Predicting Fetal Perchlorate Dose and Inhibition of Iodide Kinetics during Gestation: A Physiologically-Based Pharmacokinetic Analysis of Perchlorate and Iodide Kinetics in the Rat

Rebecca A. Clewell,*† Elaine A. Merrill,† Kyung O. Yu,‡ Deirdre A. Mahle,§ Teresa R. Sterner,† David R. Mattie,‡ Peter J. Robinson,§ Jeffrey W. Fisher‡, and Jeffery M. Gearhart§

*Geo-Centers, Inc., Wright-Patterson AFB, Ohio 45433; †Operational Technologies Corp., Dayton, Ohio 45432; ‡AFRL/HEST, Wright-Patterson AFB, Ohio 45433; and §Mantech Environmental Technology, Inc., Dayton, Ohio 45437

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Perchlorate (ClO4−) disrupts endocrine homeostasis by competitively inhibiting the transport of iodide (I−) into the thyroid. The potential for health effects from human exposure to ClO4− in drinking water is not known, but experimental animal studies are suggestive of developmental effects from ClO4− induced iodide deficiency during gestation. Normal hormone-dependent development relies, in part, on synthesis of hormones in the fetal thyroid from maternally supplied iodide. Although ClO4− crosses the placenta, the extent of inhibition in the fetal thyroid is unknown. A physiologically-based pharmacokinetic (PBPK) model was developed to simulate ClO4− exposure and the resulting effect on iodide kinetics in rat gestation. Similar to concurrent model development for the adult male rat, this model includes compartments for thyroid, stomach, skin, kidney, liver, and plasma in both mother and fetus, with additional compartments for the maternal mammary gland, fat, and placenta. Tissues with active uptake are described with multiple compartments and Michaelis-Menten (M-M) kinetics. Physiological and kinetic parameters were obtained from literature and experiment. Systemic clearance, placental-fetal transport, and M-M uptake parameters were estimated by fitting model simulations to experimental data. The PBPK model is able to reproduce maternal and fetal iodide data over five orders of magnitude (0.36 to 33,000 ng/kg-day ClO4−) and inhibition of maternal thyroid and total fetal I− uptake. The model suggests a significant fetal ClO4− dose in late gestation (up to 82% of maternal dose). A comparison of model-predicted internal dosimetrics in the adult male, pregnant, and fetal rat indicates that the fetal thyroid is more sensitive to inhibition than that of the adult.

Key Words: PBPK model; pregnancy; perchlorate; iodide; thyroid; inhibition; fetal dose.

Recent findings of perchlorate (ClO4−) contamination in groundwater and drinking water sources have resulted in widespread concern over the potential health effects from long-term ingestion of low-level perchlorate via drinking water (Motzer, 2001; Urbansky, 1998; Urbansky and Shock, 1999). Most drinking water sources reported to contain perchlorate appear to have concentrations less than 20 ppb, although concentrations as high as 3700 ppm have been reported in some Las Vegas ground water samples (Motzer, 2001). Perchlorate interferes with thyroidal iodide uptake by binding to the sodium iodide symporter (NIS) at the basolateral membrane of the thyroid follicular endothelial cell, resulting in decreased iodide uptake and decreased synthesis of thyroid hormones. However, hormone homeostasis is usually regained through upregulation of NIS via the hypothalamus-pituitary-thyroid (H-P-T) feedback mechanism (Wolff, 1998). Nevertheless, due to the identification of contaminated water sources, and the potential for interaction with thyroid iodide uptake in sensitive human subpopulations, the Environmental Protection Agency is currently in the process of evaluating human risk in order to recommend a safe water concentration of ClO4− (USEPA, 2002).

The endocrine system maintains a delicate balance of hormonal and inorganic iodide through the H-P-T feedback system, wherein the hypothalamus responds to diminished serum hormone levels by increasing production of thyrotropin releasing hormone (TRH). TRH then signals the pituitary to release thyroid stimulating hormone (TSH), which in turn increases NIS levels in the thyroid and downregulates hormone deiodination by decreasing synthesis of thyroid peroxidase (TPO). The NIS is responsible for transporting inorganic iodide (I−) into the thyroid follicular cell for the synthesis of thyroid hormones, particularly thyroxine (T4) and triiodothyronine (T3; Wolff, 1998).

In an effort to quantitatively predict ClO4−, I−, and inhibition kinetics in response to both acute and subchronic ClO4− exposure, we have developed a physiologically-based pharmacokinetic (PBPK) model in the adult male rat (Merrill et al., 2003). Because the PBPK model is based on physiological, biochemical, and mechanistic data, it allows us to determine target site dosimetry and in vivo chemical interactions (inhibition) while...
improving confidence in extrapolation between dose levels, exposure durations and routes. However, with ClO$_4^-$, as with other potential endocrine disruptors, the primary concern is not necessarily potential risk to the adult, but rather the possible developmental effects resulting from perinatal exposure. During gestation and early infancy, a critical window exists in which thyroid hormones are needed for normal physical and mental development (Bakke et al., 1976; Howdeshell, 2002; Myant, 1971; Porterfield, 1994). The most susceptible developmental periods to thyroid perturbations are not known. However, even short-term iodide deficiency in the perinatal period can result in lifelong consequences. In the human, gestational iodide deficiency has been linked to increased incidence of stillbirth, congenital abnormalities, lowered IQ, mental retardation, and impaired hearing (Delange, 2000; Haddow et al., 2001). Additionally, further extrapolation of this model to human ClO$_4^-$ exposure could allow for a more quantitative comparison of life stage differences (Clewell et al., 2001).

MATERIALS AND METHODS

Supporting Experiments

All in-house experiments were performed with timed-pregnant dams of the Sprague-Dawley strain (Crl: CD, Charles River Laboratory, Raleigh, NC). The animals used in in-house studies were handled in accordance with the principles stated in the Guide for the Care and Use of Laboratory Animals, National Research Council, 1996, and the Animal Welfare Act of 1966, as amended. GD 1 was verified by the presence of a vaginal plug. In all studies pregnant rats were housed individually in light, heat, and humidity controlled cages. Rats were kept on a 12 h light/dark cycle with access to food and water ad libitum. All rats from in-house studies (control and ClO$_4^-$ dose groups) were given the same diet (Purina #5000) containing 0.8 ppm iodine. Maternal drinking water consumption was measured daily and perchlorate drinking water levels were adjusted when necessary to ensure the accuracy of dosing levels in the drinking water studies. On GD 20, dams and fetuses were euthanized by CO$_2$ asphyxiation.

Perchlorate drinking water study.

Dams ($n$ = 6 per dose group) were exposed to drinking water treated with perchlorate from GD 2 to 20, at doses of 0.0, 0.01, 0.1, 1.0, and 10.0 mg ClO$_4^-$/kg-day. Two hours prior to euthanization, dams were given an iv dose of 33 μg/kg $^{131}$I with carrier (trace level radioiodide mixed with nonradioiodide iodide). Maternal and fetal serum samples were analyzed for free and total thyroxine ($T_4$, and $T_4$), $T_3$, and TSH. ClO$_4^-$ was also analyzed in the maternal and fetal serum, skin and gastrointestinal (GI) tract, as well as maternal thyroid, GI contents, and placenta following the methods described by Fisher et al. (2000). The same tissues were also analyzed for $^{131}$I with a gamma counter.

Radioiodide and inhibition kinetic studies.

Timed-pregnant Sprague-Dawley dams were exposed via tail vein injection to a tracer dose of $^{131}$I (average dose = 2.19 ng/kg body weight [BW]) on GD 20. Dams ($n$ = 6) were euthanized at 0.5, 1, 2, 4, 8, 12, and 24 h postdosing. Maternal and fetal serum, skin, GI tract, as well as maternal thyroid, GI content, placenta, mammary gland tissue were collected and analyzed for $^{131}$I with a gamma counter. Fetal serum was pooled by litter due to small sample volume while fetal skin and GI tract were analyzed individually. Inhibition kinetics were examined in this same manner; however, 2 h prior to the administration of $^{131}$I (average dose = 1.87 ng/kg BW), dams from the inhibition group were dosed with 1.0 mg/kg BW ClO$_4^-$ via tail vein injection. This particular ClO$_4^-$ dose was chosen to be large enough dose to significantly affect iodide uptake, based on inhibition in the male rat (Merrill et al., 2003), while being lower than the dose required to saturate the symporter, based on the drinking water study results (see Results section below). Euthanization was performed at 0.5, 1, 2, 4, 8, 12, and 24 h post-$^{131}$I dosing.

Model structure.

All model code was written in ACSL (Advanced Continuous Simulation Language, Aegis Technologies Group, Inc., Huntsville, AL). Several classical mathematical models exist for iodine kinetics in both humans and rats (Berman et al., 1968; DiStefano et al., 1982; Hays and Wegner, 1965). However, since these models are not physiologically based, they are limited in their usefulness for extrapolation across species and life stage. The PBPK model proposed here supports these types of extrapolations by accounting for physiological differences. The maternal models for both $T_3$ and ClO$_4^-$ consist of compartments for plasma, thyroid, skin, gut, kidney, liver,
fat, mammary gland, and placenta, plus two lumped compartments for the remaining slowly and richly perfused tissues. The thyroid and gut are described with three subcompartments representing the stroma, follicle, and colloid in the thyroid, and the capillary bed, GI tract, and GI contents in the gut. Skin, placenta, and mammary gland are described with two subcompartments, representing the capillary bed and tissue. Active uptake into the thyroid follicle and colloid, as well as the skin tissue, mammary gland, GI contents, and placenta, was described with Michaelis-Menten (M-M) terms for saturable processes (bold arrows in Fig. 1). Permeability area cross-products (PA) and partition coefficients were used to describe the passive movement of the anions (I⁻ and ClO₄⁻) between the capillary bed, tissue, and inner compartments (small arrows in Fig. 1), which results from the inherent electrochemical gradients within these tissues (Chow et al., 1969). The flow-limited kidney, liver, and fat compartments were described using partition coefficients and blood flows. Urinary clearance and transfer of anions between the placenta and fetal serum were represented by first order clearance rates. Binding of ClO₄⁻ to plasma proteins was described with a saturable term for association of the ClO₄⁻ anions to binding sites in the plasma and a first order clearance rate for
dissociation from plasma binding sites. However, unlike ClO₄⁻ (Merrill et al., 2003), the majority of iodide in the serum is not bound to proteins (Yu et al., 2002a,b). Thus, binding of I⁻ to plasma proteins was not included in the model. Given that the majority of thyroid iodide is organified shortly after transport into the follicle, it was necessary include a simplified description of the incorporation of iodide into thyroid hormones and hormone precursors (monoiiodothyronine, diiodothyronine), as well as the secretion of these hormones from the maternal thyroid. However, since the purpose of this present model is primarily to determine perchlorate distribution and resulting inhibition of free iodide transfer by NIS, it was possible to simplify the model by assuming that the radiodine behaved as inorganic iodide in all compartments other than the thyroid, including serum. In the thyroid compartment, the partitioning and active transport mechanisms account only for the movement of free iodide. Hormone production was modeled as a first order production rate from the thyroid follicle to an “incorporated iodine” compartment (CIProd). Clearance of this incorporated iodine from the thyroid was then described with a first order clearance rate from the incorporated iodide compartment into the serum (CISercr). Due to the dynamic nature of the fetal thyroid, the uncertainty in hormone production kinetics, and the lack of supporting data, a description of hormone production was not included in the fetal rat on GD 17–20. Rather, total iodine in the fetal thyroid was modeled using active uptake and diffusion from the serum.

The perchlorate anion was modeled in the same manner as inorganic iodide, based on the similar size and charge of the ions and their shared affinity for NIS. The thyroid, skin, GI, and mammary glands contain active NIS and were therefore defined separately in the structure of the model (Kotani et al., 1998; Spitzweg et al., 1998). The thyroid, skin, and GI contents maintain higher concentrations of ClO₄⁻ and I⁻ than the plasma (Brown-Grant and Pethes, 1959; Chow et al., 1969; Halmi and Stuelke, 1959; Wolff, 1998; Zeghal et al., 1995), requiring active transport mechanisms to work against the concentration gradient. Although other tissues, such as the salivary gland, ovary, and choroid plexus, are also known to sequester iodide and perchlorate in the rat and human (Brown-Grant, 1961; Honour et al., 1952; Spitzweg et al., 1998), small amounts of the anions in these tissues do not affect plasma concentrations. Therefore, these tissues were lumped together with the richly and slowly perfused compartments.

In addition to the reported presence of NIS, studies in our laboratory have shown the uptake of iodide in placental tissue to be inhibited by ClO₄⁻ (Mahle et al., 2002). Thus, it was necessary to include a description of NIS in the placental compartment to account for ClO₄⁻ and I⁻ concentrations and the measured ClO₄⁻ induced inhibition of placental iodide uptake. The mammary gland has also been shown to concentrate both anions during lactation and is known to contain NIS, which is regulated by hormones produced during lactation (Tazebay et al., 2000). Intralaboratory studies found mammary gland: plasma ratios of less than one for ClO₄⁻ and I⁻ during gestation. However, time-course data indicate that mammary gland levels remain elevated well into the clearance phase of the serum. In order to maintain these elevated tissue concentrations, it was necessary to include symporter activity in the mammary gland of the pregnant rat.

The kidney and liver were also separately defined in the structure of the model to describe the rapid urinary clearance of the anions and to allow future elaboration of the model to address hormone metabolism in the liver. A compartment was also included to account for the effect of changing fat volume on the kinetics of hydrophobic anions during gestation. Since kidney, liver, and fat do not maintain tissue:plasma ratios greater than one for either anion, these tissues were described as single, flow-limited compartments and do not contain terms for active uptake. Effective partitioning into these compartments is thought to result from the electrochemical gradient that moves ClO₄⁻ from serum to tissue, as in the thyroid (Chow and Woodbury, 1970).

Plasma binding of perchlorate was included in the model, in order to simulate the relatively high serum ClO₄⁻ concentrations observed in our laboratory at low administered doses (Fig. 2). This binding has been measured in human, bovine (Carr, 1952; Scatchard and Black, 1949), and rat serum (Merrill et al., 2003). At low serum concentrations (≤100 µg/l), Fisher reported approximately 99% of the anion is bound to plasma proteins and at higher concentrations (≥500 µg/l), 50% is bound (Fisher, 2002, as cited in Merrill et al., 2003). This plasma binding of perchlorate is also evidenced by the ability of the anion to interfere with T₄ binding to serum albumin and pre-albumin rates as it does in humans (Shishiba et al., 1970; Yamada, 1967). These studies suggest that perchlorate inhibits binding of T₄ to albumin by reversibly binding to the albumin via weak covalent interactions.

The structure of the fetal perchlorate model is similar to that of the pregnant rat, with the exception of the mammary gland and placenta compartments. In order to simplify the model, all fetuses from a single litter were lumped together within the model structure, essentially viewing all individual fetuses as one entity. Although there is evidence that delivered fetal dose of some toxins may vary due to position within the uterine horn, the difficulty of chemical analysis and the small sample volume obtained from the GD 20 fetus preclude the possibility of analyzing serum for each individual fetus. Thus, the model attempts to predict the available data, which consists of pooled fetal samples. Though this may present a crude estimate of a particular individual fetus, the model is able to provide a reasonably accurate estimate of the average fetal dose, thereby enabling risk estimates to be made from dosimetry in the subject of interest, the fetus, rather than the less comparable but more available adult rat. The model description of fetal dose is based on transfer of the anions between the placenta and fetal serum. Although a kidney compartment is included in the fetal model, urinary excretion is not used to identify the loss of perchlorate for the fetus, as the ability to produce urine is not well developed until after parturition. Loss of ClO₄⁻ and I⁻ from the fetus was described as clearance from the fetal serum to the placenta. The anions are then able to diffuse into the maternal serum and are, therefore, available for redistribution in the dam.

Perchlorate inhibition of iodide uptake was included in the maternal thyroid follicle and colloid, GI contents, skin and placenta, as well as the fetal GI contents and skin throughout gestation. Inhibition in the fetal thyroid follicle and colloid was also included from the onset of fetal thyroid iodide accumulation (GD 17) through parturition, based on the observation that perchlorate was present in the fetal blood and could, therefore, inhibit fetal NIS transport of iodide into the thyroid. Placental inhibition was observed in studies in our laboratory (Mahle et al., 2002) and was included in the model in order to predict the effect on iodide transfer to the fetus. Literature sources have reported inhibition in gastric juice of the male rat (Halmi and Stuelke, 1959) and intralaboratory studies showed consistent evidence of significant (>60%) inhibition of iodide uptake in both the fetal GI and skin, and slight inhibition in the maternal skin (Mahle et al., 2002). Since the release of iodide from these extrathyroidal tissues could affect the serum levels and the amount of stored iodide in the fetus, it was necessary to include iodide inhibition in the tissues with active uptake.

An additional description of thyroid perchlorate inhibition by iodide was determined to be unnecessary, as it would not affect perchlorate thyroid levels. Although both anions are transported with NIS and would therefore inhibit the other’s ability to bind to the symporter, the fact that the Kᵣ for ClO₄⁻ is an order of magnitude lower than that of I⁻ indicates that ClO₄⁻ has a much greater affinity for NIS. Additionally, even when considering dietary iodide, the relative intake of perchlorate in the drinking water dosing scenarios (on the order of mg/kg-day) is much greater than that of the trace element iodide (on the order of µg/kg-day). Competitive inhibition of ClO₄⁻ by I⁻ would be modeled by adjusting the Kᵣ by one plus the ratio of serum iodide concentration to the Kᵣ for iodide. Because the Kᵣ is large (4 × 10⁶ ng/l) and the endogenous serum iodide concentration (roughly 4000 ng/l) is approximately

FIG. 2. Perchlorate concentration in maternal (a) serum (Clewell et al., 2001), (b) thyroid (upregulated), (c) GI contents, and (d) skin as well as fetal (e) serum (Clewell et al., 2001) and (f) skin at the 0.01, 0.1, 1.0, and 10.0 mg/kg-day dose on GD 20.
FIG. 2

Figures a, b, c, d, e, and f depict the concentration of perchlorate in various compartments of the body over time. Each graph illustrates the concentration in maternal serum, maternal thyroid, maternal GI contents, maternal skin, fetal serum, and fetal skin respectively. The x-axis represents time in hours, while the y-axis represents perchlorate concentration in mg/L.
1000 times smaller than \( K_m \), it is apparent that the effect of iodide on thyroid 

**Dosing procedures.** In order to simulate the daily dosing regimen of the 

**Model parameters.** Whenever possible, physiological and kinetic param-

**Physiological parameters.** The physiological description of maternal and 

<table>
<thead>
<tr>
<th>Tissue volumes (% BW)</th>
<th>Dam</th>
<th>Fetus</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight ( BW ) and ( VJ/fet ) (kg)</td>
<td>0.280–0.361</td>
<td>0.0–0.0045</td>
<td>O’Flaherty et al., 1992</td>
</tr>
<tr>
<td>Slowly perfused ( VSc ) (% BW)</td>
<td>74.6</td>
<td>74.6</td>
<td>Brown et al., 1997</td>
</tr>
<tr>
<td>Richly perfused ( VRe ) (% BW)</td>
<td>11</td>
<td>16</td>
<td>Schneideret, 1985</td>
</tr>
<tr>
<td>Fat ( VFc ) (% BW)</td>
<td>10.0–11.0</td>
<td>0.0</td>
<td>Naismith et al., 1982</td>
</tr>
<tr>
<td>Liver ( VLc ) (% BW)</td>
<td>3.4</td>
<td>8.5–7.2</td>
<td>Schneideret, 1985</td>
</tr>
<tr>
<td>GI tract ( VGlc ) (% BW)</td>
<td>3.60</td>
<td>2.0–3.0</td>
<td>Schneideret, 1985</td>
</tr>
<tr>
<td>GI contents ( VGIJc ) (% BW)</td>
<td>7.20</td>
<td>0.8–6.2</td>
<td>Proportional to difference in adult/fetal GI</td>
</tr>
<tr>
<td>GI blood ( VGlc ) (% VG)</td>
<td>2.9</td>
<td>2.9</td>
<td>Altman and Dittmer, 1971</td>
</tr>
<tr>
<td>Skin tissue ( VSke ) (% BW)</td>
<td>19.0</td>
<td>8.8–19.3</td>
<td>Brown et al., 1997; Palou et al., 1983</td>
</tr>
<tr>
<td>Skin blood ( VSKBc ) (% VSk)</td>
<td>2.0</td>
<td>2.0</td>
<td>Brown et al., 1997</td>
</tr>
<tr>
<td>Thyroid total ( VTc ) (% BW)</td>
<td>0.0105</td>
<td>0.058–0.038</td>
<td>Malendowicz and Bednarek, 1986; Schneideret, 1985</td>
</tr>
<tr>
<td>Thyroid follicle ( VTFc ) (% VT)</td>
<td>45.9</td>
<td>61.4</td>
<td>Conde et al., 1991; Malendowicz and Bednarek, 1986</td>
</tr>
<tr>
<td>Thyroid colloid ( VTELc ) (% VT)</td>
<td>45</td>
<td>18.3</td>
<td>Conde et al., 1991; Malendowicz and Bednarek, 1986</td>
</tr>
<tr>
<td>Thyroid blood ( VTSc ) (% VT)</td>
<td>9.1</td>
<td>20.3</td>
<td>Conde et al., 1991; Malendowicz and Bednarek, 1986</td>
</tr>
<tr>
<td>Plasma ( VPlasc ) (% BW)</td>
<td>4.7</td>
<td>4.7</td>
<td>Brown et al., 1997; Altman and Dittmer, 1971</td>
</tr>
<tr>
<td>Red blood cells ( VRBCc ) (% BW)</td>
<td>2.74</td>
<td>2.74</td>
<td>Altman and Dittmer, 1971; Brown et al., 1997</td>
</tr>
<tr>
<td>Placenta ( VPlc ) (% BW)</td>
<td>0.0–2.57</td>
<td>—</td>
<td>O’Flaherty et al., 1992</td>
</tr>
<tr>
<td>Mammary gland ( VMc ) (% BW)</td>
<td>1.0–5.5</td>
<td>—</td>
<td>Knight et al., 1984; O’Flaherty et al., 1992</td>
</tr>
<tr>
<td>Blood flows (% QC)</td>
<td>Cardiac output ( Qc ) (/h/kg)</td>
<td>14</td>
<td>67.8</td>
</tr>
<tr>
<td>Slowly perfused ( QSc ) (% QC)</td>
<td>24.0</td>
<td>24.0</td>
<td>Brown et al., 1997</td>
</tr>
<tr>
<td>Richly perfused ( QRe ) (% QC)</td>
<td>76.0</td>
<td>76.0</td>
<td>Brown et al., 1997</td>
</tr>
<tr>
<td>Fat ( QFc ) (% QC)</td>
<td>7–8.1</td>
<td>—</td>
<td>Brown et al., 1997</td>
</tr>
<tr>
<td>Kidney ( QKc ) (% QC)</td>
<td>14.0</td>
<td>3.6</td>
<td>Brown et al., 1997; Rakusan and Marcinek, 1973</td>
</tr>
<tr>
<td>Liver ( QLc ) (% QC)</td>
<td>18.0</td>
<td>4.5</td>
<td>Brown et al., 1997; Rakusan and Marcinek, 1973</td>
</tr>
<tr>
<td>GI ( QGc ) (% QC)</td>
<td>13.6</td>
<td>4.6</td>
<td>Brown et al., 1997; Rakusan and Marcinek, 1973</td>
</tr>
<tr>
<td>Thyroid ( QTc ) (% QC)</td>
<td>1.6</td>
<td>1.6</td>
<td>Brown et al., 1997</td>
</tr>
<tr>
<td>Skin ( QSke ) (% QC)</td>
<td>5.8</td>
<td>10.4</td>
<td>Brown et al., 1997; Rakusan and Marcinek, 1973</td>
</tr>
<tr>
<td>Mammary ( QMc ) (% QC)</td>
<td>0.2–1.2</td>
<td>—</td>
<td>Hanwell and Linzell, 1973</td>
</tr>
<tr>
<td>Placenta ( QPlc ) (% QC)</td>
<td>0.0–12.3</td>
<td>—</td>
<td>O’Flaherty et al., 1992</td>
</tr>
</tbody>
</table>
absence of data for fetal volumes before GD 11, an exponential growth curve was used to approximate early embryonic growth, starting at zero liters and extrapolating the first available data points at GD 11. The final equation for fetal growth is dependent on the weight of the pup at the time of birth (PupBW), allowing the model to be adjusted for variations in birth weight of different rat strains.

Fetal GI, liver, kidney, and thyroid weights given for the Wistar rat (Schneideret al.,1985) on days 17 through 21 of gestation and skin volumes given for days GD 19 through PND 1 (Palou et al., 1983) were fit to exponential functions. These equations were then used in the model to describe organ growth versus fetal body weight over the course of gestation. Since organ weights are not available for earlier time points in gestation, the best-fit exponential curve to the above data was used to extrapolate to earlier time points in fetal development.

In accordance with the reported values of Schneideret al. (1985), the fetal GI and kidney increased from 2.0 and 0.3 to 3.0 and 0.44% BW, respectively, from GD 17–21. In the same time period, the relative volumes of the fetal liver and thyroid decreased (with respect to body weight) from 8.5 and 0.058 to 7.15 and 0.038% BW. Fetal skin relative volume increased more than threefold in the last three days of gestation, comprising 8.8, 13.6, and 19.3% of the body weight on GD 19, 21, and PND 1 (Palou et al., 1983). Volume fractions of fetal stroma (VTS\textsubscript{fet}), follicle (VTF\textsubscript{fet}), and colloid (VTC\textsubscript{fet}) were also significantly different than those of the dam (see Table 1) and were given values reported for the rat at birth (Conde et al., 1991). Fetal body fat (VF\textsubscript{fet}) was assumed to be negligible for the purpose of the model, based on the work of Naismith et al. (1982), who found that two-day-old rats contained only 0.16% fat and that body fat quickly increased in the neonatal period.

Temporal changes in maternal cardiac output during gestation are described in the model as the sum of initial cardiac output (Brown et al., 1997) and the change in blood flow to the placenta, mammary and fat tissues, per the approach of O’Flaherty et al. (1992), which employs changing blood flows in the placenta, mammary gland, and fat. The fraction of maternal cardiac output to the mammary gland, fat, and yolk sac change proportionally to the change in tissue volumes. Blood flow to the chorioniclantoic placenta increases more rapidly than the tissue volume.

Fetal blood flow was assumed to operate independently from the mother, increasing with fetal weight. Since regional blood flow and cardiac output data were not available for the rat fetus, the measured values could not be extrapolated back through gestation with certainty. Therefore, the values for regional blood flow to the fetal tissues were scaled allometrically (tissue weight\textsuperscript{0.75}) from the earliest available time point in the pup and cardiac output was scaled by BW\textsuperscript{0.75} from the value given by Gotshall et al. (1987) at PND 1. Fractional blood flows to fetal GI, skin, liver, and kidney (as % cardiac output) were given for the PND 1 rat in Rakusan and Marcinek (1973) and were significantly different from those of the adult rat (4.6, 10.4, 4.5, and 3.6% vs. 13.6, 5.8, 18.3, and 14.0% of the cardiac output, respectively). These changing fractions are in agreement with the time-line for maturation of organ function in the developing rat. For example, the liver and kidney are not fully functional during gestation. In fact, the kidneys do not reach full function until well after birth (glomeruli levels increase up to day 100; Bengele and Solomon, 1974). Thus, the increasing trend seen in the fractional blood flows to the fetal liver and kidney with age may, at least in part, be due to their increasing functional capability.

**Chemical-specific parameters.** Chemical-specific parameters for perchlorate and iodide are listed in Table 2. Binding of I\textsuperscript{–} to human NIS was determined to have an average Km of 4.0 \times 10\textsuperscript{-6} ng/l for iodide for the second thyroid transport mechanism at the apical membrane (K\textsubscript{mTLp}) in bovine thyroid. In the model, a slightly lower value than that measured by Golstein et al. of 1.0 \times 10\textsuperscript{-5} was used for K\textsubscript{mTLp}, based on the ability of the model to fit the later (>8 h) time-points. Like the NIS, this apical channel is also inhibited by ClO\textsubscript{4}–.

The affinity of ClO\textsubscript{4}– for NIS was not available in literature nor was it determined by experiment in our laboratory. However, since this anion competitively inhibits iodide uptake by NIS, the value for K\textsubscript{m} should be equal to its Km, Wolff and Maurey (1963) and Kosugi et al. (1996) measured the K\textsubscript{m} for ClO\textsubscript{4}– at 0.4 \times 10\textsuperscript{-5} and 1.5 \times 10\textsuperscript{-5} ng/l, respectively. Thus the K\textsubscript{m} value for binding of ClO\textsubscript{4}– to NIS (K\textsubscript{mTL}) was set between these two values (1.0 \times 10\textsuperscript{-5} ng/l) in the model. This value is further supported by various literature sources suggesting that ClO\textsubscript{4}– actually has as much as an order of magnitude greater affinity for NIS than I\textsuperscript{–} itself (Chow et al., 1969; Halmi and Stuelke, 1959; Harden et al., 1968; Lazarus et al., 1974). The Km, for the second transport mechanism in the thyroid colloid (K\textsubscript{mTC}) was set to 1.0 \times 10\textsuperscript{-4} ng/l, approximately a factor of 10 lower than the Km values used for iodide. This value for the colloid Vmax is supported by the data of Chow and Woodbury (1970), who reported saturation of this transport mechanism at 1.0 \times 10\textsuperscript{-3} ng/l ClO\textsubscript{4}–.

Unlike Km, values for Vmax vary significantly between different species and tissues with NIS (Wolff, 1998). Therefore, values for Vmax in the thyroid, GI, and skin were determined by the fit of the model to available data in the pregnant rat on GD 20. The nonlinear behavior of the ClO\textsubscript{4}– uptake in tissues with NIS suggested that the symporter was saturated between the 1.0 and 10.0 mg/kg-day doses in the perchlorate drinking water study. The model fit to data from the lower dose groups (0.01 through 1.0 mg/kg-day) in the drinking water study were used to set Vmax values for ClO\textsubscript{4}– uptake, since nonsaturable processes dominate at higher doses (10 mg ClO\textsubscript{4}–/kg-day). Kinetic data were available for radiiodide in the maternal and fetal rat; the experiments were performed at tracer doses so as to stay well below saturation of NIS. Thus, the uptake and clearance of radiiodide as measured in the kinetic study were used to determine values for Vmax in tissues with active uptake.

The ClO\textsubscript{4}– and I\textsuperscript{–} anions are not expected to partition into tissues in the classical understanding of the process. Rather, these anions are thought to respond to the electrochemical potential present across tissue membranes. Chow and Woodbury (1970) explored the relationship of these electrochemical potentials to ClO\textsubscript{4}– concentrations in the stroma, follicle, and lumen in the male rat thyroid at three different doses of ClO\textsubscript{4}–. Theoretical effective partition coefficients can be calculated (Kotyk and Janacek, 1977) from the measured differences in electrical potentials between the thyroid stroma and follicle. The approximately equal and opposite potential from the follicle to the colloid enhances passage of negatively charged species into the colloid and indicates an effective partition coefficient of greater than one. From Chow and Woodbury (1970), the potential difference for the stroma:follicle interface ranges from –58 to –51 mV; for a monovalent negatively charged ion, the resulting effective partition coefficient \( PT_{f} \), where \( \text{PT}_{f} \) is defined as

\[ \text{PT}_{f} = \frac{V_{\text{f}}}{V_{\text{st}} - V_{\text{f}}} \]

\[ \text{PT}_{f} \approx 1 \]

\[ \text{PT}_{f} \approx 1 \]

The potential difference for the stroma:colloid interface ranges from –6.8 to –7.4 mV; for a monovalent negatively charged ion, the resulting effective partition coefficient \( PT_{c} \), where \( \text{PT}_{c} \) is defined as

\[ \text{PT}_{c} = \frac{V_{\text{c}}}{V_{\text{st}} - V_{\text{c}}} \]

\[ \text{PT}_{c} \approx 1 \]

\[ \text{PT}_{c} \approx 1 \]

Theoretical effective partition coefficients can be calculated (Kotyk and Janacek, 1977) from the measured differences in electrical potentials between the thyroid stroma and follicle. The approximately equal and opposite potential from the follicle to the colloid enhances passage of negatively charged species into the colloid and indicates an effective partition coefficient of greater than one. From Chow and Woodbury (1970), the potential difference for the stroma:follicle interface ranges from −58 to −51 mV; for a monovalent negatively charged ion, the resulting effective partition coefficient \( PT_{f} \), where \( \text{PT}_{f} \) is defined as

\[ \text{PT}_{f} = \frac{V_{\text{f}}}{V_{\text{st}} - V_{\text{f}}} \]

\[ \text{PT}_{f} \approx 1 \]

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The potential difference for the stroma:colloid interface ranges from −6.8 to −7.4 mV; for a monovalent negatively charged ion, the resulting effective partition coefficient \( PT_{c} \), where \( \text{PT}_{c} \) is defined as

\[ \text{PT}_{c} = \frac{V_{\text{c}}}{V_{\text{st}} - V_{\text{c}}} \]

\[ \text{PT}_{c} \approx 1 \]

\[ \text{PT}_{c} \approx 1 \]
Partition coefficients (unitless)

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<tr>
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<th>Fetus</th>
</tr>
</thead>
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<td>Slowly perfused/plasma PS</td>
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</tr>
<tr>
<td>Rapidly perfused/plasma PR</td>
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<tr>
<td>Fat/plasma PF</td>
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<tr>
<td>Kidney/plasma PK</td>
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<td>Placenta/plasma PPI</td>
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<tr>
<td>Mammary/plasma PM</td>
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Max capacity, Vmaxc (ng/h/kg)

<table>
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<tr>
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<td>Skin VmaxcSk</td>
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<td>4.0 x 10^3</td>
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<tr>
<td>Mammary VmaxcM</td>
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<tr>
<td>Placenta VmaxcP</td>
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<td>5.5 x 10^3</td>
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Affinity constants, Km (ng/l)

<table>
<thead>
<tr>
<th>Parameters</th>
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<tbody>
<tr>
<td>Thyroid follicle KmTF</td>
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<td>1.0 x 10^5</td>
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<td>Thyroid colloid KmTL</td>
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</tr>
<tr>
<td>Skin KmSk</td>
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<td>4.0 x 10^3</td>
</tr>
<tr>
<td>Mammary KmM</td>
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</tr>
<tr>
<td>Placenta KmP</td>
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Permeability area cross-products (l/h-kg)

<table>
<thead>
<tr>
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<tr>
<td>Gastric blood to gastric tissue PAGic</td>
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<td>1.00</td>
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<tr>
<td>Gastric tissue to gastric juice PAGIic</td>
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<td>Thyroid stroma to follicle PATFc</td>
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<td>6.0 x 10^-3</td>
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<tr>
<td>Thyroid follicle to colloid PACLc</td>
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<tr>
<td>Skin blood to skin tissue PASkc</td>
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<td>1.00</td>
</tr>
<tr>
<td>Mammary blood to mammary tissue PAMc</td>
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<td>—</td>
</tr>
<tr>
<td>Placenta blood to placenta tissue PAPc</td>
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<tr>
<td>Plasma to red blood cells PARRec</td>
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</table>

Clearance values (l/h-kg)

<table>
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<tr>
<th>Parameters</th>
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<th>Fetus</th>
</tr>
</thead>
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<tr>
<td>Urinary excretion ClUc</td>
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</tr>
<tr>
<td>Incorporation of iodide into hormones ClProdc</td>
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<td>—</td>
</tr>
<tr>
<td>Incorporated iodine secretion to serum ClSecrC</td>
<td>—</td>
<td>1.0 x 10^-4</td>
</tr>
<tr>
<td>Transfer from placenta to fetus ClTrans1c</td>
<td>0.065</td>
<td>0.06</td>
</tr>
<tr>
<td>Transfer from fetus to placenta ClTrans2c</td>
<td>0.12</td>
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</tr>
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</table>

Binding constants

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Dam</th>
<th>Fetus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Association to binding sites VmaxcB</td>
<td>4.0 x 10^4</td>
<td>1.5 x 10^4</td>
</tr>
<tr>
<td>Affinity for binding sites KmB</td>
<td>1.0 x 10^4</td>
<td>1.5 x 10^4</td>
</tr>
<tr>
<td>Dissociation from plasma binding sites ClUnbc</td>
<td>0.034</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Parameters with two values indicate acute and drinking water parameters, respectively.

Fetus was given maternal values for Vmax (scaled by fetal body weight) in the absence of data.

Interest either from literature or experimental data in the rat and PA values were visually optimized to measured data. The partitioning parameters for the muscle (slowly perfused), liver (richly perfused), kidney, and red blood cells were again taken from corresponding male rat parameters (Merrill et al., 2003) and the values for the fat and mammary gland tissues were given the same values as ClO₄⁻, due to the lack of I⁻ data and the similar polarity of the anions.

Binding of perchlorate in the serum was described as a saturable process with a first order release of perchlorate from serum proteins. Because data were not available for free versus bound serum ClO₄⁻ in the pregnant or fetal rat, parameters for binding were determined by fitting the model to data at 0.01 and 0.1 mg/kg-day ClO₄⁻ from the drinking water study, since serum binding was most relevant at these lower doses. Urinary clearance of perchlorate was...
determined from the 10.0 mg/kg-day dose group, where binding had little effect on serum concentrations.

**Upregulation of thyroid NIS activity.** At the time of data collection in the drinking water study, rats had been exposed to ClO₄⁻ for 18 days. At this point, upregulation of the thyroid is evidenced by decreased T₄, and elevated TSH levels at all doses, as well as a lack of noticeable thyroid inhibition (Yu et al., 2001). Increased TSH compensates for the competitive inhibition of T₄ uptake by increasing the number and activity of NIS at the basolateral membrane of the thyroid, while the affinity of iodide for the symporter remains constant (Wolff, 1998). Thus, the value for \( V_{\text{max}} \), which corresponds to the maximum capacity for active transport at the basolateral membrane, was increased to fit the measured radiiodide concentrations in the upregulated thyroid for each dose. The resulting values for \( V_{\text{max}} \) were then plotted versus the corresponding concentrations of serum-free ClO₄⁻ and, since the ability of the thyroid to be upregulated is finite, the data were fitted to a M-M equation. This equation was then used in the model to describe the induction of NIS upregulation with time and dose, in a similar manner to the description used by Andersen et al. (1984) to describe glutathione induction.

Upregulation of thyroidal NIS also affects thyroid ClO₄⁻ uptake, and hence the measured thyroid ClO₄⁻ concentrations in the drinking water study, as both ClO₄⁻ and I⁻ are transported by the same symporter (Wolff, 1964, 1998). Thus, increased thyroid ClO₄⁻ uptake was modeled in the same manner as T₄, increasing the value for \( V_{\text{max}} \), with dose and applying the resulting M-M fit to the model.

**Sensitivity analysis of chemical-specific parameters.** A sensitivity analysis was run after setting the model parameters as described above to explore the influence of the various parameters on model predictions. The model was run to determine the change in the amount of inorganic iodide in the thyroid stroma, follicle, colloid (lumen) and the rate of change in the amount of organic or incorporated iodine in the total thyroid, respectively. \( PAT_i \), \( PATL_i \) and \( PTF_i \), \( PTL_i \) are the PAs and effective partition coefficients for the stroma:follicle and follicle:colloid membranes, respectively. \( RupTF_i \) and \( RupTL_i \) are the active uptake rates of iodide into the follicle and colloid. \( V_{\text{max}} \), \( V_{\text{max}} \) and \( V_{\text{max}} \) for iodide concentrations in the upregulated thyroid to determine the change in each of the two chosen dosimetrics, average serum ClO₄⁻ concentration (AUC: area under the curve) and total thyroid iodide uptake into the thyroid is described similarly, but without the organification rate is modified by the affinity of transport mechanism in the follicle for ClO₄⁻, which corresponds to the \( V_{\text{max}} \), which corresponds to the maximum velocity for incorporated iodine in the stroma (CTS). Inhibition of iodide uptake in other tissues with NIS is described in the same manner as the thyroid follicle inhibition.

**Model equations.** The following equations represent the distribution of iodide within the thyroid, in the absence of competitive inhibition. Perchlorate uptake into the thyroid is described similarly, but without the organification (\( CIP\text{prod} \)) and hormone secretion (\( CISeur \)) terms.

\[
RATS_i = QT \times (CA_i - CTS_i) + PATF_i \times \left( \frac{CTF_i}{PTF_i} - CTS_i \right) - RupTF_i
\]

\[
RATF_i = RupTF_i + PATF_i \times \left( \frac{CTS_i}{PTF_i} - CTS_i \right) - RupTL_i
\]

\[
+ PATLi_i \times \left( \frac{CTL_i}{PTLi_i} - CTSi \right) - (Cl \text{ Pr } odi_i \times CTF_i)
\]

\[
RATL_i = RupTL_i + PATLi_i \times \left( \frac{CTF_i}{PTLi_i} - CTS_i \right)
\]

\[
RupTF_i = \frac{V \max TF_i \times CTS_i}{KmTF_i + CTS_i}
\]

\[
RupTL_i = \frac{V \max TL_i \times CTF_i}{KmTL_i + CTF_i}
\]

\[
RABnd_i = (Cl \text{ Pr } odi_i \times CTF_i) - (CISeur \times CTF_i)
\]

\[
RAGB_i = QG \times (CA_i - CVGB_i) + PAG_i \times (CG_i/PGi - CVGB_i)
\]

\[
RAG_i = PAG_i \times (CVGB_i - CG_i/PGi)
\]

\[
+ PAGj_i \times (CGj_i/PGj_i - CG_i) - RupGji
\]

\[
RAGj_i = RupGji + PAGj_i \times (CG_j - CG_j/PGj_i) + RMR_i
\]

\[
RupGji = \frac{V \max Gj_i \times CG_i}{KmG_i \left( 1 + \frac{CG_i}{KmG_j} \right) + CG_i}
\]

Model equations for compartments without active uptake (shown for the liver, below) were modeled in the following equations, \( RAI \), is the rate of change in the amount of total iodide in the liver, \( QL \) is the fractional blood flow to the liver, \( CL \), is the concentration...
of iodide in the liver and PL, is the blood:liver partition coefficient. The kidney and fat are modeled similarly.

\[ RAL = QL \times (CA - CL/PL) \]

**RESULTS**

**Model Parameterization**

Perchlorate data from the drinking water distribution study were used to determine kinetic parameters for ClO₄⁻ in the pregnant and fetal rat. Upregulation of NIS transport of ClO₄⁻ into the thyroid was accounted for as described in the Materials and Methods section. Figure 2 shows the resulting model predictions for maternal serum, thyroid, skin, and GI content ClO₄⁻ concentrations, as well as the fetal serum and skin ClO₄⁻ concentrations, together with measured data from the drinking water study on GD 20 at 0.01, 0.1, 1.0, and 10.0 mg ClO₄⁻/kg-day. In the case of maternal GI contents, fetal serum, and fetal skin, the perchlorate levels in the 0.01 mg ClO₄⁻/kg-day were below analytical detection. In these and subsequent plots, solid lines indicate the model prediction and cross-bars indicate the mean ± SD of measured data.

Maternal mammary gland and GI tract concentrations, as well as the fetal total GI (tract plus contents), were available at the 10 mg/kg-day dose only. Placental ClO₄⁻ concentrations were detectable only at the 1.0 and 10 mg/kg-day dose. The ClO₄⁻ levels in these tissues from all other dose groups were below analytical detection limits. Therefore, these data were used to verify the applicability of assigned partition coefficients to the model. Figure 3 demonstrates that the PBPK model adequately simulates the data in these tissues.

Iodide parameters were determined by fitting the model to data from the iv radiolabeled iodide kinetic study. PA values were adjusted to describe the behavior of iodide data, where increasing PA values toward 1.0 l/h·kg generally increased the rate at which uptake and clearance in a particular tissue occurred, and decreasing PA slowed uptake and clearance. Clearance values for incorporation of inorganic iodide into hormones (CiProdCi) and clearance of the hormonal iodine (CiSecrCi) were determined from the fit of the model simulated incorporated iodine concentrations in the thyroid versus kinetic data after an iv injection of inorganic radioiodide. Figure 4 shows the simulations for various maternal tissues versus measured ¹³¹I data.

Clearance values for the transfer of iodide between the placenta and fetal blood were determined by visually optimizing the fit of the fetal serum prediction to the data, while maintaining the fit of the maternal blood, placenta, and fetal tissue data simulations. Figure 5 shows the model simulation versus fetal ¹²⁵I data. Fetal thyroid iodide uptake increases rapidly in the final days of gestation (Carpenter, 1959; Feldman et al., 1961). In order to describe this uptake, the model simulation was fit to fetal thyroid uptake data reported in Feldman et al. (1961) 24 h after administration of ¹³¹I to the dam on days 17, 18, and 19 of gestation (Fig. 5d). The time-dependent parameters were then incorporated into the model using simple linear interpolation between data points to estimate the value of the parameter at any point in time.

**Model Validation**

Model simulations were tested against data sets collected under different conditions than the data used for model parameterization to test the predictive capability of the model. Model predictions for iodide kinetics were tested against data collected in other laboratories and in different strains of rats from the literature. Although the limited data do not allow testing of the entire acute kinetic time course, they do allow validation of tissue iodide concentrations at specific time points (e.g., 2 and 24 h postdosing). Finally, the model’s ability to describe both acute perchlorate and iodide kinetics and the interaction between the anions (inhibition of thyroidal iodide uptake) was tested against acute inhibition data collected in our laboratory and in literature after iv dosing with both ClO₄⁻ and radioiodide.

**Data of Versloot et al. (1997).** A simulation was performed with the model, using the exposure conditions of Versloot et al. (1997; an iv injection of 10 μCi or 1.74 ng/kg carrier free ¹²⁵I in pregnant Wistar rats on GD 19). This study provided a data set on an additional day of gestation (GD 19 vs. GD 20), as well as an additional time point (24 h) and included measurements of the total fetal burden, which had not previously been determined. The model was able to simulate the data in the maternal thyroid, mammary gland, and placenta, and fetal thyroid (Figs. 6a–6d). The model prediction of total body burden (Fig. 6e) underpredicted the rest of body iodide content in the fetus after removal of the thyroid. However, the model simulation is within a factor of two from the measured data.

**Data of Sztanyik and Turai (1988).** A simulation of data reported in Sztanyik and Turai (1988) was performed with the model to test the ability of the model to predict I¹³¹ distribution from a lower dose than was used in model parameterization, as well as a different species of radiolabeled iodide (¹²⁵I vs. ¹³¹I). Sztanyik and Turai administered an ip injection of 0.36 ng/kg carrier free ¹³¹I to pregnant CFY albino rats on GD 20 and measured tissues at 24 h postdosing. The model reproduced the placenta data well at 24 h postdosing without changing any kinetic parameters. The model prediction of the total fetal body burden was slightly high, but was within a factor of two from the measured data. Figure 7 shows the model simulation versus the measured values of ¹³¹I in the placenta and total fetal body.

**Drinking water inhibition data.** Inhibition of thyroid iodide uptake as well as iodide distribution in several maternal and fetal tissues were measured in the perchlorate drinking water study after maternal exposure to levels of 0.0, 0.01, 0.1, 1.0, and 10.0 mg ClO₄⁻/kg-day for 18 days (see Materials and Methods). Due to upregulation, none of the thyroid iodide concentrations measured after 18 days of perchlorate dosing...
were significantly different from the control levels. However, iodide data from the control group (0.0 mg/kg-day ClO₄⁻) were useful for testing model predictions at 125I doses more than four orders of magnitude greater than those used to parameterize the model (33,000 ng/kg vs. 2.19 ng/kg). Table 3 illustrates the ability of the model to predict iodide uptake in several tissues in the dam and fetus on GD 20.

Upregulation of thyroid NIS activity was modeled as described in the Materials and Methods section. Using this equation, it was possible to describe the increase in iodide uptake based on the perchlorate dose in chronic exposure scenarios. Neither the measured data nor the model showed any inhibition in iodide concentrations in the maternal thyroid 2 h postdosing with 125I after 18 days of exposure to 0.01, 0.1, 1.0, and 10.0 mg/kg ClO₄⁻ in drinking water. Thus, the model was able to reproduce the upregulation of thyroid NIS activity resulting from subchronic perchlorate exposures in a semiempirical manner. Due to the lack of necessary data, this model does not
attempt to describe the time-dependent nature of thyroid upregulation, nor does it account for physiological changes apart from increased NIS, such as increased serum binding or decreased deiodination. Thus, the present model represents a heuristic approximation of thyroid upregulation resulting from prolonged \(\text{ClO}_4^-\) exposure. A more complete description would require considerable additional experimental data, including time course data, hormone levels, and endogenous iodine information, in order to properly simulate this time-dependent regulation.

**Inhibition of iodide uptake.** The inhibition of iodide uptake into the thyroid and placenta were simulated against data collected in our laboratory on GD 20, using the same kinetic parameters derived previously. Figure 8 shows the model prediction of thyroidal (a) and placental (b) iodide uptake with and without perchlorate inhibition. The model is able to predict
inhibition in the thyroid gland up to 24 h after dosing with iodide. The ability of the model to predict perchlorate-induced inhibition of thyroid iodide uptake is dependent on accurate simulations of both iodide and perchlorate kinetics. Thus, the appropriateness of the model perchlorate and iodide parameters is supported by the fit of the model simulation to these inhibition data.

Inhibition data of Sztanyik and Turai (1988). Further validation of model-predicted inhibition was validated against additional perchlorate dose levels (3.0 and 6.0 mg/kg KClO₄) by testing the model-predicted fetal iodide uptake with and without ClO₄⁻ against data from Sztanyik and Turai (1988). In this study, GD 20 pregnant rats were given an ip tracer dose of ¹³¹I. At 20, 60, or 120 min post-¹³¹I dosing, an ip dose of KClO₄ (either 3.0 or 6.0 mg) was administered to the dams. The uptake of radioiodide in the whole fetus was then measured 24 h after the ¹³¹I dose. The model was able to accurately predict the measured inhibition of fetal uptake in two of the three studied groups on GD 20. The third group showed only slightly less inhibition than the model predicted. Table 4 shows the model predicted inhibition of iodide uptake in the whole fetus versus the ranges (mean ± 1 SD) of measured values given in Sztanyik and Turai (1988). Mean values are given in parentheses. The ability of the model to simulate these data supports its usefulness in predicting inhibition of placental transfer and fetal thyroid uptake.

Data of Brown-Grant (1966). Brown-Grant (1966) administered 0.25 and 1% potassium perchlorate solution (approximately 200 and 800 mg/kg respectively) by oral gavage for one to five days (GD 2 to 8) in Wistar rats, and observed ¹³¹I thyroid:plasma ratios of between 1.8 and 4.1. Since these large perchlorate doses are sufficient to effectively swamp the NIS, the measured thyroid:plasma ratios primarily reflect the extent to which thyroid iodide uptake is dependent upon partitioning. As described previously, the PBPK model describes partitioning based on the measured stoichiometries of Chow and Woodbury (1970). Using these previously calculated partition coefficients and permeability area cross products, the model predicts thyroid:plasma ratios of 3.9 and 3.6, which are within the range of measured values reported by Brown-Grant (1966), thus supporting the validity of the method for calculating partition parameters.

Sensitivity analysis. Sensitivity analysis performed at 0.1 and 10.0 mg ClO₄⁻/kg-day drinking water revealed a dose-dependent difference in model sensitivity to various parameters. At 0.1 mg/kg-day, the maternal serum is primarily dependent on serum binding and urinary clearance parameters with a greater sensitivity to the serum binding. Sensitivity coefficients for all other parameters were less than 0.1. At the 10.0 mg/kg-day dose, however, only the urinary clearance remained significant, with a sensitivity coefficient of –0.84. Fetal serum is influenced by several model parameters at the 0.1 mg/kg-day dose, including placental transfer, placental active uptake, placental diffusion, fetal serum binding, and maternal urinary clearance parameters. At the higher dose (10.0 mg/kg-day), where active placental uptake and serum binding are likely saturated, only the placental transfer, placental diffusion, and maternal urinary clearance maintain significant sensitivity coefficients. Figure 9 shows the calculated sensitivity coefficients for maternal and fetal serum ClO₄⁻ AUC on GD 20. Results of the sensitivity analysis for thyroid iodide uptake (not shown) were similar, in that all of the parameter sensitivities were less than one in absolute values; however, model predictions of this metric were sensitive to a much larger number of input parameters. This result is not unexpected due to the fact that the uptake prediction is for a specific point in time after administration of the radioiodide, and the rate of distribution into all tissues can affect the time-dependent result (as compared to an average, or AUC, measure, which reflects steady-state behavior). Thus the validation of the model with data on thyroid iodine uptake and inhibition provides a stringent test of the model parameterization.

Internal dosimetrics. The validated model was used to calculate internal dosimetrics corresponding to perchlorate dosing in the pregnant rat. These internal measures of ClO₄⁻ dose include the AUC for ClO₄⁻ in the maternal serum and relative fetal dose after repeated drinking water exposure, as well as inhibition of iodide uptake in the thyroid after acute dosing. These internal dosimetrics were then compared to the ClO₄⁻ serum AUC and iodide percent inhibition in the male rat (calculated from the model of Merrill et al., 2003) to provide insight on relative exposure at different life stages. Tables 5 and 6 show the dosimetric comparisons among the adult male,
pregnant, and fetal rat for drinking water serum levels, acute iodide inhibition, and fetal ClO₄⁻ dose. The models predict a significant transfer of maternal ClO₄⁻ to the fetus. They also indicate that the greatest inhibition is predicted to be in the fetal thyroid.

DISCUSSION

The PBPK model described here successfully simulates perchlorate and radioiodide distribution kinetics in the pregnant rat and fetus by accounting mathematically for physiological changes in gestation, such as nonlinear growth. The model is able to reproduce ClO₄⁻ distribution and placental transfer resulting from drinking water exposure at doses ranging over three orders of magnitude (0.01–10.0 mg/kg-day). Although acute kinetic data for perchlorate is not available in the pregnant or fetal rats, in terms of exposure route, drinking water dosing is actually more relevant to the risk assessment. Thus, the model is able to describe distribution to the target tissues.

**FIG. 5.** Radioiodide concentration in fetal (a) serum, (b) skin, and (c) total GI after iv dose of 2.19 ng/kg I⁻ to dam. (d) Amount of radioiodide in the fetal thyroid 24 h after maternal iv dose on GD 17, 18, and 19 (Feldman et al., 1961). Cross-bars indicate the mean ± SD of data, while squares indicate reported averages.
and in the serum resulting from exposure via the route that is most applicable to that of humans.

Despite the lack of data regarding the serum binding of ClO$_4^-$, the model is able to reproduce both total serum concentrations and tissue levels resulting from only free ClO$_4^-$ transfer. The model-predicted bound perchlorate levels compare reasonably to the measured values from in vitro binding data of Fisher (2002, as cited in Merrill et al., 2003) in male rat serum. The model predicts 30 and 80% of the ClO$_4^-$ to be bound at total serum levels of 500 and 50 ng/ml ClO$_4^-$ as compared to the measured values of 50 and 100%. As expected, the sensitivity analysis suggests that at the 10.0 mg/kg-day dose, the model serum prediction is most sensitive to the value for urinary clearance, while the lower doses (0.1 mg/kg-day) are primarily determined by the binding parameters, indicating that the binding is saturated between the 1.0 and 10.0 mg ClO$_4^-$/kg-day dose groups.

In the absence of acute perchlorate kinetic data in rat gestation, we make use of the consistency of this model structure and its parameters with those of the male rat (Merrill et al.,

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**FIG. 6.** Radioiodide in maternal (a) thyroid, (b) mammary gland, and (c) placenta, plus fetal (d) thyroid and (e) total body (minus thyroid) after a single iv injection of $^{125}$I to GD 19 Wistar dam. The model simulation is shown versus the mean ± SD at the 4 and 24 h data points in the thyroid and the 24 h data point in the other tissues (Versloot et al., 1997).
which successfully describes such data. Having accounted for differences in physiology due to gestation, most chemical-specific parameters remain essentially unchanged from those of the adult male rat. Therefore, we can assume that, like the male rat model, this model is also able to adequately predict acute perchlorate kinetics. This is further supported by the ability of the model to predict acute iodide inhibition kinetics, as inhibition of thyroid uptake is dependent on free serum perchlorate levels.

The PBPK model was able to predict the ClO$_4^-$ induced inhibition of iodide uptake in the maternal thyroid and the total fetus after acute ClO$_4^-$ exposures in the pregnant rat by linking the perchlorate and iodide models via competitive inhibition at the symporter. Although some authors have suggested that ClO$_4^-$ may not be translocated into the thyroid cell via NIS (Riedel et al., 2001a,b), the weight of evidence suggests that the anion is a true competitive inhibitor of iodide uptake (Clewell and Gearhart, 2002). Based on electrogenicity studies showing that the addition of 500 μM ClO$_4^-$ to a bathing medium containing I$^-$ abolished the existing inward current in an oocyte with NIS (Eskandari et al., 1997), Riedel and coauthors (2001a,b) suggested two potential mechanisms for ClO$_4^-$ action on the thyroid: (1) that ClO$_4^-$ blocks I$^-$ transport, but is not transferred into the cell, and (2) ClO$_4^-$ competes with I$^-$ and is transferred into the cell by NIS at a 1:1 ratio with Na$^+$. In our model, we utilize the latter proposed mechanism, in which ClO$_4^-$ competes for binding sites on the NIS and is transferred into the follicle in place of iodide. This assumption is based on measured radiolabeled and cold perchlorate thyroid concentrations with tissue to blood ratios greater than one (Chow and Woodbury, 1970; Yu et al., 2002b), the consistency of ClO$_4^-$ accumulation in tissues with NIS, and studies showing that ClO$_4^-$ affects the internal thyroid iodide as well as the external uptake of iodide by NIS (Hildebrandt and Halmi, 1981). The ability of the model to reproduce experimental perchlorate, iodide, and inhibition data from different exposure scenarios (acute vs. drinking water) and over a wide range of doses supports the use of this mode of action as the foundation for the pharmacokinetic models.

The iodide model was simplified by assuming that radiola-
beled iodide could be described as free iodide in all compartments other than the maternal thyroid. Simulations were performed against total radioiodide concentrations in extrathyroidal tissues and against inorganic and incorporated iodide in thyroid. Despite this simplification of the model structure, the kinetic behavior of radioiodide in the naïve rat (no ClO₄⁻ exposure) was accurately simulated in all measured maternal and fetal tissues over a range of doses spanning nearly five orders of magnitude (0.36 to 33,000 ng/kg) 24 h after a single administration of radiolabeled iodide. Intralaboratory studies found that approximately 80% of serum iodide is in the inorganic form in the pregnant rat as much as 12 h after dosing with radioiodide (Yu et al., 2002a). Thus, the model description of extrathyroidal tissue iodide uptake based on the transfer of inorganic iodide via NIS predicts the data reasonably well without significant contribution from the uptake of incorporated iodide.

This PBPK model describes the dosimetry, distribution, kinetic behavior, and interaction between administered radioiodide and perchlorate. As such, a description of dietary iodide and interactions of endogenous and dosed iodide have not been included in the present version of the model. For the purpose of extrapolation of this animal model to human exposure scenarios, the influence of variations in dietary iodide intake will need to be included, in order to make the human predictions more applicable across populations. However, in the rat, iodide intake is easily controlled through the animal’s diet, and given that iodide is an essential nutrient, the mineral is tightly controlled by regulatory mechanisms. Therefore, slight variations in laboratory rat chow are not expected to affect model predictions of distribution or even perchlorate-induced inhibition. Although the mechanism for iodide transfer (NIS) is saturable, the high value for Kₘ dictates that very large doses of iodide would be required to inhibit its own uptake, as opposed to more effective inhibition by the higher affinity ClO₄⁻. In fact, in studies by Wolff and Chaikoff (1948), it was found that the uptake of iodide into the thyroid is linear up to serum iodide levels of 250 μg/l, which is more than six times higher than

### TABLE 3

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Radioiodide concentration (ng/l)</th>
<th>Model predicted</th>
<th>Measured (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal serum</td>
<td>4.5 × 10⁴</td>
<td>4.1 ± 0.6 × 10⁴</td>
<td></td>
</tr>
<tr>
<td>Maternal thyroid</td>
<td>1.8 × 10⁵</td>
<td>1.1 ± 0.5 × 10⁵</td>
<td></td>
</tr>
<tr>
<td>Maternal GI contents</td>
<td>1.3 × 10⁵</td>
<td>1.3 ± 0.6 × 10⁵</td>
<td></td>
</tr>
<tr>
<td>Placenta</td>
<td>4.9 × 10⁵</td>
<td>5.0 ± 1.0 × 10⁵</td>
<td></td>
</tr>
<tr>
<td>Fetal serum</td>
<td>1.8 × 10⁵</td>
<td>2.3 ± 1.7 × 10⁵</td>
<td></td>
</tr>
<tr>
<td>Fetal GI (tract + contents)</td>
<td>4.7 × 10⁵</td>
<td>4.0 ± 2.0 × 10⁵</td>
<td></td>
</tr>
<tr>
<td>Fetal skin</td>
<td>3.5 × 10⁵</td>
<td>4.0 ± 2.0 × 10⁵</td>
<td></td>
</tr>
</tbody>
</table>

Note. Two h after an iv dose of 33,000 ng/kg ¹²⁵I⁻ to the dam on GD 20.

![Graph](https://academic.oup.com/toxsci/article-abstract/73/2/235/1674339)

FIG. 8. Iodide concentration in (a) maternal thyroid and (b) placenta with and without 1.0 mg/kg ClO₄⁻ iv dose 2 h prior to an iv dose of 1.87 ng/kg ¹²⁵I⁻ to the dam (Clewell et al., 2001). The top simulation (solid line) and data (solid box) indicates the control group. The lower simulation (dotted line) and data (open circle) indicate the perchlorate-dosed inhibition group.
well as a more detailed description of hormone distribution, homeostasis, and regulation.

This model currently includes only a highly simplified description of the hormone feedback system that controls iodide uptake and upregulation of the inhibited thyroid in order to predict the dose-dependent change in thyroid iodide uptake after long-term exposure to ClO$_4^-$ in the rat. However, it is important to note that the rat thyroid has a much greater sensitivity to perturbations in the pituitary-thyroid axis than the human. In fact, evidence of upregulation is seen as early as 12 h after administration of ClO$_4^-$ to a male rat (Merrill et al., 2003), whereas human studies have found that after two weeks of ClO$_4^-$ exposure, TSH levels were not yet elevated and thyroidal iodide uptake showed inhibition similar to that of the control subjects (Brabant et al., 1992; Greer et al., 2002). Although the human shows similar inhibition to the rat (Greer et al., 2002) at equal perchlorate doses, the human system does not respond by upregulating thyroid activity. This species difference is most likely due to the fact that the human has a greater thyroid hormone storage capacity than the rat, resulting from increased thyroid colloid volume and serum binding (thyroxine binding globulin; Brown et al., 1986; Dohler et al., 1979). Therefore, small perturbations in iodide uptake do not appear to affect hormone balance to the same extent in humans as in rats. Since stable serum ClO$_4^-$ levels cannot be established in rats for more than a few hours without inducing upregulation of NIS, it is not possible to use rats directly as an animal model for human drinking water ClO$_4^-$ exposure. However, by using the rat PBPK models to extrapolate to humans, we can mathematically bridge this gap by using the existing rat data and incorporating a longer time course for upregulation in the human model.

Together with a concurrent model developed by Merrill et al. (2003), the present PBPK model is able to define and quantify kinetic differences in the male, pregnant, and fetal rat. The models account for kinetic differences through the description of physiological differences. Most of the kinetic parameters are nearly identical between the male rat and pregnant rat, with the exception of those for thyroid and skin uptake and urinary clearance of iodide. Where different kinetic parameters were necessary in the two models, literature studies were able to provide a justification for these differences. For example,

---

**TABLE 4**

<table>
<thead>
<tr>
<th>KClO$_4$ dose (mg)</th>
<th>Time of ClO$_4^-$ dose (after $^{131}$I dose) (min)</th>
<th>% Inhibition in fetus (% control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.0</td>
<td>20</td>
<td>13</td>
</tr>
<tr>
<td>3.0</td>
<td>60</td>
<td>18</td>
</tr>
<tr>
<td>3.0</td>
<td>120</td>
<td>20</td>
</tr>
</tbody>
</table>

**TABLE 5**

<table>
<thead>
<tr>
<th>ClO$_4^-$ dose (mg/kg-day)</th>
<th>Serum ClO$_4^-$ AUC (mg/l)</th>
<th>Fetal dose (% maternal dose)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male rat</td>
<td>Pregnant rat</td>
</tr>
<tr>
<td>0.01</td>
<td>0.045</td>
<td>0.0510</td>
</tr>
<tr>
<td>0.1</td>
<td>0.157</td>
<td>0.167</td>
</tr>
<tr>
<td>1.0</td>
<td>0.598</td>
<td>0.613</td>
</tr>
<tr>
<td>10.0</td>
<td>1.520</td>
<td>1.560</td>
</tr>
</tbody>
</table>

**FIG. 9.** Calculated sensitivity coefficients for model parameters with respect to serum perchlorate AUC at drinking water doses of 0.1 and 10.0 mg/kg-day.
Brown-Grant and Pethes (1959) found that the adult male and neonatal rat possess a greater iodide reserve in the skin than the pregnant dam. This difference in life stage kinetics is reflected in the parameters for skin iodide uptake in the pregnant and male rat models (e.g., $V_{\text{max}} c_{\text{SI}} = 6.0 \times 10^4$ vs. $5.0 \times 10^5$, respectively; Merrill et al., 2003). This information provided by the models, concerning the variation in perchlorate and iodide kinetics across gender and life stages in the rat, should improve the accuracy of predictions for human perchlorate exposures during gestation (Clewell et al., 2001).

A comparison of model-predicted internal dosimetries provides important information on $\text{ClO}_4^-$ distribution and inhibition kinetics across life stages. It is apparent that the fetus receives a significant portion of the maternal $\text{ClO}_4^-$ dose, while the serum AUCs are similar between the male and pregnant rats. Comparison of thyroid iodide inhibition suggests that the thyroid of the pregnant rat may be somewhat more sensitive to inhibition than that of the male rat. This may be due to the increased loss of iodine during pregnancy, resulting from loss to the fetus and increased urinary output (Versloot et al., 1997). The predicted fetal thyroid inhibition was even greater than that of the male and pregnant female, possibly due to the combined effect of inhibition at both the placenta and fetal thyroid. Inhibition in the fetal thyroid is partially offset by the increased fetal serum iodide levels resulting from inhibition in the fetal skin and GI contents. However, the actual risk to the fetus may be better characterized by looking at the effect of perchlorate on iodide levels in the total fetus, due to the fact that fetal iodide stores in extra-thyroidal tissues (e.g., skin) are important in maintaining the needed iodide supply during the transition from intra- to extra-uterine life.

A potentially informative use of this PBPK model is in the correlation of predicted internal dosimetries to periods in gestation where perchlorate exposure and/or iodide deficiency has been associated with developmental effects. The model can be used to predict tissue dosimetry in effects studies and to pinpoint specific times in gestation when fetal iodide uptake is most critical. A lactation exposure model is currently in development for use with the gestation model to provide a complete kinetic description of the developmental period. When used together, these developmental models could be used to help answer questions concerning susceptible time points and primary routes of exposure during development.

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