et al. (2007), but are values 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).

Data indicating that urinary clearance of iodide by the mother during pregnancy and the first few months postnatal may be as much as two times higher than in non-pregnant woman were also identified (Aboul-Khair et al., 1964). These data include radioiodide uptake during pregnancy and lactation. Details of the PBPK model analyses with data from the Aboul-Khair study 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). However, the review paper by Delange (2004) indicates that observations on urinary clearance changes during pregnancy are mixed and should not be considered as generally occurring (and not explanatory for the increased likelihood of maternal iodide deficiency during pregnancy). 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:non-pregnant comparisons in rats, which is the opposite of what the data in Aboul-Khair et al. (1964) suggest (but also inconsistent with conclusions by Delange 2004; see Tables 1 and 2).

Because of the differences in urinary clearance values reported in the literature, EPA considered three alternatives for pregnancy using: 1) urinary clearance values reported by Clewell et al. (2007); 2) increased urinary clearance based on the pregnant:non-pregnant ratio reported by Aboul-Khair et al. (1964); and 3) urinary clearance constants assumed to be

unchanged in pregnancy from the average adult consistent with the observations of Delange (2004) (see Table 2). In each case, the urinary clearance 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 lifestages, so there are no human data with which to validate this assumed constant proportionality.

Since there are no conclusive human pregnancy data to distinguish among these alternatives as to which is more likely, EPA selected the lower clearance values reported in the peer-reviewed, published paper by Clewell et al. (2007) for relative response estimation (lifestage sensitivity analysis). These lower clearance values were used in producing Tables 1, 3 and 4 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 inter-individual 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 about 40% of the average adult value, again based on lifestage comparisons in rats. 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); and 3) clearance equal to non-pregnant "average" values. Simulation results with all three options are shown below in Table 2. Since it does not seem biologically realistic for perchlorate clearance to be reduced while iodine clearance is not reduced, EPA decided not to use the lower perchlorate clearance of Clewell et al. (2007) (option 1). But 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), EPA chose to base the model predictions on the middle of these three possibilities, option 2. The resulting estimates of perchlorate effects are not "upper bound" values, as this analysis did not address a range of other uncertainties in the modeling, such as uncertainty thyroid NIS parameters and inter-individual variability in urinary clearance.

3.2. PARAMETER SCALING

For parameters scaled up from rodents, Clewell et al. (2007) scaled permeability-area cross products (PA) by $BW^{0.75}$. The use of such scaling is common practice for PBPK model parameters describing metabolic clearance, and for such applications has been tested through applications with a number of chemicals. 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, 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 one accepts the scaling power of 0.75 as being correct, assuming that the transport parameters in these tissues change with total BW, rather than tissue weight, may be viewed as 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 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 is" 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, which were then presumed 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 size during lactation, the equation used to adjust blood flow to the mammary in proportion to its size appeared to be in error, since the flow rate set by it at birth only reflected the mammary volume change (increase) during pregnancy, and not the

initial (pre-pregnancy) volume. The result is that the mammary 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 the authors, and the blood volume (V_B) is a constant fraction of BW (i.e., $V_B = VC_B \cdot BW$), as is assumed in the model, then the total amount of binding protein should scale as blood (i.e., $A_{bp} = C_{pb} \cdot V_B = C_{pb} \cdot VC_B \cdot BW^1$). 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, 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 using a Michaelis-Menten equation: $r_{bind} = V_{max,b} \cdot C/(K_{m,b} + C)$, 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 using a Michaelis-Menten equation would not allow for a variable rate of binding, thus limiting EPA's confidence in using these models for predictions of various intake rates of dietary iodide

3.3. POST-NATAL 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 be first run using the pregnancy model to estimate body levels at birth. However, EPA found that by post-natal day (PND) 7, the predicted fetal and maternal levels had almost no dependence on pre-birth levels versus 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.

3.3.1. BREAST-FED INFANT SUCKLING RATE

The suckling rate used by Clewell et al. (2007) was determined to be an inadequate description based on data currently available in 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 were set by the table function implemented by Clewell et al., but then fall below that table between 2.5 weeks and several months of age (Figure 1). Therefore, to improve PBPK model predictions, the suckling rate was altered from the original approach used by the authors. A smooth function of infant body weight was fit to the mean ingestion rate data from Arcus-Arth et al. (2005) and implemented in the description of infant breast-milk ingestion:

$$\text{Milk ingestion rate} = \text{KTRANS} = 28.3*(\text{BW} - 3.375)^{0.175} \text{ (mL/h)}.$$

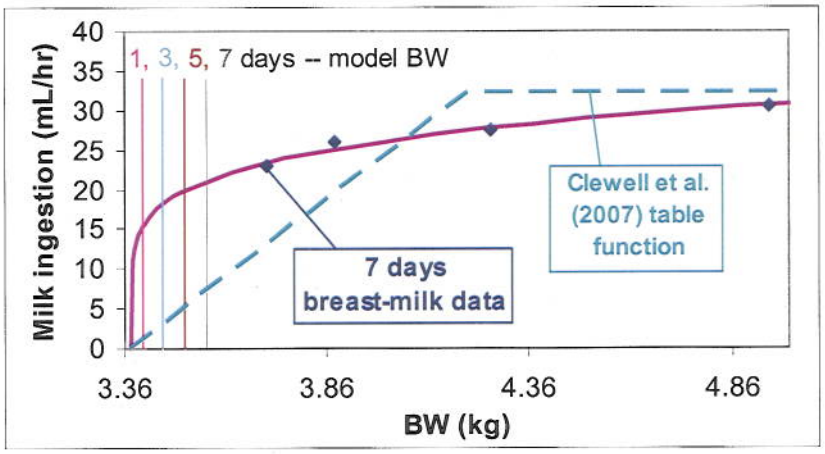

Figure 1. Breast-milk consumption values used by EPA. Data from Arcus-Arth et al. (2005).

It should also be noted that for breast-fed infant simulations, the intravenous (IV) 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 1.3-1.5% when the infant was receiving 7 μg/kg-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 close to 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 3.9 % for the breast-fed infant of the same age (both using the low 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 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 IV 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.

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, as noted above. The model code already described iodide transport to breast milk; however, EPA added perchlorate inhibition of that transport. 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 hr after simulated IV injection in the mother as the measure of effect. (No such change is needed for the bottle-fed infant, since in that case the amount of iodide in the formula is presumed to be unaffected by the presence of perchlorate.) Predicted radioiodide kinetics in infant blood is 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.

Finally, to account for the fact that water ingestion will vary with age and BW in the bottle-fed infant, a smooth function of age was fit to the results of Kahn and Stralka (2008), which had been plotted against the mid-point for each age range, as shown in Figure 2 (upper panel, solid line, quadratic equation). However, to account for the minimal ingestion occurring in the first couple days of life, the equation was multiplied by a rising exponential function: $1 - e^{-day}$. 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 the lower panel of Figure 2. (The function is only used for predictions up to 60 days of age.)

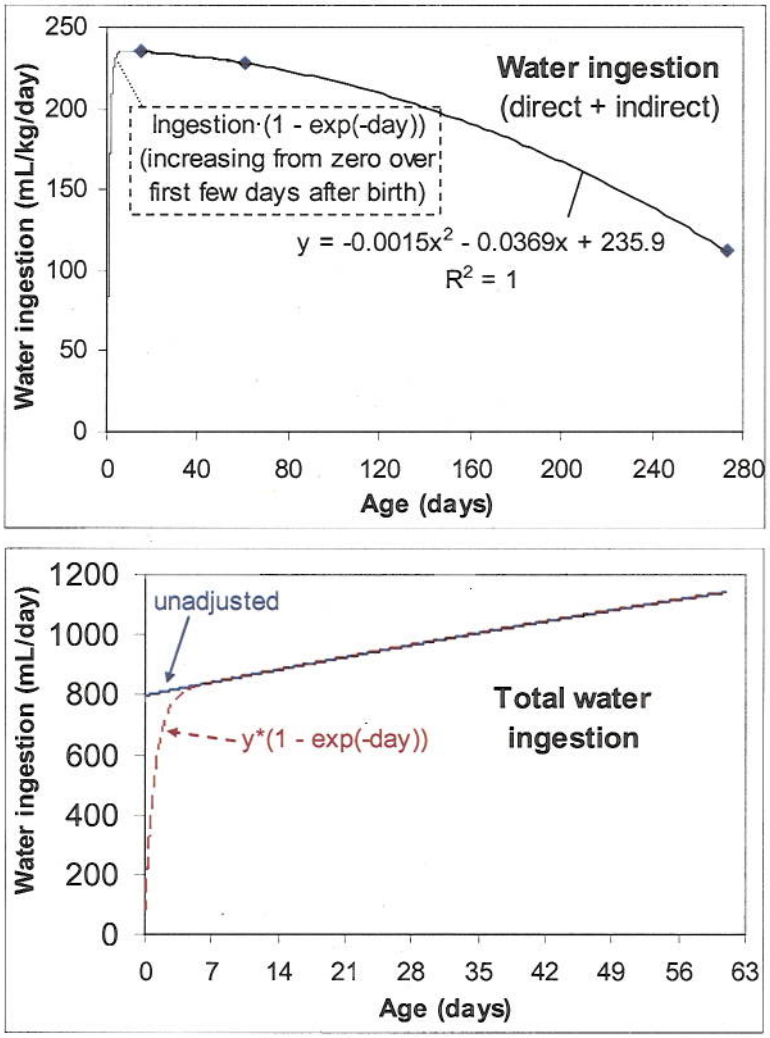

Figure 2. 90th Percentile water consumption values used by EPA for the bottle-fed infant. Upper panel: fit of body-weight-specific function to 90th percentile water ingestion data from Kahn and Stralka (2008); lower panel: total ingestion, after multiplication by body weight.

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. EPA-MODIFIED PBPK MODEL RESULTS AND LIFESTAGE ANALYSIS

4.1. EPA-MODIFIED PBPK MODEL RESULTS

Model predictions obtained with the model as modified by EPA are compared to published values by Clewell et al. (2007) in Table 1. 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 attributed 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. Addition of 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 1: Comparison of Clewell et al. (2007) published model predicted percent inhibition of thyroidal radioiodide uptake across lifestages with EPA modified versions.*

External Dose (mg/kg-day)	Fetus[a] (% Inhibition)		Breast-fed Neonate[b] (% Inhibition)		Child[c] (% Inhibition)		Pregnant Woman[a] (% Inhibition)		Lactating Woman[d] (% Inhibition)	
	Clewell	EPA	Clewell	EPA	Clewell	EPA	Clewell	EPA	Clewell	EPA
0.001	1.1	1.3	0.9	1.3	0.3	0.3	1	0.95	1.1	0.3
0.01	10	12	8	12	3	2.9	9	8.9	10	2.9
0.1	49	52	34	56	21	23	50	50	54	25
1	84	86	63	92	72	75	91	90	92	78

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

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

[b] Breast-fed neonate shown at post-natal month 1.5; suckling rate was set to the ingestion rate-function fit to the data of Arcus-Arth et al. (2005); external dose is that ingested by the mother; neonate ingestion (mg/kg-day) is 2.2, 2.1, 1.6, and 0.56 times maternal 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.

[c] Child shown at 7 years of age and EPA prediction uses "medium" estimate for urinary clearance.

[d] Lactating woman shown at post-natal day (PND) 7.

Table 2 compares the effects of alternate urinary clearance parameters on EPA-modified PBPK model predictions of RAIU inhibition for the different lifestages. A decrease in the PBPK model urinary clearance rate of iodide and perchlorate resulted in increases of RAIU inhibition predictions for all lifestages. The largest effect was seen for the near-term fetus (GW40), such that as the prediction of fetal RAIU inhibition increased from 3.3% inhibition at the highest rate of clearance to 11% inhibition at the lowest rate of clearance (Table 2). The detailed effects on RAIU inhibition resulting from EPA model modifications, as described in section 3, are provided in Appendix A.

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

Urinary Clearance Rate	Gestation Model [b]		Lactation Model						Bottle-Fed Infant[d]			Older Child[e]	
	Pregnant Woman	Fetus	Lactating Woman[c]			Breast-Fed Infant[d]						10 kg	14 kg
	GW 40	GW 40	7d	30d	60d	7d	30d	60d	7d	30d	60d	0.97yr	2yr
High	1.7%	3.3%	1.4%	1.6%	1.8%	3.0%	2.9%	2.8%	1.5%	1.5%	1.6%	1.4%	1.4%
Medium [a]	3.0%	5.3%	2.1%	2.0%	1.9%	3.3%	2.7%	2.6%	2.0%	1.5%	1.3%	1.9%	1.9%
Low	6.3%	11%	4.3%	4.0%	3.9%	6.5%	4.8%	3.5%	4.4%	3.0%	2.5%	2.3%	2.3%

[a] Average adult value for urinary clearance was used as a "medium" estimate for the pregnant and lactating woman and the older child, where cluc_i = 0.11 and cluc_p = 0.125.

[b] "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 Vmax for thyroidal uptake of iodide and perchlorate were adjusted (VmaxTc × 1.8631) to fit Aboul-Khair et al. (1964) maternal thyroid uptake data. 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 Vmax 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.

[c] The dose of 7 μg/kg-day was provided to the lactating woman, and the breast-fed infant received a concentration in breast milk that corresponded to maternal intake of 7 μg/kg-day. High clearance rate for the lactating woman was set equal to that of the average adult values from Merrill et al. (2005), but the Vmax for thyroid perchlorate and iodide uptake was adjusted to fit literature data. Central estimate did not include Vmax 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).

[d] The breast-fed infant received perchlorate dose from maternal milk that was estimated by the model following maternal ingestion of 7 μg/kg-day. The bottle-fed infant was simulated using a constant 7 μg/kg-day perchlorate dose rate. "High" is estimated 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 (~40%).

[e] "High" value was estimated as published in Clewell et al. (2007) 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; this scaling described the average renal excretion of cimetidine (Lloyd et al., 1985) better. The "low" clearance estimate was estimated by scaling by BW^1 and multiplying by the ratio (0.76) of the lower 95% confidence bound to the mean for the Lloyd et al. (1985) data.

4.2. LIFESTAGE RELATIVE RESPONSE ANALYSIS

For this document and analysis, *sensitivity* is defined as the predicted response in percent RAIU inhibition 24 hours after iodide intravenous 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, non-pregnant adult, where response is the percent RAIU inhibition 24 hours after iodide IV 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 point of departure (POD) of 7 μg/kg-day, which was identified by the National Research Council (NRC, 2005) as a no-observed-effect- level (NOEL) for the derivation of the RfD and adopted by EPA. Table 3, column 3 shows the PBPK model predictions of percent inhibition of RAIU at the 7 μg/kg-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 3, column 4).

EPA's model predictions may generally be considered central estimates for each subgroup (at the consumption levels modeled) that account for PK differences, and do not take into account within-group variability in pharmacokinetics, uncertainty in model parameters and predictions, or population differences in PD. Fetal simulations are only reported for the end of gestation (GW 40) as key fetal parameters are considered to be too uncertain for reliable use earlier in gestation. However, maternal parameters are considered to be more reliable, so maternal predictions are also shown for GW 13 and 20.

Table 3. Model-predicted radioactive iodide uptake (RAIU) inhibition and relative sensitivity of different subgroups compared to the average adult at a dose equal to the point-of-departure (POD) based on EPA's modified PBPK models.

Population or lifestage	Body weight (kg) [a]	RAIU inhibition at the POD (7 μg/kg-day)	Relative sensitivity vs. average adult @ the POD
Average Adult	70	2.1%	1
Woman (child-bearing age)	68	3.1% [b]	1.5
GW13	Mom: 69	6.6% [c]	3.1
GW20	Mom: 71	6.5% [c]	3.1
GW 40	Mom: 78	6.3% [c]	3.0
	Fetus: 3.5	11% [c]	5.3
Mother and breast-fed infant (7 d)	Mom: 74	2.1% [d]	0.99
	Infant: 3.6	5.9% [d, e, f]	2.9
Mother and breast-fed infant (30 d)	Mom: 73	2.0% [d]	0.95
	Infant: 4.2	4.3% [d, e, f]	2.1
Mother and breast-fed infant (60 d)	Mom: 72	1.9% [d]	0.93
	Infant: 5.0	3.9% [d, e, f]	1.9
Bottle-fed infant (7 d)	Infant: 3.6	4.4% [e]	2.1
Bottle-fed infant (30 d)	Infant: 4.2	3.0% [e]	1.4
Bottle-fed infant (60 d)	Infant: 5.0	2.5% [e]	1.2
Child (0.97 yr) [g]	Child: 10	1.9% [h]	0.9
Child (2 yr)	Child: 14	1.9% [h]	0.9

[a] The body weight (70 kg) for the average adult is the default weight used by the Office of Water (OW). All other body weights are generated by the model.

[b] Results were obtained using modified code, in which fetal and placental compartments were removed from the code for pregnancy. Maternal body weight 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.

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

[d] Results are based on setting the maternal clearance rates of both perchlorate and iodide during lactation equal to that 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.

[e] %RAIU inhibition given for the infant is provided based upon 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 glomerular filtration rates (see bullet in text for further details). Clewell et al. (2007) scaled urinary clearance by $BW^{0.75}$, rather than adjusting based on GFR.

[f] These %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 2 notes), as follows: 7 and 30 days – 3.1 μg/kg-day; 60 day – 3.4 μg/kg-day. These doses differ due to changes in body weights and other PK factors with age.

[g] Because OW typically uses a 10 kg child as a default assumption for its health advisories, the model was run for a child at 0.97 yr, the age at which the model-simulated body weight for a child is 10 kg.

[h] Results obtained by setting urinary clearance constants for the older child equal to the average adult (Merrill et al., 2005) and scaling by BW^1.

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 conservative 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 5-fold higher for the fetus at gestational week 40 than for the average adult. (Simulations at earlier gestation weeks 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 two-fold higher for the breast-fed and bottle-fed infant (7-60 days) than for the average adult, and is slightly lower for the 1-2 year old child compared to the average adult.

To the extent that predictions of percent RAIU inhibition for the different subgroups are close to the average adult, this provides greater confidence in applying the existing RfD for these subgroups, which is based on an uncertainty factor of 10 to account for intra-species variability. 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. LIFESTAGE COMPARISON FOR THREE DRINKING WATER CONCENTRATIONS

EPA evaluated the percent of RAIU inhibition at various water concentrations, with and without perchlorate intake from food sources, for the different lifestages. The EPA-adjusted models based on Clewell et al. (2007), as described earlier, were used to simulate three (15, 20, 24.5 ppb) drinking water concentrations (Table 4). Available literature was used to estimate water intake rates for the different lifestages, 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, non-pregnant woman, and pregnant woman are based on normalized 90th percentile values for total (direct and indirect) consumers-only water intake multiplied by the age- or gestation-week-dependent BW. The water intake rates used to estimate daily perchlorate exposure from drinking water were 0.032 L/kg/day (Kahn and Stralka, 2008; U.S. EPA, 2004) for the average adult and non-pregnant 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, 90th 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 and 1- to 2-year-old children, the water intake rates of 0.971 L/kg-day and 0.674 L/kg-day, respectively, were set based on 90th percentile values for direct and indirect water consumers-only intake (Kahn and Stralka, 2008). Additionally, to calculate L/day for these age groups, the corresponding age group mean body weights obtained from NHANES 1999-2006 were used: 9.2 kg for 6- to 12-month 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. Refer to sections 3.3.1 and 3.3.2 for detail 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 FDA 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 result from the combined food and water dose to the mother providing breast milk to the infant.

EPA used perchlorate concentrations in infant formula based on perchlorate data from the FDA TDS. The data gathered for 2005-2006 resulted in detection (level of detection (LOD) 1.0 ppb) of perchlorate in 8 of the 12 samples (soy- and- milk based formulas) with a detected concentration mean of 1.875 ppb (http://www.cfsan.fda.gov/~dms/clo4dat2.html). Using ½ 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 4 times a year in 4 geographical locations for 5 week period and in 3 cities in each region. 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.

Assuming a 90th 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 body weight, the pregnant woman's dose of perchlorate was estimated to not exceed the reference dose if she consumed less than 15 ug/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 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 90th percentile community water-consumers intake-rate data (intake per unit BW) of Kahn and Stralka (2008), 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 90th percentile water intake rate of 0.84 L/day for the 7-day bottle fed infant and 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, 2008). The values estimated 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 1-30 day-old infants from Kahn and Stralka (2008; 0.137 L/kg-day N=40).

Table 4. Predicted percent radioactive iodide uptake (RAIU) inhibition and corresponding perchlorate intake (μg/kg-day) at three different water concentrations with and without food intake.

	Body weight (kg)[a]	90th Percentile Water intake (L/day)[b]	% RAIU Inhibition Perchlorate intake (μg/kg-day) Water only			TDS Food (μg/kg-day)[c]	% RAIU Inhibition Perchlorate intake (μg/kg-day) Food + Water		
			15 μg/L	20 μg/L	24.5 μg/L		Food + 15 μg/L	Food + 20 μg/L	Food + 24.5 μg/L
Average adult	**70**	**2.24**	0.15	0.20	0.24	**0.1**	0.18	0.23	0.27
μg/kg-day			0.48	0.64	0.78		0.58	0.74	0.88
Non-pregnant woman	**66**	**2.11**	0.21	0.28	0.35	**0.1**	0.26	0.33	0.39
μg/kg-day			0.48	0.64	0.78		0.58	0.74	0.88
Pregnant woman									
Mom -- GW 13	***69***	**2.18**	0.49	0.65	0.80	**0.1**	0.59	0.75	0.90
μg/kg-day			0.50	0.66	0.81		0.60	0.76	0.91
Mom -- GW 20	***71***	**2.34**	0.49	0.65	0.79	**0.1**	0.59	0.75	0.89
μg/kg-day			0.50	0.66	0.81		0.60	0.76	0.91
Mom -- GW 40	***78***	**2.57**	0.47	0.63	0.77	**0.1**	0.57	0.72	0.86
μg/kg-day			0.50	0.66	0.81		0.60	0.76	0.91
Fetus -- GW 40	***3.5***	--	0.90	1.2	1.5		1.1	1.4	1.6
			--	--	--		--	--	--
Breast-fed infant									
Mom -- 7 d	***74***	**2.96**	0.18	0.24	0.29	**0.1**	0.21	0.27	0.32
μg/kg-day			0.60	0.80	0.98		0.70	0.90	1.1
Infant -- 7 d	***3.6***	**0.52[d]**	1.1	1.5	1.8	**--[d]**	1.3	1.6	2.0
μg/kg-day			1.4	1.8	2.2		1.6	2.0	2.4
Mom -- 60 d	***72***	**2.96**	0.17	0.23	0.28	**0.1**	0.20	0.25	0.30
μg/kg-day			0.61	0.82	1.0		0.71	0.92	1.1
Infant -- 60 d	***5***	**0.74[d]**	0.73	0.97	1.2	**--[d]**	0.84	1.1	1.3
μg/kg-day			1.3	1.7	2.1		1.5	2.0	2.3
Bottle-fed infant									
Infant -- 7 d	***3.6***	**0.84[e]**	2.0	2.7	3.3	**1.42 μg/L**	2.2	2.9	3.5
μg/kg-day			3.5	4.7	5.8		3.9	5.0	6.1
Infant -- 60 d	***5***	**1.14[e]**	1.3	1.7	2.0	**1.42 μg/L**	1.4	1.8	2.2
μg/kg-day			3.4	4.6	5.6		3.7	4.9	5.9

Older child									
6-12 mo[f]	**9.2**	**1.03**	0.46	0.61	0.75	**0.275**	0.53	0.68	0.82
µg/kg-day			*1.68*	*2.24*	*2.74*		*1.96*	*2.52*	*3.02*
1-2 yr[f]	**11.4**	**0.64**	0.23	0.31	0.38	**0.37**	0.33	0.41	0.48
µg/kg-day			*0.84*	*1.12*	*1.37*		*1.21*	*1.49*	*1.74*

[a] Calculations for a 70 kg "average" adult are shown, while the body weight (BW) for the non-pregnant 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 (2008). BWs in italics are predicted weights (functions of age or gestation week) using growth equations from Gentry et al. (2002) as implemented in the PBPK models (Clewell *et al.* 2007; non-pregnant value is BW at day 0 of gestation).

[b] Water intake levels for adults other than the lactating mother are based on normalized 90th percentile values for total water intake (direct and indirect) multiplied by the age- or gestation-week-dependent BW, as follows: 32 mL/kg-day for average adult and non-pregnant 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 (2008), with the exception of the values for women, which come from U.S. EPA (2004).

[c] 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 an average of available FDA TDS data, with ½ LOD included in the average for the samples in which perchlorate was not detected

[d] 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 by fitting an age-dependent function to the breast-milk ingestion data (L/kg-day) from Arcus-Arth et al. (2005). Urinary clearance rates for the lactating woman equal to that of the average adult were used, consistent with data presented in Delange (2004).

[e] 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 (2008), and multiplied by BW(age). FDA has suggested an alternate approach, using the caloric intake requirement of a 7-day old infant as the basis for calculating consumption (FDA, 2008). This would likely yield a lower estimate of intake than the 0.84 L/day EPA has used in the model.

[f] For the 6- to 12-month 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-yr olds (to represent 6- to 12-month-old children) and 1.285-yr olds (to represent 1- to 2-year-old children).

5. 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 lifestage. 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 that the most expedient course was not to 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 was 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) (Table 1), the EPA evaluation determined that, with modifications as described herein, Clewell et al. (2007) is acceptable to calculate the lifestage 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 between 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 showing the impact of each change or set of changes on model predictions of inhibition of radio-iodide uptake given exposure at the point-of-departure (7 μg/kg-day), to illustrate the quantitative results of these corrections.

Perchlorate Inhibition of Iodide Transport

While reviewing the acsl CSL code 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) 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, "Inhibition 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) lead 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 lifestages.

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

(3) (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, one would expect a dual impact on the breast-fed infant, due to reductions in iodide it receives 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 1e6 ng/L for the lactating woman in breast milk 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 hr after maternal ^{125}I-dosing (along with lactational transfer of perchlorate) to obtain the percent inhibition in the breast-fed infant.

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 in proportion to its size appeared to be in error, since the flow rate set by it 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 mammary 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.

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 the existing direct-dose rate, either set PDOSE_N was set > 0 for fixed μg/kg-day rates and/or added to a fixed concentration multiplied by the suckling rate (KTRANS; change in 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 IV dose of iodide to the infant was simulated in order to calculate percent of thyroidal radioiodide uptake (%RAIU) inhibition in the bottle-fed infant, in contrast to the iodide dose being received via breast-milk for the calculation if %RAIU inhibition in the breast-fed infant.

Model Code Errors, Little to No Quantitative Impact on Model Predictions

The binding equation for iodide in the blood for the pregnant woman, lactating woman, and older child models had the term for concentration of iodide in the arterial blood (Ca_i or Ca_ni) twice in the denominator. This effectively reduced the Km and Vmax for blood binding by half, which does not affect model predictions at the concentrations tested (within linear range of blood binding). The extra Ca_i term was removed in these models.

Mass balance 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 (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 Km and the perchlorate inhibition term; ie.

[Vmax_Bi*CA_i)/(CA_i+Km_Bi+(1_+ Ca_p/Km_Bp))]

instead of

[Vmax_Bi*CA_i)/(CA_i+Km_Bi*(1_+ Ca_p/Km_Bp))].

(The equation for binRAbnd_i equation has the correct operator. EPA changed the + to a * and this had minimal impact on model predictions.

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 (MM) 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 MM binding equation. EPA notes that Ca_i and Cv_i are typically very close if not equal to one another, so this was expected to have minimal impact on model predictions. That was found to be the case when the term was moved to the arterial equation. However, making this change did seem to improve computation speed and stability.

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 larger changes were noted. For each of these lifestages/populations, other technical corrections that are not described here and had only minimal effects were also made.

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

- Average (non-pregnant) adult – original model predictions assumed perchlorate in 4 equal doses at 4-hour intervals, corresponding to the Greer et al. study protocol:
  - > 3.3% – original value from Table 2 (1st 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)

- Pregnant woman:
  - > 6.4% – original value from Table 2 (Mattie, 2006), GW 38
  - > 5.7% – multiple, small corrections (e.g., + inhibition in mammary), continuous exposure

- 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

- Lactating woman – original model used 4 doses at 4-hr intervals:
  - > 6.9% – original value, postnatal 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)

- Breast-fed neonate (exposure is to mother, per maternal BW):
  - > ~ 7% – original value (interpolated from Table 1, Mattie (2006)), age 1 month
  - > 11.6% – including inhibition of iodide transfer in breast milk & maternal skin, other fixes
  - > 18.5% – revised (reduced) urinary clearance to scale as GFR vs. adult (Appendix B)
  - > 17.6% – revised lactation expression (Appendix C)
  - > 20.2% – age 7 days

- 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

- Older child:
  - > 2.1% – original value Table 5 (Clewell et al. 2007); 7 year-old
  - > 1.7% – w/ inhibition in GI tract and skin, steady-state exposure simulation
  - > 2.0% – revised clearance for perchlorate (scale as BW^1, Appendix B), 7 year-old
  - > 1.9% – revised clearance, 2 year-old
  - > 1.9% – revised clearance, 1 year-old

APPENDIX B

EVALUATION OF URINARY CLEARANCE PARAMETERS

In the PBPK models of Merrill et al. (2005) and Clewell et al. (2007) the urinary clearance of perchlorate and iodide are described using a common form of allometric scaling by body-weight (BW) raised to the ¾ power. The actual urinary clearance constant for an individual of a given BW is given by:

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

where CLU_k has units of L/h 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/h/kg, but clearly this should be L/h/kg$^{0.75}$ to be consistent with this mathematical formulation, which is how the CLU values are calculated in the computer code. Moreover the tables list $CLUC_P = 0.13$ (± 0.05 in Merrill et al.) and $CLUC_I = 0.11$ (Merrill et al.) or 0.1 (Clewell et al.). 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 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 Vmax 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, 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 ~ 10% of iodide is actively resorbed, but a much smaller faction of perchlorate, such a difference would be predicted.

The current model does not include active renal transport per se, but takes renal excretion to be:

$$rCLU_k = CLUC_k \times CVK_k, \qquad \text{(Eq. B2)}$$

where 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 pharmacokinetic (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, one should be mindful of the fact that not all of the inter-individual 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 non-specific fluid resorption, the expectation is that the relative clearance for iodide and perchlorate, i.e., CLU_I/CLU_P, should be constant across ages, body weights, and lifestages. In EPA's evaluation for the child and "average" (non-pregnant, non-lactating) adult, this proportionality has been maintained.

Note 3: In Clewell et al. (2007), "The maternal urinary clearance value (*Cluci*) 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 $CLUC_I$ and $CLUC_P$ were set to 0.05 in the pregnant woman (both reduced by about the same proportion), but in the lactating woman, only $CLUC_p$ was so reduced while $CLUC_I$ was not. These maternal lactation values go against the argument given just above that the proportionality should be maintained, but EPA chose to use the maternal values as so 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.

Urinary Clearance in Adults (outside of pregnancy/lactation)

Using the calculations as indicated above, the clearance rate from the Merrill et al. (2005) model for "average" adults is $CLU_P = CLUC_P \times 70^{0.75} = 3.025$ L/h = 50.4 mL/min. The average glomerular filtration rate (GFR) in adults is 125 mL/min = 7.5 L/h, so $CLU_P/GFR = 40\%$. For iodide the values are 2.662 L/h or 44.3 mL/min, 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 mL/min, 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 mL/min, 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 becomes zero. (The slope of the dashed line is about 10 mL/min.)

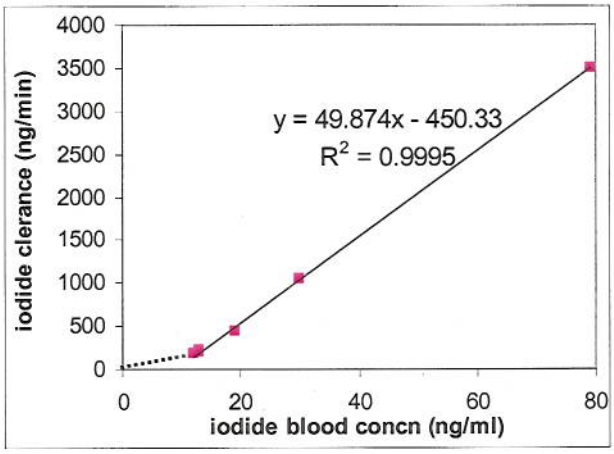

Figure B-1: Iodide clearance vs. blood levels in men, from Gardner et al. (1988)

What these data and regression indicate is that there is a non-linearity in clearance at low levels, which could well be due to active re-absorption 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 and thereby describe 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 lifestages, 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.

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. Further, for each alternate value of CLU_k evaluated, the Vmax for NIS-mediated uptake of both iodide and perchlorate in the follicle (from blood to follicle tissue) was adjusted to maintain model fits to radio-iodide uptake (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 is shown in Figure B-1. 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/body weight is 3.557 mL/min or 0.21 L/h. 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/h. 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 one would obtain at 3.6 kg BW is 0.085 L/h, almost 4 times lower than the default extrapolation.

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, as this scaling has been found to explain much of inter-individual 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 mean value to adult, plotted vs. mid-point of each age range) are shown in Figure B-3, along with a simple power-function curve fit, $SGR = 0.2087 \times day^{0.23333}$, where "day" is the child's age in days. This function was used, together with $BW^{2/3}$ scaling to estimate urinary clearance for perchlorate in the infant as:

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

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

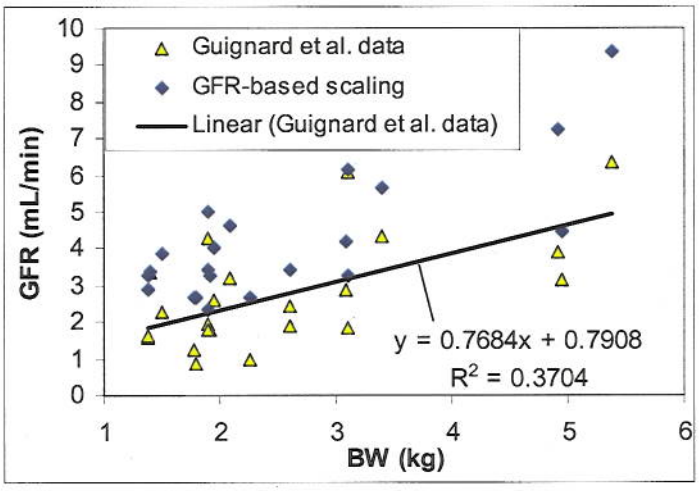

Figure B-2: Glomerular filtration rate (GFR) vs. body weight (BW) in infants (Guignard et al., 1975). GFR-based scaling uses equation (B1 with total adult GFR of 125 mL/min vs. 3.025 L/h perchlorate clearance) with individual age and BW values of Guignard et al. (1975).

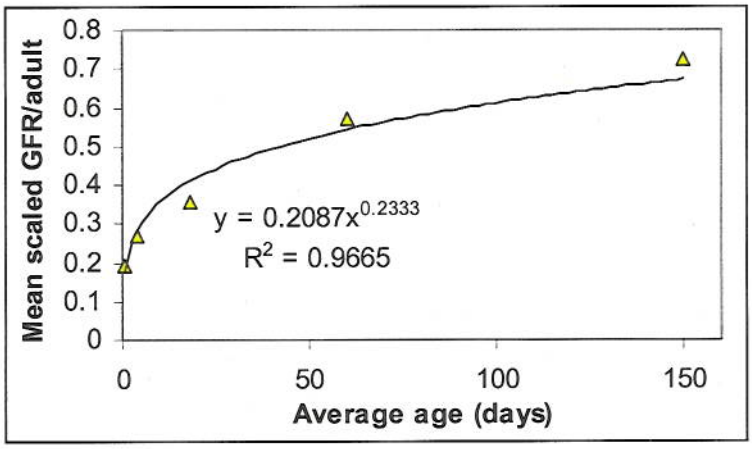

Figure B-3: Ratio of surface-area normalized glomerular filtration rate (GFR, ml/min/SA) in infants vs. adults, as a function of age. Data from DeWoskin and Thompson (2008).

The result of applying equation (B1) to the individual data of Guignard et al. (1975), but using the total adult GFR of 125 mL/min (7.5 L/h) instead of the iodide clearance of 3.025 L/h, 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 for a 7-day-old, 3.6-kg infant, one obtains:

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

Compared to the GFR of 0.21 L/h, this seems reasonable, although it is 67% of GFR rather than 40%. At 60 days, when an average child is 5 kg, equation B3 yields 0.28 L/h or 4.7 mL/min, which again 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 therefore 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.

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 close-head injuries ("secondary to motor-vehicle accident"), and EPA restricted the Lloyd et al. data (larger data-set) analysis to only that injury category (excluding a few cases of sepsis, for example) and ages less than 12 years (youngest was 4.1 years). The Chin et al. (1982) data are included because it includes children as young as 1 year old, though fewer subjects. Unlike neonates, from these data it appears that either direct BW scaling, or 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 (constant, dash-dot horizontal line with this normalization), $BW^{0.75}$ and $BW^{2/3}$ scaling from adult values.

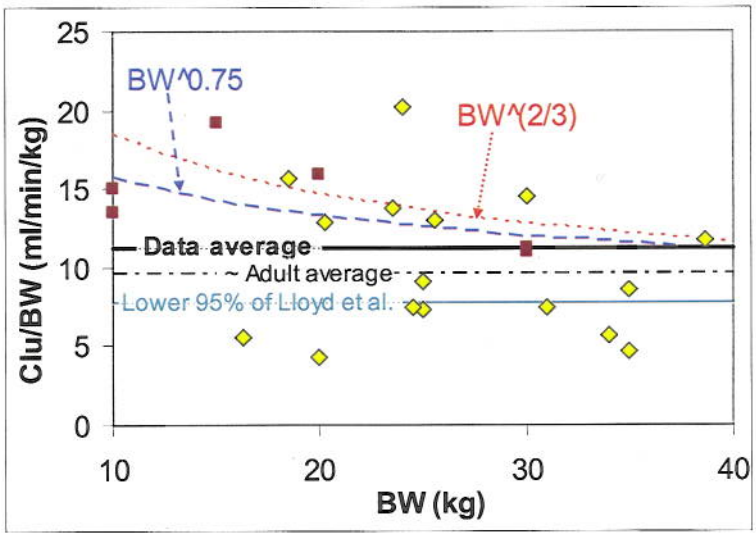

Figure B-4: Cimetidine clearance data (Chin et al., 1982 [red squares] and Lloyd et al., 1985 [yellow diamonds]) and possible scaling relationships. $BW^{0.75}$ and $BW^{2/3}$ curves are normalized to BW after applying this scaling.

Lloyd et al. (1985) state that the range of clearance rates in adults is 9-10.3 ml/min/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. (normalized to BW^1) 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 is 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 clearance (Clu) by BW^{1} 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}, 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) were also shown in Table 2 as representing a "high" clearance values, and the results of scaling by BW^{1} 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.

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 lead to half of theaverage adult for perchlorate, but a value for iodide equal to adults. The data for iodide clearance in humans of Aboul-Khair et al. (1964) during pregnancy and early postnatal times, shown below, was also considered.

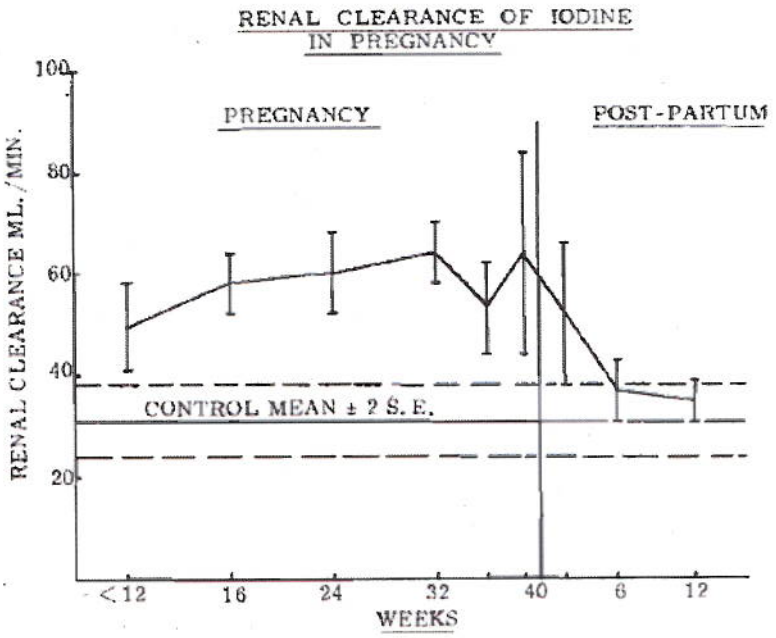

Figure B-5: Renal clearance of iodine (mean ± 2 S.E.) in pregnancy and post-partum period compared with non-pregnant values. From Aboul-Khair et al. (1964) Figure 2.

These data show that renal (urinary) clearance for iodide is elevated to as much as 2-times control (non-pregnant) values during pregnancy, and while this declines fairly rapidly towards control after birth, it is still elevated in the first couple of months, where EPA's analysis on neonatal clearance has focused attention. Keeping with the assumed proportionality between perchlorate and iodide, based on these data the same relationship would be expected to hold: higher clearance rather than reduced. A dilemma occurs in considering the data of Aboul-Khair et al. (1964); however, in that the control iodine clearance as measured by them is 31.05 ± 3.66 mL/min (mean ± SE), while the value determined by Merrill et al. (2005) for non-pregnant adults is 44.3 mL/min. Likewise Aboul-Khair et al. (1964) report thyroid iodide uptake at 2.5 hr post-injection as 21.4 ± 1.4 % of the administered dose, but the amount predicted by the Merrill et al. (2005) model (in the absence of perchlorate) is 7.78%. Therefore, the data of Aboul-Khair et al. (1964) was normalized to their own controls for both urinary clearance and iodide uptake, and then use that relative change as a model input (for clearance, multiplying the non-pregnant 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 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.

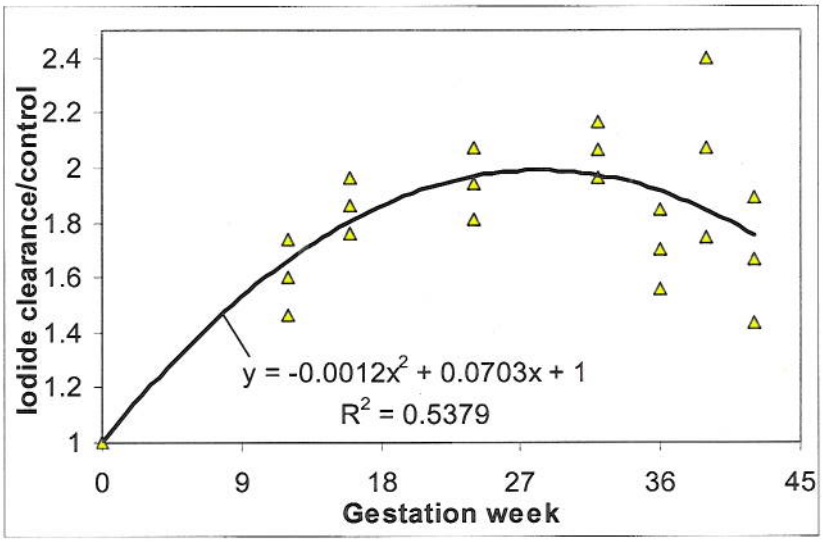

Figure B-6. Relative pregnant:non-pregnant iodide clearance values from Aboul-Khair et al. (1964), with quadratic interpolation function. Points are mean ± SE.

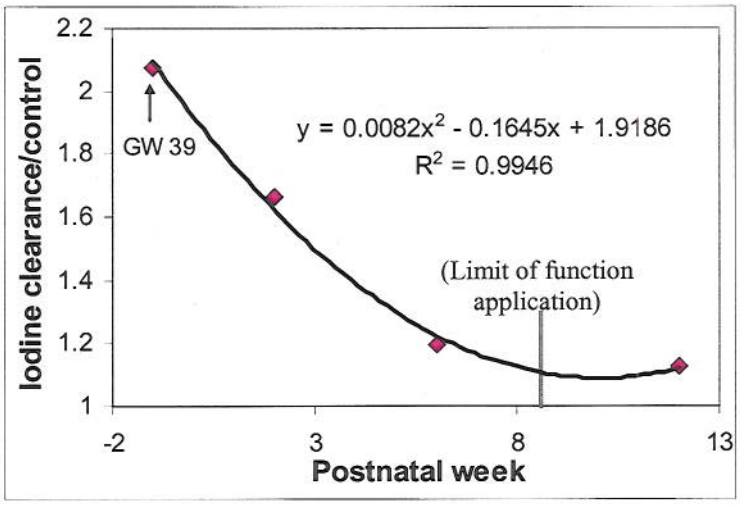

Figure B-7: Maternal iodine urinary clearance and approximation function for early postnatal period; data from Aboul-Khair et al. (1964).

For pregnancy, the Vmax 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,

$$RVmax(pregnancy) = 0.0009 \cdot GW^2 - 0.054 \cdot GW + 2.6, \quad \text{(Eq. B5)}$$

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.

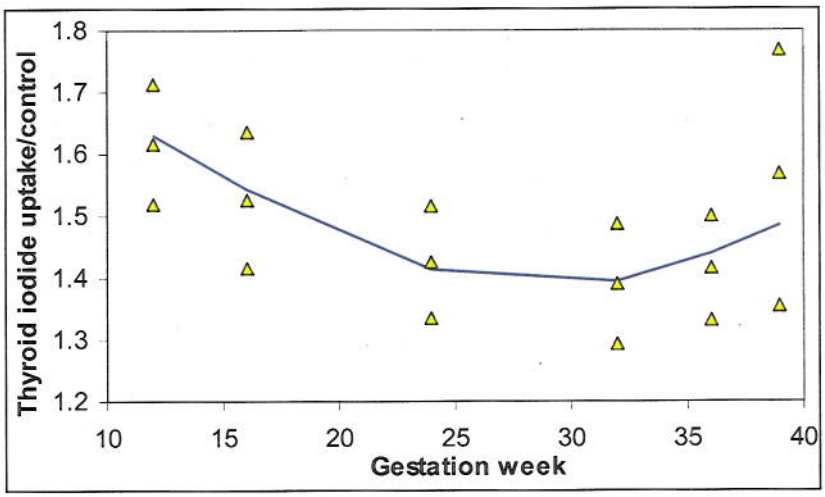

Figure B-8: Thyroid iodide uptake at 2.5-hr post IV injection relative to control. Data (points) are from Aboul-Khair et al. (1964) (mean ± SE). Line is model simulation. Note that while values drop from GW 12 to 32, they are consistently greater than one.

During the post-partum period the urinary clearance and iodide uptake data of Aboul-Khair et al. (1964) are both falling towards control values, but again there is the situation that the control uptake measured, 21.4% of the IV dose at 2.5 hr post-injection, is well above the value estimated by the PBPK model for an average adult: 7.6%. Further, a number of the physiological parameters differ in the lactating woman model vs. the average adult, as well as over time. Therefore, EPA first ran the lactating woman model, using the average adult clearance constants (that scale by $BW^{0.75}$) at postnatal week 50 to estimate the model-control maternal uptake at 2.5 hr post iodide injection: 6.57%. 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 2 times control values at GW 39 (1 week prior to birth) towards control, the NIS Vmax 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 Vmax was multiplied by:

$$RVmax(lactation) = (RCLF - 1)^2 + 1, \qquad \text{(Eq. B6)}$$

Thus, for RCLF = 2.09 (from the equation in Figure B-7) at GW 39 (postnatal week "-1"), RVmax equals 2 and as RCLF falls towards 1.0 (i.e., clearance approaches control values), RVmax also falls to 1.0, so Vmax 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 is shown in Figure B-9, where both have been normalized by their respective control values.

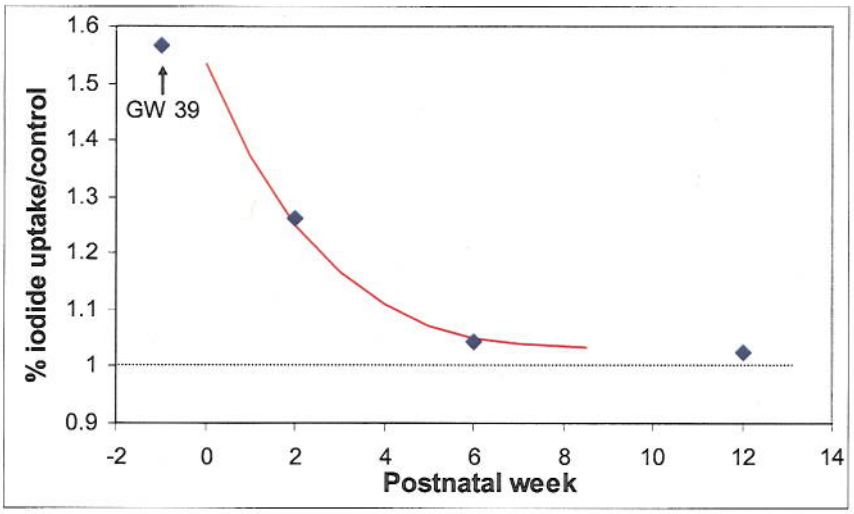

Figure B-9: Radio-iodide uptake in late pregnancy and early postnatal period. Data are from Aboul-Khair et al. (1964). PBPK simulations are with adjusted lactation model (see text above).

APPENDIX C

MODEL REVIEW FINAL REPORT FROM EPA CONTRACTOR

Battelle
The Business of Innovation

Center for Biological Monitoring & Modeling

September 25, 2008
Report for Work Assignment 4-5
Evaluation of Perchlorate PBPK Model

Submitted To:

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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. 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 30 hour after dosing (for each dose group), only this timepoint 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 5 Clewell 2007

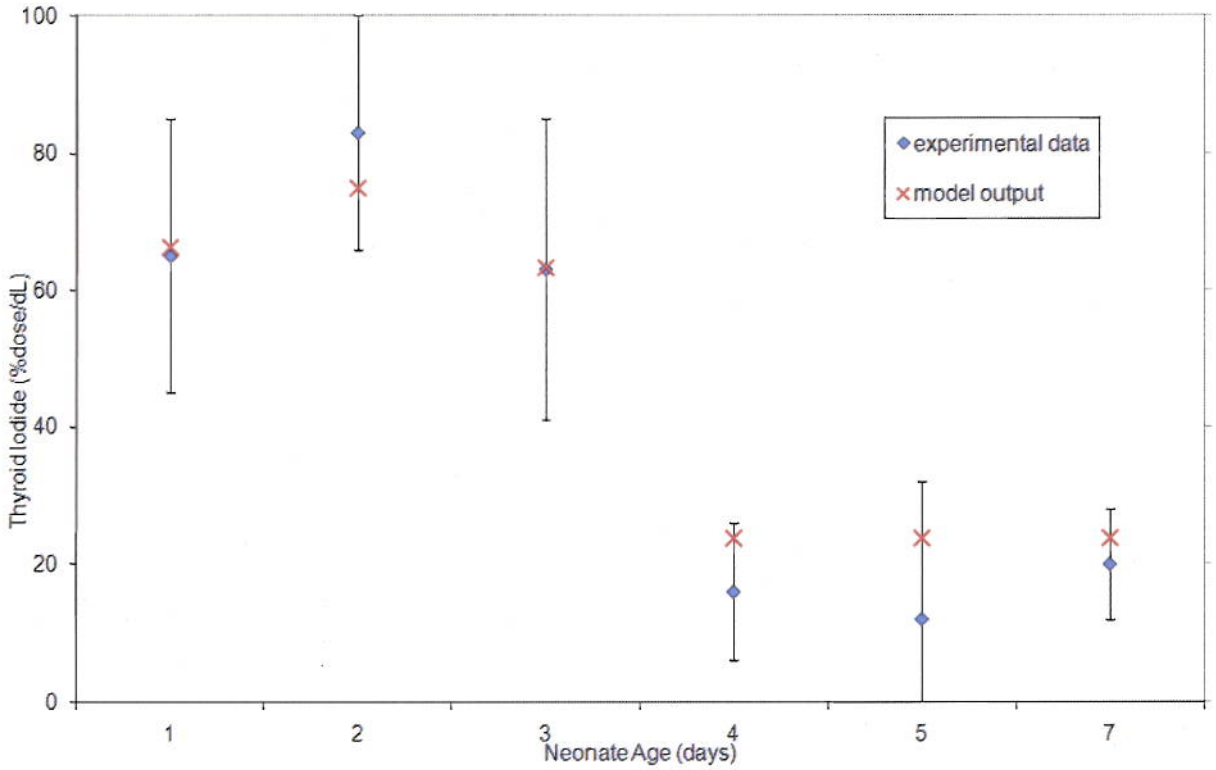


Figure 6 – Maternal Concentrations

- 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

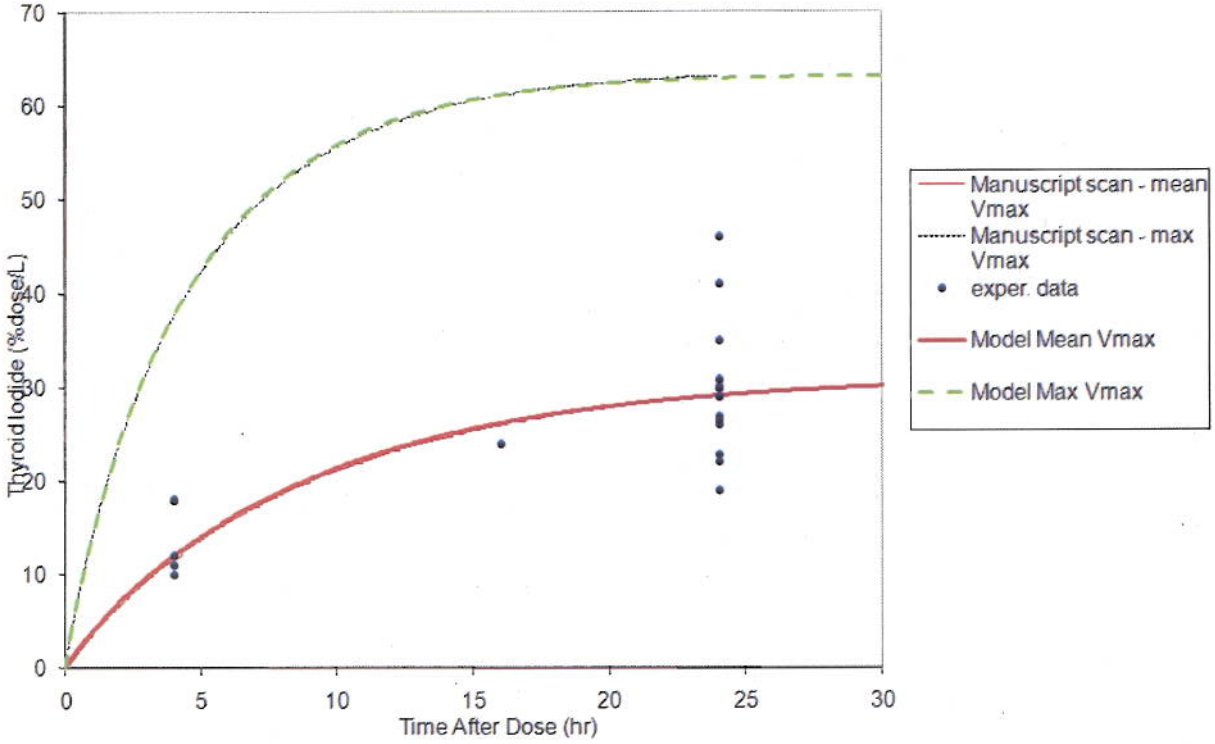


Figure 6b Clewell 2007

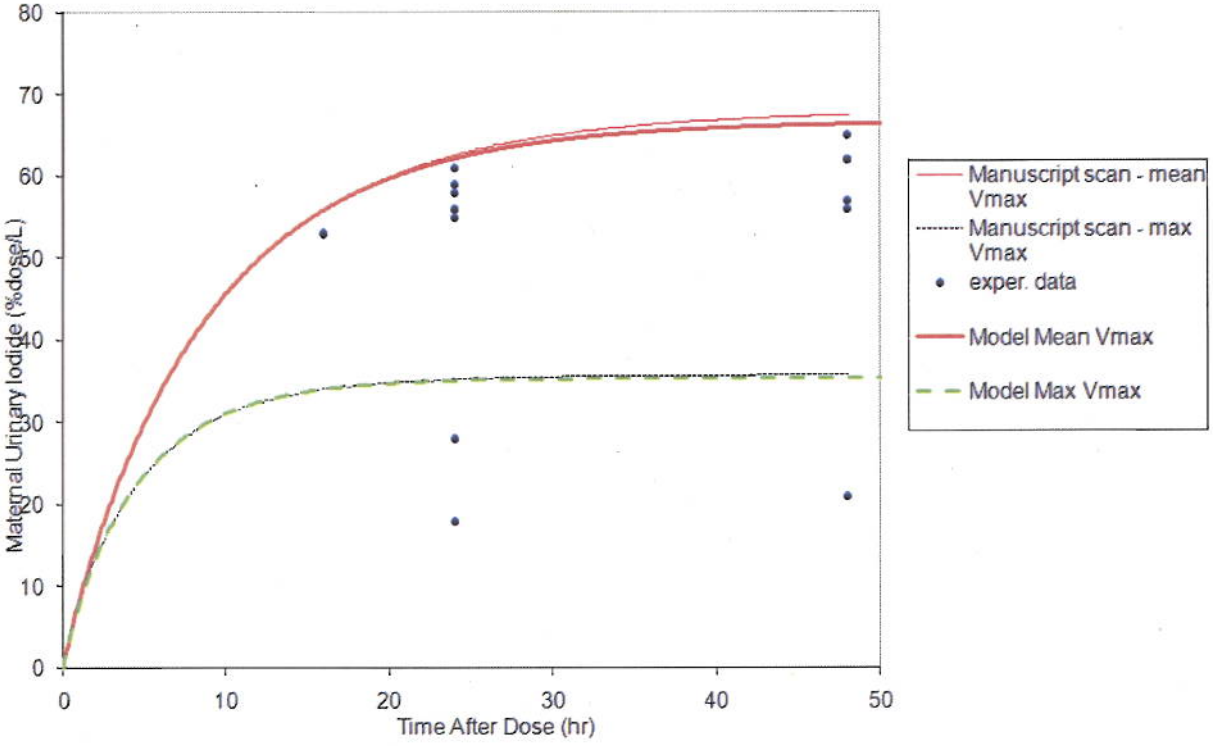


Figure 6c Clewell 2007

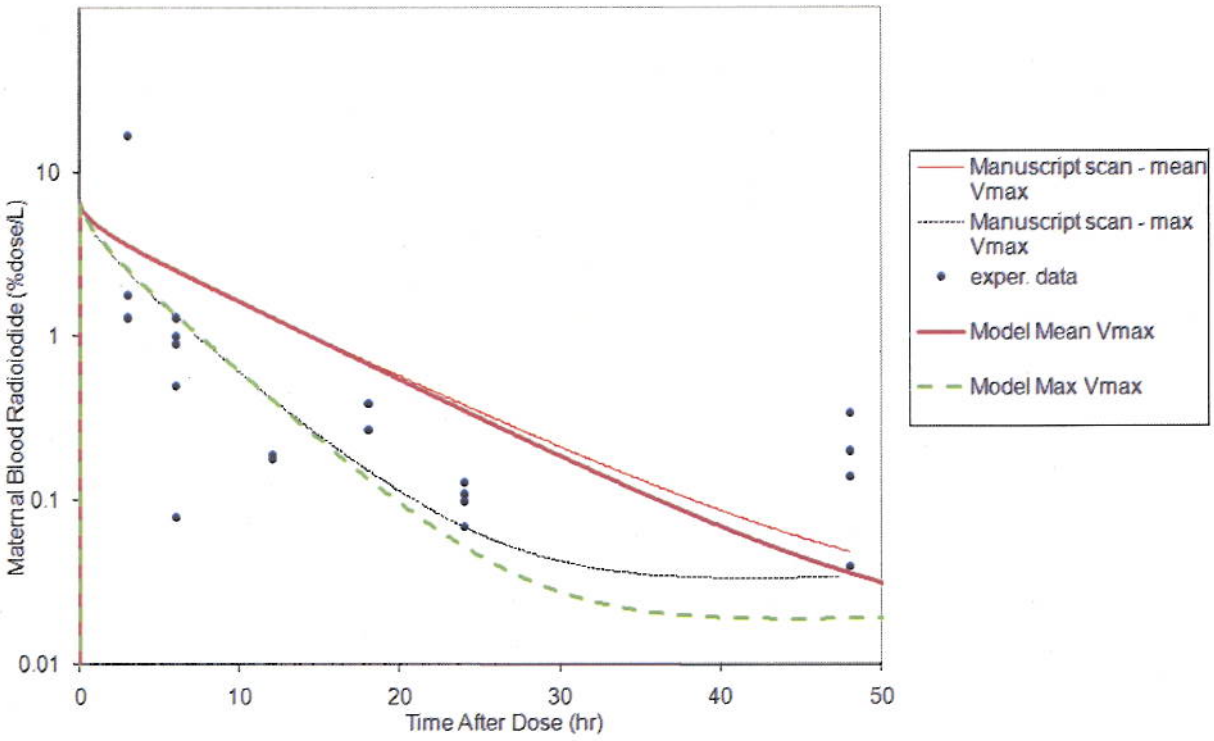


Figure 6d Clewell 2007

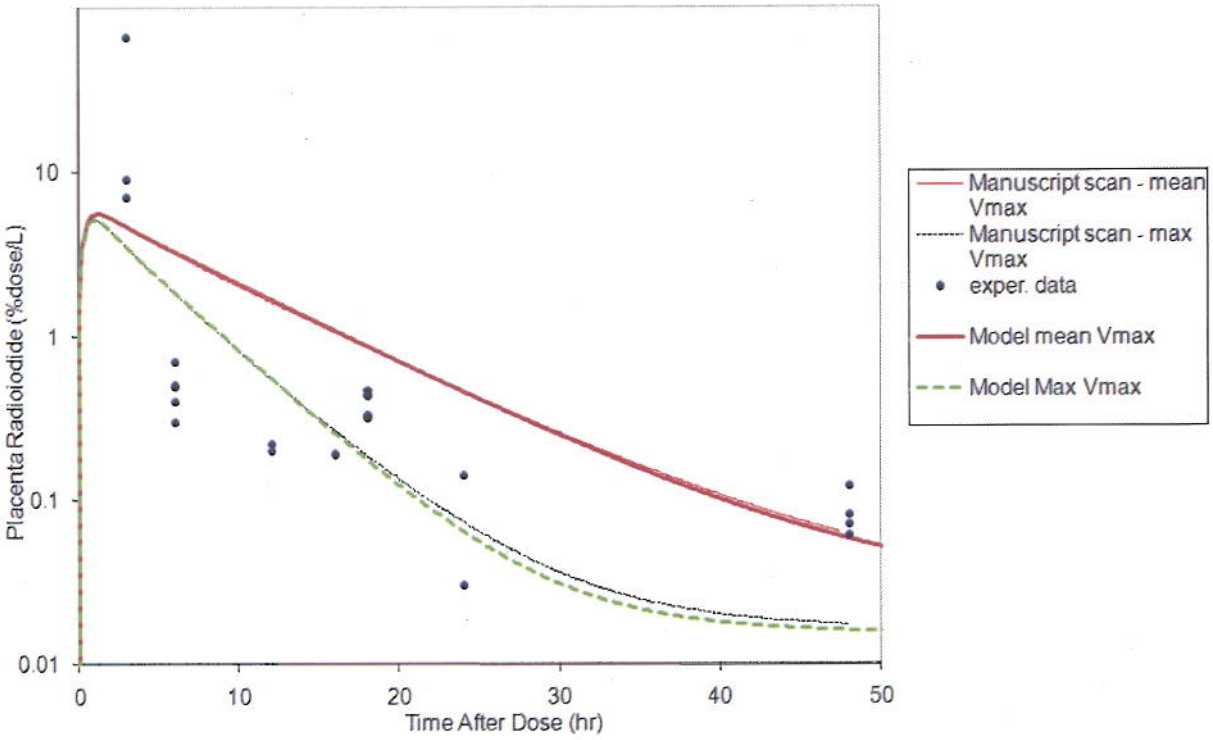


Figure 7 – Total fetal burden

- Produced using the **pregnancy** model and the **Fig7.m** file.
  - The figure presents Total fetal $^{131}I^-$ 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 acslXtreme code showing slightly faster elimination.

Figure 7 - GW 13, Clewell 2007

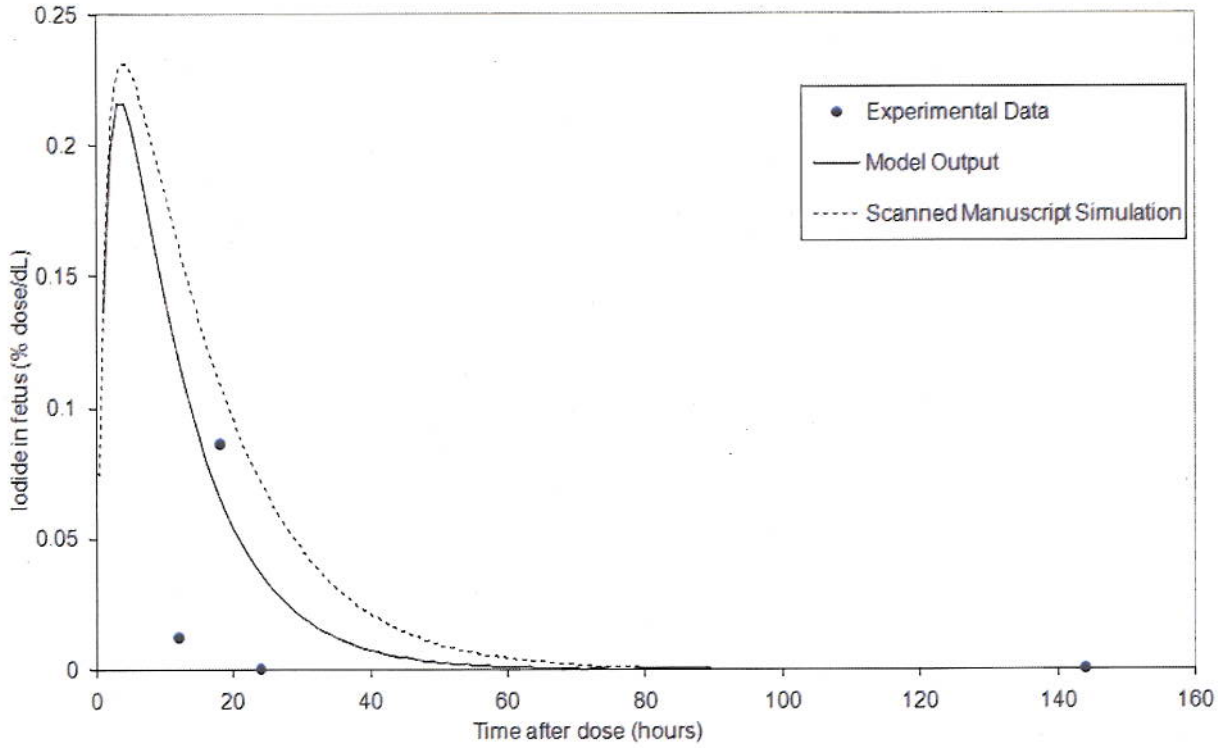


Figure 7 - GW 14, Clewell 2007

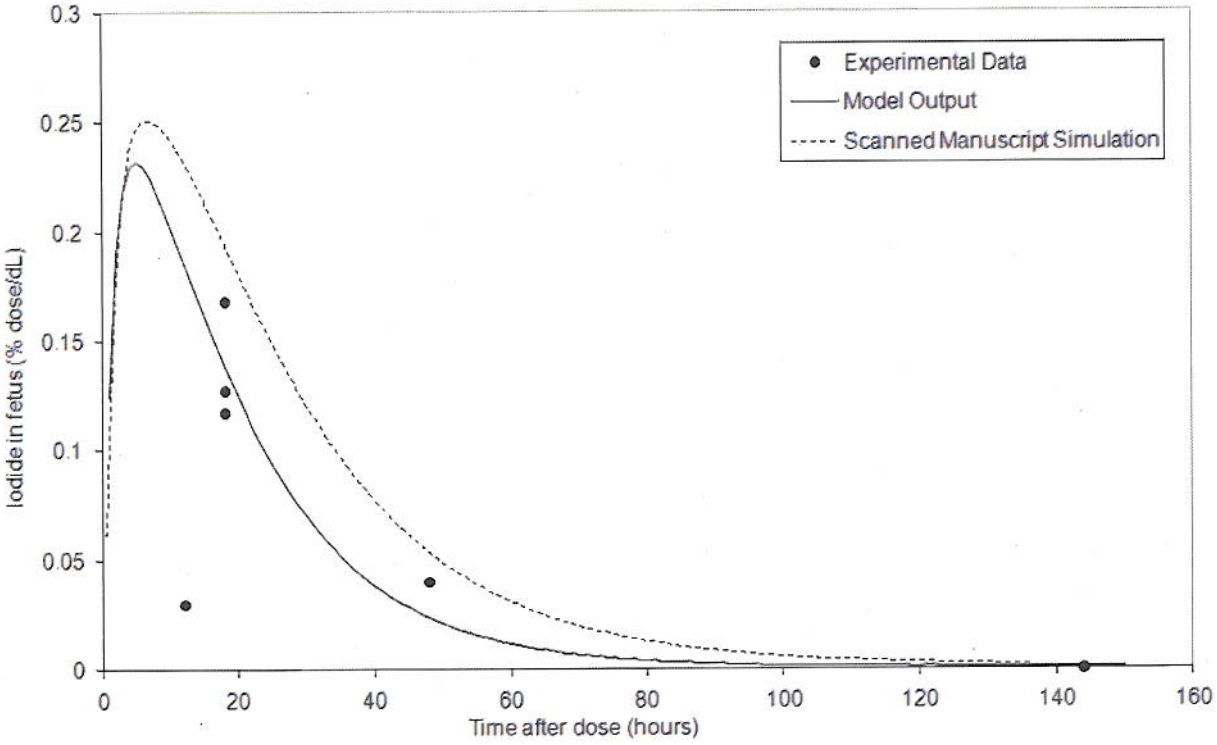


Figure 7 - GW 15, Clewell 2007

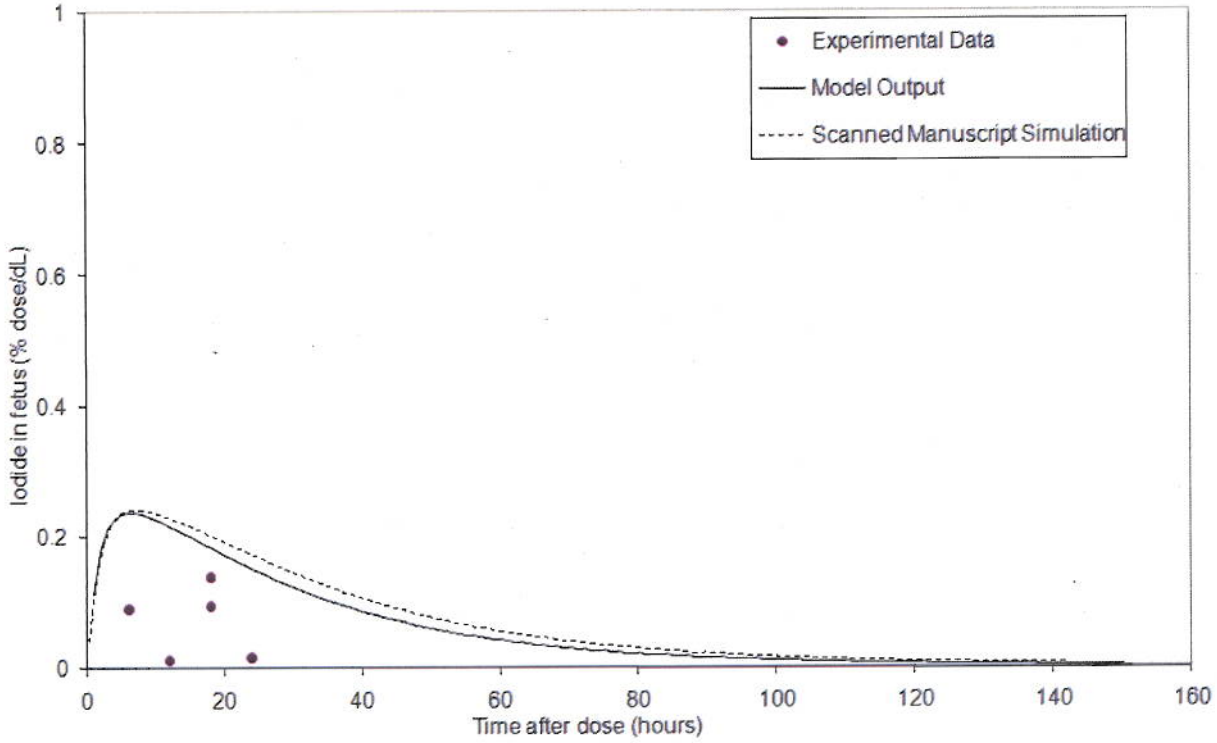


Figure 8 – Lactating women

- 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 is 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.

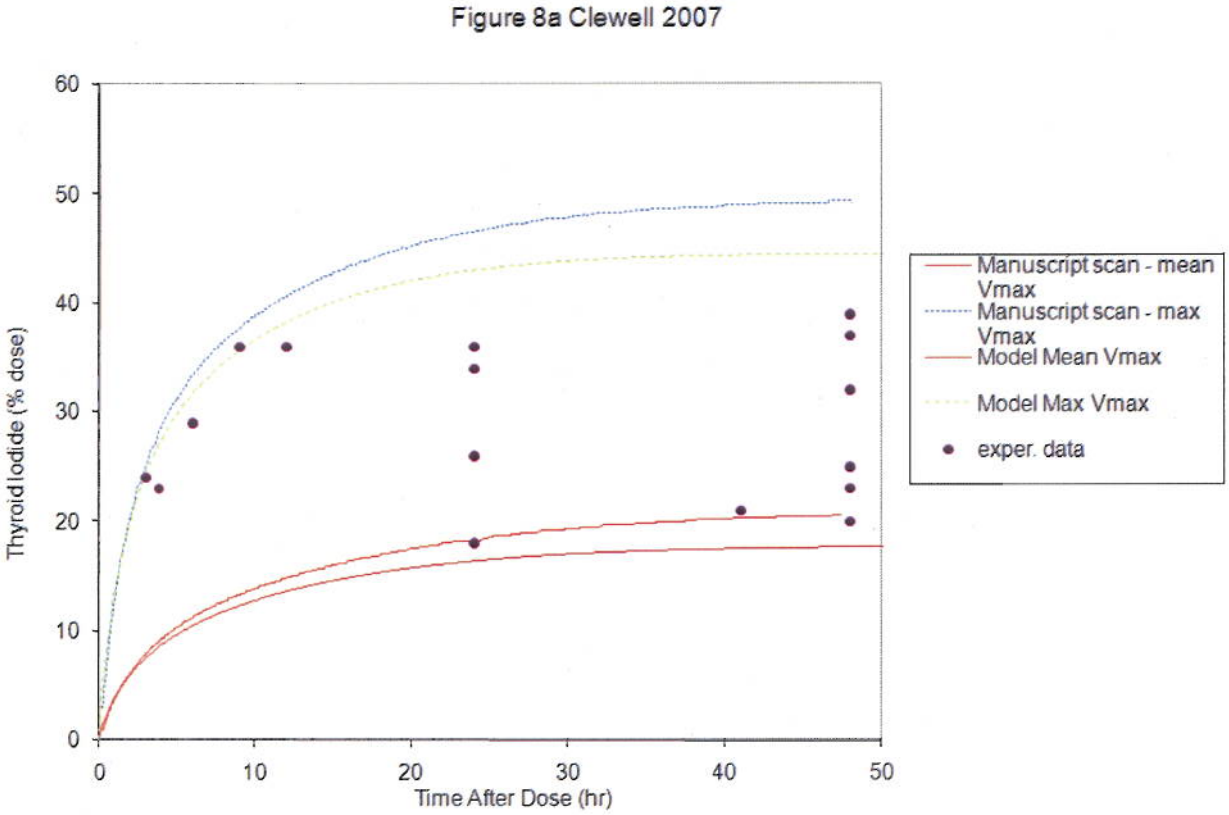


Figure 8b Clewell 2007

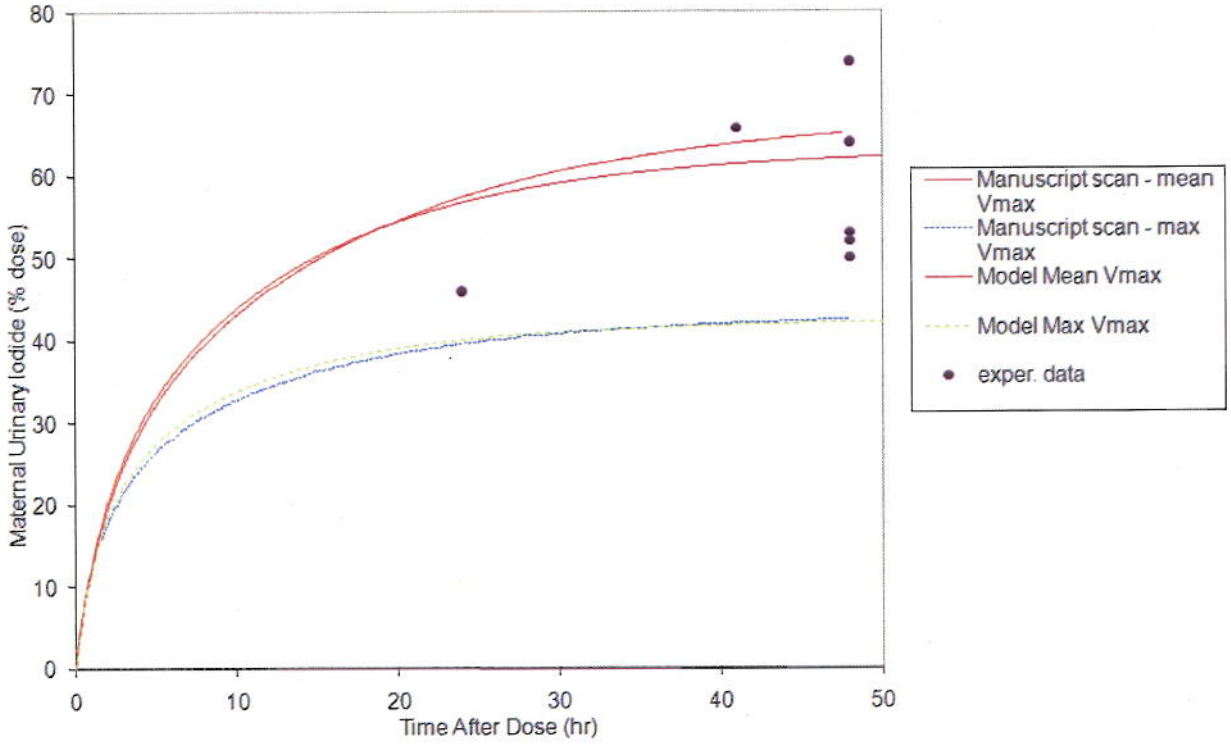


Figure 8c Clewell 2007

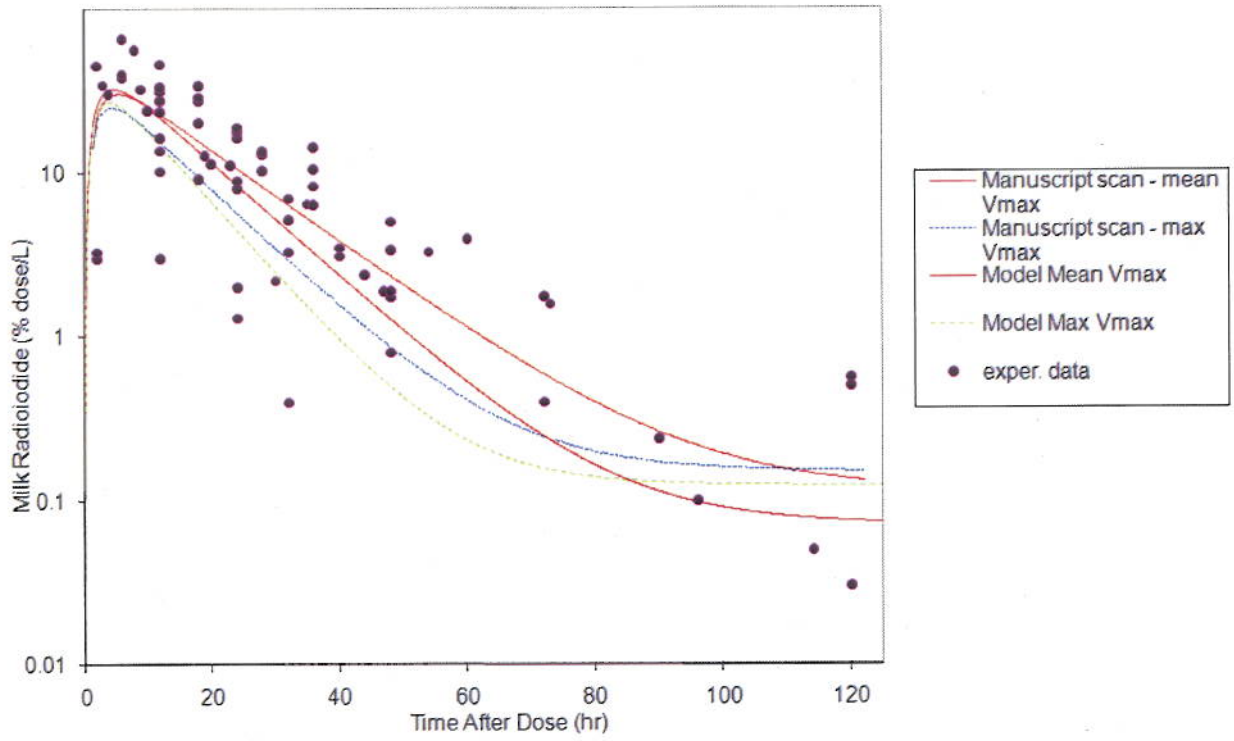
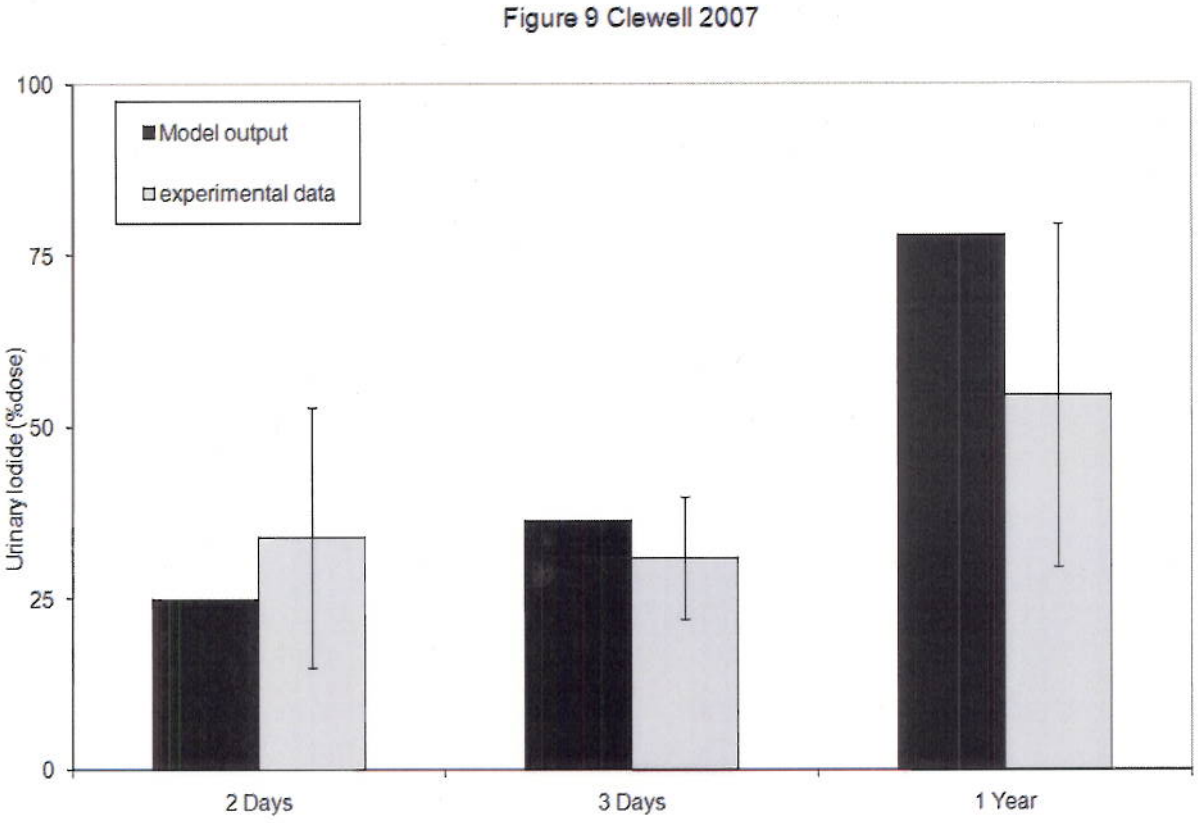


Figure 9 – Neonatal Urine

- Produced using the **lactational** model and the **Fig9_GFR.m** file.
  - The figure presents Predicted radioiodide in neonatal urine after a direct oral $^{131}I^-$ 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.



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 ClO4 (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 CTTOT_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
3243       NaN      NaN       NaN
3243       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
3246       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
```