Biologically Based Dose Response Models for the Effect of Perchlorate on Thyroid Hormones in the Infant, Breast Feeding Mother, Pregnant Mother, and Fetus: Model Development, Revision, and Preliminary Dose-Response Analyses

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NOTICE

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LIST OF ABBREVIATIONS[1]

Abbreviation	Definition
BBDR	Biologically based dose response
BW	Body weight
CHO	Chinese hamster ovary
ClO_4	Perchlorate
EPA	Environmental Protection Agency
FDA	Food And Drug Administration
fT4	Free thyroxine
GI	Gastrointestinal
HRCCA	Health Risk Reduction And Cost Analysis
Km	Michaelis Menten saturation constant
LMS	Lambda-mu-sigma transformed normal distribution
MCLG	Maximum contaminant level goal
MOA	Mode of action
NHANES	National Health And Nutrition Examination Survey
NIS	Sodium-iodide symporter
NPDWR	National Primary Drinking Water Regulation
PBPK	Physiologically based pharmacokinetic
PBPK-PD	Physiologically based pharmacokinetic-pharmacodynamic
PND	Postnatal day
POD	Point of departure
RAIU	Radio-iodide uptake
RfD	Reference dose
SAB	Science Advisory Board
SD	Standard deviation
SDWA	Safe Water Drinking Act
T3	Triiodothyronine
T4	Thyroxine
TH	Thyroid hormone
TSH	Thyroid stimulating hormone

[1]Table 3 lists model parameter abbreviations and their definitions

EXECUTIVE SUMMARY

The U.S. EPA is developing a National Primary Drinking Water Regulation (NPDWR) for perchlorate. The 1996 Safe Drinking Water Act Amendment (SDWA) regulatory efforts require assessment of effects of the contaminant on potentially sensitive subgroups of the general population. The SAB (2013) recommended that the EPA focus the application of physiologically based pharmacokinetic (PBPK) modeling on the condition of hypothyroxinemia in which free thyroxine (fT4) levels are decreased to the lower end of the normal range with TSH levels maintained in the normal range. Hypothyroxinemia is considered a more sensitive and therefore better "indicator of the potential adverse health effects than the more pronounced decreases in thyroid function associated with hypothyroidism" (SAB, 2013), where T4 is reduced to low levels and TSH is elevated. To meet the regulatory requirement and to follow the 2013 Science Advisory Board (SAB) perchlorate report recommendations, EPA and FDA worked collaboratively to develop and integrate PBPK models for perchlorate and iodide[1] with biologically based dose response (BBDR) models for thyroid hormones to predict the effect of perchlorate on the thyroid gland in formula-fed and breast-fed infants, as well as lactating women. (BBDR models describe biologically processes and how they change as a result of chemical interaction, while the PBPK model describes the delivery of a chemical to the biological site of action). The model developed is limited to the range of perchlorate doses and dietary iodine intakes for which thyroid homeostasis is expected to be auto-regulated. Under this condition, TSH is in its normal range and thyroid hormone excretion is only a function of organified iodide content in the thyroid gland.

Model development efforts focused on: 1) the lactating mother; and 2) the breast-fed and formula-fed infant for the postnatal period from postnatal day (PND) 7 to 90. With a few modifications, the existing PBPK models for perchlorate dosimetry in the infant (formula- or breast-fed) and lactating mother (U.S. EPA, 2009), which were used in previous work predicting inhibition of radiolabeled iodide uptake (RAIU), were integrated with PBPK models for iodide and BBDR models for thyroid hormones, which were based on the Lumen et al. (2013) model for pregnant women and fetuses and Fisher et al. (2013) for lactating rats and nursing pups. The new, integrated infant and maternal models predict the effects of perchlorate on serum thyroid hormone concentrations in the lactating mother exposed to perchlorate in the diet and in infants exposed via ingestion of perchlorate in formula or breast milk.

[1] Elemental iodine can be present in many forms, including ionic, non-bound iodide, and as incorporated into thyroid hormones (often referred to as "organified" or "organified iodine"). Since iodine nutrition is determined by the total amount of available forms in the diet, dietary levels (amount ingested) are referred to as "iodine" in this report. Since model-predicted levels of un-bound iodine in the blood, breast milk, and urine are assumed to be in the ionic form, these *concentrations* are usually referred to as "iodide". However, when discussing the nutritional levels and intake for the breast-fed infant, the term "iodine" will be used, to be consistent in that regard, even though it is assumed to be present in breast milk as iodide.

Lifestage-specific model parameters were obtained from the literature, when available, or calibrated either by empirical estimates or model fits to literature data. While there was a paucity of data in the literature for the population targeted by these models, data were available to compare with model predictions for 1) infant urinary iodide and perchlorate concentrations for both breast-fed and formula-fed infants; 2) breast milk iodide and perchlorate concentrations; and 3) maternal and breast-fed infant free T4 (fT4) and total bound and free T4 (T4) serum concentrations. Overall, the model predictions when compared with literature data were considered adequate.

Dose-response analyses for perchlorate effects on thyroid hormones were performed with the newly developed models, as well as with the pregnant woman and fetus model developed previously (Lumen et al., 2013)[2]. For the analyses, maternal iodine intakes ranged from 75 to 250 µg/day and formula iodine concentrations ranged from 108 to 384 µg/L, with perchlorate doses up to 20 µg/kg/d. In initial analyses for formula-fed infants, perchlorate reduced fT4 levels at PND 7 but not PND 30 to 90 for all formula concentrations of iodine. For all postnatal days and formula iodine concentrations, the model predicted fT4 would be within the reference intervals (i.e., the range considered to be normal and healthy) for infants (Lem et al., 2012).

For the breast-fed infant, the model predicted that maternal perchlorate exposure would reduce serum fT4 concentrations from PND 7 to 90, with the magnitude of the decrease dependent upon the maternal iodine intake. The model predicted that breast-fed infants from lactating mothers with iodine intake $\geq$ 150 µg/d and perchlorate exposure up to 20 µg/kg/d would have serum fT4 levels within the reference intervals for infants (Lem et al., 2012) from PND 30 to 90; however, the lactating mothers were predicted to become hypothyroxinemic or hypothyroid at perchlorate exposures below 20 µg/kg/d, even with daily iodine intakes of 250 µg/d. For PND 7, serum fT4 levels in breast-fed infants would be below the reference intervals at lower perchlorate doses < 12 µg/kg/d, depending on maternal iodine intake.

The difference in the predicted effect of perchlorate on the breast-fed vs. formula-fed infant is due primarily to a larger perchlorate dose from breast milk versus formula, which is exacerbated by low iodide levels in breast milk when the mother's iodine ingestion is inadequate. The local sensitivity analysis performed with the model showed that, in addition to the kinetic parameters governing the formation and metabolism of thyroid hormones and urinary clearance of iodide, maternal parameters controlling iodide concentration in milk and the ingested amount of milk were influential on predictions of fT4 and T4 in the breast-fed infant.

Dose-response analyses were conducted for the pregnant mother and fetus, since the fetus in particular is considered a sensitive lifestage for developmental effects, and results were compared with those for the lactating mother and breast-fed infant. The pregnant mother was predicted to have somewhat lower base-line fT4 levels (in the absence of perchlorate) than the

[2] As detailed in this report, the affinity of perchlorate for the sodium-iodide symporter (NIS), a key parameter in the PBPK/BBDR models, was revised based on a re-evaluation of original data. The revised value was substituted into the model of Lumen et al. (2013) in the analyses conducted here.

lactating mother at PND 7 for the same iodine ingestion levels. However, the steepness of the perchlorate dose-response for the pregnant mother is about the same as for the PND 7 lactating mother. And from PND 7 to 90, fT4 levels in the breast-feeding mother were predicted to decrease as a result of postnatal physiological changes and the demands of breast-feeding, making the lactating mother potentially more vulnerable than during pregnancy. On the other hand, the estimated variance in serum fT4 levels for the pregnant mother, hence the width of the reference range used to define normal vs. hypothyroxinemic conditions, was somewhat smaller than that estimated for the lactating mother. The model predicts that a pregnant mother and a lactating mother at PND 60 or 90 who only ingest 75 µg/d of iodine would be hypothyroid with no perchlorate exposure. With an iodine intake of 100 µg/d (considered medically insufficient), both pregnant and lactating mother would be barely within or just below the reference range. The model does predict that at 150 µg/d iodine or higher, the mother stays within the reference range during both pregnancy and lactation as long as perchlorate exposures are below 4 µg/kg/d.

The late-gestation fetus is predicted to be potentially more sensitive than nursing infants, since compared with the breast-fed infant, base-line fT4 levels are lower and the decrease in fT4 levels with increasing perchlorate dose is greater even with moderate to high maternal iodine intakes of 150-250 µg/d. However, because there is not a well-established thyroid hormone reference range for fetuses, an absolute determination of the relative risk to the fetus vs. the breast-fed infant cannot be made.

INTRODUCTION

Regulatory Background & Needs for Perchlorate

On February 11, 2011, EPA published a determination to regulate perchlorate (ClO_4^-) under the Safe Drinking Water Act (SDWA) (U.S. EPA, 2011) based on the following:

- perchlorate may have an adverse effect on the health of persons;
- perchlorate is known to occur or there is a substantial likelihood that it will occur in public water systems with a frequency and at levels of public health concern; and,
- regulation of perchlorate, in the sole judgment of the Administrator, presents a meaningful opportunity for health risk reduction for persons served by public water systems.

EPA is now in the process of developing a National Primary Drinking Water Regulation (NPDWR) for perchlorate. The Safe Drinking Water Act (SDWA) requires EPA to propose a Maximum Contaminant Level Goal (MCLG) simultaneously with the NPDWR. The MCLG is a non-enforceable goal defined under the SDWA (§1412.b.4.B) as *"the level at which no known or anticipated adverse effects on the health of persons occur and which allows an adequate margin of safety."* In addition to the MCLG, EPA must also prepare a Health Risk Reduction and Cost Analysis (HRRCA) when proposing a regulation that includes an assessment of the quantifiable and non-quantifiable health risk reduction benefits likely to occur as a result of treatment to remove the contaminant. SDWA further requires that the HRRCA include an assessment of the effects of the contaminant on the general population and on groups within the general population such as infants, children, pregnant women, the elderly, individuals with a history of serious illness, or other populations that are identified as likely to be at greater risk of adverse health effects.

In support of SDWA regulatory efforts, on May 29, 2012, EPA evaluated the evidence relating perchlorate exposures and health effects in humans since the NRC 2005 report (NRC, 2005) including the EPA's 2009 PBPK model findings and derived a range of possible MCLGs for different life-stages in a white paper, titled, "*Life Stage Considerations and Interpretation of Recent Epidemiological Evidence to Develop a Maximum Contaminant Level Goal for Perchlorate*" (U.S. EPA, 2012). In the report, EPA developed a range of potential MCLGs for different life stages based on the reference dose of 0.7 µg/kg/day and relevant exposure assumptions/parameters that are needed as part of the formula to derive the MCLG. The RfD of 0.7 µg/kg/day was developed by NRC and later adopted by EPA (U.S. EPA, 2012; NRC, 2005). It is based on the No Observed Effect Level of 7 µg/kg/day and corresponds to a RAIU inhibition of 1.8 percent reported in healthy human adults (Greer et al., 2002) with application of an uncertainty factor of 10 to account for differences in sensitivity between the healthy adults in the

study and the most sensitive population, namely “fetuses of pregnant women who might have hypothyroidism or iodide deficiency.” The exposure parameters and assumptions included body weights and drinking water intake specific to those life stages as well as relative source contribution which are needed as part of the formula to derive the MCLG.

The Science Advisory Board (SAB) evaluated the white paper in 2012 and provided recommendations to EPA (SAB, 2013). Specifically, the SAB recommended that the EPA derive a perchlorate MCLG that addresses sensitive life stages through use of a physiologically based pharmacokinetic/pharmacodynamic (PBPK-PD) model which incorporates perchlorate’s mode of action, rather than the default MCLG equation that uses the RfD and a limited number of exposure parameters (e.g., body weight, drinking water intake). PBPK-PD modelling is referred to as Biologically Based Dose Response (BBDR) modeling in this document. This is the first time that EPA has considered a BBDR model to set an MCLG rather than the traditional algebraic equation using the RfD. The SAB further found “that hypothyroxinemia (i.e., [moderately] low levels of thyroid hormone) is a more appropriate indicator of the potential adverse health effects than the more pronounced decreases in thyroid hormones associated with hypothyroidism.” SAB’s recommendation is based on a biological endpoint, i.e., hypothyroxinemia, that is different from the endpoint used to derive the RfD for perchlorate in 2005, i.e., the inhibition of radioactive iodide uptake into the thyroid, used to derive the RfD for perchlorate. SAB also recommended that EPA should consider sensitive populations including fetuses of hypothyroxinemic pregnant women and infants exposed to perchlorate through either water-based formula preparations or the breast milk of lactating women.

The mode of action (MOA) of perchlorate is well understood and involves perchlorate’s ability to disturb thyroid homeostasis by limiting iodide uptake by the thyroid, which in turn can lead to reduced production of thyroid hormones (THs) (SAB, 2013). Developing fetuses are considered particularly sensitive to the potential effects of *in utero* exposure. From the SAB (2013):

> "Children exposed gestationally to maternal hypothyroxinemia (without hypothyroidism) show reduced levels of global and specific cognitive abilities, as well as increased rates of behavior problems including greater dysregulation in early infancy and attentional disorders in childhood (Kooistra et al., 2006; Pop et al., 2003; Pop et al., 1999; Man et al., 1991). Notably these effects are correlated with both degree (Henrichs et al., 2010; Pop et al., 1999) and duration (Pop et al., 2003) of maternal hypothyroxinemia. The Henrichs et al. (2010) study, which stratified children into severe (<5th percentile) and mild (5-10th percentile) maternal hypothyroxinemia subgroups, showed that while effects were stronger and broader in the severe subgroup, the mild subgroup still showed delayed language development, thus suggesting that any factor that lowers maternal fT4, even slightly, can affect the offspring."

Li et al. (2010), Henrichs et al. (2010)**,** Román et al. (2013), and Min et al. (2016) provide further evidence and discussion of the effects of hypothyroxinemia *in utero*.

While Costeira et al. (2011) and Kooistra et al. (2006) evaluated neurodevelopmental effects in infants associated with iodine deficiency and low maternal fT4 (hypothyroxinemia), the association was with iodide nutrition and fT4 levels during gestation. Hence these studies do not provide information on the sensitivity of the neonate to iodine/fT4 decrements occurring after birth. Other studies of neurodevelopmental endpoints either focused on premature infants or severe thyroid hormone changes such as hypothyroidism. Thus adverse effects of moderate postnatal thyroid hormone decrements (hypothyroxinemia) on neurodevelopment in full term (born healthy), nursing or bottle-fed infants have not been studied. However, as described in a detailed review by U.S. EPA (2012),

> "A reduction in the storage of thyroid hormone and iodine, as well as rapid thyroid hormone turnover (i.e., shorter half-life) in the fetus, neonates and children makes these life stages more sensitive to thyroid disrupting agents such as perchlorate compared to adults. Furthermore, the physiological demand for thyroid hormones is far greater in the developing fetus, neonate and child compared to the adult, again increasing the vulnerability to thyroid disrupting agents."

The conclusion that infants are also particularly sensitive to perchlorate exposure is strongly supported by the SAB (2013) report (section 3.1) and introduction to Fisher et al. (2016). On the other hand, because the previous analysis based on the initial iodide uptake effect of perchlorate, quantified as RAIU inhibition, indicated that older children would be less sensitive than infants (also because they ingest less fluid/kg BW than infants) (U.S. EPA, 2009), EPA decided that the current modeling effort and analysis could be limited to infants between birth and 3 months of age, and the analysis was not extended to older children.

Within this exposure/MOA framework, a BBDR model can provide a tool for integrating exposure (e.g., different drinking water consumption scenarios), perchlorate and iodide pharmacokinetics, thyroid function description predicting serum thyroid hormone levels, and dose-response relationships for perchlorate effects on thyroid function at the different life stages. The SAB concluded that a data-driven approach with a BBDR model represents a more rigorous way to address differences in biology and exposure between adults and sensitive life stages than is possible with the default approach for deriving an MCLG.

In 2009, EPA reviewed and revised the available perchlorate life stage PBPK models (U.S. EPA, 2009; Clewell et al., 2007; Merrill et al., 2005). In discussion with the original authors, EPA improved the PBPK model by addressing the minor errors in mathematical equations and model code and inconsistencies among model code files and reflecting the biology in the models. The resulting model (U.S. EPA, 2009) predicted radioactive iodide uptake inhibition for different life stages in response to perchlorate exposures. The model did not include dietary iodine or address the effects of perchlorate under iodine sufficient or deficient conditions. The SAB reviewed this model and recommended that the EPA PBPK model for different life stages (U.S. EPA, 2009) needed to be expanded to include THs in order to predict hypothyroxinemia as a critical endpoint. Such a model integrating perchlorate, dietary iodine, and THs could then be

used to predict what levels of perchlorate could result in hypothyroxinemia for various life stages.

Shortly before the SAB provided their recommendations to EPA, Lumen et al. (2013) reported development of a TH/perchlorate BBDR model for pregnant women and fetuses. Specifically, the model predicted perturbations in serum THs during human pregnancy due to dietary iodine and perchlorate exposures. In order to implement the SAB recommendations, EPA expanded the Lumen et al. model to address formula-fed infants and lactating mothers and breast-fed infants.

The purpose of this document is to describe the perchlorate BBDR model development, including calibration, to predict the TH perturbations (i.e., free T4 and total T4 in serum) for the lactating mother, breast-fed infant, and formula-fed infant upon exposure to various perchlorate dose levels under different iodine intake conditions. In addition, this document describes the model application and preliminary dose-response predictions for the pregnant mothers and fetuses, lactating mothers and breast-fed infants and formula-fed infants. This document provides support for verification of model coding, evaluation of model calibration, and evaluation of the accuracy of model predictions for different perchlorate doses and iodide intake levels to determine if the model is adequate for perchlorate MCLG derivation.

Technical Background for Model Development

Model Introduction

The new BBDR model is intended to predict and evaluate the potential combinatory effects of iodine nutrition and exposure to perchlorate on thyroid function in the infant and lactating mother. The infant, 7 to 90 days of age, and lactating mother were selected for BBDR model development to address the most sensitive period of thyroid function maturation (SAB, 2013).

To make the modeling efforts more efficient in responding to SAB recommendations, EPA and FDA worked collaboratively and integrated the perchlorate PBPK models developed by EPA (U.S. EPA, 2009) for the infant (formula- or breast-fed) and lactating mothers with newer models for iodine nutrition and thyroid hormone (TH) production in those life stages, developed from Lumen et al. (2013) and the thyroidal iodide binding description from Fisher et al. (2013).

Figure 1 shows the overall workflow for creation and calibration of the models for the infant and lactating mother. The existing EPA peer-reviewed model (McLanahan et al., 2014; U.S. EPA, 2009) was incorporated as the perchlorate sub-model for the formula-fed and breast-fed infant and lactating mother with the modifications as noted below in Integration of Thyroid BBDR Model with Perchlorate PBPK Model. Physiological parameters were set to the life-stage-specific human values. Iodide and TH parameters for the lactating human mother were either: 1) adapted from the human pregnancy model (Lumen et al., 2013); 2) set based on literature references to the extent possible for this life-stage; or 3) fit to available iodide and thyroid hormone data. More published data on thyroid function during infant development was available for setting model parameters than for the lactating mother, allowing 5 of 19 infant iodide and TH parameters to be identified directly from those data. In the absence of literature references, 14 of 19 parameters were adapted from the fetal model (Lumen et al., 2013), the rat lactation model (Fisher et al., 2013), or visually fit to available data (blood and urine levels of thyroid hormones and iodide). The fitting was accomplished by first calibrating the BBDR model for dietary iodine, perchlorate, and TH kinetics for the formula-fed infant, and then expanding it to include the lactating mother with exposure to the infant through breast milk.

The model code is available separately on U.S. EPA's HERO website via the following citations for the acslX implementation of the pregnancy (U.S. EPA, 2016a) and lactation (U.S. EPA, 2016c) models and R/MCSim versions of the pregnancy (U.S. EPA, 2016b) and lactation (U.S. EPA, 2016d) models. The models were originally developed in acslX (AEgis Technologies,

Huntsville, Alabama) and model simulation plots shown in this report were produced with that software (packages cited/lined above). However AEGis has discontinued sales and support of the software, so the models have been converted to also run as MCSim models within the open-source R programming language. Instructions for downloading and installing R and MCSim are included in the HERO citations, (U.S. EPA, 2016c) and (U.S. EPA, 2016b). The R/MCSim versions reproduce the corresponding acslX versions to within 1% accuracy, although the formatting of the plots produced is not identical.

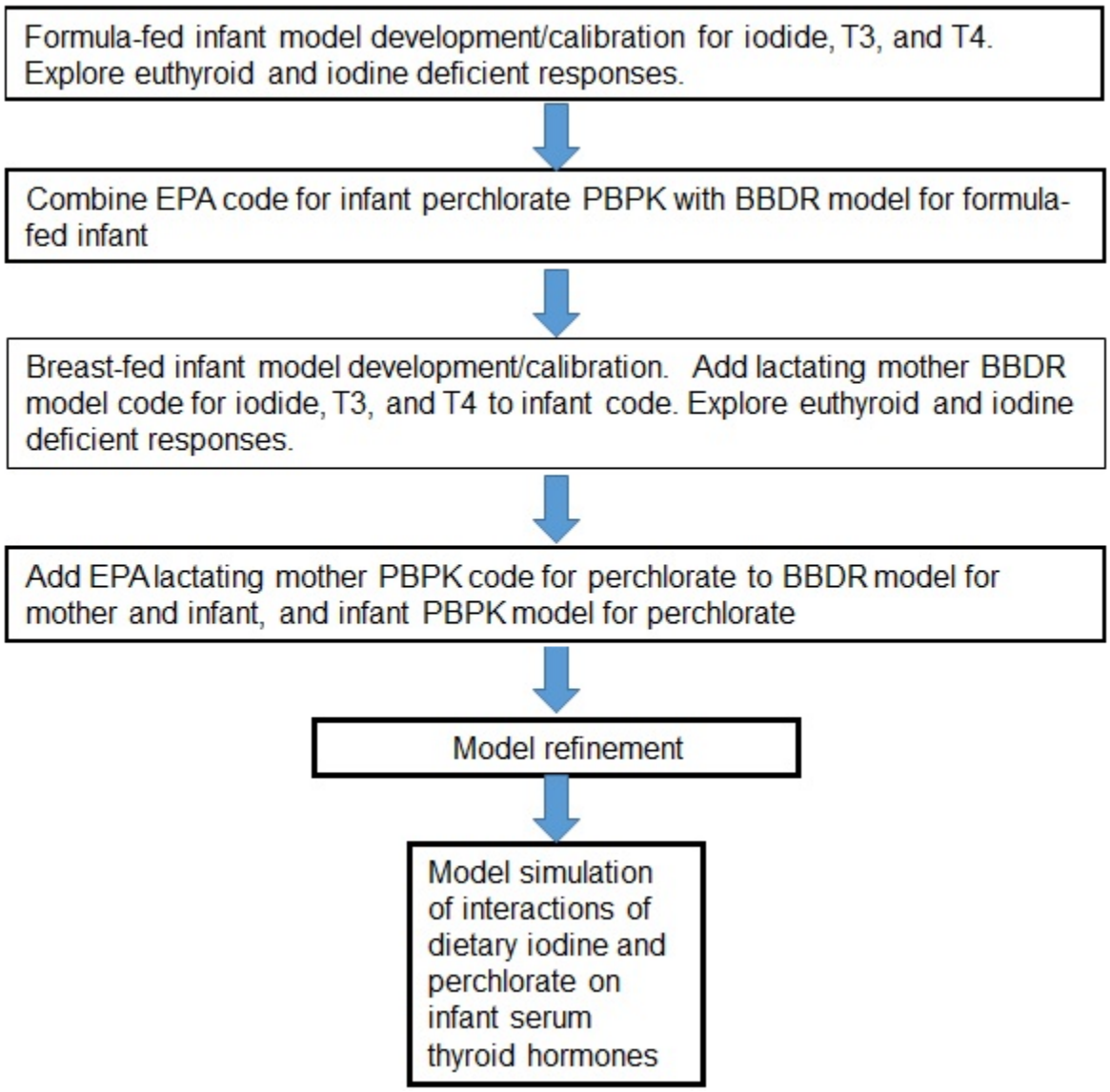

Figure 1. Work flow for model development and calibration.

Domain of Model Applicability

At the suggestion of the SAB (2013), the developed maternal lactation and infant models are limited to the range of iodine dietary concentrations within which the thyroid's function is effectively autonomous; i.e., both euthyroid conditions, where T4 is within the normal (reference[3]) range, and hypothyroxinemic conditions, where T4 is decreased below the reference range but with TSH within its reference range (Silva and Silva, 1981). Therefore, these newly developed models do not address conditions at which the hypothalamic-pituitary-thyroid axis[4] is involved with regulating thyroid hormone concentrations in the lactating mother or infant. In the range of evaluation, it is assumed that there is insufficient feedback control of the T4 concentrations on TSH levels to warrant model description. Hence, the dynamic ranges where the models are considered valid for predicting thyroid hormone disruption are, qualitatively, the reference intervals for serum T4 and fT4 concentrations in lactating women and in infants that are aged 7 to 90 days (Lem et al., 2012) (i.e., the range for these hormones that is normal for healthy individuals) and a range just below the reference range where there is a clinically significant reduction in T4 or fT4, but TSH is expected to remain within its normal range (i.e., the hypothyroxinemic range)[4]. Likewise predictions of the late-gestation pregnant mother and fetus models (Lumen et al., 2013) are only assumed valid for T4 and fT4 levels in

[3] Here we use "reference range" to refer to the range of a hormone that is considered normal and healthy for a population. Hence some in the population will have hormone levels above or below that reference range and be identified as having an adverse clinical condition. The "population distribution" refers to the distribution of hormone levels in the entire population, including those outside of the reference range.

[4] The model does not describe TSH levels, but assumes they stay within the normal reference range. Clinically, significant elevations in TSH can be observed whether or not T4 of fT4 are in the reference range. Thus there are not specific levels of T4/fT4 that are definitively associated with TSH elevation. This may be due to inter-individual variability in hormonal control mechanisms. But mechanistically it is known that TSH production and release are stimulated by decreasing T4 levels. Hence for the purpose of this model, specific, life-stage-specific fT4 levels are identified below which TSH levels are expected to be sufficiently stimulated as to rise above their reference range and introduce significant feedback control on the thyroid, which would make model predictions invalid.

the reference interval and expected hypothyroxinemic range for that life stage.

While the preceding paragraph discusses the qualitative distinction between euthyroid and hypothyroxinemic condition, and the corresponding range in which the model is considered valid, there are no unified quantitative definitions for reference ranges or hypothyroxinemia during pregnancy (Moleti et al., 2011), lactation (maternal), or infancy. For example, within the context of pregnancy, some authors use the 2.5th percentile of the fT4 population distribution while others use the 10th percentile and the reference ranges vary between populations and studies (Moleti et al., 2011). Elevated TSH levels, the marker of hypothyroidism, can occur in women with fT4 levels in the same range as other women with normal TSH levels (e.g., see Figure 1 in Moleti et al. (2008)), so there is also not a specific fT4 level below which this transition occurs.

Given the lack of a unified clinical definition, for the purpose of this report it is assumed that euthyroid conditions occur for fT4 levels between the 10th and 90th percentile of the entire population in a given life stage, and that hypothyroxinemia occurs for fT4 levels between the 2.5th and 10th percentile for the entire population. When fT4 falls below the 2.5th percentile for the entire population in a life stage, it is assumed that TSH levels would be sufficiently elevated to trigger hormonal feedback, invalidating the model predictions. While model simulation results are shown beyond this range for the purpose of illustration, simulation curves for these results are shown in grey (vs. black) to indicate that the confidence in those model predictions is low (e.g., **Figure 10.** Free T4 (fT4) levels as a function of perchlorate exposure and dietary iodine ingestion in the gestation-week 40 pregnant mother and fetus.

The actual range of model accuracy is uncertain, due to the fact that the data on urinary and breast milk iodide levels, and TH blood levels, are generally not correlated with specific iodine intake levels (doses). Hence model predictions of fT4 as a function of iodine dose cannot be

evaluated or calibrated against corresponding dose-response data. The pregnancy model (Lumen et al., 2013) was primarily developed and calibrated using an assumed iodine intake of 200 µg/d, the level recommended for pregnant women. Because fT4 levels are predicted to only decline slightly as iodine intake drops to 150 µg/d, and data for pregnant women (compared to urinary iodide, a measure of nutrition) show a similar pattern (Silva and Silva, 1981), pregnancy model dose-response predictions for ≥ 150 µg/d iodine intake are judged to be quite reliable. Model predictions are also shown for 100 and 75 µg/d iodine, but the results for 100 µg/d are near the bottom of the provisional reference range, and are much more uncertain. At 75 µg/d the pregnant mother is predicted to be below the provisional 2.5th population percentile, hence in the hypothyroid range, even without perchlorate exposure (Figure 10, lowest curve, shown in grey). Those results are included, even though the entire 75 µg/d curve is currently considered uncertain, in the event that it is later determined that model predictions are valid for lower levels of fT4.

The infant model was first developed using iodine levels reported for infant formulas, which have iodine concentrations in the range of 100-300 µg/L. These concentrations effectively saturate the infant thyroid's capacity to produce THs and model dose-response predictions are judged to have relatively low uncertainty for the formula-fed infant.

Iodine Ingestion & T4 Reference Ranges

The lactating mother model was primarily calibrated or tuned assuming iodine ingestion of 250 µg/d, the level recommended for breast-feeding women. However, given that the dependence on iodine nutrition is quite similar to that predicted for the pregnant mother and that only a 6-14% difference is predicted in fT4 levels at 150 vs. 250 µg/d iodine (depending on postnatal day (PND) and perchlorate exposure), the model predictions are likewise judged to be reliable for ≥ 150 µg/d iodine, but to have increasing uncertainty at 100 µg/d iodine (where maternal fT4 approaches the bottom of the reference range). At 75 µg/d iodine, maternal fT4 is predicted to

be at or below the provisional 2.5th percentile (the point where hypothyroidism is expected to begin), similar to the pregnant mother. Results for 75 µg/d iodide are included because they may be relevant if the provisional reference range is adjusted downwards, but are considered highly uncertain. Since maternal predictions provide the nutritional intake for the breast-fed infant, the same degree of uncertainty is assumed for that life stage.

The fT4 reference range reported in different studies varies depending on the size, ethnicity, and iodide nutrition of the population examined, the analytic method, and laboratory where the analyses are conducted. As a result, the mean for a given population might not correspond to the control level of fT4 predicted by the model given the recommended iodine intake (e.g., 200 µg/d during pregnancy). Therefore, a normalization procedure was used to avoid apparent discrepancies and resulting bias. If the model predicts control fT4 levels above the actual population mean, this normalization adjusts the reference range upward to the same extent, and vice-versa.

Specifically, fT4 reference ranges or population percentiles from a given study or analysis were normalized to the mean fT4 for that study/analysis (or median, if the mean is not available), resulting in a conversion to a fraction of the mean. The model-predicted results for an iodine intake of 200 µg/d and no perchlorate intake for both pregnancy and lactation was used to set the model-mean fT4 for the pregnant mother, lactating mother, and breast-fed infant. (At 200 µg/d, fT4 levels in the lactating mother were 1-6% lower than at 250 µg/d, and this choice was simpler than simulating a woman who changes dietary intake from 200 to 250 µg/d just after giving birth.) Since results for the lactating mother and breast-fed infant changed with PND, the assumed population mean for each life stage therefore varied with PND. The relative fT4 level (fraction of that mean) for the lactating mother estimated as the 10th and 2.5th population percentile did not depend on PND, due to limited data for lactation, but the infant percentiles did vary, based on a larger data set.

For the formula-fed infant, the age-specific mean was defined by model predictions for an infant ingesting formula with an average concentration of 159 µg/L iodine (average of 8 formulas reported by Pearce et al. (2004)), born to a mother who ingested 200 µg/d iodide during pregnancy; i.e., an infant with fully sufficient iodine levels and intake.

For the pregnant mother, results from 6 studies characterizing T4 reference intervals during pregnancy (Khalid et al. (2014); Wang et al. (2011); Yan et al. (2011); Gong and Hoffman (2008); Stricker et al. (2007); Panesar et al. (2001)) were combined by first normalizing the reported percentiles (2.5^{th}, etc.) from each study to the respective mean or median. Because the resulting percentiles clearly showed a right-skewed normal distribution, the lambda-mu-sigma transformation of the normal distribution (LMS, Box and Cox (1964)) was then fit to the resulting collection of normalized percentiles (Appendix E). From the resulting distribution, the 10^{th} and 2.5^{th} population percentiles were then calculated to be 83.5% and 75.7% of the mean, respectively. Since the Lumen et al. (2013) model predicts an fT4 level of 14 pM for a 3^{rd}-trimester pregnant mother consuming 200 µg/d iodide, the corresponding 10^{th} and 2.5^{th} fT4 percentiles were estimated to be 11.7 and 10.6 pM, respectively.

For the lactating mother, an analysis of fT4 levels in 48 lactating women from the NHANES (2007-2008 and 2009-2010) was used to directly estimate the 10^{th} and 2.5^{th} percentiles for fT4 as 81% and 74% of the group mean relative values close to those estimated for the pregnant mother (Appendix F). As mentioned above, mean fT4 levels are predicted to change with PND in the lactating mother, but *not* the relative variation around the mean, so these percentiles are applied to the PND-specific fT4 levels from the model (assumed to represent the mean, for a woman consuming 200 µg/d iodide) to obtain PND-specific percentiles.

For the infant, an age-dependent reference analysis was conducted by Lem et al. (2012), who fit their data using the LMS-transformed normal distribution with age-dependent parameters. While

Lem et al. (2012) did not provide specific parameters for their statistical fit, it was possible to identify a single value for the shape parameter (λ) and age-specific values for the coefficient of variation (σ), consistent with their summary statistics (**Table 1**). For example, the hypothyroxinemic fT4 range for the 1-month, breast-fed infant, for example, is assumed to be 61-66% of the predicted fT4 in a 1-month infant of a mother who ingests 200 µg/d iodide.

Table 1: Provisional Reference Intervals for fT4 in Normal-Term Infants[5]

Infant age	-2 SDs	-1 SD	Mean	+1 SD	+2 SDs	λ†	σ†	Ratio of 2.5th%** to mean	Ratio of 10th%** to mean
1 week*	12.32	15.91	21.61	31.65	52.54	-0.64	0.34	0.58	0.62
1 month*	12.81	16.12	21.12	29.28	44.33	-0.64	0.29	0.61	0.66
2 months	--	--	--	--	--	-0.64‡	0.266‡	0.64	0.68
3 months*	13.41	16.28	20.31	26.29	36.83	-0.64	0.24	0.66	0.71

† Selected to match values for +/- 1 and +/- 2 SD in the preceding columns, given the reported mean for 1 week, 1 month, and 3 months.
* Data from Lem et al. (2012); range of levels for normal, healthy infants.
‡ Average of 1- and 2-month values, used for this analysis
** Calculated using the LMS-transformed normal distribution (Box and Cox, 1964) with mean = 1, λ and σ as identified for each age.

The Fisher et al. (2013) BBDR model for iodide nutrition in the suckling rat pup and lactating dam served as the foundation for development of a model to predict serum TH concentrations in the lactating mother and breast- and formula-fed infant, with many parameters taken from the BBDR model for thyroid hormones in the 3rd trimester pregnant woman and fetus Lumen et al. (2013). The infant BBDR model was calibrated for PND 7 to 90 in the euthyroid, full-term infant (> 37 weeks of gestation). Changes in TH kinetics have been noted in the first few days after birth (Fisher and Klein, 1981; Jacobsen et al., 1977; Erenberg et al., 1974; Abuid et al., 1973). Apparent surges in TSH and T4 production result in high serum levels of TSH and T4 at birth.

[5] All of the ranges proposed here for reference intervals and hypothyroxinemia should be considered preliminary, pending review and comments from the proposed peer review.

Serum TSH and T4 concentrations decline in the newborn over the first week after birth. By PND 7 the rate of change in infant serum TSH is much slower (Lem et al., 2012), and thus model predictions beginning at PND 7 are more reliable. As stated above, TH production in these models was calibrated based on euthyroid conditions (normal iodine intake, serum TSH and TH levels).

BBDR MODEL DEVELOPMENT AND PARAMETERIZATION

Literature Data for Model Development

A PubMed search was conducted in December, 2013, using the following strategy, and the resulting papers were entered into EPA's HERO database. Other relevant papers known to the various project contributors (see list of contributors on title page), or later identified as potentially relevant where subsequently added, resulting in a set of 1471 references, listed in the available spreadsheet "ReviewHERO416.xlsx", from which data might be obtained.

Search	Query	Items found	Time
#13	**Search (#4) AND #12 Filters: Humans**	**1408**	8:47:19
#12	Search ((((#7) OR #8) OR #9) OR #10) OR #11 Filters: Humans	161800	8:40:58
#11	Search (iodine) OR iodide Filters: Humans	61544	8:40:08
#10	Search (triiodothyronine) OR triiodothyronine[MeSH Terms] Filters: Humans	14122	8:40:08
#9	Search (thyroid) OR thyroid[MeSH Terms] Filters: Humans	110777	8:40:08
#8	Search (thyroxine) OR thyroxine[MeSH Terms] Filters: Humans	26695	8:40:08
#7	Search (thyroid hormone) OR thyroid hormone[MeSH Terms] Filters: Humans	46330	8:40:08
#6	**Search (#3) AND #4 Filters: Humans**	**22**	8:22:19
#5	Search (#3) AND #4	48	8:13:57
#4	Search (#1) OR #2	92212	8:13:47
#3	Search perchlorate	4826	8:13:47
#2	Search postpartum	74486	8:13:22
#1	Search (lactat*) NOT lactate	51482	8:13:22

The literature search was performed with the objective of identifying sufficient data to calibrate the BBDR model. Additional citations were also identified from reference lists from reviewed articles, general internet searches, and the EPA HERO database. Of the papers identified in the searches, 566 were reviewed for inclusion. Criteria for inclusion included that the data be specifically identifiable for infants or breast feeding mothers from 7-90 days after birth. Also, data were only included from studies with full-term, healthy infants and healthy mothers that were not being treated for thyroid or other diseases while breast-feeding, and who were considered to have sufficient iodine in their diets. Of the papers reviewed, 403 articles were excluded because the titles indicated that these inclusion criteria were not met, or that they were unlikely to include useful information on thyroid hormone levels. Of the 163 articles that were reviewed more thoroughly, 21 articles had no useful information, 10 were review articles, and data from 70 articles was excluded for specific reasons, such as disease or not having subjects specifically identified as being in the range of 7 to 90 days postnatal. The remaining 62 articles had data that were used for calibration, validation or setting model parameters. Information for these 62 specific articles are provided in ReviewHERO416.xlsx. The review process was halted after it was judged that the objective had been met; i.e., sufficient data for model calibration had been identified.

In-vivo data were collected from studies for lactating mothers, breast-fed infants, formula-fed infants, and infants for which the nutrition source was not indicated. The in-vivo, life-stage specific studies identified with iodide, perchlorate, or thyroid hormone data for calibrating the lactation BBDR model, are summarized in **Table 2**.

Table 2: Publications providing life-stage-specific, in-vivo data for calibration and evaluation of the BBDR models

Reference	Infant diet*	Maternal			Infant	
		Plasma	Urine	Breastmilk	Plasma	Urine
Cao et al. (2010)	FF, BF				Iodide, fT4	Iodide, ClO4, fT4
Gordon et al. (2014)	FF, BF					Iodide
Kirk et al. (2013)	BF		ClO4	ClO4		
Lem et al. (2012)	NS				T3, T4, fT4	
Leung et al. (2012)	BF		Iodide, ClO4	Iodide, ClO4	fT4	Iodide, ClO4
NHANES†	BF	T3, T4, fT4	Iodide, ClO4			

*BF=Breast-fed, FF=Formula-fed, NS=Nutrition source not specified.
† http://wwwn.cdc.gov/nchs/nhanes/2007-2008/THYROD_E.htm, http://wwwn.cdc.gov/nchs/nhanes/2009-2010/THYROD_F.htm

Physiological parameters are life-stage specific, and were taken from appropriate sources (identified in the data tables) where possible. However many of the iodide/thyroid hormone and perchlorate parameters where not available for specific life-stages, and so were extrapolated from other life-stages or in-vitro data.

The parameters that were calibrated are listed with their respective definitions in **Table 3**. **Table 4** and **Table 5** list the data sources and description of the method of estimation (empirical or model fit to the data) for the infant and lactating mother, respectively. Parameter values (with units) are provided in **Table 6**-**Table 8**. The primary data sets used to calibrate the model to predict serum THs for the lactating mother and formula-fed or breast-fed infant were taken from Leung et al. (2012), NHANES (NHANES, 2007-2008, Thyroid Profile[6] and NHANES 2009-2010, Thyroid Profile[7]), Caldwell et al. (2011), Cao et al. (2010), Gordon et al. (2014), and Lem et al. (2012). Some of these authors also measured iodide in infant and mother's urine, and breast

[6] http://wwwn.cdc.gov/nchs/nhanes/2007-2008/THYROD_E.htm
[7] http://wwwn.cdc.gov/nchs/nhanes/2009-2010/THYROD_F.htm

milk, which were used for model calibration or validation. In cases where limited or no data are available for aspects of iodine and TH homeostasis in the lactating mother, some model parameter values for the lactating mother were taken from the pregnant woman (Lumen et al., 2013)

Table 3: Symbols and definition for calibrated parameters*

PARAMETER		DEFINITION
Lactating Mother	Infant	
CTGIPOOL	CTGIPOOL_N	Euthyroid organified iodide stores
CLUX_I	CLUCX_NI	Urinary clearance of iodide; scaled table for infant
CLFECESFT3C	KFECEST3C_N	Scaled fecal clearance of T3
CLFECESFT4C	KFECEST4C_N	Scaled fecal clearance of free T4 (mother);T4 (infant)
CLMILKT3C	---	Scaled clearance of T3 to milk
CLMILKFT4C	---	Scaled clearance of free T4 to milk
CLUT3C	---	Scaled urinary clearance of T3
CLUFT4C	CLUFT4C_N	Scaled urinary clearance of free T4
FRCONVT4	FRCONVT4_N	Ratio of serum free T4 to total T4
KBINDC_I	KBINDC_NI	2^{nd} order scaled rate constant for organification of free iodide in thyroid
KMETT3C	CLMETT3CX_N	1^{st} order scaled T3 degradation rate constant (mother) or T3 metabolic clearance (infant)
KMETT4C	CLMETT4CX_N	1^{st} order scaled T4 degradation rate constant (mother) or T4 metabolic clearance (infant)
KM_MKI	---	M-M constant for iodide uptake to milk
KMTHY_I	KMTHY_NI	M-M constant for iodide uptake in thyroid
KPRODT3C	VPRODT3F_N	1^{st} order scaled rate constant for production of T3; ratio of T3:T4 production in infant
KPRODT4C	KPRODT4CX_N	1^{st} order scaled rate constant for production of T4(mother); Scaled production rate table (infant)
PATHYC_I	PATHYC_NI	Scaled permeability- area (PA) product for iodide
PAMKC_I	--	PA product for iodide into milk
---	PBODY_NI	Rest of body tissue: plasma partition coefficient (PC) for iodide
PMK_I	---	Milk: mammary plasma PC for iodide
PRP_I	---	Richly perfused: plasma PC for iodide
PSP_I	---	Slowly perfused: plasma PC for iodide
PTHY_I	PTHY_NI	Thyroid tissue: plasma PC for iodide
T3FRAC	T3FRAC_N	Fraction of T4 metabolized to T3
VDT3C	VDT3C_N	Volume of distribution for T3
VDT4C	VDT4C_N	Volume of distribution for T4
VMAXCX_MKI	---	Scaled Vmax for NIS: iodide into milk
VMAXTHYX_I	VMAXTHYCX_NI	Vmax table for NIS: iodide into thyroid

* "X" in the parameter name indicates that it is a time-dependent table function.

Table 4: Calibration of Parameter Values for Infants

Parameter	Data Source and parameter calibration
Iodide	
CTGIPOOL_N	Fisher et al. (2016): set so that the total pool equals amount predicted in fetus at end of pregnancy (Lumen et al., 2013) for 250 µg/d maternal iodide.
CLUCX_NI	Ponchon et al. (1966): Set table values to 0.044 L/h/kg at birth, increasing to 0.055 L/h/kg at 3 weeks (n=3), and then to 0.6 L/h/kg at 6 weeks (n=4).
VMAXTHYCX_NI	Ponchon et al. (1966): Estimated based on reported clearance of 2.5 ml/min/kg (n=3, birth-21 days of age) and 1.1 ml/min/kg (n=4, 21-90 days of age), for healthy infants from Belgium, VmaxthyC_Ni = Clearance (L/h)×Km_NISi for NIS (3.15×10^4 nmol/L)
KMTHY_NI	Lumen et al. (2013):Values reported in Gluzman and Niepomniszcze (1983)
KBINDC_NI	Fitted in Fisher et al. (2013); scaled to (thyroid weight)$^{0.75}$ for growth.
PATHYC_NI	(Delange, 1998): Visually Fitted to maintain ATGIPOOLC_N.
PBODY_NI	Body/plasma value of 0.21 is intermediate between slowly perfused (0.18) and richly perfused (0.4) from Lumen et al. (2013) for pregnant mother
PTHY_NI	Lumen et al. (2013): Set to maternal values
Thyroxine (T4)	
VDT4C_N	Cottino et al. (1961): 0.31 L/kg
VPRODT4CX_N	Fisher (1996): Estimated from reported T4 utilization per day for infants in 3 age groups up to 12 months: 10 µg T4/kg/day from birth to 4 days of age, 7 µg T4/kg/day at 28 days, and 6 µg T4/kg/day from 1-12 months of age. (Data originally from Nelson et al. (1993).)
CLMETT4CX_N	Model fit to visually predicted plasma T4 and fT4 from Lem et al. (2012).
T3FRAC_N	Qualitative pattern based on ratio of T3 to T4 serum concentration profiles over 30 days in 18 infants from the United States in Chopra et al. (1975), Lumen et al. (2013) estimates in the term fetus, and Richard et al. (1998) reported ontogeny of iodothyronine deiodinases. Fit to T3 data from Lem et al. (2012), Elmlinger et al. (2001), Franklin et al. (1985), and Williams et al. (2004).
FRCONVT4_N	Model fit to visually predict Lem et al. (2012) data with Gemelli et al. (1990) (n = 11 newborns) (ratio of fT4/T4 = 9.6×10^{-4}) as initial estimate.
CLUFT4C_N	Based on Cao et al. (2010) and Sillén (2001), infant urinary excretion estimated to be < 0.1% of total synthesis and ingestion; hence this rate was set to zero.
CLFECEST4C_N	Set value based on adult humans- 15% of administered ^{131}I-T4 was found in feces from Oddie et al. (1964), and targeted 7.8% of T4 formed per day excreted based on estimates for maturation from Saghir et al. (2012).
Triiodothyronine (T3)	
VDT3C_N	Lumen et al. (2013): Set to fetal value, which was fit to data of Hume et al. (2004)
VPRODT3F_N	Lumen et al. (2013): Used assumption from Lumen model that thyroidal production of T3 vs. T4 is similar to the adult value of 1/11 of T4 production rate, based on Thorpe-Beeston et al. (1992) and Hume et al. (2004)
CLMETT3CX_N	Model fit to describe visually T3 time course data from Lem et al. (2012).
CLFECEST3C_N	Set value based on 10.3% of administered ^{131}I-T3 was found in feces of adults from the United States (n=18) from Fisher and Oddie (1964b) and assumed that 4.5% of T3 formed per day is excreted in feces based on immature status reported by Saghir et al. (2012).

Table 5: Calibration of Parameter Values for Lactating Mother*

Parameter	Data Source and Calibration
Iodide	
CTGIPOOL	Lumen et al. (2013): Set to target 15 mg for thyroidal iodide stores
CLUX_I	Fisher et al. (2016): set to vary over time, model fit to central tendency for Leung et al. (2012) data; values initially from Aboul-Khair et al. (1964).
VMAXCX_MKI	Leung et al. (2012): Fit to iodide concentrations in breast milk
VMAXTHYX_I	Aboul-Khair et al. (1964) reported thyroidal clearance of systemic iodide equal to 2.25, 1.48, and 1.22 L/h at 2, 6 and 12 weeks. Converted to Vmax (clearance (L/h)×Km (nmol/L)). Last time-point value coded as occurring at 90 days rather than 84 day. Value at birth similar to pregnant woman (Lumen et al., 2013).
KBINDC_I	Dunn and Delange (2005): Fit value to predict thyroid stores near the maximal concentration for euthyroid adults (10-20 mg/thyroid)
KM_MKI	Lumen et al. (2013): Set equal to the KMNIS_I based on commonality of NIS molecule; binding affinity not expected to vary with site of expression.
KMTHY_I	Set equal to pregnancy values that were fit in Lumen et al. (2013) based on commonality of NIS molecule; binding affinity not expected to vary with site of expression.
PAMKC_I	Fitted parameter to Leung et al. (2012)
PATHYC_I	Lumen et al. (2013): Chosen to be consistent with expected thyroidal iodide concentration stores in adults; i.e., very low levels of free iodide. (No data are available for pregnant or lactating women.)
PMK_I, PRP_I, PSP_I, PTHY_I	Lumen et al. (2013); McLanahan et al. (2014): Set to values used previously in pregnancy model
Thyroxine (T4)	
CLMILKFT4C	van Wassenaer et al. (2002): Model fit to predict < 1 µg/day of T4 ingested in milk (0.6 µg/day)
CLFECESFT4C	Model fit to target 10% of T4 excreted in feces for euthyroid lactating woman. Oddie et al. (1964) reported that in adults 15% of administered ^{131}I-T4 was found in feces.
CLUFT4C	Habermann et al. (1976): Model fit to target of less than 2 µg/day of T4 in urine, 1.6 µg/day euthyroid lactating woman. Habermann et al. (1976) reported excretion of 1.4 µg/day of T4 in urine over 24 h in 20 subjects
FRCONVT4	Fisher et al. (2016): Slightly increased from pregnancy value (0.9×10^{-4}; Lumen et al. (2013)) to 1.0×10^{-4}
KPRODT4C	Decrease in model-fit value from Lumen et al. (2013) for pregnant women (2.45×10^{-6} $h^{-1}kg^{-0.75}$) to 1.1×10^{-6} for lactating woman
KMETT4C	Reduced to 1.4×10^{-4} $h^{-1}kg^{-0.75}$ to fit data for lactating woman vs. 1.9×10^{-4} used for pregnant women Lumen et al. (2013)
VDT4C	Slight decrease in model-fit value from Lumen et al. (2013) for pregnant women (8.4% BW for lactating woman vs. 12.1% for pregnant women)

* "X" in the parameter name indicates that it is a time-dependent table function.

Table 5 (cont.): Calibration of Parameter Values for Lactating Mother

Parameter	Data Source and Calibration
Triiodothyronine (T3)	
CLFECEST3C	Fisher and Oddie (1964b): Model fit to target < 8% of T3 excreted in feces, Fisher and Oddie (1964b) reported 10.3% of administered ^{131}I-T3 was found in feces of adults from the U.S. (n=18)
CLUT3C	Model fit to target < 3 µg/d excreted in urine, lactating woman was 2.3 µg/d; Habermann et al. (1976) reported excretion of 1.7 µg/day of T3 in urine over 24 h in 20 adults.
CLMILKT3C	Fit to predict < 1 µg/day of T4 ingested in milk (0.6 µg/day reported in van Wassenaer et al. (2002))
T3FRAC	Lumen et al. (2013): Set to pregnancy value
KMETT3C	Lumen et al. (2013): Set to upper limit of pregnancy value
KPRODT3C	Increased from 1/11 of T4 production to reflect thyroidal metabolic shift which occurs when iodine intake is low
VDT3C	Slight increase from pregnant woman fitted value (0.46 L/kg) from Lumen et al. (2013) to 0.47 L/kg for lactating woman

Infant temporal reference intervals for serum thyroid hormones reported over the course of 90 days of age (Lem et al., 2012; Elmlinger et al., 2001) were used to set the distribution of serum thyroid hormone concentrations for normal euthyroid thyroid function in infants, regardless of the method of feeding. Reference intervals (percentiles) for serum THs were not found in the peer-reviewed literature for lactating women; however, individual data at time-points from PND 1 to 90 were available from the NHANES studies and were used as guidance for model development. These NHANES data were used to calibrate the model for the lactating mother with lactation-day-specific information. While other sources report thyroid hormone data during the postpartum period, they were deemed less useful for a variety of reasons, including failure to specifically identify results for lactating vs. non-breast-feeding mothers, failure to specify the postnatal age (day) on which the data were collected, or only providing data for a portion of the 90-day period for which data was needed (e.g., Ardawi et al. (2002) only provides data for a single time-point). Hence these other data sets were not used.

Model Structure

The iodide and perchlorate models assume that iodide does not distribute into red blood cells (RBCs) and distribution of perchlorate into RBCs is diffusion-limited. Therefore transport of iodide between tissues occurs only in the plasma, and while perchlorate is carried in both RBCs and plasma, uptake of perchlorate into tissues is only assumed to occur from the plasma.

The infant iodide sub-model is a physiologically based pharmacokinetic model (**Figure 2**) with the same compartments as the iodide sub-model for the fetus (Lumen et al., 2013): including plasma, rest of body, thyroid plasma, and thyroid gland tissue. The model structure is both simpler and distinct from the previous radiolabeled PBPK models (McLanahan et al., 2014; U.S. EPA, 2009). Differences include the reduction of the thyroid gland to two compartments (plasma and tissue instead of plasma, follicle, and lumen), input of ingested iodine directly into the plasma (GI tract not specified for iodide), and lumping of adipose and skin with tissues in the rest-of-body compartment.

The model compartments for the mother (**Figure 3**) consist of plasma, slowly perfused, richly perfused, thyroid gland and thyroid plasma, and mammary gland plasma and milk. Active iodide uptake is modeled between the thyroid plasma and thyroid tissue and between the mammary plasma and milk. Two structural changes made for this model compared with the previous kinetic models for radiolabeled iodide are: 1) a reduction in the thyroid subcompartments (plasma and tissue instead of plasma, follicle, and lumen); and 2) direct transfer of iodide from the mammary plasma to the milk (with mammary gland tissue not specifically included). Similar to the infant, the dietary intake of iodine is treated as infusion directly into the plasma (GI tract not specifically described), and the adipose and skin compartments are lumped into the slowly perfused compartment.

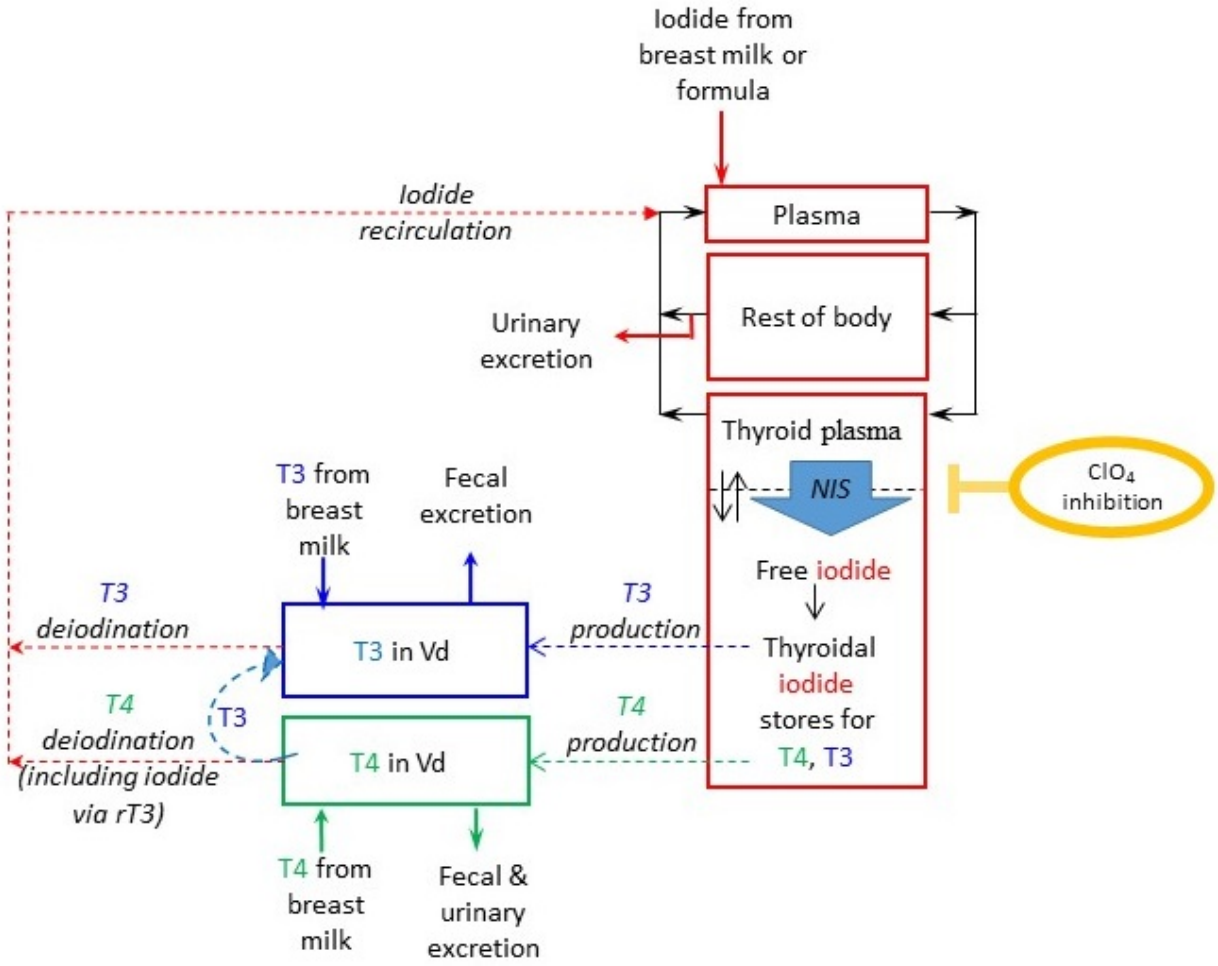

Figure 2. Infant iodide/thyroid hormone sub-model.

In mammary and thyroid, free diffusion between plasma and tissue is indicated by ↓↑ and active uptake by NIS is indicated by ⇩. Vd denotes volume of distribution

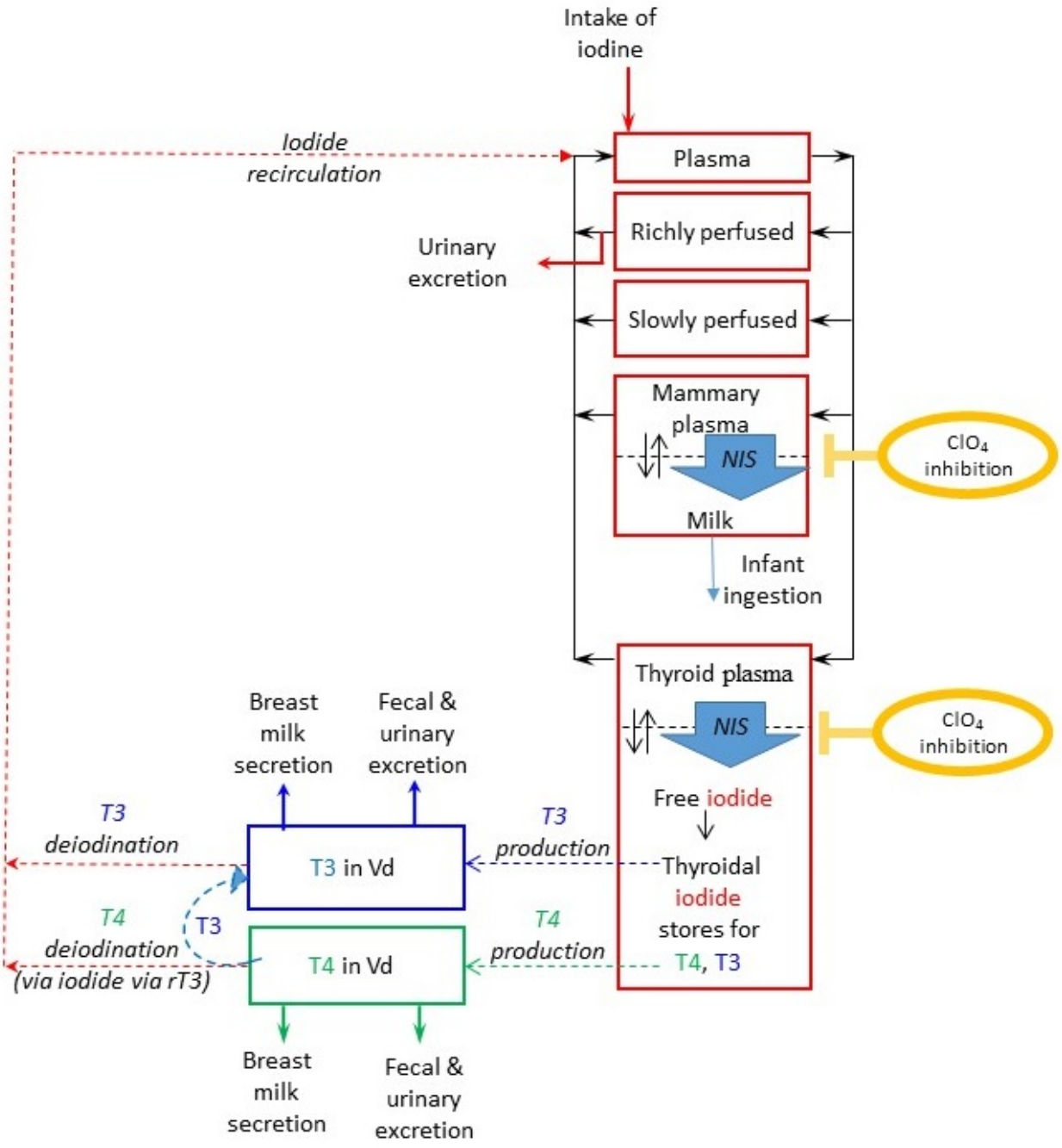

Figure 3. Maternal iodide/thyroid hormone sub-model.

In mammary and thyroid, free diffusion between plasma and tissue is indicated by ↓↑ and active uptake by NIS is indicated by ⇓. Vd denotes volume of distribution

Physiological Model Parameters

Values and units for physiological parameters for the infant and lactating mother in comparison with non-pregnant and pregnant women, when available, are listed in **Table 6**. A description of the literature sources for the values is given below.

Infant

Edginton et al. (2006) reported reference ranges from published literature for cardiac output (L/min) versus age in children, which was used to set up a table function to linearly increase the cardiac output over the 3 month simulation period. Whole blood cardiac output was converted to plasma flow based on the ratio of red blood cell volume to plasma volume (i.e., the hematocrit) in the infant (Brines et al., 1941). Plasma volume was calculated with information from Brines et al. (1941), who estimated plasma volume in 4 infants, age 7 to 21 days old, and the red blood cell volume was set assuming the adult hematocrit value from Altman and Dittmer (1971). The current model used set values for the hematocrit, while the U.S. EPA (2009) model used a time-dependent function. The plasma flow rate to the thyroid gland was set to the adult value, 1.6% of cardiac output, based on sources used for the Lumen et al. (2013) model (i.e., Brown et al. (1997), Kiserud et al. (2006)).

Infant body weight (BW_N) growth was expressed by an algebraic equation for infants aged less than 11 months (Clewell et al. (2007); originally from Clewell et al. (2004)). Thyroid gland weight changes in the infant over the course of 90 days were taken from Clewell et al. (2007) and verified with data from Ogiu et al. (1997), while thyroid plasma volume (fraction of total thyroid mass) was set using the thyroid blood fraction used for the pregnant mother and fetus in Lumen et al. (2013), which obtained the estimate from Brown et al. (1986), and circulating blood hematocrit. Sillén (2001) reported urine production of 5 ml/h/kg BW for the newborn based on 4-

hour experimental periods. To better predict urinary excretion of iodide, urine production was assumed to be constant at 5 ml/h/kg from birth to 90 days of age.

Lactating Mother

The compartment volumes and plasma flow rates were calculated with equations from McLanahan et al. (2014). The plasma flow rate to the mammary gland was set to a constant rate from birth to postpartum day 90, based on data reported by Geddes et al. (2012). The previous U.S. EPA (2009); McLanahan et al., 2014 model used a flow rate relative to the size of the mammary gland, which was modeled as increasing from birth to 2 weeks postpartum and then held constant up to 6 months postpartum. The excretion rate for breast milk was described with a non-linear function fitted to milk yield data (Arcus-Arth et al., 2005; Casey et al., 1986; Hofvander et al., 1982), and the residual volume of milk was set to mean value reported in Kent et al. (2006) and Daly et al. (1992).

Iodide and Thyroid Hormone Kinetics

Infant Iodide

Infant-specific, iodide model parameters (**Table 7**) included urinary excretion of iodide, active uptake of iodide into the thyroid gland, organification of iodide, storage in the thyroid gland, and tissue/plasma partition coefficient values for the thyroid gland and body. Values include those obtained from literature reference and calibrated values (**Table 4**).

Urinary excretion of iodide has been reported to increase over the first 90 days in the infant (Ponchon et al., 1966) with an excretion rate of 0.74 ml/h/kg from birth to 13 days (n=3), followed by an increase to 1.1 ml/h/kg over the next few months (n=4). The maximal velocity for thyroidal uptake of iodide (VMAXTHYCX_NI) was derived from thyroidal clearance calculations (Ponchon et al., 1966). Ponchon et al. (1966) reported that thyroidal clearance of iodide

decreased with age (2.5 ml/min-kg for the first 3 weeks of life, then 1.1 ml/min-kg for up to 6 months of age), therefore, VMAXTHYCX_NI decreased with postnatal age in the model. The Michaelis-Menten constant for the infant thyroid (KMTHY_NI) was set to the maternal value. Passive, bi-directional diffusion of iodide into and out of the infant thyroid gland was described with a fitted permeability coefficient value of 0.01 L/h/kg $BW^{0.75}$ based on euthyroid, organified iodide stores. Once iodide was translocated into the thyroid gland the organification of iodide was described with a second order constant, which increases with age, fitted to provide thyroidal iodide stores near the maximal value for euthyroid infants. The maximal organified iodide stored in the thyroid gland of the newborn (n=13, average 292 µg iodide) was expressed as a concentration, 1716.7 nmol iodide/g thyroid weight, based on 13 healthy newborns from Canada (Delange, 1998). The thyroid/plasma partition coefficient (0.15) was set equal to that used by Clewell et al. (2007) and Merrill et al. (2005), as was done by Lumen et al. (2013), and the rest-of-body: plasma partition coefficient (0.21) to an intermediary value between maternal slowly (0.18) and richly perfused (0.4) values.

Intake of iodide from formula was described as the concentration of iodide in the formula times the rate of formula ingested. Iodide concentrations in formula were taken from measurements reported in Pearce et al. (2004) for 8 commercial formulas: 17.9, 22.2, 17.9, 17.3, 56.8, 16.2, 24.2 and 15.9 µg per 5 oz. The average of these iodide concentrations is 159 µg/L of formula, with a range of 108-384 µg/L. The 50^{th} percentile volume of formula ingested daily decreased with age from 0.199 L/day/kg for the first month of life (n=56, days 8-30), to 0.189 (n=62) and 0.174 (n=69) L/day/kg for the second and third months of life (Fomon et al., 1964).

Intake of iodide from breast milk or formula was described as the breast milk or formula concentration times the ingestion rate, which was described with a non-linear function fitted to milk ingestion data. (The model code also allows use of a table function going through the same data.) The data were calculated from mean daily milk ingestion rates reported by Casey et al.

(1986) over a 5-day period. Nursing rates for birth, 12 h, and days 1, 2, 3, 4, and 5 were 13, 13, 13, 40, 98, 140 and 155 g milk per kg BW of infant per day (g/kg/d). For lactation months 1 and 2, Hofvander et al. (1982) reported milk ingestion rates of 154 and 148 g/kg/d and, at lactation month 3, Dewey et al. (1991) reported 130 g/kg/d. Arcus-Arth et al. (2005) also summarized milk ingestion data, including the sources identified here.

Infant Thyroid Hormones

The model structures for T4 and triiodothyronine (T3) are one compartment volumes of distribution (**Figure 2**) similar to Lumen et al. (2013) and for the lactating rat and breast-fed pup (Fisher et al., 2013) with losses from metabolism by deiodination or conjugation, and excretion into breast milk, urine (T4 only), and feces. T4 is formed in the thyroid gland and secreted into systemic circulation, while T3 is primarily formed from deiodination of T4 in the body and to a much lesser degree from formation in the thyroid gland.

The thyroid endocrine system in the body of an infant is highly active compared to an adult, with high throughput or turnover in the infant thyroid gland (Fisher, 1996). Consequently, several model parameter values associated with T4 were age-dependent in the infant, where data were available. **Table 4** lists data sources that were used for fitting or setting the value of the parameters in the model. **Table 8** contains the thyroid hormone model parameters for infants compared with the lactating and pregnant mother.

Maximum T4 secretion (production) from the thyroid gland from birth to day 4 was set to 0.535 nmol/h/kg BW; it then decreased linearly from 0.535 nmol/h/kg at day 4 to 0.375 nmol/h/kg at day 7, linearly from there to 0.322 nmol/h/kg at day 28, and then remained constant at 0.322 nmol/h/kg for up to 6 months based on the data reviewed by Fisher (1996).

For the current model, whole body estimates of deiodination rates of T4 were estimated by visually fitting the model-predicted plasma T4 concentrations with literature data for T4 and fT4

plasma concentrations (Lem et al. (2012) resulting in age-dependent rates of 3.5 ml/h/kg at birth, declining to 2.3 ml/h/kg at 10 days, then to 2.0 ml/h/kg at 30 days, where it stayed constant to 90 days of age.

Conversion of T4 to reverse T3 (rT3), an inactive form of T3, was described as an age-dependent function. Deiodinase III is present at birth at relatively high levels and decreases with age (Richard et al., 1998; Chopra et al., 1975). Chopra et al. (1975) collected serum profiles in 18 infants for rT3. From birth to 5 days of age, 50% of the T4 was assumed to be converted to rT3 (1–T3FRAC_N), declining to 45% on day 6, 40% on day 7, 30% on day 30, and ultimately to 20% at 6 months. This time-dependence was coded into the model by defining the parameter T3FRAC_N (the fraction of T4 metabolized to T3), the remainder going to the inactive form of T3 (rT3) with a time-dependent table function (linear interpolation between defined time-points). rT3 was not tracked in the model but was assumed to be completely deiodinated, releasing three iodide atoms.

A small fraction of T4 was assumed to be secreted into feces (up to 8% in the infant) based on adult experimental findings (Oddie et al., 1966). These authors found 15% of administered ^{131}I-thyroxine excreted in feces of adults. For the infant, the maturation of uridine 5'-diphospho-glucuronosyltransferase (UGT) enzymes (Saghir et al., 2012) responsible for conjugation of T4 was assumed to be nearly 50% (or less) of adult values (e.g., for UGT1A1). No data were found for the infant to verify this assumption.

For urinary excretion of T4 in the infant, information was derived from adults. Habermann et al. (1978) collected 24-h urine samples from 20 adults and found trace amounts of T4 in urine (1-2 µg), which is nearly 1% of the T4 formed each day for the adult. For the infant, 0.3% or less of T4 was assumed to be excreted in urine. No data were found for the infant to verify this assumption. The free fraction of total T4 in serum (fT4) was set to an initial constant value of

9.6×10^{-4} (i.e., [fT4] = $9.6\times10^{-4}\times$ [T4], based on Gemelli et al. (1990), n=21), but was then fitted to measured and free T4 in serum (Lem et al., 2012); FRCONVT4_N = 1.7×10^{-4}.

The volume of distribution of T4 in the infant was set to 0.31 L/kg based on an analysis of 7 children up to 12 months of age, who were injected with radiolabeled thyroxine (Cottino et al., 1961). The volume of distribution for T3 in the infant was set equal to the fetus (Lumen et al., 2013) and the production rate was set equal to 1/11 of the infant T4 production rate, similar to the T3:T4 production ratio of 1/11.8 used for the fetus by Lumen et al. (2013).

As with T4, T3 metabolic rates were estimated visually and set to age-dependent parameter values of 0.12 L/h/kg from birth to 10 days, then 0.09 L/h/kg for 30-90 days of age, which allowed the model to predict the mean serum T3 values reported by Lem et al. (2012). Fisher and Oddie (1963) reported that fecal excretion of radiolabeled T3 was about 10% in adults. For the infant the model value targeted 4.5% of the adult T3 excretion rate because of the immature status for conjugation (Saghir et al., 2012). Urinary excretion of T3 was not included in the model.

Lactating Mother Iodide

Several iodide-specific model parameters were obtained from the literature for the lactating mother. **Table 5** lists data sources that were used for fitting or setting the value of the parameters for the iodide sub-model for the lactating mother. Values of the kinetic parameters for the iodide sub-model, compared with the pregnant female and infant, are listed in **Table 7**.

Urinary excretion of iodide was found to decrease over time in 7 lactating women (Aboul-Khair et al., 1964) for postpartum weeks 2, 6, and 12. These urinary iodide excretion clearance values (L/h) and the estimated urinary iodide excretion clearance value from the 3rd trimester mother at birth (Lumen et al., 2013) were used for the lactating mother model for iodide.

In a similar trend, the calculated thyroidal uptake of iodide declined over 12 weeks of lactation (Aboul-Khair et al., 1964). Thyroidal clearance of iodide was converted to a maximum velocity term (VMAXTHYX_I) for NIS mediated uptake of thyroidal iodide by multiplying the clearance values by the affinity constant, KMTHY_I; it was not scaled by body weight but was estimated to decrease with time. At birth, the lactating mother maximal velocity was set equal to the 3rd trimester pregnant mother. The maximal velocity for NIS mediated uptake of iodide into the breast milk was fit visually to measured iodide levels in breast milk (Leung et al., 2012); it was scaled to body weight and assumed to be constant during the 3 months postpartum.

Partition coefficients for the slowly and richly perfused tissue groups, and the thyroid gland were set to previously used values (McLanahan et al., 2014; Lumen et al., 2013).

While generally assumed to represent diffusion-limited transport, in reality the permeability-area (PA) coefficients are empirical terms which allow for the probable movement of the charged anions across membranes by transporters other than NIS, including ion gates, and through gaps in cell membranes. Because there are no measures of free iodide in the human thyroid, the value of the thyroidal PA coefficient is not validated in any strict sense, but is selected to allow the system of model equations to work without numerical errors. The diffusion-limited term is included in the model to make it more biologically realistic, but because model results are not sensitive to the value of the thyroidal PA, the exact choice of its value has a negligible impact on model predictions.

Iodide Transfer via Breastmilk

The mammary gland is capable of concentrating iodide in breast milk via the sodium iodide symporter (NIS), a membrane bound transport protein that is under the control of prolactin and other hormones (Micali et al., 2014; Semba and Delange, 2001). There are likely several mechanisms involved, including other transporters such as pendrin, but data by which their

contribution to iodide transport could be quantified (vs. that of NIS) is lacking. Therefore the model structure only includes NIS-mediated transport between blood plasma and breast milk.

Semba and Delange (2001) and Dorea (2002) reviewed reports for low breast milk concentrations of iodide in regions of the world with goiter (13-18 µg/L) compared to those with breast milk iodide concentrations exceeding 100 µg/L. Breast milk measurements of iodide in the United States are limited. Leung et al. (2012) compared three reports of mean or median iodide measurements in breast milk for Boston, MA (155 µg/L,178 µg/L and 45.5 µg/L) with Texas (34 to 52 µg/L). The range of iodide concentrations in spot breast milk samples can exceed two orders of magnitude, so factors other than variation in dietary intake must influence this range.

The volume of mammary plasma (VMB) was set to 27.6% of the volume of the whole mammary gland. Reported breast volumes range from 0.2 to over 1.0 L (Xu et al., 2014; Ramsay et al., 2005; Vandeweyer and Hertens, 2002). A mean value of 1.74% of pre-pregnancy body weight was used for the total mammary tissue (VMAMC) (ICRP, 1975), which yields a volume of 0.59 L. The volume of breast milk (VMK) was based on reports of milk yield and was considered to be a constant volume (0.369 L). This simplifying assumption is suitable because the synthesis rate of milk (11-58 ml/hr/breast, n=6, Daly et al. (1992)) rapidly replaces the volume of milk suckled by the infant (29 to 33 ml/hr during the first months of life). The residual amount of breast milk remaining in the breast immediately after nursing (mean value of 109 g with high variability; Dewey et al. (1991)) helps ensure an ample supply of milk each nursing. This residual breast milk (109 ml per breast) plus average feeding volume of 84 ml for productive breast and 67 ml for less productive breast (Kent et al., 2006) equals a total theoretical milk volume of 0.369 L or 0.1845 L per breast. The mammary glands will hold up to 193 ml of breast milk for a productive breast and 164 ml for a less productive breast (Kent et al., 2006).

Iodide was assumed to be transported unidirectionally by the NIS protein into breast milk and to a limited extent by passive diffusion. The NIS in the mammary gland on the basolateral membrane of alveolar cell mediates transfer of iodide from the plasma into milk. NIS expression is stimulated by the hormones oxytocin, prolactin, and estrogens (Micali et al., 2014). The maximal velocity for NIS mediated uptake of iodide into the breast milk (VMAXCX_MKI) was fit to predict both measured iodide levels in breastmilk (Pearce, 2007) and infant urinary iodide (Leung et al., 2012). No in vitro or in vivo data were found for NIS protein expression in the mammary gland as a function of postpartum days.

The mammary blood: milk PA coefficient values were adjusted in the course of describing breast milk iodide concentration and thyroid total iodine storage data for iodine intake from about 50-100 µg iodide/d to about 400 µg iodide/d, to maintain sufficient thyroidal iodine stores. The permeability-area coefficient for thyroid blood: tissue bidirectional transport (PATHYC_I) was set to 10^{-5} L/h/kg$^{0.75}$ compared to 10^{-4} L/h/kg$^{0.75}$ used by Clewell et al. (2007), Lumen et al. (2013) and McLanahan et al. (2014), because the current model was based on the lactating rat model (Fisher et al., 2013). The permeability-area coefficient value for the milk: mammary plasma bidirectional transport (PAMKC_I) was set to 0.001 L/h/kg$^{0.75}$ to better predict transfer to the infant, which determines the infant urinary iodide concentration, compared to 0.02 L/h/kg$^{0.75}$ used for both plasma: mammary tissue and mammary tissue: milk by Clewell et al. (2007) and McLanahan et al. (2014).

Lactating Mother Thyroid Hormones

A list of the kinetic parameters for the thyroid hormone sub-model and their values compared with the pregnant female and infant is found in **Table 8**. **Table 5** list data sources that were used for fitting or setting the value of the parameters for the thyroid hormone sub-model for the lactating mother.

As shown in Table 5, the primary data set used to calibrate the model to predict serum thyroid hormones for the lactating mother was taken from NHANES. Measurements of iodide in infant and mother's urine, and breast milk, were also used for model calibration or validation. For comparison, Soldin et al. (2007) reported postpartum fT4 levels for a number of other countries, all with values between 13 and 14 pM. In contrast, the mean levels for U.S. lactating women, with the combined NHANES 2007-2008 and 2009-2010 data sets, was 9.3 pM (SE = 0.3 pM, *n* = 39). It is not clear if these differences are due to differences in analytical methodology or represent true population differences. Iodine levels in the diet vary with geographic region and there may also be ethnic differences in hormone levels. Whether the differences are analytical or demographic, careful interpretation is needed when comparing predictions with the model described here, having been calibrated to the NHANES data, to reported thyroid hormone values from other countries.

At parturition, the lactating mother's Vmax for iodide uptake in the thyroid (VMAXTHY_I) was set equal to the value used for the 3rd trimester pregnant mother Lumen et al. (2013). Organification, the process of formation of precursor thyroid hormones in the thyroid gland, was a second order process with a rate constant (KBINDC_I) used in a similar manner as the adult rat (Mclanahan et al., 2008) and lactating rat (Fisher et al., 2013). KBINDC_I was fitted to predict data on near maximal concentrations of thyroidal iodide stores (CTGIPOOL), which were reported by Dunn Dunn and Delange (2005). Bidirectional diffusion of iodide between the thyroid gland and plasma (PATHYC_I) was assumed to be minimal to predict very low concentrations of free thyroidal iodide in the thyroid gland.

Several other model parameters for the thyroid hormones in the lactating mother were derived from Lumen et al. (2013) for the pregnant woman, which were calibrated from baseline values obtained from adult studies in men and non-pregnant women. For T4, the volume of distribution and secretion (production) rate from the thyroid gland were adjusted slightly from those reported

in Lumen et al. (2013) (**Table 6**); VDT4C = 0.084 L/kg in the lactating mother, VDFT4_MI = 0.121 L/kg in the pregnant mother (Lumen et al., 2013); KPRODT4C = 1.1×10^{-6} $h^{-1}kg^{-0.75}$ in lactating mother and KPRODT4F_MI = 2.45×10^{-6} $h^{-1}kg^{-0.75}$ in the pregnant mother (Table 8). Fifty percent of deiodinated T4 was assumed to be converted to rT3 and 50% to T3, similar to Lumen et al. (2013). In the model rT3 was not tracked, but was assumed to be completely deiodinated, releasing three iodide atoms. The iodide atoms were immediately available for systemic circulation. The clearance rate constant for metabolism or deiodination of T4 was set equal to the value for the pregnant woman. The concentration of fT4 was set to a small fraction of total T4 (Lumen et al., 2013). Fecal and urinary elimination clearance constants for T4 were fitted to predict less than 2 µg/d of T4 in urine (Habermann et al., 1976) and for feces, 10% excretion as hepatic conjugates (Oddie et al., 1964). Excretion of T4 in milk was described with a fitted clearance term to predict transport of less than 1 µg/d of T4 (van Wassenaer et al., 2002).

For T3 the volume of distribution for the lactating woman was increased from the pregnant woman (Lumen et al., 2013), while the production rate of T3 and its deiodination rate were set to values reported in Lumen et al. (2013). Fecal clearance of T3 was estimated to target elimination of less than 8% of the T3 formed (Fisher and Oddie, 1964a). Fisher and Oddie (1964a) reported 10% of radiolabeled T3 was secreted in feces of adults. Urinary clearance of T3 was estimated to target excretion of less than 3 µg/d (Habermann et al., 1976). For euthyroid mothers with healthy, full-term infants, very limited data was found on excretion of T3 into milk. Specifically, Sato and Suzuki (1979) measured 0.14 ± 0.11 and 0.07 ± 0.07 µg T3/L breast milk for postnatal ages of 0-1 and 1-2 months, respectively. (Other sources report T3 in breast milk, but for mothers with thyroid disease (Mizuta et al., 1983) or pre-term infants (van Wassenaer et al., 2002).) Based on these data, the rate constant for T3 clearance to milk was estimated to target less than 0.1 µg secreted per day.

Integration of Thyroid BBDR Model with Perchlorate PBPK Model

For perchlorate, the previously evaluated and modified models (McLanahan et al., 2014; U.S. EPA, 2009) were integrated into the new lactating mother and infant model code for dietary iodine and thyroid hormones. The details of the EPA perchlorate and radioiodide PBPK models for the lactating mother and breast-fed and formula-fed infant can be found in (McLanahan et al., 2014; U.S. EPA, 2009) and are not discussed in this document except for changes that were made to the model structure and parameters during the current process of model evaluation and refinement, as discussed below.

As described earlier under *Model Structure*, the structure in the iodide and thyroid hormone BBDR model differs slightly from the radioiodide and perchlorate PBPK models (McLanahan et al., 2014; U.S. EPA, 2009). Therefore, the perchlorate PBPK model was changed in parallel to match the iodide model structure for the thyroid and mammary gland. The thyroid was reduced to two subcompartments, the plasma and tissue, and the mammary gland was simplified to a one-step process with transport occurring directly from mammary plasma to the milk.

Since the perchlorate PBPK model structure was simplified to match the iodide model structure, it was necessary to determine if the revised perchlorate model still matched the human perchlorate exposure radioiodide data (Greer et al., 2002) or breast milk data, or whether some of the parameters needed to be revised. Also, preliminary results with the current lactating mother/breastfeeding infant model indicated that higher levels of perchlorate than previously considered (above 7 µg/kg/d) could have negligible effect on thyroid hormone levels, but performance of the model at these higher exposure levels had not been evaluated.

Key Perchlorate PBPK Model Parameter Changes

Evaluation of the simplified perchlorate PBPK model revealed the need to update some model parameters. Values of two key model parameters were changed from those used in previous

PBPK models (U.S. EPA, 2009). Details of the analyses conducted are provided in Appendix B, "Model Structure Changes and Additional Evaluation." The most important changes are summarized here. Based on re-evaluation of the in vitro data of Kosugi et al. (1996), the affinity constant (Km) for binding of perchlorate to the NIS (KM_TPNG) was reduced from $1.6x10^5$ ng/L (McLanahan et al., 2014; Clewell et al., 2007; Merrill et al., 2005) to $6x10^4$ ng/L (Schlosser, 2016). This lower value was shown to provide model predictions much more consistent with the radioiodide uptake inhibition data of Greer et al. (2002) for doses up to 100 µg/kg/d than those obtained with the former value for the Km.

Similar to the revision of the Km for perchlorate on the NIS in the thyroid, the Km for perchlorate transport in other tissues in which it is expressed and incorporated into the model, for the lactating mother and infant, and for the pregnant mother and fetus, were revised to match the new maternal value noted above. Other tissues with NIS-mediated transport described in the model include the skin, GI tissue, and mammary. The molecular form of NIS is not known to vary between the tissues in which it is expressed and the NIS-iodide Km is assumed to be the same in all tissues, hence a single value for NIS-perchlorate Km was used to be consistent with both the underlying biology and the iodide sub-model.

The second parameter to be revised, Vmax for perchlorate transport from the mammary plasma to the milk (VMAXC_MKP), also had a significant impact on model predictions. Analysis of perchlorate levels in human breast milk vs. urinary excretion from Kirk et al. (2013) showed a range in the milk:urine ratio of perchlorate among the subjects in that study. Based on the mean value of the ratio of milk concentration to 24-hour urinary excretion from Kirk et al. (2013), the VmaxC for perchlorate expression in breast milk, VMAXC_MKP, was increased by a factor of 2.24 from that used by Clewell et al. (2007) and McLanahan et al. (2014).

Perchlorate Clearance – Uncertainty

An uncertainty that was identified in the process of model evaluation, but appears to be of minor consequence, is that the terminal clearance half-life for perchlorate in the pregnancy and lactating mother PBPK models (McLanahan et al., 2014; Clewell et al., 2007) is much longer than that for the "average adult" (Merrill et al., 2005): ~ 56 h vs. 7 h. As shown in Appendix D, the difference in distribution parameters significantly affects the time course of perchlorate after exposure begins or ends, but does *not* affect the predicted blood perchlorate concentration at steady-state, which is the level used in evaluating the dose-response for perchlorate. Therefore, while we are uncertain about the validity of this significant life-stage difference in humans, that uncertainty has little impact on model predictions shown here. This difference could significantly impact the results of meal-by-meal ingestion simulations, vs. the continuous ingestion assumed here for simplicity.

Table 6: Physiological parameters

Parameter	Symbol [a]	Units	Value or Range	Source/notes
Initial Body Weight				
Nonpregnant female		kg	67.8	Clewell et al. (1999), Table 1, mean BW
Pregnant	BW_M	kg	72.3	Lumen et al. (2013)
Lactating	BWPOST	kg	74.6	Clewell et al. (2007); Gentry et al. (2002)
Infant	BW_N0	kg	3.512	Clewell et al. (2007; 2004), males
Body weight over time				
Pregnant female	BW_M	kg	72.3	Gestation week 40; Lumen et al. (2013); Hytten and Leitch (1971)
Lactating female	BW [a]	kg	74.6 – 71.6	Clewell et al. (2007); BW = BWPRE + Vft (increased fat) + (Vwholem-Vmampre) (increased mammary tissue)+ VMK (residual milk)
Infant	BW_N [a]	kg	3.512 – 5.6	Clewell et al. (2007; 2004), growth equation for males
Cardiac output				
Nonpregnant		L/h/kg$^{0.75}$	13.2	Adapted from Brown et al. (1997); provided for comparison
Pre-pregnancy	QCPRE	L/h	Calculated	Pre-pregnancy plasma flow, 55.7% of total blood flow; QCPRE = 0.557*QCC*BWPRE$^{0.75}$
Pregnant	QFC_M	L/h/kg$^{0.75}$	15.6	Lumen et al. (2013); Hytten and Leitch (1971)
Lactating	QCC	L/h/kg$^{0.75}$	15.35	QC = plasma flow then matches average results of Robson et al. (1987a) & Robson et al. (1987b), using plasma = 55.7% of total blood; QC varies in proportion to VF & VM, weighted by QFC and QMC, respectively
Infant	QCx_N [b]	L/h	Age (d) / Cardiac output (L/h): 0 / 30; 14 / 40.8; 60 / 55.8; 135 / 66; 180 / 66	Edginton et al. (2006); linear interpolation of QCC_N between points; infant plasma is 57.83% of total blood volume, so: QC_N = 0.5783*QCC_N(t);
Fractional blood flows				
Fat				
Nonpregnant		Unitless	0.085	Brown et al. (1997); Table 27, Females
Lactating	QFC [a]	Unitless	0.085	Brown et al. (1997);QF = QFC*QCPRE*VF/(VFC*BWPRE); QF/QC range = 0.094-0.104
Infant	Calculated [c]	Unitless	0.064-0.074	Clewell et al. (2007; 2004); QF_N = QFC*QC_N*VFC_N/VFC
Gastric tissue				
Nonpregnant		Unitless	0.21	Brown et al. (1997); Table 27, "Portal Vein" flow for Females
Lactating	QGC	Unitless	0.21	Brown et al. (1997); QG = QGC*BWPRE
Infant	Calculated [c]	Unitless	0.021-0.022	Clewell et al. (2007; 2004); QG_N = QGC*QC_N*VGC_N/VGC

Table 6: Physiological parameters, **Fractional Blood Flows** (cont'd)

Kidney				
Nonpregnant female		Unitless	0.17	Brown et al. (1997); Table 27, Females
Lactating	QKC	Unitless	0.17	Brown et al. (1997); QK = QKC*BWPRE
Infant	Calculated [c]	Unitless	0.27-0.31	Clewell et al. (2007; 2004); QK_N = QKC*QC_N*VKC_N/VKC
Liver				
Nonpregnant female		Unitless	0.06	Brown et al. (1997); Table 27, total liver flow minus portal vein flow for Females
Lactating	QLC	Unitless	0.06	Brown et al. (1997), Clewell et al. (2007); QL = QLC*BWPRE
Infant	Calculated [c]	Unitless	0.09-0.10	Clewell et al. (2007; 2004); QL_N = QLC*QC_N*VLC_N/VLC
Mammary gland (not present as separate compartment in infant or nonpregnant adult)				
Lactating	QM [a]	L/h	9.83	Geddes et al. (2012)
Rapidly perfused (RP) *(not present as separate compartment for iodide in the infant)*				
Nonpregnant female		Unitless	0.695	Brown et al. (1997); Table 27, Females, 100% minus % flow to slowly perfused tissue;
Pregnant	QFRP_M	Unitless	0.76	Lumen et al. (2013); QRP_M = (QFRP_M–QFTHY_M – QFPLC)*QCM; QRP_M/QCM = 0.644
Lactating-perchlorate	QRC	Unitless	0.695	Brown et al. (1997); Table 27, Females, 100% minus % flow to slowly perfused tissue. Flow to remaining RP tissue is: QR = (QRC–QMC)*QCPRE – QG – QT– QL– QK. Range of fraction flow (QR/QC) is 0.21-0.2125
Lactating-iodide	QRC	Unitless	0.695	Remaining RP tissue flow is: QR_I = (QRC–QMC)*QCPRE – QT, since thyroid is the only separate RP tissue for iodide. Range of fractional flow (QR_I/QC) is 0.64-0.65.
Infant (perchlorate only)	QRC_N	Unitless	0.657	Brown et al. (1997); Table 27, "PCMT", 100% minus % flow to slowly perfused tissue; Remaining RP flow is: QR_N = QRC_N*QC_N – QG_N – QTHY_N – QL_N – QK_N; Range of fractional flow (QR_N/QC_N) is: 0.20-0.26
Slowly perfused (SP) *(not present as a separate compartment for iodide in the infant)*				
Nonpregnant female			0.305	Brown et al. (1997); Table 27, Females, sum of adipose, bone, muscle, and skin
Pregnant-iodide	QFSP_M	Unitless	0.24	Lumen et al. (2013)
Pregnant-perchlorate	QFSP_M	Unitless	0.24	Lumen et al. (2013); Qsp_mp = QFSP_M – QFSK_MP since skin is separate
Lactating-perchlorate	QSC	Unitless	0.305	Brown et al. (1997); Clewell et al. (2007); Remaining SP flow (QS) is: (QSC – QFC – QSKC)*QCPre. Range of fractional flow (QS/QC) is 0.166-0.168
Lactating-iodide	QSC	Unitless	0.305	Brown et al. (1997); Clewell et al. (2007); fat & skin included for iodide, so QS_i = (QSC-QFc)*QCPre + QF. Range of fractional flow (QS_i/QC) is 0.311-0.319
Infant-perchlorate	QSC_N	Unitless	0.343	Brown et al. (1997); Table 27, "PCMT", Sum of flows for adipose, bone, muscle, & skin; QS_N = QSC_N*QCN – QSK_N – QF_N; Range of QS_N/QC_N = 0.19-0.21
Skin				
Nonpregnant female		Unitless	0.05	Brown et al. (1997); Table 27, Females
Pregnant-perchlorate	QFSK_MP	Unitless	0.058	Clewell et al. (2007)
Lactating	QSKC	Unitless	0.05	Brown et al. (1997); Table 27, Females; QSK=QSKC*QCPRE; QSK/QC ~ 0.049
Infant	--	Unitless	0.074-0.078	Clewell et al. (2007); QSK_N = QSKC*VSKC_N/VSKC

Table 6: Physiological parameters, **Fractional Blood Flows** (cont'd)

Thyroid				
Nonpregnant female		Unitless	0.015	Brown et al. (1997); Table 27, Females
Pregnant	QFTHY_M	Unitless	0.016	Lumen et al. (2013)
Lactating	QTC	Unitless	0.015	Brown et al. (1997); QT = QTC*QCPRE; Range of QT/QC = 0. 0146-0.0148
Infant	QTC_N	Unitless	0.016	Brown et al. (1997); Table 27, PCMT
Fractional Volumes				
Rest of body *Infant*	VbodyC_N	Unitless	0.92	Lumen et al. (2013); Used for iodide in infant to determine the rest of body volume: VBODY_N = VBODYC_N*BW_N – VTHY_N – VPLASMA_N
Fat				
Nonpregnant female	VFC	Unitless	0.327	Brown et al. (1997); Table 14, Female
Lactating (increase from nonpregnant) [a]	VFT	Unitless	T(h) / VFT: 0 / 0.0836; 4380 / 0	Gentry et al. (2002) for day 0; Gentry et al. (2003) for 6 months (4380 h); VF = (VFC + VFT(t))*BWPRE; Range of VF/BW = 0.35-0.37
Infant [a]	VFC_N	Unitless	0.244-0.286	Clewell et al. (2007), VF_N(t) from Haddad et al. (2001); VFC_N = VF_N(t)/BW_N
GI tract				
Nonpregnant female		Unitless	0.021	Brown et al. (1997); Tables 7 & 21; whole GI tract plus spleen & pancreas, for consistency with use of hepatic portal flow, which comes from all of those tissues
Lactating	VGC	Unitless	0.021	Brown et al. (1997); VwholeG = VGC*BWPre; range of VwholeG/BW = 0.019-0.02. For tissue only VG = VGC*(1-VGBC)*BWPRE; Range of VG/BW = 0.018-0.019
Infant (stomach only)	VGC_N	Unitless	0.0021	Clewell et al. (2007), Haddad et al. (2001); empirical function for VWHOLEG_N(t); VG_N(t) = VWHOLEG_N(t)*(1-VGBC_N); Range of VG_N/BW_N = 0.002-0.0021
GI tissue blood *(all)*	VGBC, VGBC_N	Unitless	0.041	Clewell et al. (2007), Merrill et al. (2005), extrapolated from Altman and Dittmer (1971); fraction of whole GI volume
Gastric juices *(all)*	VGJC	Unitless	0.00071	Clewell et al. (2007), Merrill et al. (2005), Licht and Deen (1988) (0.05 L/70 kg); VGJ = VGJC*BWPRE; VGJ_N = VGJC*BW_N
Kidney				
Nonpregnant female		Unitless	0.0044	Brown et al. (1997)
Lactating	VKC	Unitless	0.0044	Clewell et al. (2007);VK = VKC*BWPRE; Range of VK/BW = 0.0040-0.0042
Infant [a]	VKC_N	Unitless	0.007-0.008	Clewell et al. (2007), VK_N(t) from Haddad et al. (2001); VKC_N = VK_N/BW_N
Liver				
Nonpregnant female		Unitless	0.026	Brown et al. (1997)
Lactating	VLC	Unitless	0.026	Clewell et al. (2007);VL = VLC*BWPRE; Range of VL/BW = 0.024-0.025
Infant [a]	VLC_N	Unitless	0.039-0.045	Clewell et al. (2007), VL_N(t) from Haddad et al. (2001); VLC_N=VL_N/BW_N

Table 6: Physiological parameters, **Fractional Volumes** (cont'd)

Volume of milk	VMK	L	0.357	Kent et al. (2006); Daly et al. (1992)
Mammary tissue	VMAMC	Unitless	0.0174	Fraction of pre-pregnancy body weight (ICRP, 1975); lumped with other richly perfused for distribution, but used in tissue blood volume calculation
Mammary blood	VMBC	Unitless	0.276	Fraction of total mammary tissue volume; set equal to blood fraction in thyroid (VTBC), from (Brown et al., 1986)
Plasma				
Nonpregnant female		Unitless	0.044	Altman and Dittmer (1971)
Pregnant	VFPLS_M	Unitless	0.052	Lumen et al. (2013)
Lactating	VPLASC	Unitless	0.044	Clewell et al. (2007), Altman and Dittmer (1971); VPLAS = VPLASC*BWPRE; VPLAS/BW range = 0.040-0.042
Infant	VPLASC_N	Unitless	0.048	Clewell et al. (2007); Brines et al. (1941)
Red blood cells				
Nonpregnant female		Unitless	0.035	Merrill et al. (2005); Marieb (1992); Altman and Dittmer (1971)
Pregnant-perchlorate	VRBCC_MP	Unitless	0.035	Clewell et al. (2007)
Lactating	VRBCC	Unitless	0.035	Clewell et al. (2007); VRBC = VRBCC*BWPRE; VRBC/BW range = 0.032-0.033
Infant [a]	VRBCCC_N	Unitless	0.035	Clewell et al. (2007)
Richly perfused (RP)				
Nonpregnant female		Unitless	0.192	Brown et al. (1997), Tables 21: Sum of brain, GI, heart, kidneys, liver, lung, "rest of body" and blood
Pregnant	VFRP_M	Unitless	0.19	Brown et al. (1997), all RP
Lactating	VRC	Unitless	0.192	Brown et al. (1997), Clewell et al. (2007; 2004); VR = VRC*BWPRE –VTTOT–VL–VK – VWHOLEG–VMAMPRE–VBLOOD; VR/BW range=0.051-0.053
Infant-perchlorate (remaining RP only) [a]	VRC_N	Unitless	0.032-0.033	Clewell et al. (2007; 2004); Calculated, VRC_N = 2.596*VGIT_N/BW_N, where VGIT_N is the age-based volume of the total GI tract from Haddad et al. (2001)
Slowly perfused (SP)				
Nonpregnant female		Unitless	0.794	Brown et al. (1997); Table 21, sum of adipose, bone, muscle, and skin
Pregnant	VFSP_M	Unitless	0.793	Brown et al. (1997), all SP
Lactating	VSC	Unitless	0.794	Brown et al. (1997), Clewell et al. (2007);VS = VSC*BWPRE – VWHOLESK –VFPRE; VS/BW range = 0.39-0.41
Infant (remaining SP only) [a]	VSC_N	Unitless	0.40-0.46	Clewell et al. (2007); Calculated, VSC_N = 0.92 – (all other tissues, including remaining richly perfused, liver, kidney, blood, thyroid, stomach, stomach contents, skin, & fat)

Table 6: Physiological parameters, **Fractional Volumes** (cont'd)

Skin				
Nonpregnant female		Unitless	0.037	Brown et al. (1997)
Pregnant-perchlorate	VFSK_MP	Unitless	0.037	Brown et al. (1997)
Lactating	VSKC	Unitless	0.037	Clewell et al. (2007); VwholeSk = VSkC*BWPre, VwholeSk/BW range = 0.034-0.035; skin tissue only VSK = VSKC*(1 – VSKBC)*BWPRE; VSK/BW range = 0.031-0.032
Infant [a]	VSKC_N	Unitless	*0.053-0.055*	VWHOLESK_N(t) from Haddad et al. (2001); VSKC_N = VWHOLESK_N(t) /BW_N; skin tissue only, VSK_N = VWHOLESK_N(t)*(1 – VSKBC_N), Range of VSK_N/BW_N range = 0.051-0.053
Blood fraction of skin				
Nonpregnant female		Unitless	0.08	Brown et al. (1997)
Pregnant-perchlorate	VFSK_MP	Unitless	0.037	Clewell et al. (2007)
Lactating	VSKBC	Unitless	0.08	Brown et al. (1997); VSKB = VSKBC*VSKC* BWPRE
Infant	VSKBC_N	Unitless	0.08	Assumed same as adult
Thyroid (total volume)				
Nonpregnant female		Unitless	3×10^{-4}	Brown et al. (1997)
Pregnant	VFTHY_M	Unitless	2.2×10^{-4}	Lumen et al. (2013)
Lactating	VTTOTC	Unitless	2.5×10^{-4}	Yokoyama et al. (1986); VTTOT=VTTOTC*BWPRE, VTTOT/BW range = $2.27\text{-}2.36\times10^{-4}$; thyroid tissue only: VT=VTTOTC*(1 – VTBC)*BWPRE; VT/BW range = $1.64\text{-}1.71\times10^{-4}$
Infant [a]	VTTOTC_N	Unitless	$2.82\text{-}3.95\times10^{-4}$	Clewell et al. (2007); Ogiu et al. (1997); calculated from empirical function, VTG_N = $1.3857\times10^{-3} + 6.3\times10^{-5}\times$months (grams); VTTOTC_N = VTG_N$\times10^{-3}$ /BW_N; for tissue only, VT_N= VTG_N*(1-VTHYBC_N); Range of VT_N/BW_N = $3.34\text{-}2.39\times10^{-4}$
Blood fraction of thyroid				
Rat		Unitless	0.18	Brown et al. (1997)
Pregnant (human)	VFTHYB	Unitless	0.276	Lumen et al. (2013)
Lactating (human)	VTBC	Unitless	0.276	Brown et al. (1986), Table 2; average for decades 3-6 (i.e., ages 20-59) of the sum of mean % volume for stroma & vessels; VTB = VTBC* VTTOTC*BWPRE
Infant	VTHYBC_N	Unitless	0.153	Clewell et al. (2007)
Urine volume				
Pregnant	VURINE	L/d	1.5	Lumen et al. (2013); Thorp et al. (1999)
Lactating	VURINE	L/d	1.453	Fisher et al. (2016), Table S1 based on NHANES 2009-12[e]
Infant [a]	VURINEC_N	L/h/kg	0.005	Sillén (2001); Estimated based on 4-h infant void volume at 3 months of age

[a] Calculated by equation over time.

[b] Entered in model code as table function. Values between time points are linearly interpolated.

[c] Calculated based on neonate to adult volume of tissue

[d] Changes are based on changes in body weight over time relative to tissue

[e] Centers for Disease Control & Prevention (CDC), National Center for Health Statistics (NCHS). National Health and Nutrition Examination Survey Data Hyattsville, MD: U.S. Department of Health and Human Services, Centers for Diseases Control and Prevention; 2009-2012. Available from: http://www.cdc.gov/nchs/nhanes/nhanes_questionnaires.htm.

Table 7: Iodide kinetic parameters

Parameter	Model Symbol	Units	Value		Source
Ingestion					
Formula ingestion rate	VFORMULAC	L/h/kg	Time (d)	Value	Fomon et al. (1964)
			0	0.00829	
			31	0.00789	
			61	0.00725	
			91	0.00658	
Breast milk ingestion rate	KTRANS	L/d/kg	Time (d)	Value	Arcus-Arth et al. (2005); Casey et al. (1986); Hofvander et al. (1982)
			0	0.013	
			1	0.013	
			2	0.04	
			3	0.098	
			4	0.14	
			5	0.155	
			30	0.154	
			60	0.148	
			90	0.13	
Formula concentration	FORMULAUGL_I	µg/L	~ 100-400		Pearce et al. (2004)

Table 7: Iodide kinetic parameters (cont'd)

Urinary clearance				
Pregnant mother	CLF_UIM	L/h/kg$^{0.75}$	0.17	Lumen et al. (2013) (model fit)
Lactating	CLUx_I [a]	L/h	Time (d) / Value: 0 / 5; 14 / 4; 42 / 4; 90 / 3	Fisher et al. (2016); model fit to predict central tendency for Leung et al. (2012) data; values initially from Aboul-Khair et al. (1964), 3.9±0.6 L/h (mean ± SE) at GW 39, then 3.1±0.4, 2.2±0.2, and 2.1±0.1 (mean ± SE) at postnatal weeks 2, 6, and 12, respectively.
Infant	CLUCx_NI [a]	L/h/kg	Time (d) / Value: 0 / 0.044; 12.5 / 0.05; 20.8 / 0.055; 89.5 / 0.06; 180 / 0.066	Fisher et al. (2016): values at d=0 and d=180 calculated from Ponchon et al. (1966); functions predictions were smoothed out by adding transitions between the two urinary excretion estimates.
Thyroid Euthyroid Iodide Stores				
Pregnant mother	(n/a)	--	--	Term not included in model
Lactating	CTGIPOOL [a]	nmol/kg thyroid	1.13×10^{7}	Fisher et al. (2016): set to yield > 15 mg thyroidal iodide stores
Infant	CTGIPOOL_N [b]	nmol/g thyroid	1717	Fisher et al. (2016): set so that the total pool equals amount predicted in fetus at end of pregnancy (Lumen et al., 2013) for 250 µg/d maternal iodide.
Binding for organified iodide in thyroid				
Pregnant mother	(n/a)	--	--	Binding rate set = net uptake of iodide
Lactating	KBINDC_I [a]	L^{1}nmol^{-1} h^{-1} kg-thyroid$^{-0.75}$	1×10^{-4}	Model fit to predict thyroid stores near the maximal concentration for euthyroid condition [a]
Infant	KBINDC_NI [b]	L^{1}nmol^{-1} h^{-1} kg-thyroid$^{-0.75}$	0.1	Fisher et al. (2013); model fit, scaled to thyroid weight because of growth of the thyroid gland

Table 7: Iodide kinetic parameters (cont'd)

Maximal thyroidal uptake				
Pregnant mother	VmaxNISF_THY_mi	nmol/h/kg$^{0.75}$	3800	Lumen et al. (2013) (model fit)
Lactating	Vmaxthyx_i [a]	nmol/h	Time (d) / Value: 0 / 96500; 14 / 70875; 42 / 46620; 90 / 38430	Aboul-Khair et al. (1964) for PND 14, 42, and 90. Value at PND 0 calculated using the allometric coefficient from Lumen et al. (2013) and the post pregnancy body weight for the lactating mother: 3800x(BWpost^0.75).
Infant	VmaxthyCx_Ni [b]	nmol/h/kg	Time (d) / Value: 0 / 4763; 21 / 2060; 90 / 2060	Estimated from Ponchon et al. (1966) [b]
Thyroid Michaelis-Menten constant				
Pregnant mother	KMNIS_I	nmol/L	3.15×10^4	Lumen et al. (2013) (literature data)
Lactating	KMTHY_I [a]	nmol/L	3.15×10^4	Set to pregnancy value [a]
Infant	KMTHY_NI [b]	nmol/L	3.15×10^4	Set to pregnancy value [b]
Maximal milk uptake (n/a for pregnant female and infant)				
Lactating	VMAXCx_MKI [a]	nmol/h/kg	1.05×10^4	Model fit [a]
Milk Michaelis-Menten constant (n/a for pregnant female and infant)				
Lactating	KMMk_I [a]	nmol/L	3.15×10^4	Set to thyroid value [a]

Table 7: Iodide kinetic parameters (cont'd)

Scaled permeability surface area				
Thyroid				
Pregnant mother	PAFTHY_MI	L/h/kg$^{0.75}$	1×10^{-4}	Lumen et al. (2013) (previous model fit)
Lactating	PATHYC_I [a]	L/h/kg$^{0.75}$	1×10^{-5}	Model fit [a]
Infant	PATHYC_NI [b]	L/h/kg$^{0.75}$	1×10^{-2}	Model fit [b]
Milk				
Lactating mother	PAMKC_I [a]	L/h/kg$^{0.75}$	1×10^{-3}	Model fit I [a]
Partition Coefficients				
Milk: plasma	PMK_I	unitless	1.0	Clewell et al. (2007); Merrill et al. (2005)
Thyroid: plasma				
Pregnant mother	PTHY_MI	unitless	0.15	Clewell et al. (2007); Merrill et al. (2005)
Lactating	PTHY_I	unitless	0.15	Clewell et al. (2007); Merrill et al. (2005)
Infant	PTHY_NI	unitless	0.15	Set to maternal value
Rest of body: plasma (n/a for pregnant and lactating *mother*)				
Infant	PBODY_I [b]	unitless	0.21	Estimated; between maternal richly and slowly perfused values
Richly perfused: plasma (n/a for infant)				
Pregnant mother	PRP_MI	unitless	0.4	Lumen et al. (2013) (model fit)
Lactating	PRP_I	unitless	0.4	Clewell et al. (2007); Merrill et al. (2005)
Slowly perfused: plasma (n/a for infant)				
Pregnant mother	(n/a)	unitless	0.18	Lumen et al. (2013) (model fit)
Lactating	PS_P	unitless	0.18	Set to pregnancy value

[a] Information on data source and calibration in table 5
[b] Information on data source and calibration in table 4

Table 8: Thyroid hormone kinetic parameters

Parameter	Model Symbol	Units	Value	Source
Thyroxine (T4)				
Volume of Distribution				
Pregnant mother	VDFT4_MI	L/kg	0.121	Lumen et al. (2013) (model fit)
Lactating	VDT4C [a]	L/kg	0.084	Estimated based on Lumen et al. (2013)
Infant	VDT4C_N	L/kg	0.31	Cottino et al. (1961)
T4 Production in thyroid				
Pregnant Female	KPRODT4F_MI	$h^{-1}kg^{-0.75}$	2.45×10^{-6}	Lumen et al. (2013) (model fit)
Lactating mother	KPRODT4C[a]	$h^{-1}kg^{-0.75}$	1.1×10^{-6}	Estimated based on Lumen et al. (2013)
Infant	VPRODT4CX_N [b]	nmol/h/kg	Time (d) / Value: 0 / 0.535; 4 / 0.535; 7 / 0.375; 28 / 0.3218; 30 / 0.3218; 180 / 0.3218	Set equal to T4 utilization reported from Fisher (1996)
Total T4 metabolism				
Pregnant mother	KDEGT4F_MI	$h^{-1}kg^{-0.75}$	1.9×10^{-4}	Lumen et al. (2013) (model fit)
Lactating	KMETT4C	$h^{-1}kg^{-0.75}$	1.4×10^{-4}	Model fit
Infant	CLMETT4CX_N [b]	L/h/kg	Time (d) / Value: 0 / 0.0035; 10 / 0.0023; 30 / 0.0020; 90 / 0.0020	Estimated clearance

Table 8: Thyroid hormone kinetic parameters (cont'd), Fractional conversion of T4 to T3

Pregnant Female	(set in equations)	unitless	0.5	Lumen et al. (2013) (model fit)
Lactating mother	T3FRAC	unitless	0.5	Set to pregnancy value
Infant	T3FRAC_N [b]	unitless	Time (d) / Value: 0 / 0.5; 5 / 0.5; 6 / 0.55; 7 / 0.6; 30 / 0.7; 180 / 0.8	Estimated, fraction of T4 metabolism going to T3 vs. rt3. Day 0 value from Lumen et al. (2013) for fetus; days 5-30 fit to infant T3 data (Lem et al., 2012; Williams et al., 2004; Elmlinger et al., 2001; Franklin et al., 1985) and to qualitatively match declining trend for rT3/T4 from Chopra et al. (1975); day 180 value set based on ontogeny of iodothyronine deiodinases (near adult activity at 6 months of age) from Richard et al. (1998).
Free fraction of total T4				
Pregnant Female	FRCONVT4_MI	unitless	9×10^{-5}	Lumen et al. (2013) (literature data estimate)
Lactating mother	FRCONVT4	unitless	1×10^{-4}	Fisher et al. (2016): Slightly increased from 0.9×10^{-4}; (Lumen et al., 2013) to 1.0×10^{-4}
Infant	FRCONVT4_N [b]	unitless	1.7×10^{-4}	Fit to data from Lem et al. (2012)
Urinary clearance fT4 (not specified in pregnant female; composite urinary/fecal clearance given below)				
Lactating mother	CLUFT4C [a]	L/h/kg$^{0.75}$	0.6	Fisher et al. (2016): Estimated for free T4
Infant	CLUFT4C_N [b]	L/h/kg$^{0.75}$	0	Amount not quantitatively significant
Fecal elimination (not specified in pregnant female; composite urinary/fecal clearance given below)				
Lactating mother	CLFECESFT4C[a]	L/h/kg$^{0.75}$	2	Fisher et al. (2016): Estimated for *free* T4
Infant	CLFECEST4C_N [b]	L/h/kg$^{0.75}$	2×10^{-4}	Fisher et al. (2016): Estimated for *total* T4
Milk clearance fT4				
Lactating mother	CLMILKFT4C[a]	L/h/kg$^{0.75}$	0.3	Fisher et al. (2016): Estimated for *free* T4
Composite urinary/fecal clearance for Total T4				
Pregnant Female	CLFT4_mi	L/h/kg$^{0.75}$	1.85×10^{-4}	Lumen et al. (2013) (model fit)
Lactating mother	(n/a)	L/h/kg$^{0.75}$	2.61×10^{-4}	Sum corrected for free fraction
Infant	(n/a)	L/h/kg$^{0.75}$	2×10^{-4}	Sum corrected for free fraction

Table 8: Thyroid hormone kinetic parameters (cont'd),
TRIIODOTHYRONINE (T3)

Volume of Distribution				
Pregnant mother	VDFT3_MI	L/kg	0.46	Lumen et al. (2013) (model fit)
Lactating	VDT3C [a]	L/kg	0.47	Fisher et al. (2016)
Infant	VDT3C_N	L/kg	0.304	Lumen et al. (2013) (model fit for fetus)
T3 Production in thyroid				
Pregnant mother	KPRODT3F_MI	$h^{-1}kg^{-0.75}$	2.2×10^{-7}	Lumen et al. (2013) (model fit)
Lactating	KPRODT3C [a]	$h^{-1}kg^{-0.75}$	4×10^{-7}	Increased from 1/11 T4 production [a]
Infant	$VPRODT3F_N * VPRODT4Cx\_N$ [b]	nmol/h/kg	Time (d) / Value: 0: 0.0487; 4: 0.0487; 7: 0.0341; 28: 0.0293; 30: 0.0293; 180: 0.0293	Assumed 1/11 production ratio T4:T3 from Lumen (2013)
Total T3 metabolism				
Pregnant mother	KDEGT3F_MIt	$h^{-1}kg^{-0.75}$	Pdoseμg_i (μg/d) / Value: 50: 9.9×10^{-4}; 75: 2.6×10^{-3}; 100: 1.425×10^{-3}; 150: 1.6×10^{-3}; 200: 1.67×10^{-3}; 250: 1.705×10^{-3}; 300: 1.72×10^{-3}; 350: 1.725×10^{-3}	Lumen et al. (2013) (model fit); *function of iodide dose-rate*
Lactating	KMETT3C	$h^{-1}kg^{-0.75}$	2.5×10^{-3}	Model fit
Infant	CLMETT3CX_N [b]	L/h/kg	Time (d) / Value: 0: 3.5×10^{-3}; 10: 2.3×10^{-3}; 30: 2.0×10^{-3}; 90: 2.0×10^{-3}	Fisher et al. (2016): Estimated [b]

Table 8: Thyroid hormone kinetic parameters (cont'd),

Urinary clearance T3 (not specified in pregnant female; composite urinary/fecal clearance given below; not included for infant)				
Lactating mother	CLUT3C_I [a]	L/h/kg$^{0.75}$	3×10^{-3}	Fisher et al. (2016): Estimated [a]
Fecal elimination (not specified in pregnant female; composite urinary/fecal clearance given below)				
Lactating mother	CLFECEST3C [a]	L/h/kg$^{0.75}$	5×10^{-3}	Fisher et al. (2016): Estimated [a]
Infant	CLFECEST3C_N [b]	L/h/kg$^{0.75}$	9×10^{-4}	Fisher et al. (2016): Estimated [b]
Milk clearance				
Lactating mother	CLMILKT3C [a]	L/h/kg$^{0.75}$	2×10^{-4}	(*Lactating mother only*) Estimated
Composite clearance for Total T3				
Pregnant mother	CLFT3_MI	L/h/kg$^{0.75}$	2.7×10^{-3}	Lumen et al. (2013) (model fit)
Lactating	(n/a)	L/h/kg$^{0.75}$	8.1×10^{-3}	Sum milk, urine, fecal
Infant	(KFECESCT3_N)	L/h/kg$^{0.75}$	9×10^{-4}	Fecal only

[a] Information on data source and calibration in table 5.
[b] Information on data source and calibration in table 4.

MODEL CALIBRATION RESULTS

The figures below show the model comparison with the data that was used for calibrating the model parameters in both the formula-fed and breast-fed infant exposure scenarios. The parameters that were calibrated from model fits are listed in **Table 4** for the infant and **Table 5** for the lactating mother. The values for the parameters are given in **Table 7** and **Table 8** for iodide and the thyroid hormones, respectively. While comparisons of model predictions to data for infants less than 7 days old are shown, the model was not calibrated to those data because of the rapid changes in TSH levels which occur during this period (Lem et al., 2012). Overall, the model predictions were considered adequate.

First, model predictions of urinary iodide concentrations for the formula-fed infant were compared with data from Gordon et al. (2014) and Cao et al. (2010). The model simulations were performed based on formula concentrations of iodine reported by Pearce et al. (2004) and perchlorate by Schier et al. (2010). Urinary iodide predictions were not sensitive to perchlorate exposure levels, so for these simulations, the geometric mean perchlorate concentration (0.92 µg/L) of all formulas analyzed by Schier et al. (2010) was used for the iodide comparisons (**Figure 4**, top panel). The model-predicted urinary iodide concentration for formula-fed infants with iodine concentrations at the lowest reported value of 108 µg/L by Pearce et al. (2004) were within the range of literature-reported values (**Figure 4**) from Gordon et al. (2014) and from Cao et al. (2010) for both cow milk-based and soy-based formula. Model-predicted urinary concentrations of iodide for the average formula iodine concentration (159 µg/L) also were consistent with Gordon et al. (2014) and cow formula data from Cao et al. (2010) but were higher than the majority of values for soy formula. However, the reported iodine concentrations for soy formula were 108 and 117 µg/L from Pearce et al. (2004), and therefore, the average iodine concentration of 159 µg/L likely does not reflect the iodide intake of infants consuming

soy formula in the Cao et al. (2010) study. The model-predicted urinary iodide concentrations with formula iodide = 384 µg/L, the highest reported iodine formula concentration from Pearce et al. (2004), were only within the higher range of values for infants consuming cow formula from Cao et al. (2010). This higher formula concentration of iodine was greater than 2 standard deviations from the mean of the 8 formulas analyzed by Pearce et al. (2004).

For perchlorate, data for urinary concentrations were only available for formula-fed infants from Cao et al. (2010). To cover the range of literature-reported perchlorate concentrations, simulations are shown for 0.18 µg/L (minimum measured by Schier et al. (2010)), 0.92 µg/L (geometric mean of values for both soy and cow milk formulas from Schier et al. (2010)), and 4.1 µg/L (maximum value from Pearce et al. (2004), which is higher than the maximum measured by Schier et al. (2010)). Model-predicted concentrations of perchlorate were within the reported values for cow and soy formulas (**Figure 4**, bottom panel).

For the breast-fed infant, model predictions of urinary iodide were compared with literature data (**Figure 5**) from Gordon et al. (2014) and Cao et al. (2010). Because the actual breastmilk iodide concentrations were not reported in the studies, the data was compared with a range of iodine intakes. Model simulations only varied slightly for perchlorate exposures up to 350 ng/kg/d (1/2 the RfD; results not shown); very small differences could be seen in simulation plots but these were well within the variation among the data. Since average (mean) perchlorate exposures were previously estimated to be between 80 and 123 ng/kg/d for women of childbearing age in different subgroups (U.S. EPA (2008)[8], **Table 4**), an approximate overall average perchlorate intake of 100 ng/kg/d was used while evaluating fits to the iodine data. The

[8] https://www.gpo.gov/fdsys/pkg/FR-2008-10-10/html/E8-24042.htm

model-predicted urinary iodide concentrations for breast-fed infants were lower than the majority of the literature reported values at postnatal days < 45 but were within the range of reported values for later simulated postnatal days.

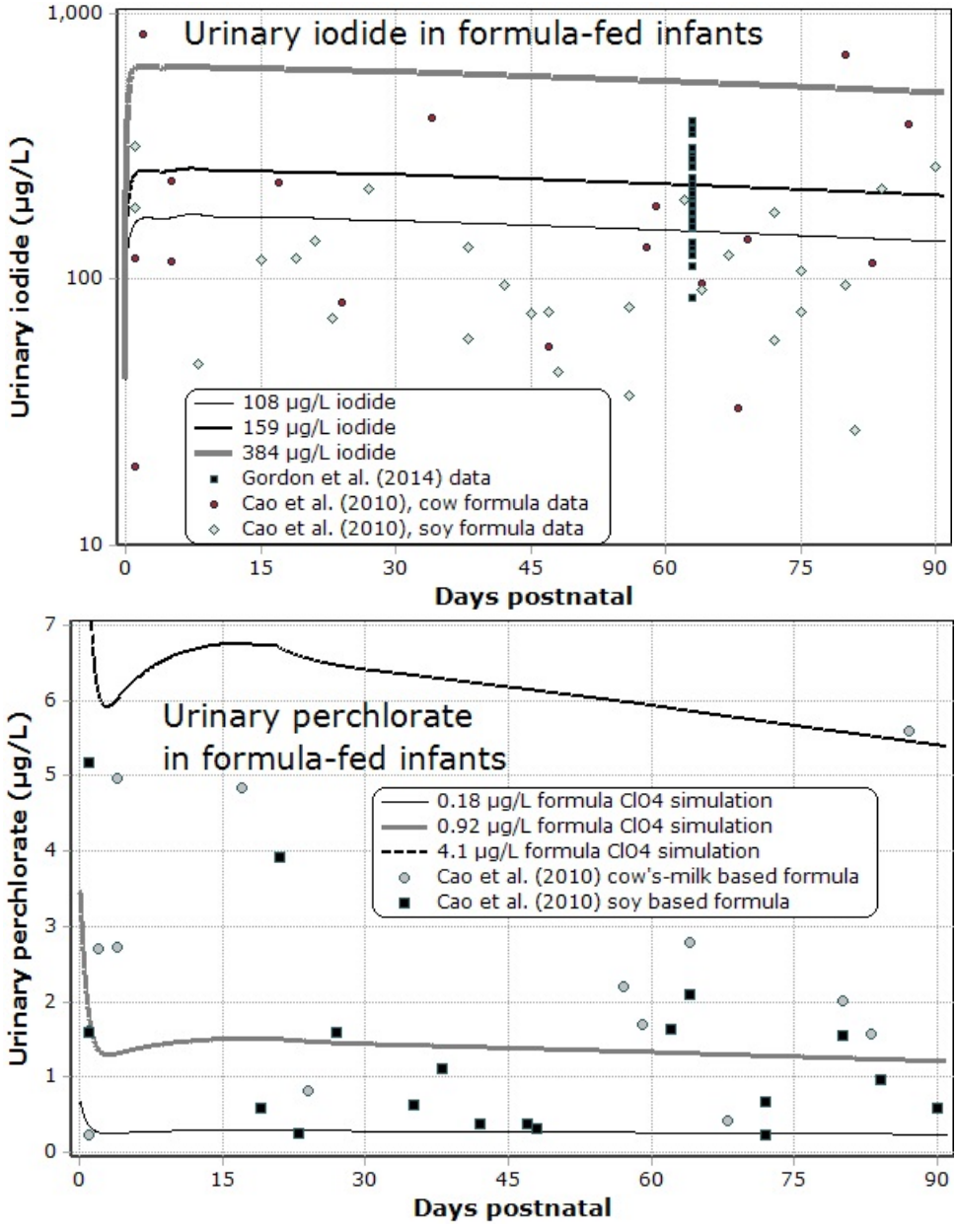

Figure 4. Comparison of formula-fed model simulations of urinary iodide and perchlorate with literature data.

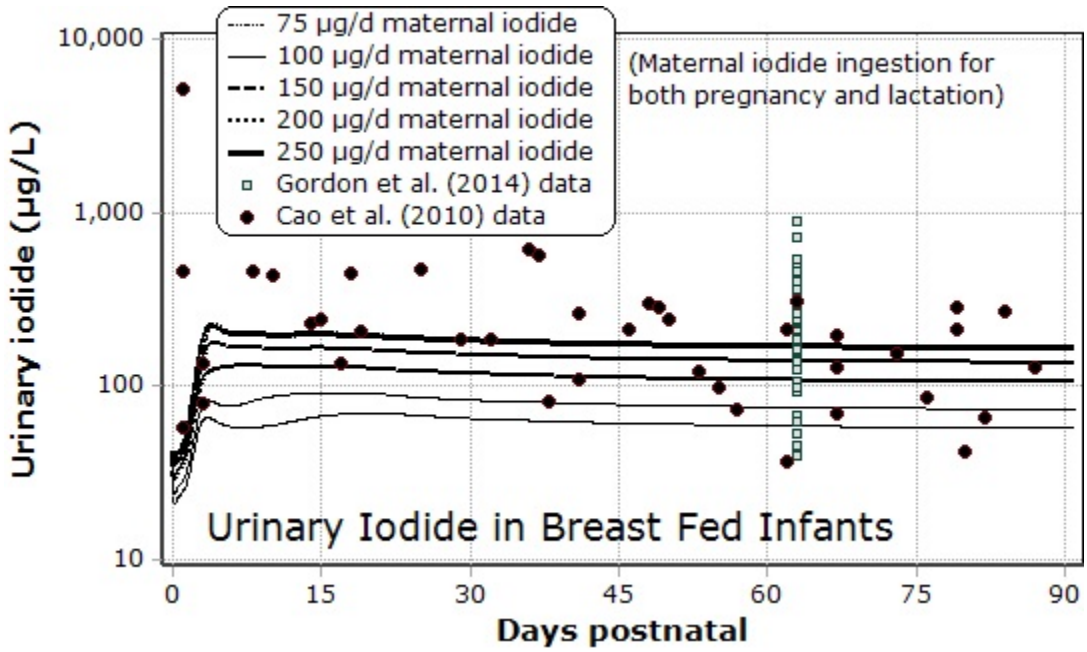

Figure 5. Model simulations for breast-fed infant from a range of maternal iodine intakes and 100 ng/kg/d perchlorate compared with literature data for urinary concentrations of iodide.

Maternal iodine and perchlorate exposures from Cao et al. (2010) and Gordon et al. (2014) are unknown. Data from Cao et al. (2010) are weekly averages obtained separately for boys (*n* = 1-5 per point) and girls (n = 2-6). Data from Gordon et al. (2014) are for 30 individual formula-fed children. Iodine and perchlorate exposures are unknown for both studies.

The model-predicted urinary perchlorate concentrations were within the range of reported values for birth to 90 days for both formula-fed and breast-fed infants, when perchlorate daily intake was assumed to vary between 0.001 and 1 µg/kg/d (**Figure 6**; data from Leung et al. (2012) and Cao et al. (2010)). Since iodine intake between 75 and 250 µg/day had no effect on predicted urinary perchlorate levels, the iodine intake by the lactating mother was assumed to be 150 µg/day for the perchlorate comparison: a low level within the plausible range for which the model is assumed valid. Since iodine intake has very little effect on predicted perchlorate kinetics, the exact choice of iodine level is not important for this comparison.

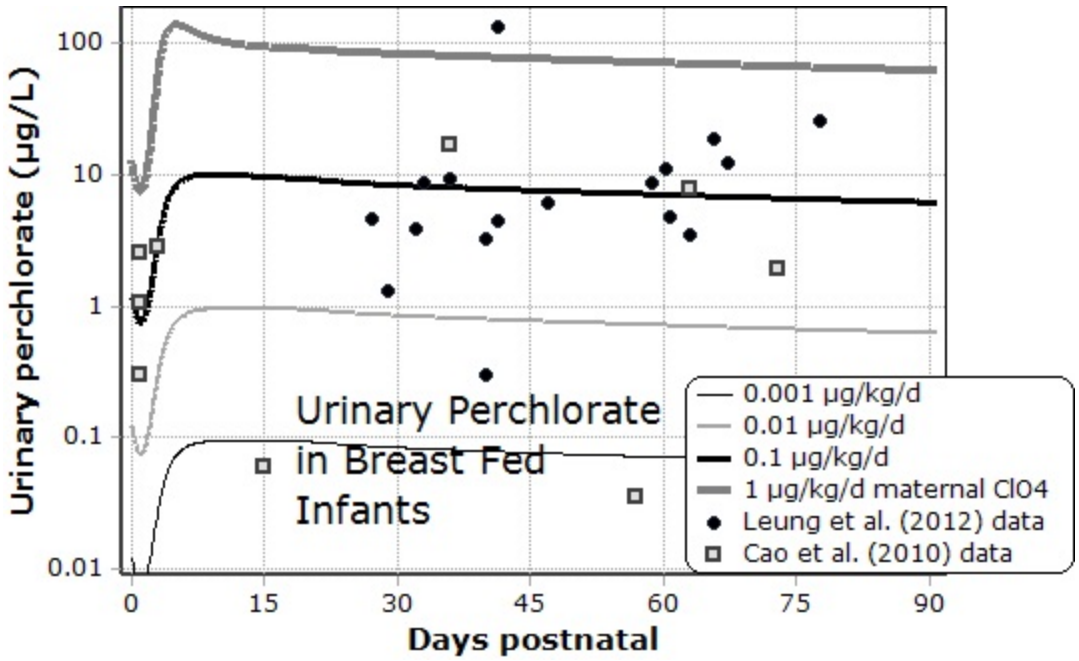

Figure 6. Model simulations for the breast-fed infant from a range of maternal perchlorate levels (with 150 µg/d dietary iodine) compared with literature data for urinary perchlorate.

Maternal iodine and perchlorate exposures from Cao et al. (2010) and Leung et al. (2012) are unknown.

Note: it has been suggested that iodide levels are not stable in breast-milk samples (Dr. Benjamin Blount, CDC, personal communication), which may explain in part why many of the data in **Figure 7** (also see **Figure 17**) are well below the model simulations for 150 and 250 µg/d maternal iodine intake. The predictions for the lowest iodine intake of 75 ug/d are closer to the middle of the range of the data.

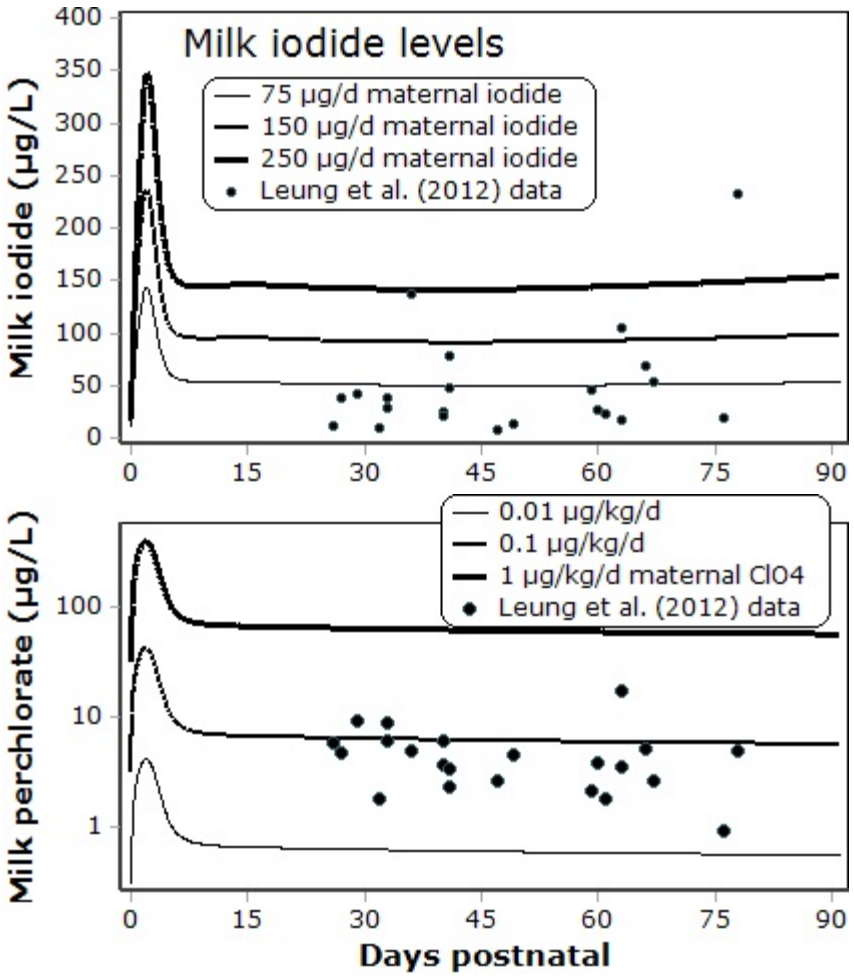

Figure 7. Model simulated iodide and perchlorate breast milk concentrations compared with data of Leung et al. (2012).

Data are from an observational study where the iodine and perchlorate exposures are unknown.

For comparison with literature data on thyroid hormones, the model predictions were performed only for lactating mothers (**Figure 8**) and breast-fed infants (**Figure 9**) because literature data was not specifically available for formula-fed infants. The model predictions of T4 and fT4 were

compared with data obtained from NHANES (NHANES, 2007-2008, Thyroid Profile[9] and NHANES 2009-2010, Thyroid Profile[10]) for the lactating mother. Model-predicted infant serum T4 concentrations were compared with data from Lem et al. (2012), and model-predicted serum fT4 were compared with data from Lem et al. (2012), Leung et al. (2012), and Cao et al. (2010). Because the maternal intakes of iodine from the literature sources are not known, the model simulations were performed at maternal iodine intakes ranging from 75 to 250 µg/d with perchlorate exposure of 100 ng/kg/d. Model-predicted concentrations of T4 and fT4 in both lactating mothers and breast-fed infants were within the range of literature reported values. Model-predicted concentrations of T4 and fT4 for maternal intake of 75 µg/d iodine differed significantly from the iodine intakes of 150-250 µg/d. As shown in the dose-response section, for maternal iodide intake of 100 µg/d, maternal fT4 was predicted to be near or slightly below the bottom of the reference range (when the reference range is calculated relative to model predictions for 200 µg/d iodide). And for maternal intake of 75 µg/d iodide, maternal fT4 is predicted to be in or below the hypothyroxinemic range; i.e., in the range of hypothyroidism. However, infant fT4 levels were predicted to be still within the reported reference intervals for normal infants, ages 7 to 90 days (Lem et al., 2012), for maternal intake as low as 75 µg/d iodide.

[9] http://wwwn.cdc.gov/nchs/nhanes/2007-2008/THYROD_E.htm
[10] http://wwwn.cdc.gov/nchs/nhanes/2009-2010/THYROD_F.htm

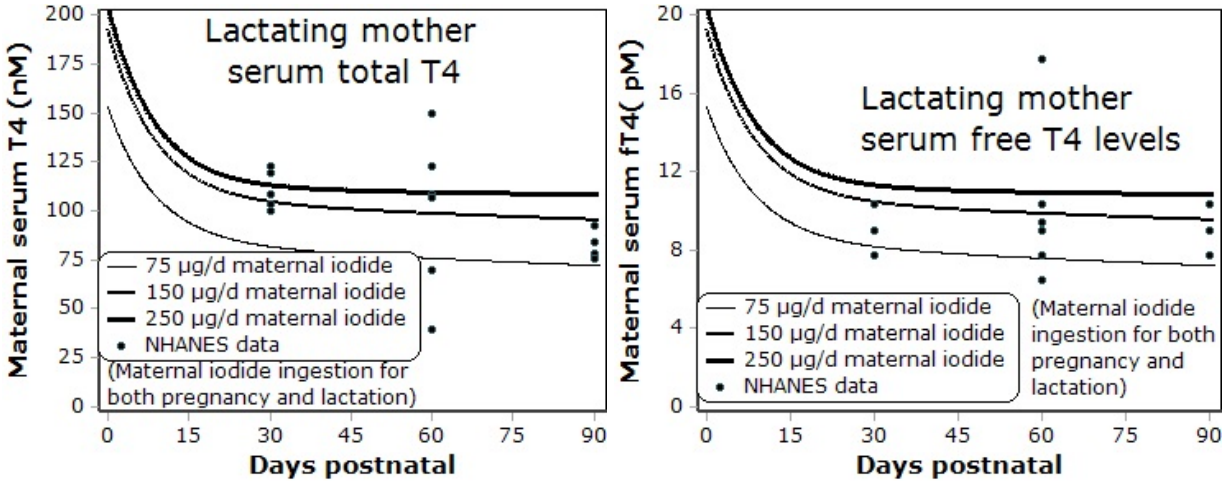

Figure 8. Comparison of model-simulated plasma T4 concentrations with experimental data for the lactating mother.

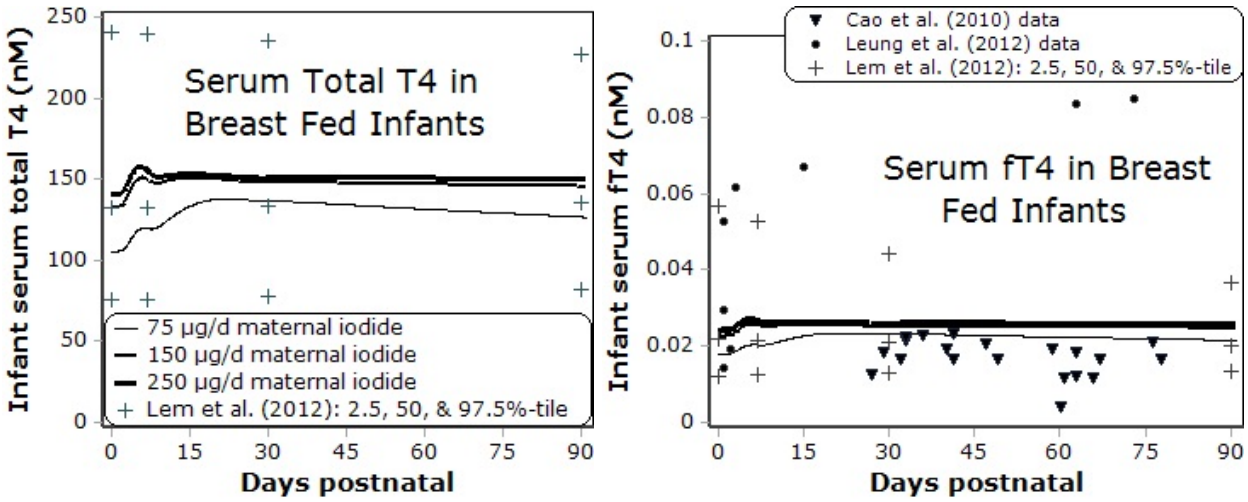

Figure 9. Comparison of model-simulated plasma T4 concentrations with experimental data for the breast-fed infant.

Data are from observational studies where the iodine and perchlorate exposures are unknown. Simulated iodine intakes are the same for both plots.

SUMMARY OF CALIBRATION RESULTS

Overall, the model predictions were considered adequate based on the comparison of model simulations to data in **Figure 4**-**Figure 9**. Because the only source of variability evaluated was iodide and perchlorate dietary intake, it is not expected that the model will capture the entire observed variability. Variability in physiological factors, such as urinary excretion rate and body

weight, and biochemical factors, such as NIS expression and thyroid hormone synthesis activity, would also have significant effects on the metrics evaluated. However model predictions should roughly match average values in each data set and variability in ingestion should account for some of the observed variability. Variability should be more completely described for perchlorate than iodide, since there is a wider range of possible perchlorate ingestion levels and perchlorate metabolism is not a source of variability.

The urinary iodide concentrations for formula-fed infants were within the upper range of literature-reported values (**Figure 4**, top panel). Compared with iodide, fewer urinary perchlorate data were available; however, the model-predicted perchlorate urinary concentrations were within the range of reported values for birth to PND 90 for both formula-fed and breast-fed infants (**Figure 4**, bottom panel, and **Figure 6**). The model-predicted urinary iodide concentrations were within the range of reported values for PND 45 to PND 90 for breast-fed infants, although the predicted values were generally in the upper range of reported values for earlier postnatal days (**Figure 5**). The model-predicted iodide concentrations in milk for maternal intake of ≥ 150 µg/d iodine were within the observed range of literature-reported values; although the majority of values were lower than model predictions (**Figure 7**, top panel).

The model-predicted concentrations of serum T4 and fT4 in both lactating mothers and breast-fed infants were within the range of literature reported values (**Figure 8** and **Figure 9**). The model-predicted concentrations of serum T4 and fT4 for maternal intake of 75 µg/d iodide were clearly lower than those predicted for maternal iodine intakes of 150-250 µg/d, but were still within the reported ranges for normal infants, PND 7 to 90 (**Figure 9**).

DOSE-RESPONSE EVALUATION

This section provides the results for the model's behavior, specifically dose-response predictions for the effect of perchlorate on thyroid hormones. Because the model sets the ratio of T4 to fT4 in serum at a constant proportion, the dose-response for one will parallel the other. Therefore, serum fT4 levels were evaluated as a measure of response. Provisional reference and hypothyroxinemic ranges for each life stage, and postnatal day, were identified as described in the Technical Introduction. Hypothyroxinemia is assumed to ensue when fT4 drops to the 10^{th} population percentile for each life stage (and PND), and hypothyroidism to ensue at the 2.5^{th} percentile. These ranges, including the specific values for the percentiles, require evaluation during the peer review, and revision may occur. They are used here to illustrate how the BBDR model can be used in conjunction with identified fT4 levels that mark the boundary of adversity.

The range of perchlorate exposures over which the model is valid was discussed in the Technical Introduction, and can be evaluated based on both model predictions of radio-iodide uptake inhibition and perchlorate-induced changes in T4 or fT4. Comparing the predictions with the perchlorate sub-model to the radio-iodide uptake data of Greer et al. (2002) (for adults, the lactating mother, see Appendix B) indicated that the model is only accurate for prediction of iodide uptake inhibition up to an exposure level of ~ 100 µg/kg/d perchlorate. However, dose-response analyses were restricted to 0-20 µg/kg/d perchlorate, since that is the range of interest and likely application, and because predicted ft4 levels for many of the life-stages and iodine intake levels fell below their respective reference levels in this range. Predicted fT4 levels below the hypothyroxinemic range (i.e., to the point that TSH is expected to be significantly elevated) will be shown with grey, to emphasize their uncertainty. *When model predictions fall below that*

range, hypothyroid conditions are assumed to occur and TSH feedback is activated, at which point the model is no longer valid.

For this evaluation, perchlorate doses were set at specific normalized ingestion rates, µg/kg/d of perchlorate for the formula fed infant or breast feeding mother (the amount of perchlorate transferred to the breast-fed infant is a model prediction), rather than being set based on a given water concentration and water ingestion rate. The analysis provided here is a straightforward dose-response evaluation separated from the issues of water ingestion or exposure. This approach allows the results to be compared directly with the existing RfD (0.7 µg/kg/d) and point-of-departure (7 µg/kg/d).

Pregnant Mother and Fetus

First, dose-response results for the pregnant mother and fetus at gestation week 40 are shown in **Figure 10**. As described previously, the Km for perchlorate interaction with NIS was changed in the pregnancy model to match the value now used for the lactating mother and infant. Therefore these results do not match those reported by Lumen et al. (2013).

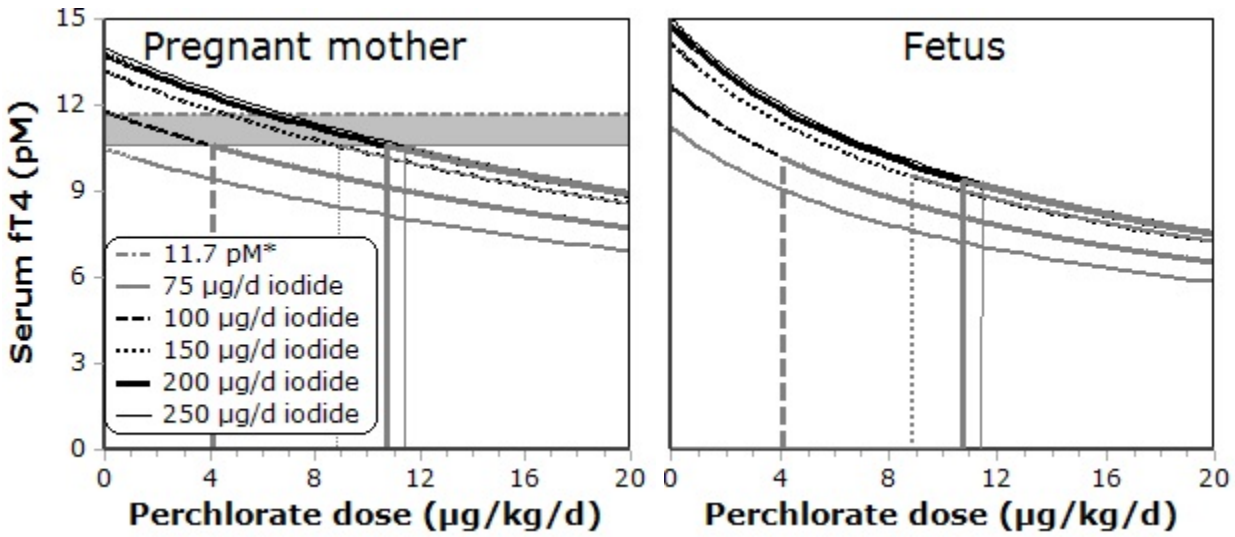

Figure 10. Free T4 (fT4) levels as a function of perchlorate exposure and dietary iodine ingestion in the gestation-week 40 pregnant mother and fetus.

The horizontal line at 11.7 pM marks the bottom of the applied reference range for the pregnant mother. The grey band (11.7-10.6 pM) shows the assumed range for maternal hypothyroxinemia. (There is not a defined range for the fetus.) Results are shown in grey for both mother and fetus when the maternal level is below 10.6 and vertical lines mark the points at which maternal fT4 falls below 10.6 ppm, hence the model becomes uncertain. These are drawn at the same perchlorate concentrations for the fetus as the mother, since the predictions for the fetus also become uncertain at those concentrations. Simulation results are shown in grey for both the mother and fetus when the maternal level is below 10.6 pM.

Formula Fed Infant

The next set of results are for the formula-fed infant (**Figure 11** and **Figure 12**) with an iodine concentration of 108 µg/L (the lowest level measured among 8 infant formulas by Pearce et al. (2004). In **Figure 11** the maternal iodide refers to the level of the mother's ingestion during pregnancy, which was used to set the initial condition. For these simulations, the pregnant mother also was assumed to have received the same level of perchlorate exposure (in µg/kg/d) as the infant did after birth; for example the results for 4 µg/kg/d perchlorate exposure in infants in **Figure 11** assumed that the mother was exposed to 4 µg/kg/d perchlorate during pregnancy, and the results from that pregnancy simulation were used to set the initial condition for these infant simulations. The bottom of the reference range is defined as the predicted 10^{th} percentile using the lambda-mu-sigma (LMS) transformed Gaussian (normal) distribution derived from Lem et al. (2012) as described in the Technical Introduction (Table 1) and Appendix E. The age-specific mean was defined by model predictions for a formula-fed infant ingesting formula with an average concentration of 159 µg/L iodine (average of 8 formulas reported by Pearce et al. (2004)), born to a mother who ingested 200 µg/d iodide during pregnancy; i.e., an infant with fully sufficient iodine levels and intake. The skewness (λ) and age-specific variance (σ) estimated from the results of Lem et al. (2012) (**Table 1**) were applied with this model-derived age-dependent mean.

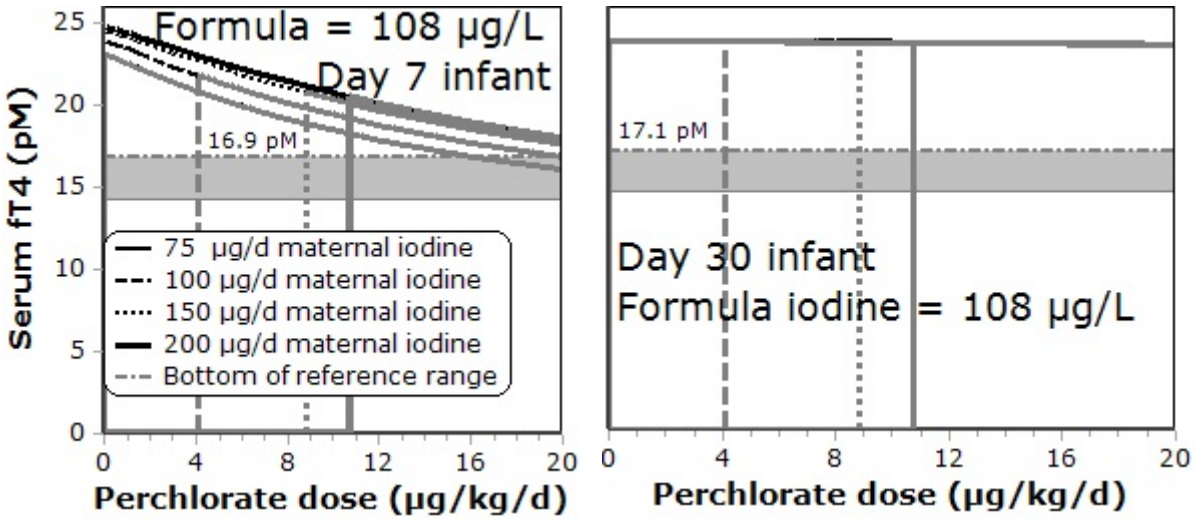

Figure 11. Free T4 in the formula fed infant at 7 and 30 days of age, given 108 µg/L iodine in formula, as a function of perchlorate exposure and maternal iodine ingestion during gestation.

The vertical lines mark the perchlorate exposure from Figure 10 where the pregnant mother's fT4 levels were predicted to fall below 10.6 pM (below the range of hypothyroxinemia) and model simulations are shown in grey beyond those points to emphasize their uncertainty. For 75 ug/d maternal iodine the pregnant mother's fT4 is below 10.6 pM with no perchlorate exposure, so the vertical line is at the y axis and the entire line is grey. For day 30 the results for all maternal iodine levels are indistinguishable, so the uncertainty with regard to the initial condition is reduced, assuming there is no long-term effect on the infant's thyroid function if the mother becomes hypothyroid during pregnancy. The grey band shows the assumed range for infant hypothyroxinemia.

However, as seen in **Figure 10**, a range of perchlorate exposures was predicted to cause the pregnant mother's fT4 levels to fall below the range of hypothyroxinemia for iodine intake between 100 and 250 µg/d, at which point the BBDR model is not valid. Hence the initial conditions for an infant whose mother ingested 150 µg/d iodine and was exposed to 9 µg/kg/d perchlorate, for example, is uncertain. The vertical lines in **Figure 11** mark the corresponding perchlorate exposure levels, and the model curves for 100-250 µg/d iodide to the right of these points are colored grey to emphasize this uncertainty. Since the pregnant mother is predicted to be hypothyroid when ingesting only 75 µg/d iodine, even with zero perchlorate exposure, the entire corresponding infant curve in **Figure 11** is colored grey.

Assuming that there are no longer term effects on infant's thyroid function, **Figure 11** illustrates that maternal iodine ingestion during pregnancy has only a modest effect on the results for the formula-fed infant at day 7, and by day 30 the initial iodide condition has almost no effect. The simulation results are sufficiently above the bottom of the infant's reference range to accommodate some uncertainty. Results for days 60 and 90 are likewise independent of initial iodide (not shown). For consistency with the analyses for other life stages, the subsequent plots (for higher formula iodine levels) used the 200 µg/d maternal iodine initial condition to set the initial condition for the formula-fed infant.

Figure 12 shows that the range of iodine levels in formula is sufficient to almost entirely offset the effects of perchlorate exposure at 30, 60, and 90 days: the predicted serum concentrations of fT4 were essentially independent of perchlorate exposure and there was very little difference among predictions for 108-384 µg/L iodine. As in **Figure 11**, the initial condition in these simulations was set assuming the same perchlorate exposure in the pregnant mother, hence to the fetus, as to the formula-fed infant, but with a fully sufficient maternal iodine intake (200 µg/d). The steeper dose-response for the day 7 infant was therefore the result of that in utero exposure with border-line inadequate maternal iodine ingestion.

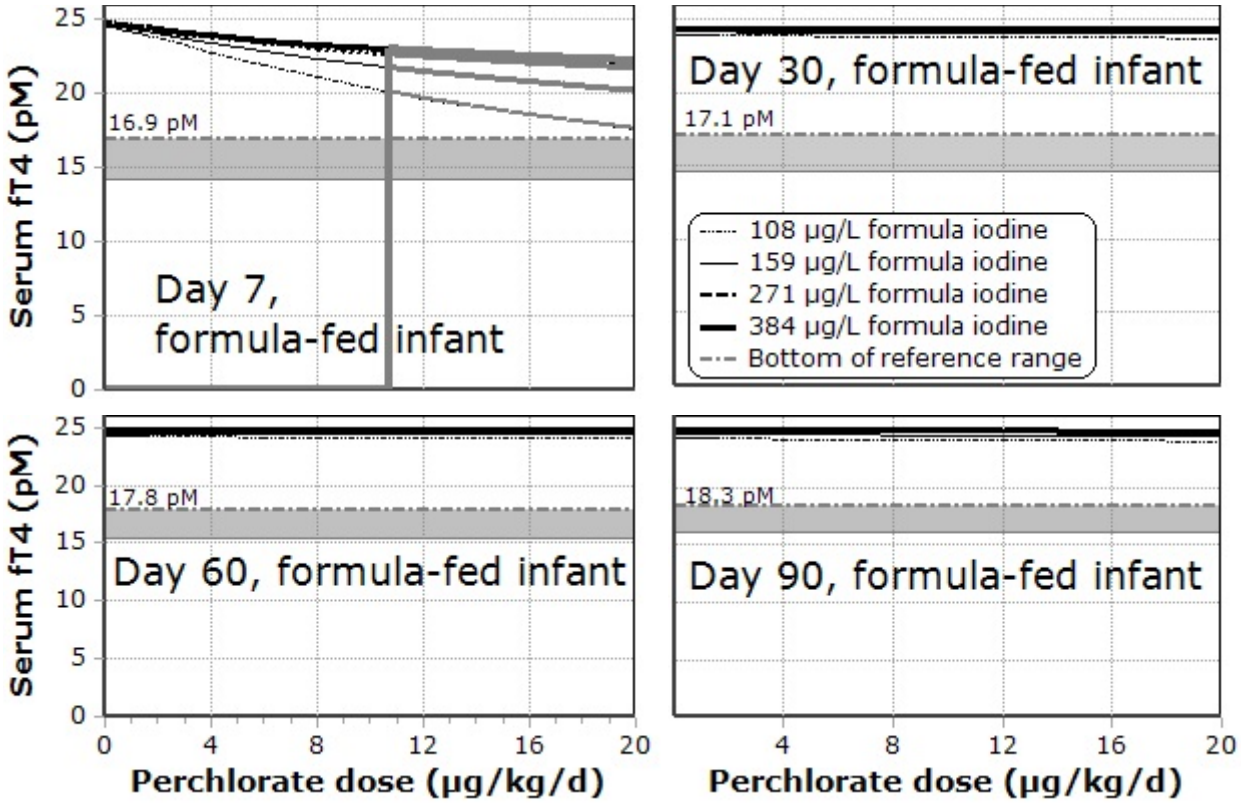

Figure 12. Free T4 in the formula fed infant, given varying iodine in formula, as a function of perchlorate exposure.

Levels of perchlorate, iodine, and thyroid hormones at birth are set assuming the same perchlorate dose to the pregnant mother and 200 µg/d iodine. The vertical line at 8.85 µg/kg/d on the day 7 plot is the level at which the pregnant mother's fT4 level falls below her reference range (i.e., below 10.6 pM), making model predictions for higher perchlorate levels uncertain. So predictions for higher perchlorate levels above that point are shown in grey. Initial conditions had little effect on model predictions for days 30-90. The grey band shows the assumed range for infant hypothyroxinemia.

Breast-Fed Infant

Results for the breast-fed infant are shown in **Figure 13**. The iodine (and perchlorate) levels given are for the mother both during pregnancy and lactation.

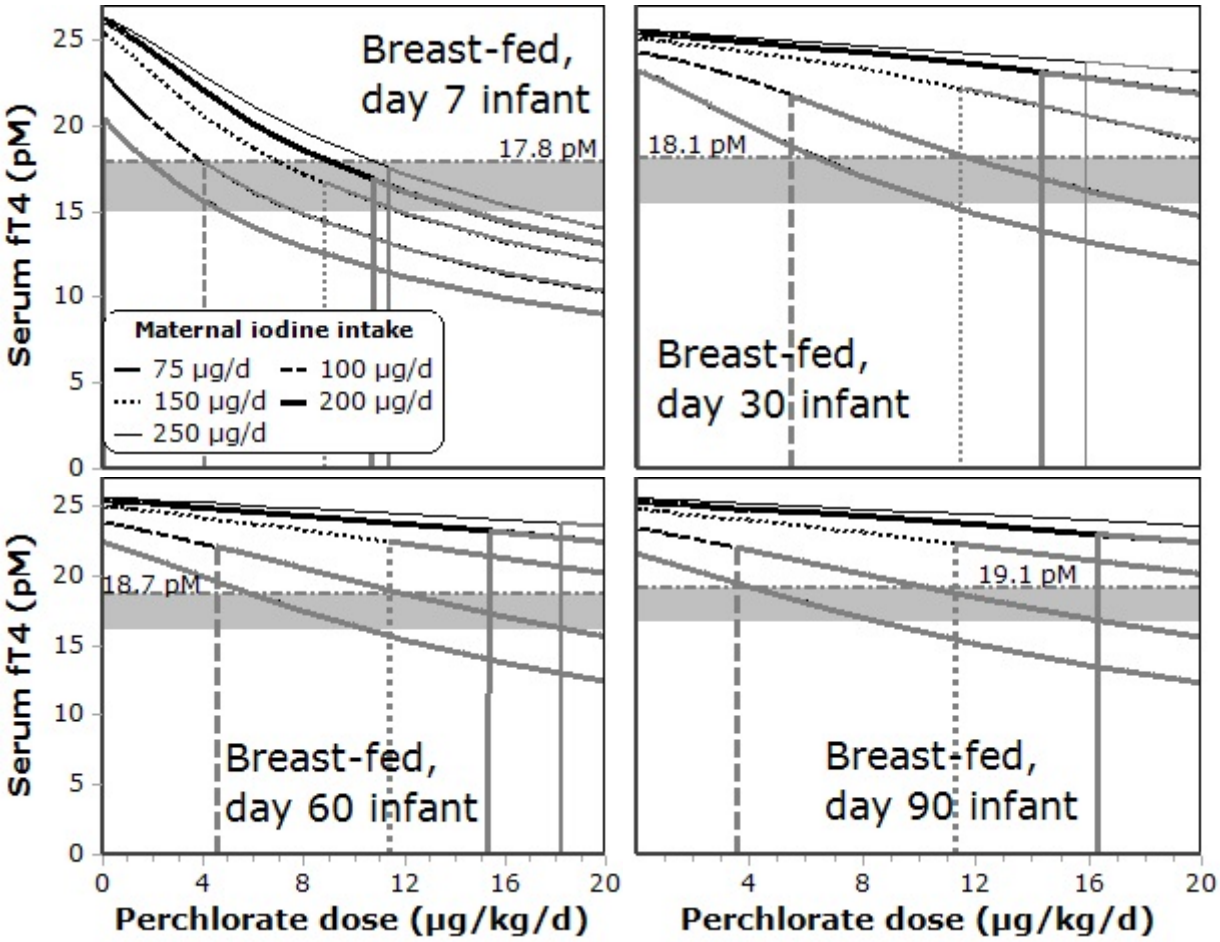

Figure 13. Free T4 (fT4) in the breast-fed infant, as a function of maternal perchlorate exposure and iodine ingestion.

Levels of perchlorate, iodide, and thyroid hormones at birth were set assuming the same perchlorate and iodide doses to the mother (and fetus) when pregnant. The grey horizontal band shows the range over which hypothyroxinemia is assumed to occur, from the 10th percentile of the age-dependent reference distribution (labelled by that fT4 level) down to 2.5th percentile. The vertical lines mark the perchlorate level beyond which the lactating mother falls below her hypothyroxinemic range, making the model predictions uncertain for perchlorate exposure above that point. Simulation results for the out-of-range conditions (perchlorate levels higher than the vertical lines) are in grey.

For the day *7* infant the vertical lines mark the perchlorate exposures above which model predictions become uncertain, because fT4 levels in the pregnant mother are predicted to be below the hypothyroxinemic range. For days 30-90 the vertical lines are the exposure levels at which the lactating mother's fT4 levels fall below her hypothyroxinemic range (details in next section). Hence the predicted iodide (and T4) levels in the ingested breast milk become uncertain above those perchlorate levels, and the model curves are shown in grey. Since the infant's fT4 levels are predicted to fall into the infant's hypothyroxinemic range (and below) for some of the iodine and perchlorate levels that were simulated, the potential impact of this uncertainty is more significant.

To better understand why the breast-fed infant shows such a strong dose-response compared to the formula-fed infant, the time-evolution of serum fT4 levels in the breast- and formula-fed infants for various levels of maternal and formula iodine ingestion, respectively, with no perchlorate exposure is shown in **Figure 14**. The formula-fed infant simulations assumed a low level of iodine nutrition during pregnancy, 75 µg/d. Despite that low initial value, even the lowest formula iodine that was simulated (108 µg/L) is sufficient for a quick recovery of the infants to levels near those for breast-fed infants whose mothers consume higher iodine levels: 150-250 µg/d (**Figure 14**). But breast-fed infants of mothers who consume 100 µg/d or less iodine have slightly lower predicted levels of fT4, which declines incrementally from about PND 30 onward (**Figure 14**). The predicted decrement is modest when the mother consumes 100 µg/d iodine, but the iodide levels are no longer saturating thyroid hormone production: the decrease from 150 to 100 µg/d iodine (50 µg/d difference) has very little effect on fT4, but a further decrease of only 25 µg/d iodine (from 100 to 75 µg/d) more significantly affects the infant's fT4 levels (**Figure 14**). Thus, the iodide levels and thyroidal stores for infants whose mothers ingest only 100 µg/d iodine are already compromised (though they may be sufficient for healthy

development in the absence of perchlorate), hence these infants are more sensitive to any further decrease due to inhibition by perchlorate (Figure 13).

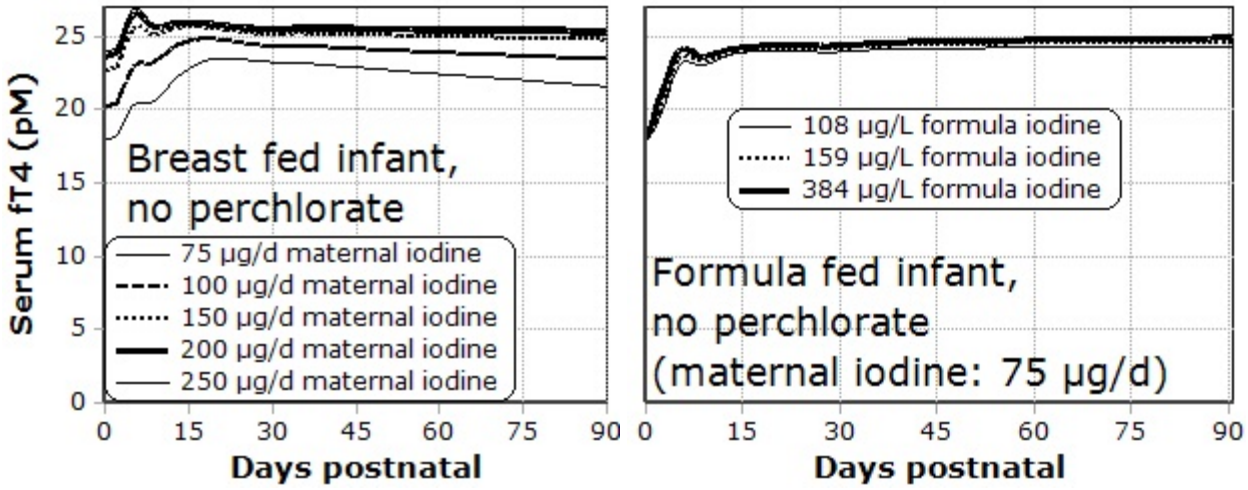

Figure 14. Evolution of serum fT4 over time in breast-fed and formula fed infants with no perchlorate exposure, but varying levels of iodine ingestion.

Another way to understand the sensitivity of formula- vs. breast-fed infants is to compare their intake of iodine vs. perchlorate on a µg/kg/d basis. For infants ingesting formulas with iodine concentrations of 108 µg /L or 384 µg/L the average iodine dose over the 90 day postnatal period is predicted to be 20 or 69 µg/kg/d, respectively. This iodine dose rate is equal or > 3 times a perchlorate exposure of 20 µg/kg/d (maximum evaluated here). For breast-fed infants whose mothers ingested 20 µg/kg/d perchlorate, the predicted average daily doses of perchlorate is 183 µg/kg/d; i.e., 9 times higher than direct maternal exposure to 20 µg/kg/d. Further, while the predicted iodide concentration in breast milk was ~ 140 µg/L for a mother ingesting 250 µg/d iodine averaged, comparable to the infant formulas, when a mother ingests only 75 µg/d iodine, the predicted iodide concentration in her milk was only ~ 50 µg/L, much lower than available in formulas.

Perchlorate exposure in the range of 0-20 µg/kg/d also reduces breast milk iodide levels by a few percent. When the mother ingests 75 µg/d iodine and 20 µg/kg/d perchlorate, the model predicts that the infant only gets an average of 7.6 µg/kg/d iodine and that between birth and day 90 the infant ingests 25 ng perchlorate per µg of iodide. When the mother ingests 200 µg/d iodine and 20 µg/kg/d perchlorate, the model predicts that the infant gets an average of 17.5 µg/kg/d iodine and that the infant ingests 11 ng perchlorate per µg of iodide between birth and day 90. While this difference in relative dose is only a bit more than 2-fold, for breast milk or formula iodine concentrations above 100 µg/L infant T3 and T4 production becomes saturated, so a small decrement in iodine uptake by the formula- or breast-fed infant thyroid has little effect on T3 and T4 levels. But when the milk (or formula) concentration is below 100 µg/L iodine, the infant's TH levels become more sensitive to perchlorate.

Lactating Mother

Predicted changes in free T4 in the lactating mother due to perchlorate exposure are shown in **Figure 15**. The control maternal fT4 levels (i.e., for zero perchlorate exposure) in **Figure 15**, depended on maternal iodide intake, including iodine ingestion during pregnancy. There was a clear decrease in control maternal fT4 between day 7 and 30; control maternal fT4 levels increased somewhat between days 30, 60, and 90, but remained below the day 7 levels. This reduction in control levels was likely due to iodide excretion in breast-milk.

Figure 15 also shows that iodine ingestion levels during pregnancy and lactation (75-250 µg/d) have a larger effect on maternal fT4 levels than the range of formula iodine concentrations (108-384 µg/L) had on the PND 30-90 formula-fed infant (**Figure 12**). This difference in sensitivity to the iodine ranges exists across the range of perchlorate doses analyzed. (Recall that the internal iodide and TH levels at PND 0 are determined by ingestion during pregnancy.) The

predicted sensitivity of the lactating mother to perchlorate exposure exists precisely because her fT4 levels are sensitive to iodine intake, hence iodide availability to the thyroid.

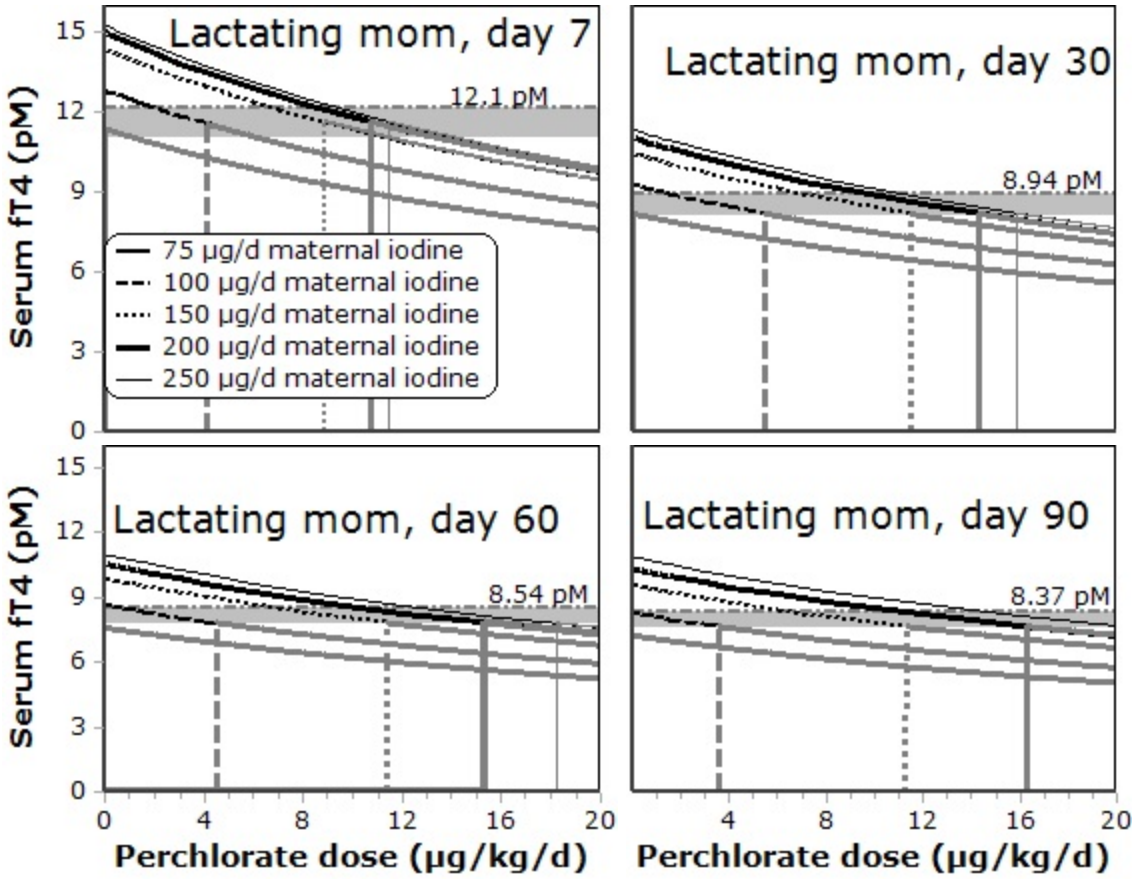

Figure 15. Free T4 (fT4) in the lactating mother, as a function of maternal perchlorate exposure and iodine ingestion.

Levels of perchlorate, iodide, and thyroid hormones at birth were set assuming the same perchlorate and iodine doses to the mother when pregnant. The annotated horizontal lines mark the bottom of the provisional reference range for each PND. They grey bar marks the assumed range for hypothyroxinemia.

For day 7 the vertical lines mark the perchlorate levels for which the *pregnant mother* is predicted to fall below 10.6 pM, i.e., below the range of hypothyroxinemia, making the initial conditions for the lactation model uncertain. This uncertainty is assumed to impact the day 7 predictions, so simulation curves above the corresponding perchlorate levels are shown in grey.

For days 30-90 the vertical lines mark perchlorate levels where the *lactating mother* falls below the range of hypothyroxinemia (varies with PND), so the model is no longer valid and simulations become uncertain. Simulations above those levels are shown in grey.

While the pregnant mother's predicted fT4 levels in the absence of perchlorate (**Figure 10**) were higher than the lactating mother, except for PND 7 (**Figure 15**), the dose-response curve was steeper for the pregnant mother than for the lactating mother on PND 30-90. The dependence on iodine intake (i.e., the degree to which the curves for different iodine levels are separated in **Figure 10** and **Figure 15**) was similar for both life-stages, however. Hence it is not immediately clear whether the pregnant mother or the lactating woman is the more "sensitive" life-stage. The dose-response for the lactating mother on PND 7 likely reflects the effect of exposure during pregnancy to a large extent, hence the similarity in the shape of the relationship to perchlorate exposure: it is transitional between that for pregnancy and later in lactation. The fetus' dose-response was slightly stronger than the mother. This likely occurred because the model contains three avenues of impact on fetal T4: 1) perchlorate inhibits the uptake of iodide and hence T4 production by the fetal thyroid; 2) perchlorate inhibits the transfer of iodine into the placenta (hence iodine availability to the fetus); and 3) perchlorate reduces T4 in mother and thus the transfer of T4 to the fetus[11].

A detailed analysis of the dosimetry of perchlorate and iodide to the lactating mother and breast-fed infant vs. the formula-fed infant is provided in Appendix C. In summary, the µg/kg/d of iodine ingested by the lactating mother at her intake levels (less than 250 µg/d) is about 7-fold lower than the formula-fed infant. The much stronger effect predicted for the breast-fed infant compared with a formula fed infant receiving the same perchlorate dose (µg/kg/d) as the breast-feeding mother is assumed to be due to two factors: 1) significant concentration of perchlorate

[11] The pregnancy model predicts that the late-gestation fetus gets ~ 20% of its T4 from the mother.

in breast-milk; and 2) lower iodine ingestion by the infant from breast-milk when maternal iodine ingestion is low (which is further reduced by perchlorate, Figure 16) vs. from formula.

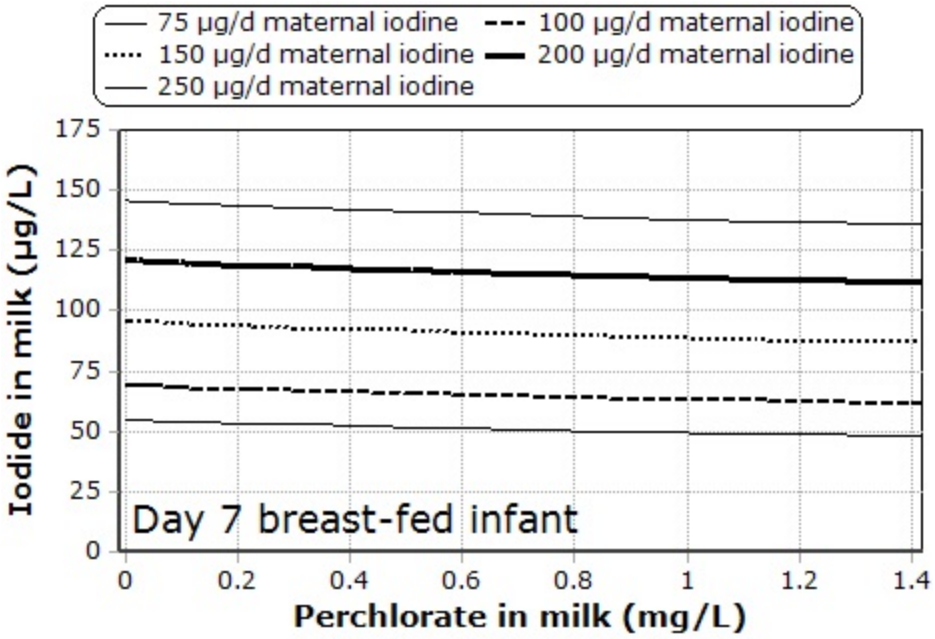

Figure 16. Predicted perchlorate vs. iodide concentrations in breast milk for varying maternal iodide ingestion levels.

While the dose-effect of perchlorate on lactational transfer of iodide described in Appendix C (Figure C-2; **Figure 16**) may seem stronger than expected, compared to epidemiological sampling studies (e.g., Leung et al. (2012)), it should be noted that the perchlorate dose-range evaluated here is much larger than most environmental exposures.

To confirm that the model predicts reasonable levels of breast milk iodide and is consistent with epidemiological studies, model results were compared to the data of Leung et al. (2012) who measured perchlorate and iodide in breast milk samples from breast-feeding mothers, mostly in the range of 30-90 days postnatal in **Figure 17**. The left-hand panel of Figure 17 shows milk iodide vs. milk perchlorate, with simulations for PND 60. In the range of perchlorate levels observed by Leung et al. (2012) the model predicts almost no effect of perchlorate on milk iodide levels, which agrees with the lack of trend obtained for those data.

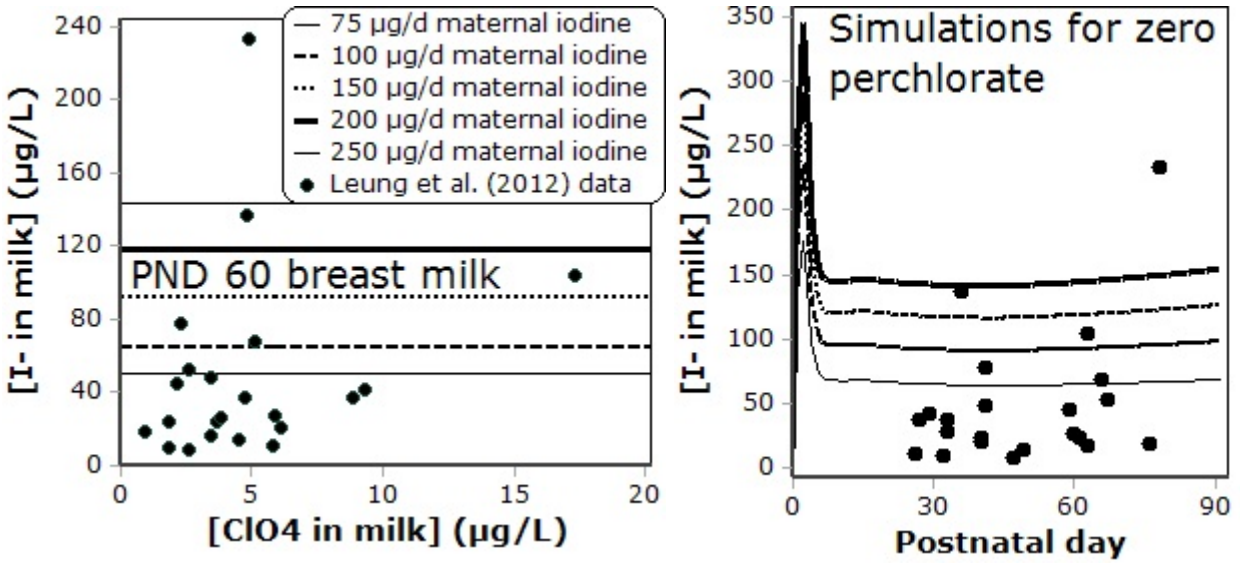

Figure 17. Model predictions of iodide vs. perchlorate concentrations in breast milk at low perchlorate exposure levels (left panel) and iodide in milk vs. PND with zero perchlorate exposure (right panel), for varying levels of maternal iodide ingestion.

The right-hand panel of **Figure 17** shows model predictions vs. PND for zero perchlorate, to illustrate that neither do the data show nor does the model predict a significant time-dependence in milk iodide after ~ PND 7. The median urinary iodine level of the subjects was 101.9 µg/L, but the range (27-570 µg/L) (Leung et al., 2012) indicates that many of the subjects were well below the level of sufficiency, 100 µg/L. It is possible that the subjects were more iodine deficient than the levels evaluated with the model. Alternatively, the stability of iodine in breast milk declined as much as 50% after three days of storage at 4°C (observed by Dr. Benjamin Blount, CDC; personal communications) which may explain the reported low iodide levels (**Figure 17**).

LOCAL SENSITIVITY ANALYSIS OF BBDR MODEL WITH PERCHLORATE

A local sensitivity analysis was conducted for the formula-fed and breast-fed infant to determine which parameters were most influential on the plasma concentrations of T4 and fT4 in the infant. The infant and mother's initial iodide and perchlorate concentrations were set based on the end of pregnancy values for the Lumen et al. (2013) model according to the maternal exposure conditions given in **Table 9** and **Table 10**, but the limited exposure-dependence in the values obtained for the cases evaluated here indicates that the additional information one might gain from further analyses is limited. Therefore analyses were limited to either low maternal iodine with low infant iodide exposure or high maternal iodine with high infant iodide exposure (**Table 9** and **Table 10**,). The BBDR model described in this report was used to run exposure scenarios in the infant from birth through 90 days, and the normalized sensitivity coefficients were calculated for all parameters in the model based on a 1% change from default values.

Table 9: Initialization conditions and formula-fed exposures for sensitivity analysis

Maternal Pregnancy Exposure for Initial Values		Infant exposure	
Iodide (µg/d)	Perchlorate (µg/kg/d)	Iodide formula (µg/L)	Perchlorate (µg/kg/d)
75	0	108	0
75	1	108	1
75	20	108	20
200	0	384	0
200	1	384	1
200	20	384	20

Table 10: Initialization conditions and breast-fed exposure scenarios for sensitivity analysis

Maternal Pregnancy Exposure for Initial Values		Breastfeeding Mother Exposure	
Iodide (µg/d)	Perchlorate (µg/kg/d)	Iodide (µg/d)	Perchlorate (µg/kg/d)
75	0	75	0
75	1	75	1
75	20	75	20
200	0	200	0
200	1	200	1
200	20	200	20

The sensitivity analysis results for total T4 and fT4 are identical, except that total T4 is not sensitive to FRCONVT4 (mother) or FRCONVT4_N (infant), which define the relationship between fT4 and T4. Therefore only results for fT4 are explicitly described here. The sensitivity coefficients for fT4 in the formula- and breast-fed infants are listed in **Table 11** and **Table 12**, respectively, and shown graphically in **Figure 18** (formula-fed infant), **Figure 19** (infant parameters' influence on breast-fed infant), and **Figure 20** (maternal parameters' influence in breast-fed infant). As seen in these graphs, the influential parameters were time-dependent, iodine-dependent, and perchlorate-dependent. The influence of perchlorate on iodine was more significant in the breast-fed exposures because the low predicted iodide transferred in milk at the lower maternal exposure of 75 µg/d.

Figure 18. Normalized sensitivity coefficients for fT4 in the formula-fed infant at Day 7, 30, 60, and 90.

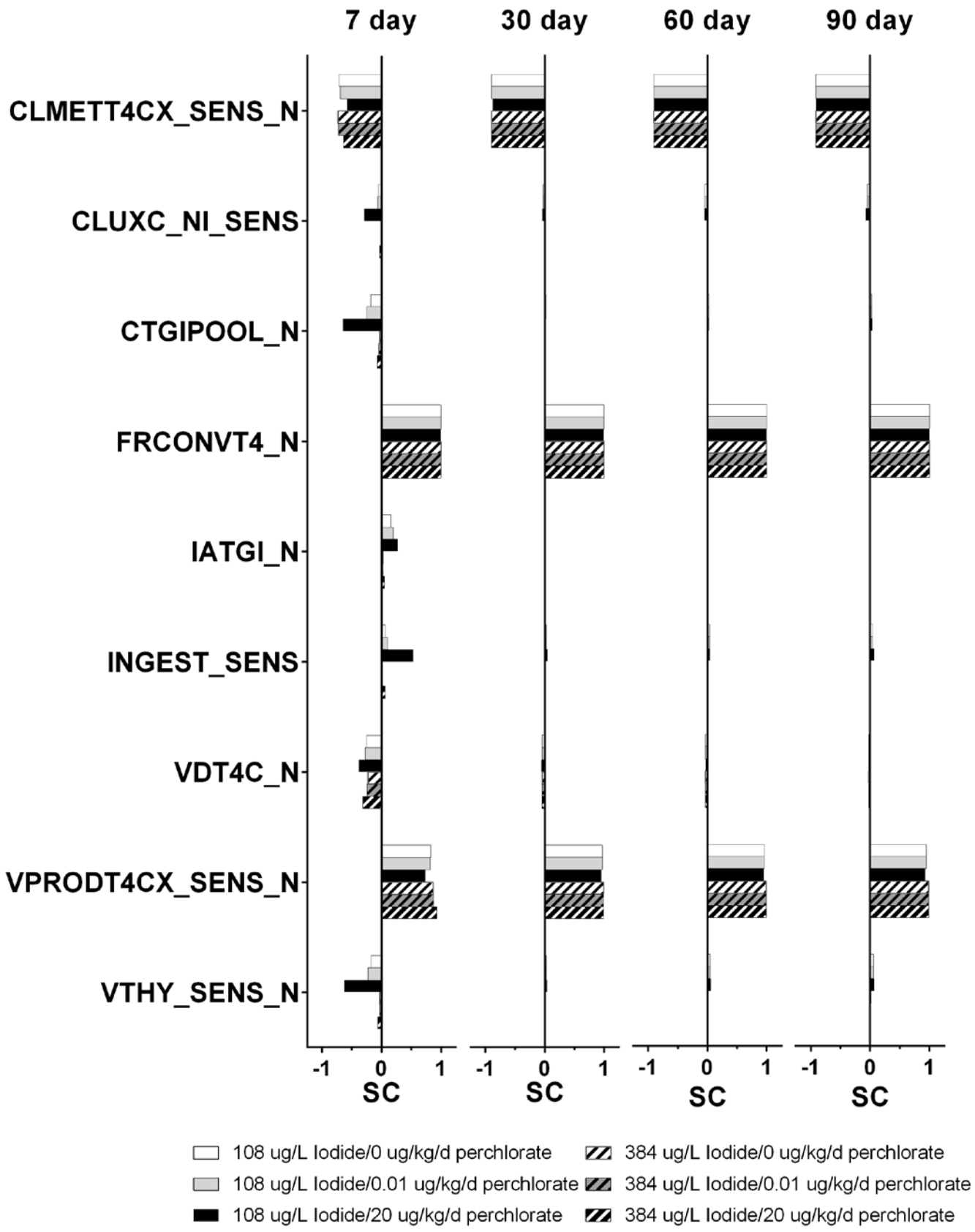

Figure 19. Normalized sensitivity coefficients for infant parameters on fT4 in the breast-fed infant at Day 7, 30, 60, and 90.

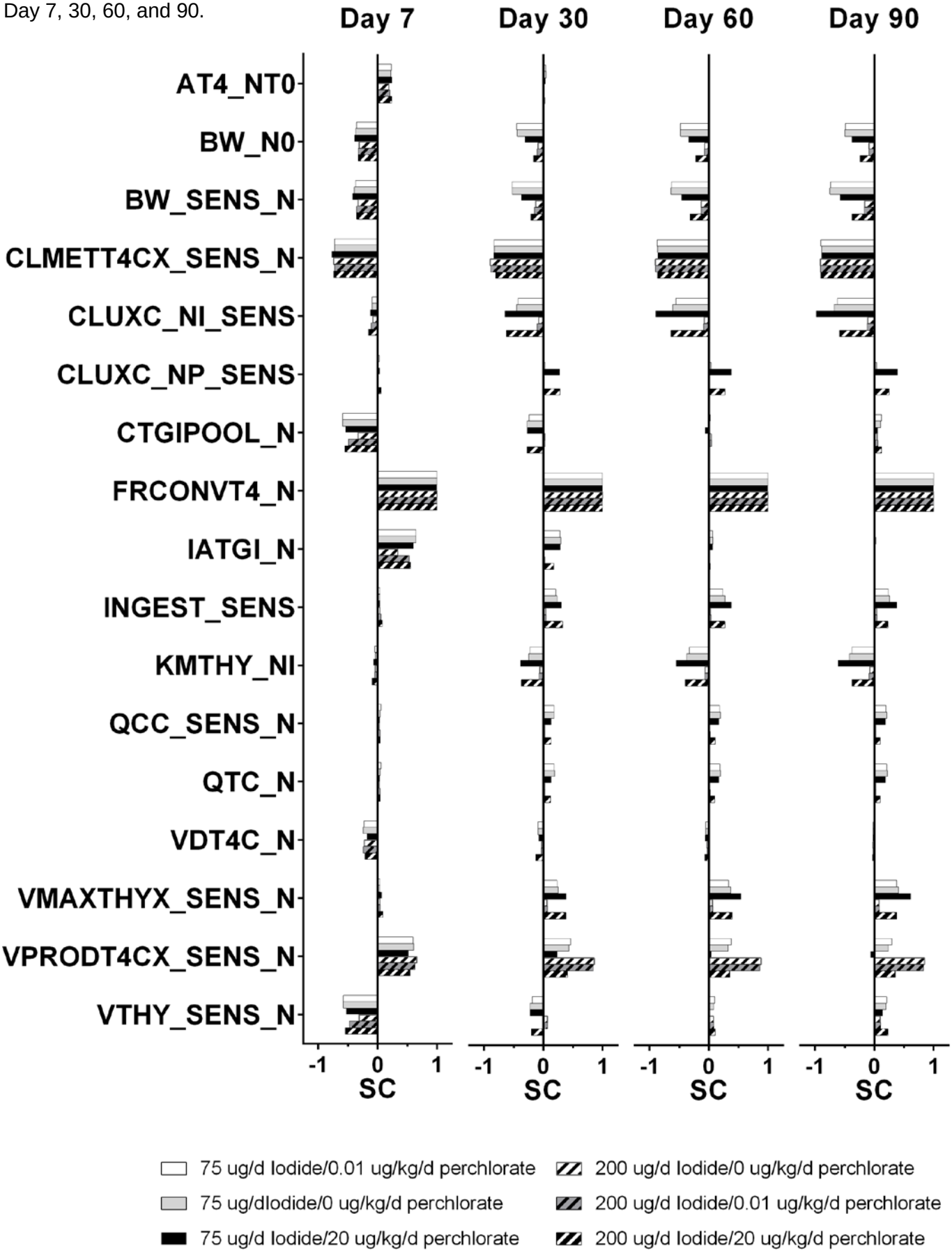

Figure 20. Normalized sensitivity coefficients for maternal parameters on fT4 in the breast-fed infant at Day 7, 30, 60, and 90.

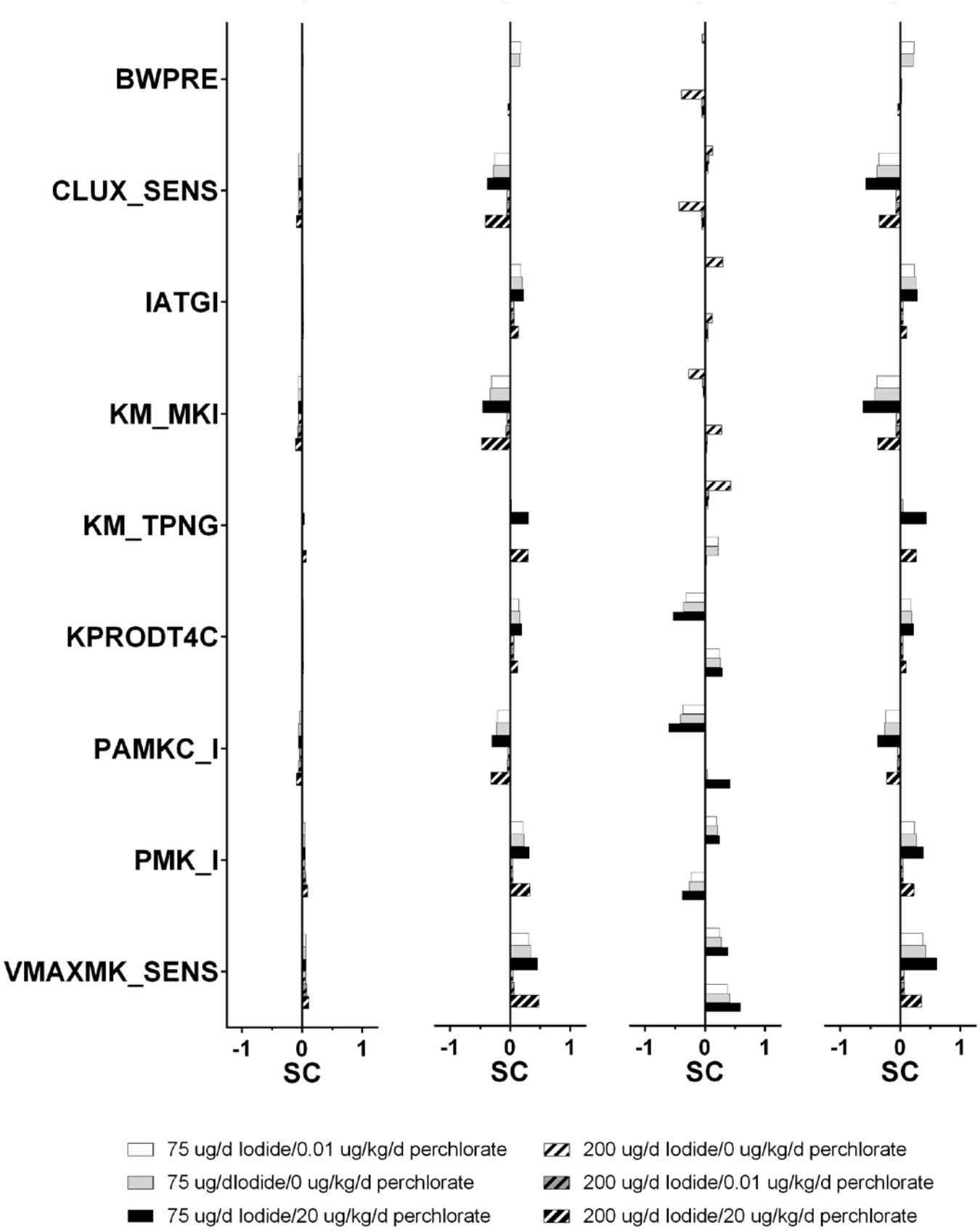

Table 11: Normalized sensitivity coefficients for fT4 in formula fed infant

Parameter	Normalized Sensitivity Coefficients						
		Formula iodide concentration (µg/L)					
		108			384		
		Infant perchlorate dose (µg/kg/d)					
	Day	0	0.01	20	0	0.01	20
CLMETT4CX_SENS_N	7	-0.71	-0.69	-0.58	-0.73	-0.72	-0.64
	30	-0.89	-0.89	-0.88	-0.89	-0.89	-0.89
	60	-0.9	-0.9	-0.9	-0.9	-0.9	-0.9
	90	-0.91	-0.91	-0.91	-0.91	-0.91	-0.91
CLUXC_NI_SENS	7	-0.05	-0.06	-0.29	0	0	-0.03
	30	-0.03	-0.03	-0.04	0	0	0
	60	-0.04	-0.04	-0.05	0	0	0
	90	-0.05	-0.05	-0.07	0	0	0
CTGIPOOL_N	7	-0.18	-0.25	-0.65	-0.03	-0.04	-0.07
	30	0.02	0.02	0.02	0	0	0
	60	0.03	0.03	0.03	0	0	0
	90	0.03	0.03	0.04	0	0	0
FRCONVT4_N	7	1	1	1	1	1	1
	30	1	1	1	1	1	1
	60	1	1	1	1	1	1
	90	1	1	1	1	1	1
IATGI_N	7	0.16	0.2	0.28	0.03	0.03	0.05
	30	0	0	0	0	0	0
	60	0	0	0	0	0	0
	90	0	0	0	0	0	0
INGEST_SENS	7	0.07	0.11	0.54	0	0.01	0.06
	30	0.03	0.03	0.05	0	0	0
	60	0.04	0.04	0.05	0	0	0
	90	0.05	0.05	0.07	0	0	0
VDT4C_N	7	-0.25	-0.27	-0.38	-0.23	-0.24	-0.31
	30	-0.04	-0.04	-0.06	-0.04	-0.04	-0.04
	60	-0.03	-0.03	-0.03	-0.03	-0.03	-0.03
	90	-0.02	-0.02	-0.02	-0.02	-0.02	-0.02
VPRODT4CX_SENS_N	7	0.83	0.82	0.74	0.87	0.88	0.93
	30	0.97	0.97	0.96	0.99	0.99	0.99
	60	0.96	0.96	0.95	0.99	0.99	0.99
	90	0.95	0.95	0.93	0.99	0.99	0.99
VTHY_SENS_N	7	-0.17	-0.23	-0.62	-0.03	-0.03	-0.06
	30	0.03	0.03	0.04	0	0	0
	60	0.05	0.05	0.06	0.01	0.01	0.01
	90	0.06	0.06	0.07	0.01	0.01	0.01

Table 12: Normalized sensitivity coefficients for fT4 in the breast fed infant

Parameter	Day	Normalized Sensitivity Coefficients					
		Maternal iodide intake (µg/d)					
		75			200		
		Maternal perchlorate dose (µg/kg/d)					
		0	0.01	20	0	0.01	20
AT4_NT0	7	0.22	0.23	0.25	0.19	0.21	0.24
	30	0.04	0.05	0.04	0	0	0.02
	60	0	0.01	0.01	0	0	0
	90	0	0	0	0	0	0
BWPRE	7	0.01	0.02	0	0	0.01	-0.01
	30	0.17	0.16	0	0.01	0.01	-0.04
	60	0.22	0.22	0.03	0.01	0.01	-0.04
	90	0.23	0.22	0.02	0.02	0.02	-0.04
BW_N0	7	-0.37	-0.35	-0.39	-0.31	-0.33	-0.33
	30	-0.45	-0.44	-0.31	-0.09	-0.1	-0.16
	60	-0.48	-0.48	-0.34	-0.07	-0.08	-0.22
	90	-0.49	-0.5	-0.38	-0.09	-0.09	-0.24
BW_SENS_N	7	-0.39	-0.37	-0.42	-0.33	-0.35	-0.35
	30	-0.53	-0.52	-0.37	-0.13	-0.15	-0.21
	60	-0.63	-0.64	-0.46	-0.13	-0.13	-0.31
	90	-0.74	-0.75	-0.58	-0.16	-0.17	-0.38
CLMETT4CX_SENS_N	7	-0.72	-0.72	-0.77	-0.74	-0.72	-0.74
	30	-0.83	-0.82	-0.84	-0.9	-0.89	-0.8
	60	-0.87	-0.86	-0.86	-0.9	-0.9	-0.86
	90	-0.9	-0.89	-0.89	-0.91	-0.91	-0.9
CLUXC_NI_SENS	7	-0.09	-0.09	-0.12	-0.08	-0.1	-0.15
	30	-0.42	-0.45	-0.65	-0.08	-0.1	-0.62
	60	-0.55	-0.61	-0.9	-0.08	-0.09	-0.64
	90	-0.62	-0.68	-0.98	-0.11	-0.12	-0.59
CLUXC_NP_SENS	7	0	0.03	0.04	0	0	0.06
	30	0	0.03	0.28	0	0	0.28
	60	0	0.04	0.38	0	0	0.27
	90	0	0.04	0.4	0	0	0.25
CLUX_SENS	7	-0.05	-0.05	-0.06	-0.05	-0.06	-0.09
	30	-0.26	-0.28	-0.39	-0.05	-0.06	-0.41
	60	-0.32	-0.36	-0.53	-0.05	-0.06	-0.39
	90	-0.36	-0.39	-0.58	-0.07	-0.08	-0.35
CTGIPOOL_N	7	-0.59	-0.59	-0.54	-0.33	-0.49	-0.55
	30	-0.24	-0.27	-0.27	0.03	0.03	-0.27
	60	0.02	0	-0.06	0.04	0.05	0
	90	0.12	0.11	0.06	0.05	0.06	0.12

Table 12: Normalized sensitivity coefficients for fT4 in the breast fed infant (cont'd)

FRCONVT4_N	7	1	1	1	1	1	1
	30	1	1	1	1	1	1
	60	1	1	1	1	1	1
	90	1	1	1	1	1	1
IATGI	7	0.03	0.03	0.03	0.02	0.03	0.03
	30	0.18	0.2	0.23	0.06	0.07	0.14
	60	0.24	0.26	0.3	0.05	0.06	0.13
	90	0.24	0.26	0.29	0.05	0.05	0.11
IATGI_N	7	0.65	0.65	0.61	0.34	0.53	0.56
	30	0.28	0.3	0.29	0	0.02	0.17
	60	0.06	0.07	0.07	0	0	0.02
	90	0.01	0.02	0.02	0	0	0
INGEST_SENS	7	0.04	0.04	0.05	0.04	0.06	0.08
	30	0.21	0.23	0.31	0.04	0.05	0.32
	60	0.24	0.27	0.38	0.03	0.04	0.27
	90	0.24	0.26	0.38	0.04	0.05	0.23
KMTHY_NI	7	-0.04	-0.05	-0.07	-0.04	-0.05	-0.09
	30	-0.23	-0.25	-0.39	-0.06	-0.07	-0.37
	60	-0.33	-0.37	-0.55	-0.06	-0.07	-0.4
	90	-0.38	-0.42	-0.61	-0.08	-0.09	-0.38
KM_MKI	7	-0.06	-0.06	-0.07	-0.06	-0.07	-0.11
	30	-0.31	-0.33	-0.46	-0.05	-0.07	-0.48
	60	-0.37	-0.41	-0.6	-0.05	-0.06	-0.43
	90	-0.39	-0.42	-0.62	-0.06	-0.07	-0.37
KM_TPNG	7	0	0	0.05	0	0	0.07
	30	0	0.02	0.31	0	0	0.3
	60	0	0.04	0.42	0	0	0.3
	90	0	0.04	0.44	0	0	0.27
KPRODT4C	7	0.02	0.02	0.03	0.02	0.02	0.03
	30	0.15	0.16	0.2	0.06	0.06	0.13
	60	0.19	0.21	0.25	0.05	0.05	0.12
	90	0.18	0.19	0.23	0.04	0.05	0.1
PAMKC_I	7	-0.04	-0.05	-0.06	-0.04	-0.06	-0.09
	30	-0.21	-0.23	-0.31	-0.04	-0.05	-0.32
	60	-0.23	-0.26	-0.38	-0.03	-0.04	-0.27
	90	-0.24	-0.26	-0.38	-0.04	-0.05	-0.23
PMK_I	7	0.05	0.05	0.06	0.05	0.06	0.09
	30	0.21	0.23	0.32	0.04	0.05	0.33
	60	0.24	0.27	0.39	0.03	0.04	0.28
	90	0.24	0.27	0.39	0.04	0.05	0.23
QCC_SENS_N	7	0.05	0.06	0.04	0.03	0.05	0.05
	30	0.18	0.18	0.14	0.02	0.02	0.13
	60	0.18	0.2	0.17	0.01	0.02	0.11
	90	0.2	0.21	0.19	0.02	0.02	0.1

Table 12: Normalized sensitivity coefficients for fT4 in the breast fed infant (cont'd)

QTC_N	7	0.05	0.06	0.04	0.03	0.05	0.05
	30	0.18	0.19	0.14	0.02	0.02	0.12
	60	0.19	0.2	0.17	0.01	0.02	0.1
	90	0.21	0.22	0.19	0.02	0.02	0.1
VDT4C_N	7	-0.24	-0.23	-0.18	-0.22	-0.24	-0.21
	30	-0.09	-0.09	-0.08	-0.03	-0.04	-0.13
	60	-0.05	-0.05	-0.06	-0.03	-0.03	-0.07
	90	-0.02	-0.02	-0.03	-0.02	-0.02	-0.03
VMAXMK_SENS	7	0.06	0.06	0.07	0.06	0.08	0.11
	30	0.31	0.34	0.46	0.05	0.07	0.48
	60	0.37	0.41	0.6	0.05	0.06	0.43
	90	0.38	0.42	0.62	0.06	0.07	0.36
VMAXTHYX_SENS_N	7	0.04	0.04	0.07	0.04	0.05	0.09
	30	0.23	0.25	0.39	0.06	0.07	0.38
	60	0.33	0.37	0.55	0.06	0.07	0.4
	90	0.38	0.41	0.62	0.08	0.09	0.38
VPRODT4CX_SENS_N	7	0.61	0.6	0.52	0.66	0.63	0.55
	30	0.46	0.43	0.24	0.86	0.84	0.41
	60	0.38	0.32	0.05	0.88	0.86	0.36
	90	0.3	0.24	-0.06	0.85	0.83	0.36
VTHY_SENS_N	7	-0.58	-0.58	-0.53	-0.31	-0.48	-0.54
	30	-0.19	-0.22	-0.22	0.07	0.07	-0.2
	60	0.1	0.08	0.02	0.08	0.09	0.11
	90	0.21	0.2	0.15	0.1	0.11	0.23

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