External Letter Peer Review of EPA's Draft Report, Inhibition of the Sodium-Iodide Symporter by Perchlorate: An Evaluation of Lifestage Sensitivity Using Physiologically-based Pharmacokinetic (PBPK) Modeling

Contract EP-C-07-024
Task Order 54

Submitted to:
U.S. Environmental Protection Agency
Office of Research and Development
National Center for Environmental Assessment
Research Triangle Park, NC 27711

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November 12, 2008

QUALITY NARRATIVE STATEMENT

ERG selected reviewers according to selection criteria provided by EPA. EPA confirmed that the scientific credentials of the reviewers proposed by ERG fulfilled EPA's selection criteria. Reviewers conducted the review according to a charge prepared by EPA and instructions prepared by ERG. ERG checked the reviewers' written comments to ensure that each reviewer had provided a substantial response to each charge question (or that the reviewer had indicated that any question[s] not responded to was outside the reviewer's area of expertise). ERG organized reviewer comments by charge question, however, since this is an independent external review, ERG did not edit the reviewers' comments in any way, but rather transmitted them unaltered to EPA.

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Contents

Responses to General Charge Questions

(G1) Is EPA's analysis logical, clear and appropriate in depth and length? Has EPA accurately, clearly and objectively represented and synthesized the scientific evidence for the changes made to or specification of the model code and input parameters?

Janusz Byczkowski	The reviewed EPA draft document is logical, clear and concise. In general, reasons for changes in code and input parameters and most of their their consequences are objectively described. However, given a large number of abbreviations and acronyms, a glossary listing these abbreviations and acronyms would be helpful.
Brian Cummings	EPA's analysis is logical and clear. The length is appropriate and the appendix aid in understanding their approach while adding to depth. Care is taken to explain what changes were made to the model, why there were made, the scientific evidence and literature used, the implications of these changes. In many cases EPA has simulated these changes and found little, to no effect on the model.
Panos Georgopolous	The document under review, "Inhibition of the Sodium-Iodide Symporter (NIS) by Perchlorate: an Evaluation of Lifestage Sensitivity Using Physiologically-Based Pharmacokinetic (PBPK) Modeling", has a very specific, and deliberately narrow, objective. Indeed it defines (p. 21) sensitivity "as the predicted response in percent RAIU (radioactive iodide uptake) inhibition 24 hours after iodide intravenous injection for an average individual within a specific subgroup (e.g., bottle-fed infants) relative to the predicted response in percent RAIU inhibition for an average, non-pregnant adult, where response is the percent RAIU inhibition 24 hours after iodide IV injection." Though this is rather constrained as a sensitivity metric, it can be reasonably argued that it addresses adequately the biological issue of concern here. The emphasis of the analysis is on sensitivity with respect to "lifestage". Parameters and processes, related to different lifestages, were modeled based on assumptions that are discussed in rather extensive detail in the document under review. The lifestages evaluated in the document correspond to "average" adult, non-pregnant woman of child-bearing age, pregnant woman, lactating woman, fetus, breast-fed infant, bottle-fed infant, 1 year old child, and and 2 year old child. The tools employed for the analysis were the PBPK models of Clewell et al. (2007) for the pregnant woman/fetus and for the lactating woman/breastfed infant. Results for the "bottle-fed" neonate were obtained by altering the dose specification in the model for the breast-fed infant. The PBPK model for the average adult was that of Merrill et al. (2005), while the model for the non-pregnant woman of childbearing age was a direct modification of the model for the pregnant woman, obtained by removing the placental and fetal compartments, but retaining the mammary compartment. The above PBPK models, with various corrections and adjustments (that are discussed in detail in the appendices of the document) were used to estimate the predicted percent RAIU inhibition for the average adult and different specific ("average") individuals representing potentially sensitive subgroups. It should be mentioned here that the actual text of the document under review states that the calculations were made for "subgroups, including potentially sensitive subgroups"; however population-based modeling (with considerations of inter-individual and intra-individual variability) was not actually

pursued.

"Base" calculations were made assuming a dose equal to the point of departure (POD) of 7 μg/kg-day, (consistent with the recommendations of the National Research Council - NRC, 2005) and were summarized in Table 3 of the document under review. The relative sensitivity of different subgroups was determined by comparing the percent RAIU inhibition of each subgroup to the percent RAIU inhibition for an average adult at a dose equal to the POD.

The document states that the "model predictions may generally be considered central estimates for each subgroup (at the consumption levels modeled) that account for PK (pharmacokinetic) differences, and do not take into account within-group variability in pharmacokinetics, uncertainty in model parameters and predictions, or population differences in PD." It should be noted that fetal simulations were reported for only the end of gestation (Gestation Week 40).

The analysis presented in the document concluded that urinary clearance was a "key" parameter (i.e., model predictions were highly sensitive to the values of this variable). Though for modeling pregnancy and early infancy a conservative parameterization was adopted, the document emphasizes that "a full population analysis of urinary clearance was not conducted, and given that variability in other PK parameters was not addressed, these estimates should not be considered a true upper confidence bound on RAIU inhibition" (p. 23). The document also identified the fetus as the most sensitive subgroup with respect to percent RAIU inhibition at a dose equal to the POD, in general agreement with earlier PBPK modeling (Clewell et al., 2007) and estimating approximately 5-fold higher percent RAIU inhibition for the fetus at gestational week 40 than for the average adult. In fact it is also stated that "simulations at earlier gestation weeks indicate that the fetus is more sensitive than the adult throughout pregnancy, but are considered too quantitatively uncertain to assign exact relative sensitivities" (p. 23).

Overall it can be stated that EPA's analysis is clear and with sufficient discussion of assumptions involving model and parameter specification (including adjustments and corrections to the original models and their codes). It should be noted, however, that in multiple instances (discussed further in the answers to the following questions) the rationale behind specific assumptions and parameterizations relates more to "convenience" rather than to scientific defensibility. Although this may not necessarily affect the general conclusions, it is nevertheless a weakness of the analysis presented in the document under review.

References Cited in Answer to Question G-1:

Clewell, R.A., Merrill, E.A., Gearhart, J.M., Robinson, P.J., Sterner, T.R., Mattie, D.R., and Clewell, H.J., 3rd. 2007. Perchlorate and radioiodide kinetics across life stages in the human: using PBPK models to predict dosimetry and thyroid inhibition and sensitive subpopulations based on developmental stage. J Toxicol Environ Health A 70 (5):408-28.

Merrill, E.A., Clewell, R.A., Robinson, P.J., Jarabek, A.M., Gearhart, J.M., Sterner, T.R., and Fisher, J.W. 2005. PBPK model for radioactive iodide and perchlorate kinetics and perchlorate-induced inhibition of iodide uptake in humans. Toxicol Sci 83 (1):25-43.

NRC. 2005. Health Implications of Perchlorate Ingestion. National Research Council of

	the National Academies, National Academies Press. Washington, D.C. http://www.nap.edu/catalog/11202.html
Sean Hays	Yes
Frederick Kaskel	Physiologically based pharmacokinetic (PBPK) models were modified to predict inhibition of the sodium-iodide symporter (NIS)5 for pregnant and lactating women, nursing infants, and for the subsequent stages of childhood. The published models were modified by EPA to fix errors and incorporate new data, particularly data on lifestage variability in the urinary clearance of pechlorate, to which NIS inhibition is sensitive. The models are suitable to provide quantitative predictions to the Agency on the lifestyle variability of perchlorate NIS inhibition of thyroidal iodide uptake. EPA's analysis is logical, clear and appropriate in depth and length and EPA has accurately, clearly and objectively represented and synthesized the scientific evidence for the changes made to or specification of the model code and input parameters.
Kannan Krishnan	• The EPA analysis of perchlorate-mediated inhibition of the NIS in humans is based on Merrill et al. (2005) and Clewell et al. (2007) PBPK models, and specifically addresses the variability of NIS inhibition as a function of lifestage. The document is clear and concise. The depth and legnth of presentation are appropriate, given the objective. • The structure of the PBPK models published by Merrill/Clewell has not been altered; rather some of the input parameters as well as equations have been modified either to correct an error or to reflect current state of knowledge more appropriately.
Chensheng Lu	The Reviewer is convinced that EPA has performed an outstanding job in improving the PBPK model so the codes written in the model are consistent to the physiology of iodide uptake in the thyroid glands and the uptake inhibition by perchlorate. It is also clear that EPA has tried to perfect the input parameters to increase the predictability of the model.
Lauren Zeise	In general EPA's analysis is clear and logical, and succinct, although there are several suggestions for improvement in the comments below. With regard to depth, the limited treatment of variability and uncertainty is problematic. Pharmacokinetic models can provide a structure for exploring and integrating variability, but this was not done in this analysis. This major limitation is recognized by EPA (page 25). EPA points out the model predictions apply to "a subgroup average for typical, healthy individuals, and effectively describe the RAIU inhibition relative to that same individual as his/her own control." EPA further points out that "These models were not designed to account for whether the pregnant women are hypothyroid or iodine deficient." Analysis of such large, susceptible populations is a critical aspect of understanding the potential health impact of perchlorate drinking water exposure. A more rigorous and explicit treatment of variability is needed to get a better handle on intra-human variability in response to perchlorate exposure. The analysis would also be improved by more rigorous statistical and quantitative treatment of uncertainty. The degree to which the analysis for the GW

40 fetus may or may not represent the first and second trimester fetus needs explicit and careful treatment.

On a smaller point, it would help if greater motivation was provided for some of the statistical fits to data. Some statistical fits provided an expedient and practical way forward in the analysis but appeared to introduce logical inconsistency. It would be preferable for a more expanded discussion to provide a context for the approach taken.

(G2) Please identify any additional studies or other data sources that should be considered in the assessment of the specific parameters addressed below, including urinary clearance (of iodide and/or perchlorate) and ingestion rates (breast milk, formula and water), especially in neonates.

Janusz Byczkowski

It seems, that most of the published relevant data have been already reviewed and/or included in the modeling and analysis.

However, this Reviewer could not identify the reference posted in the EPA Perchlorate human lactation model code (pp. 74 and 76, lines 67 and 131, respectively): *Gentry et al. (2001)*. It is suggested, that for the *VMk* parameter ("Residual milk volume", see the answer to G-3 below), another data source could be used: Dewey K.G., Heinig M.J., Nommsen L.A., and Lonnerdal B.: Maternal versus infant factors related to breast milk intake and residual milk volume: the DARLING study. Pediatrics 1991: 87: 829–837.

Brian Cummings

Zuckier et. al., Journal of Nuclear Medicine, 45(3), 500-507, 2004. This study mainly assesses perrhenate, but also studies the interaction of iodide and perchlorate in NIS tissues, both in vivo and in vitro. It specifically studies biodistributions of these compounds in the presence and absence of each other. It takes into account the effect of NIS on this distribution. It may prove helpful.

A search of pubmed did not reveal any references to iodide or perchlorate clearance not already mentioned by the authors. The most recent article I could find on either subject was DeWoskin and Thompson, 2008, which the authors use.

Why was a compartment analysis figure not shown for this revised model? Such figures are useful to readers in conceptualizing the model. These were included in the literature on which the current model was based.

Panos Georgopolous

The scientific literature relevant to NIS inhibition by perchlorate is currently growing fast; the same holds true for related literature areas covering fields such as demographics and exposure informatics and modeling, Physiologically-Based Pharmacokinetic and Pharmacodynamic modeling methods, etc. Though the document under review is not expected to provide a thorough literature review of the subject of perchlorate inhibition of NIS and of related exposure and risk issues, it could certainly provide a more complete picture to its readers, by incorporating some of the references suggested below.

These suggestions are grouped in three categories: (a) "general references," that cover various aspects of perchlorate exposure and effect, (b) references that focus on studies of human exposure to perchlorate, and (c) references that focus on biological (physiological and biochemical) issues, either directly specific to perchlorate and NIS inhibition or indirectly related, such as e.g. references on information for urinary clearance related parameters or on information for PBPK modeling specific to infants.

It should be noted in particular that USFDA (The US Food and Drug Administration) has developed PBPK modeling recommendations, as well as computer software that implements them, for early life stages (Luecke et al., 2007, 2008); at a minimum, it would be useful to examine how these parameterizations compare to the ones adopted in the analysis presented in the document under review. (Similarly, it would be useful to

compare exposure-related parameter selections used in the reviewed work to corresponding relevant recommendations in USEPA's Child-Specific Exposure Factors Handbook).

General:

ATSDR. 2008. Toxicological Profile for Perclorates. Agency for Toxic Substances and Disease Registry. Atlanta, GA. http://www.atsdr.cdc.gov/toxprofiles/tp162.pdf

Charnley, G. 2008. Perchlorate: Overview of risks and regulation. Food and Chemical Toxicology 46 (7):2307-2315.

De Groef, B., Decallonne, B.R., Van der Geyten, S., Darras, V.M., and Bouillon, R. 2006. Perchlorate versus other environmental sodium/iodide symporter inhibitors: potential thyroid-related health effects. Eur J Endocrinol 155 (1):17-25.

Gu, B., and Coates, J.D. 2006. Perchlorate: Environmental Occurrence, Interactions and Treatment. New York: Springer.

Kirk, A.B. 2006. Environmental perchlorate: why it matters. Anal Chim Acta 567 (1):4-12.

Kirk, A.B., Dyke, J.V., Martin, C.F., and Dasgupta, P.K. 2007. Temporal patterns in perchlorate, thiocyanate, and iodide excretion in human milk. Environ Health Perspect 115 (2):182-6.

Kirk, A.B., Martinelango, P.K., Tian, K., Dutta, A., Smith, E.E., and Dasgupta, P.K. 2005. Perchlorate and iodide in dairy and breast milk. Environ Sci Technol 39 (7):2011-7.

Wang, R.Y., and Needham, L.L. 2007. Environmental chemicals: from the environment to food, to breast milk, to the infant. J Toxicol Environ Health B Crit Rev 10 (8):597-609.

Exposure:

Baier-Anderson, C., Blount, B.C., Lakind, J.S., Naiman, D.Q., Wilbur, S.B., and Tan, S. 2006. Estimates of exposures to perchlorate from consumption of human milk, dairy milk, and water, and comparison to current reference dose. J Toxicol Environ Health A 69 (3-4):319-30.

Blount, B.C., Valentin-Blasini, L., Osterloh, J.D., Mauldin, J.P., and Pirkle, J.L. 2007. Perchlorate exposure of the US Population, 2001-2002. J Expo Sci Environ Epidemiol 17 (4):400-7.

Ginsberg, G.L., Hattis, D.B., Zoeller, R.T., and Rice, D.C. 2007. Evaluation of the U.S. EPA/OSWER preliminary remediation goal for perchlorate in groundwater: focus on exposure to nursing infants. Environ Health Perspect 115 (3):361-9.

Zender, R., Bachand, A.M., and Reif, J.S. 2001. Exposure to tap water during pregnancy. J Expo Anal Environ Epidemiol 11 (3):224-30.

Physiological/Biochemical:

Brandt, J.R., Wong, C.S., Hanrahan, J.D., Qualls, C., McAfee, N., and Watkins, S.L. 2006. Estimating absolute glomerular filtration rate in children. Pediatr Nephrol 21 (12):1865-72.

Clewell, R.A., and Gearhart, J.M. 2002. Pharmacokinetics of toxic chemicals in breast milk: use of PBPK models to predict infant exposure. Environ Health Perspect 110 (6):A333-7.

Dohan, O., De la Vieja, A., Paroder, V., Riedel, C., Artani, M., Reed, M., Ginter, C.S., and Carrasco, N. 2003. The Sodium/Iodide Symporter (NIS): Characterization, Regulation, and Medical Significance. Endocrine Reviews 24 (1):48-77.

Hawcutt, D.B., and Smyth, R.L. 2008. One size does not fit all: getting drug doses right for children. Archives of Disease in Childhood 93 (3):190-191.

Ito, S., and Alcorn, J. 2003. Xenobiotic transporter expression and function in the human mammary gland. Adv Drug Deliv Rev 55 (5):653-65.

Johnson, T.N. 2008. The problems in scaling adult drug doses to children. Arch Dis Child 93 (3):207-11.

Kurz, H., Sandau, K., Dawson, T.H., Brown, J.H., Enquist, B.J., and West, G.B. 1998. Allometric ccaling in biology. Science 281 (5378):751a-.

Lewandowski, T.A., Seeley, M.R., and Beck, B.D. 2004. Interspecies differences in susceptibility to perturbation of thyroid homeostasis: a case study with perchlorate. Regul Toxicol Pharmacol 39 (3):348-62.

Luecke, R.H., Pearce, B.A., Wosilait, W.D., Slikker, W., Jr., and Young, J.F. 2007. Postnatal growth considerations for PBPK modeling. J Toxicol Environ Health A 70 (12):1027-37.

Luecke, R.H., Pearce, B.A., Wosilait, W.D., Doerge, D.R., Slikker, W., Jr., and Young, J.F. 2008. Windows based general PBPK/PD modeling software. Comput Biol Med 38 (9):962-78.

McManaman, J.L., and Neville, M.C. 2003. Mammary physiology and milk secretion. Adv Drug Deliv Rev 55 (5):629-41.

Packard, G.C., and Birchard, G.F. 2008. Traditional allometric analysis fails to provide a valid predictive model for mammalian metabolic rates. J Exp Biol 211 (Pt 22):3581-7.

Spitzweg, C., Dutton, C.M., Castro, M.R., Bergert, E.R., Goellner, J.R., Heufelder, A.E., and Morris, J.C. 2001. Expression of the sodium iodide symporter in human kidney. Kidney Int 59 (3):1013-23.

Strawson, J., Zhao, Q., and Dourson, M. 2004. Reference dose for perchlorate based on thyroid hormone change in pregnant women as the critical effect. Regulatory Toxicology

	and Pharmacology 39 (1):44-65. West, G.B., Brown, J.H., and Enquist, B.J. 1997. A general model for the origin of allometric scaling laws in biology. Science 276 (5309):122-126.
Sean Hays	N/A
Frederick Kaskel	Additional studies that should be considered in the assessment of the specific parameters such as urinary clearance of iodide and/or perchlorate and ingestion rates (breast milk, formula and water) in neonates and these include data on maturation of tubular transport rates. On page 39 of the report, the issue of the role of pendrin transporter for iodide during development is addressed. One cannot assume that perchlorate and iodide are handled similarly by the developing kidney based on their similar charge and diameter; more data is needed in the investigation of tubular maturation of the transporters that regulate the clearance of iodide and perchlorate. This is also addressed on page 40, second paragraph in the report where it is stated that one cannot assume that the relative clearance for iodide and perchlorate should be constant across all ages and life stages. Additionally on page 42 the EPA states that there is no data on renal transporters during infancy to suggest the level and pattern of expression changes required to change clearance/GFR. Thus, the report used DeWoskin and Thompson's published data for scaling of renal excretion for infants by body weight and on page 44 the EPA extended its extrapolation to a 60-day-old, 5 kg child is sound. These assumptions are reasonable but indicate the importance of additional investigations in newborn models and in humans.
Kannan Krishnan	This reviewer is not aware of any studies in neonates that would provide better estimates of urinary clearance of perchlorate and iodide. Even though isolated studies reporting ingestion rates (breast milk, formula and water) in infants in other parts of the world could be obtained from the literature, such studies probably would only introduce further uncertainty. However, the study of Kirk et al. (2005). Perchlorate and iodine in dairy and breast milk. Environ Sci technol 39: 2011-17 may used to corroborate the findings of the present study – as it relates to the relationships between drinking water concentration and breast milk concentration.
Chensheng Lu	Considering the elevated inhibition of RAIU in bottle-fed infants, EPA should seek for additional data to re-affirm the water ingestion rates that are used by EPA, particularly the use of the 90^{th} percentile values in which the situations exceed the expectation of the fundamental knowledge. The Reviewer has no knowledge of whether there are studies or data sources that EPA could use.
Lauren Zeise	Additional possible studies and data sources are identified in response to specific charge questions below.

(G3) Besides the specific parameters identified below, please comment on any other parameter or model choices described in the document that you think are incorrect or require further explanation or where other available information could provide better estimates.

Janusz Byczkowski

The numerical value of the *VMk* parameter, identified in the EPA Perchlorate human lactation model code (p. 76), line 131: "*CONSTANT VMk = 0.6320 ! Residual milk volume (L) (Gentry et al 2001)*" seems to be unrealistically high. It is closer to the low daily breast milk intake by infant rather than to the residual milk volume (< 650 g/day *vs* 109 g/day; Dewey et al., 1991).

Since the mammary glands respond to feeding stimuli by secreting breast milk on demand, the "residual milk volume" usually refers only to the small volume of unconsumed milk. Without suckling stimulation, even lower void volume of milk remains in alveoli, lactiferous ducts and sinuses between the feeding sessions, and it stays in equilibrium with blood under near steady-state conditions (Byczkowski, J.Z. in *U.S. EPA (2002): Final Report "EXPLORATION OF PERINATAL PHARMACOKINETIC ISSUES"*, EPA/630/R-01/004, May 10, 2001. On-line: http://oaspub.epa.gov/eims/eimscomm.getfile?p_download_id=120867). While milk intakes vary with caloric demand of the infant (represented by *KTrans* in the EPA model), as reported by Dewey *et al*. (1991), infants with low intakes left as much milk unconsumed as those with higher intakes, which justifies the residual milk volume to remain constant (109 g/day).

On the other hand, the EPA PBPK modeling algorithm suggests that the *VMk* parameter corresponds rather to the initial volume of milk (632 g), further linked to the growth function of infant's body weight by *KTrans,* which infant receives in each "*pulse*". Even though this approach may adequately describe the volume of breast milk actually ingested by growing infant, it is not physiologically accurate and does not allow for any interspecies extrapolation. Since *VMk* affects concentrations of both iodide and perchlorate in breast milk, it is difficult to predict if and how the suggested change in *VMk* description would change the RAIU inhibition in the breast-fed neonate. Before any change, a sensitivity analysis should be performed with varied *VMk* value, to evaluate how *VMk* parameter affects the PBPK model output and to decide if and how this potential problem should be addressed.

Brian Cummings

The methods used for scaling of clearance to body weight, age and surface area are appropriate; however, such scaling is most accurate for clearance when the substance in question is not reabsorbed or secreted. Given that fact that both pendrin and NIS are reported to act on perchlorate, and given reports that NIS expression does not scale to bodyweight in some tissues (see below), do the authors feel that their approach is still valid? Do alterations in NIS expression need to be included in this model? If they are, would this increase the risk for children age 10-14, when NIS expression is believed to altered?

GFR in children is typically scaled according to muscle mass, which scales well with the cube of height in boys and girls from 6 months to adult (see Check, DB et al., Am. J. Clin. Nutr, 30:851, 1977). Scaling formulas have even been derived for children based on creatinine levels (See Diseases in the Kidney, Chapter 80, Seventh Edition, Editor =

	Schrier, Page 2355). Could these formulas be used to more accurately reflect GFR in children when calculating perchlorate and iodide clearance?
Panos Georgopolous	As discussed in more detail in the answer to the questions regarding the characterization of urinary clearance processes, there is a need to develop and thoroughly test a consistent framework for modeling these processes for different lifestages. "Correcting" the inconsistencies, that are in fact identified in Appendix B of the document under review, would be a first step towards the implementation of such a framework.
Sean Hays	N/A
Frederick Kaskel	There are no other parameters or model choices described in the document that are incorrect or require further explanation or provide better estimates.
Kannan Krishnan	The parameters of this model consist of: • Physiological parameters • Intake/contact rates • Partition coefficients • Permeability-area cross product • Urinary clearance • Binding parameters • Maximal velocity and affinity constants The parameter values found in the original reports and refined following EPA's evaluation would appear to be supported by available literature. However, focused data collection might facilitate the improvement of the partition coefficient values used in the model as well as the urinary clearance values for perchlorate and iodide in the various lifestages.
Chensheng Lu	EPA should explain the rationale of using the 90^{th} percentile values in the analysis. It seems to the Reviewer that such choice is deemed to create an upper bound limit, however, throughout the document, EPA has stated that this is not the purpose due to the uncertainties involved in the model simulation and other reasons.

Lauren Zeise

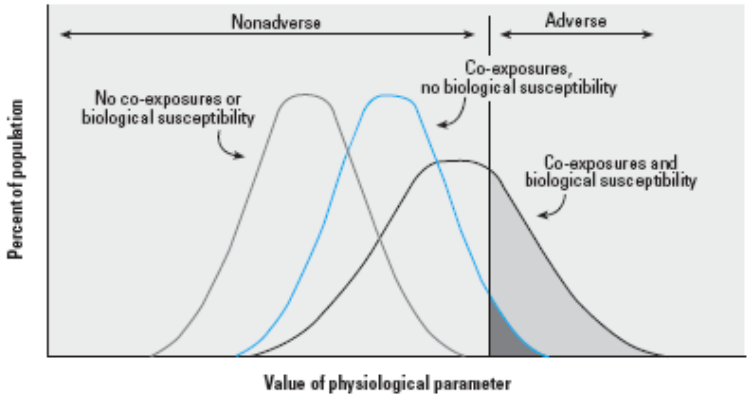

Figure 2. Distribution of a typical physiological parameter within the population and how that may vary depending on the influence of chemical and biologic background.

The above figure, taken from Woodruff et al. (2008; EHP 116:1568), illustrates the main limitation in the analysis and approach to modeling. The question in evaluating the potential risks from perchlorate in drinking water is about the extent to which the incremental exposure from water results in adverse effects to the mom, her baby, her developing fetus or others. In the above figure it is above the extent to which the perchlorate drinking water exposure, in the presence of coexposure and biological sensitivity, is creating adverse outcomes in the population. The fidelity of the analysis depends on whether individuals with biological susceptibility have been adequately addressed and also whether coexposures that affect iodide inhibition have been adequately considered.

EPA analysis enables biological susceptibility and coexposures to be partially addressed in the assessment, but it needs to move further to enable a fuller treatment. With regard to biological susceptibility EPA considers susceptible subgroups – the infant, fetus, mom – and an important factor that increases susceptibility in these groups – low renal clearance. But the analysis does not enable the agency to consider the extent of impact on other sensitive subgroups in these populations, such as those with clinical and subclinical hypothyrodism, those that may be genetically predisposed (see e.g., Scinicariello, EHP 113(11):1479-84), and those that are iodine deficient. The EPA analysis also considers an important coexposure – perchlorate intake via food. However, the analysis does not consider the combined impact with thiocyanate, which also affects iodide uptake at the NIS. Thiocyanate is also found in breast milk (see e.g., Kirk et al. 2007, EHP, 115:182-186), cigarette smoke, and common foods. The recent finding in women who smoked, that those with low urinary iodine levels had decreasing T_4 with increasing perchlorate (Steinmaus et al. 2007, EHP, 115:1333-1338) as well as reduced content of iodine in breast milk and the urine of breast feeding infants of smokers (Laurberg et al. 2004, J Clin Endo Met 89:181-187) indicates the importance of considering coexposures to thiocyanate. Nitrate, ubiquitous though far less potent than perchlorate, should also be considered (see e.g., DeGroef et al., 2006, Eur J Endocrin 155: 17-25).

(G4) Please discuss research that you think would be likely to increase confidence in these models and their use in predicting RAIU inhibition by perchlorate in different life stages (for an average individual within each life-stage).

Janusz Byczkowski	If the overall model output is sensitive to *VMk* parameter, the confidence in the PBPK modeling of "*Breast-fed neonate*" could be increased by a better description of "residual milk volume" (see answers to G-2 and G-3, above).
Brian Cummings	Studies directly assessing the effect of perchlorate on the clearance of NIS substrates are needed. Further, data on the mechanisms of perchlorate inhibition of NIS is lacking. Research investigating the toxicity of substrates of NIS, in the presence and absence of perchlorate, is needed. Finally, urinary clearances of environmental pollutants in infants and neonates are needed.
Panos Georgopolous	The main challenge related to this question is "defining" an "average individual within each lifestage." Equally challenging would be the identification of an "average exposure" within each lifestage. EPA should consider the merits of a probabilistic sensitivity/uncertainty analysis and eventually the feasibility of population-based PBPK modeling (with explicitly defined sensitive subpopulations). Such an approach will provide a more realistic assessment of the actual ranges of the outcomes considered in the analysis, will eventually improve risk characterization efforts, and help explain both inter-individual and intra-individual variability within a (sub) population.
Sean Hays	The results of this modeling effort should be classified as theoretical since no validation exercises have been performed and EPA should be clear to state this. There are existing data which could help to validate the model predictions, especially related to the most sensitive scenario (e.g., the nursing infant of the exposed mother). In particular, Pearce et al. (2007) provides matched data on perchlorate and iodine in breast milk and urine samples from nursing mothers. EPA should obtain this data from the study authors. This will greatly help to test the model predictions. Furthermore, the authors found no correlation (either positive or negative) between perchlorate and iodine in breast milk samples. The authors of this study indicate this is consistent with other researchers. This may raise questions about the results of EPA's modeling efforts. Since no results for the concentrations of perchlorate and iodine in milk as a function of perchlorate dose are provided in EPA's report, it is impossible to determine the validity of this issue.
Frederick Kaskel	Newer estimates of renal function have been provided by Schwartz which should be evaluated
Kannan Krishnan	• In the iodide/perchlorate models, the chemical concentration entering the tissues corresponds to the arterial PLASMA concentration whereas the flow rate to tissues corresponds to BLOOD (RBC + Plasma) FLOW rates. Either the influx in all mass balance equations should correspond to whole blood concentration or the flow rate should correspond to plasma flows – since the RBC:plasma partition

	coefficient (PRBC_p) is not always equal to 1 (see for example lines 112 on page 38, or line 115 on page 4 of the EPA model code file), and the chemical movement between plasma and RBC is diffusion-limited and no flow-limited. The consequence of this modeling assumption may be verified to ensure confidence in the use of these models. For example, if the simulations indicate that the concentration profile of perchlorate is identical in RBC and plasma compartments, qualitatively and quantitatively, then the above observation has no consequence. • Further, consideration should be given to the possibility of being able to simulate iodine-deficient (or hypothyroid) situation in pregnant women by modulating specific parameters of the model. • Both the response to the previous question and the comments under general overview are all applicable here.
Chensheng Lu	What will significantly increase the confidence in these PBPK model is to use the real-world data (such as perchlorate in drinking water and the level of iodide in blood or thyroidal functions in population) liking perchlorate exposure and iodide inhibition. The article published by Blount et al. (EHP 2006 114(12) 1865-1871) would be an ideal application for these PBPK models. Unfortunately, data used in Blount et al. study (NHANES) do not include children ages 6 and below.
Lauren Zeise	Research to get a better handle on renal clearance of iodine and perchlorate during pregnancy and postpartum; biomonitoring of perchlorate, iodide, thiocyanate and thyroid hormone during and after pregnancy during lactation in smoking and non-smoking women. Measurements of perchlorate in baby formula – in non-composited samples.

(G5) Does EPA accurately characterize the strengths and limitations of the analysis? Please comment on any particular strengths that may not be mentioned or adequately characterized for the estimates of RAIU for different life stages. Please also comment on any specific sources of uncertainty that you believe have been overlooked in EPA's analysis or that require further discussion, and which might be significant to EPA's estimates of RAIU for different life stages

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Janusz Byczkowski

The reviewed EPA draft document adequately describes strengths and limitations of the analysis. However, the overall strength of any PBPK modeling used for risk assessment is its ability to simulate adequately the experimental data. While a partial validation of the unmodified iodide PBPK model was performed and reported by Clewell et al., in the EPA document, only limited comparisons of some variables to the actual data have been presented (in Appendix C). The strength of the EPA modeling analysis could be better presented by including comparisons of the PBPK model simulations to all significant real-life experimental data.

Brian Cummings

EPA does a good job of characterizing the strengths and limitations of the model, but needs a separate paragraph at the end directly addressing these points.

Did EPA take into account any hormone effects on NIS or thyroid function? The previous literature, on which this model is based, devotes some discussion to this subject. This is particularly important when discussing susceptibility during puberty.

Panos Georgopolous

In this reviewer's opinion the main strength of the analysis is the explicit listing of unresolved issues (and inconsistencies) in the modeling described in the document under review.

The main limitations involve:

- the emphasis on point estimates rather than on pursuing a distributional (probabilistic) approach for characterizing exposures (with explicit variability and uncertainty of activities for each individual and across a sub-population,
- the emphasis on "average" individuals for each lifestage rather than on pursuing population-based modeling with explicit characterization of inter-individual and intra-individual pharmacokinetic (physiological and biochemical) variabilities, and
- the consideration of iodide and perchlorate exposure "in isolation" and not in a context of "total" exposure that would consider other NIS inhibitors (thiocyanate, nitrates).

Sean Hays

In places, EPA adequately highlights the strengths and weaknesses of their analysis. However, there are other areas where the limitations have not been adequately addressed (e.g., lack of validation).

Frederick Kaskel	EPA accurately characterized the strengths and limitations of the analysis. However, as indicated in the report, additional information on the possible effects of maturation of glomerular filtration, tubular reabsorption and secretion, and changes in body composition during the neonatal period is indicated in order to more confidently apply EPA's estimates of RAIU for different life stages.
Kannan Krishnan	• The strength relates to the use of PBPK model to assess the lifestage sensitivity to inhibition of the sodium-iodide symporter by perchlorate; the use of fetus as a subgroup to evaluate the relative sensitivity to adults; consideration of relevant route/source of exposure (drinking water); • The weaknesses are related to the fact that the analysis did not include certain subgroups (e.g., elderly, foetus during early gestation periods, iodine-deficient or hypothyroid status during pregnancy) and did not address variability of parameter values within subgroups in the simulations (i.e., with the use of Bayesian or Monte Carlo type methods).
Chensheng Lu	This document is well written and has highlighted what EPA has accomplished in assessing RAIU inhibition at different life stages resulting from perchlorate exposure. The Reviewer thought EPA has thoroughly discussed the strengths and limitation of this analysis, including the uncertainty analysis. One uncertainty, however, has not been addressed by EPA is the use of direct IV dose of radioiodide to the bottle-fed infants in order to determining iodide uptake inhibition caused by perchlorate in formula. Although this approach seems intuitive, it may not reflect the real-world scenario in which iodide intake is usually taking place by oral ingestion. Pharmacokinetically speaking, the absorption of chemicals in humans could vary significantly between oral ingestion and bolus iv injection. EPA needs to conduct an uncertainty analysis to assure that such approach would not impact the outcomes significantly.
Lauren Zeise	EPA does not sufficiently elaborate on the limitation of focusing on "healthy" individuals, and the lack of consideration of the large susceptible populations. Some parts of the analysis are scenario based, using 90th percentile values, while other parts use mean values. With over 4 million infants born in the US each year, scenario analyses should be added. These would be directed at ascertaining the inhibition levels for the some plausible higher susceptibility cases, such as infant and fetus exposures associated with a mom with relatively high thiocyanate exposure (e.g., from broccoli consumption or smoking), low renal clearance, who got all her fluids directly or indirectly from tap water.

(G6) As recommended in EPA's 2000 Risk Characterization Handbook, is the analysis transparent in terms of the steps, logic, key assumptions, limitations, and decisions? Specifically, does characterization of the results of EPA's work fully explain: a) the analysis approach employed; b) the use of assumptions and their impact on the analysis; c) the use of extrapolations and their impact on the analysis; d) plausible alternatives and the choices made among those alternatives; e) the impacts of one choice vs. another on the analysis; f) significant data gaps and their implications for the analysis; g) the scientific conclusions identified separately from default assumptions and policy decisions, if any; and h) the major conclusions, and the discussions of EPA's confidence and uncertainties in the conclusions?

Janusz Byczkowski

It seems that the reviewed EPA draft document complies with guidelines and recommendations of the EPA 200 *Risk Characterization Handbook*. As stated in the answer to G-1 (above), it is logical, clear and concise. The alternatives are presented and the decision points are adequately explained.

Perhaps, the contribution of specific PBPK uncertainties to the overall uncertainty of modeling RAIU inhibition by perchlorate could be summarily discussed and presented in a separate section.

Brian Cummings

The analysis conforms to EPA guidelines on transparency with regards to steps and logic. The key assumptions of the model are clearly listed, as well as the decisions involved in parameters changes and impact of these changes. The limitations of the model could be more clearly listed (see above).

The analysis approach employed is adequately explained as are the basis for assumption used in this model. EPA goes to great lengths to test the impact of these assumptions by determining the sensitivity for each parameter changed.

The extrapolations used are clearly outlined and well as their rationale for them; however, for the most part, only the theoretical impact of these extrapolations are discussed. Some validation is presented, but this mostly uses previously validated data. Was the model applied to any data sets in the literature not previously studied? Are such data available at this time?

The impacts of choice for most of the critical parameters are discussed. This is particular true when discussing choices for BW, clearance and scaling these values.

Plausible alternatives for some of the parameters could be more clearly listed. This is particularly true of the impact on altering the level of water ingestion to 90%. What were the alternatives to this value and how did they affect them model?

Alternative choices for scaling to BW and clearance are clearly listed as well as their impacts.

The major and scientific conclusions for this work are clearly stated and appear to be separated from any grand statements on policy decisions.

The authors do discuss data gaps throughout the manuscript, but a specific section is needed, towards the end, listing these gaps in itemized, or table form. This should be

	followed by paragraph that list major perceived weaknesses and uncertainty of this model, which need to be more, clearly stated.
Panos Georgopolous	The document and - in particular the appendices – are quite explicit ("transparent") in listing and discussing all the assumptions and approximations involved in the analysis. This takes place at a level of detail that exceeds what is typically expected in the peer reviewed literature and the authors of the document under review should be commended for this. However, the justifications of what can be called "emergency solutions" to various problems discussed in the document, especially those related to inconsistencies in modeling urinary clearance processes (including inconsistencies in scaling factors as well as "ad hoc" adjustments to achieve agreement for predicted values) are often weak. It can, probably reasonably, be argued that, for the ranges of concentrations and exposures considered, the effect of correcting the above inconsistencies will not have a substantive impact on calculated outcomes; however, it is still very important to develop a "fully defensible" model that incorporates up-to-date scientific information and assumptions that are consistent "across lifestages". Clearly, resolving the inconsistencies that have been identified through the efforts presented in the document under review will be useful in numerous other applications involving exposures to chemicals *in utero* and during infancy.
Sean Hays	Yes, the analysis is transparent.
Frederick Kaskel	The analysis is transparent in terms of the steps, logic, key assumptions, limitations, and decision. The characterization of the results of EPA's work fully explains: a) the analysis approach employed; b) the use of assumptions and their impact on the analysis; c) the use of extrapolations and their impact on the analysis; d) plausible alternatives and the choices made among those alternatives; e) the impacts of one choice vs. another on the analysis; f) significant data gaps and their implications for the analysis; g) the scientific conclusions identified separately from default assumptions and policy decisions, and h) the major conclusions and the discussions of EPA's confidence and uncertainties in the conclusions.
Kannan Krishnan	The analysis is transparent and the assumptions as well as alternative approaches are generally described in sufficient detail. The conclusions are essentially scientific in nature, based on data obtained from PBPK model simulations. The following improvements are suggested: 1. The reason for limiting the present analysis to eight sub-groups (i.e., pregnant woman,fetus, lactating woman, breast-fed infant, bottle-fed infant, 1 year old and 2 year old child,"average" adult, and non-pregnant woman of child-bearing age) may be specified at the outset. In this regard, it may be useful to clarify as to why the elderly and teens were not part of the sub-groups analysed in this study. 2. Clarify as to why the results of this analysis are also applicable to chronic exposure exposure situations (compared to typically acute (short-term) simulations)

	3. The justification of the choice of 24-hr RAIU as the endpoint should be included. Was 24-hr AUC considered as an alternative measure ? What was the scientific basis for basing the analysis on a single RAIU value in infants and adults obtained at one specific time point (i.e,. 24 hr). Some consideration/discussion of the sensitivity of that time point to the key input parameters as a function of age might be useful.
Chensheng Lu	EPA has clearly explained their approaches employed including the assumptions, alternatives, and the use of extrapolations and their impacts on the analyses. Apparently, there are significant data gaps, particularly for newborn infants that lead to some limitations of using this revised PBPK model. However, it is rather common for many PBPK modeling work, and therefore should NOT be considered a major limitation of this analysis. It is apparent that the outcome of the PBPK model prediction is dictated by the use of urinary clearance of perchlorate and iodide. Other parameters have somewhat less impacts on the results. EPA has taken the right approach focusing on the parameters related to perchlorate exposure and iodide intakes. The revised PBPK model that EPA modified has demonstrated the importance of those parameters, and the Reviewer agrees with the EPA's scientific conclusion in which the modified Clewell et al. model is acceptable to calculate the lifestage differences in the degree of NIS inhibition of thyroidal radioiodide uptake at a given level of perchlorate exposure.
Lauren Zeise	EPA does a reasonably good job laying out the logic, key assumptions, limitations and decisions. But for the most part, it is done in a manner that will be understandable to someone with a modeling background. It will be difficult to follow and very accessible to a more general reader. More motivation of the forms for the statistical fits is needed, and a more quantitative and rigorous treatment of uncertainty. EPA reasoning for using 90th percentile values for some parameters and mean values for others is not explained well. Failure to address certain large susceptible populations and the possible sizes of these populations should be discussed. The degree to which the analysis for the GW 40 fetus may or may not represent the first and second trimester fetus needs explicit and careful treatment.

Responses to Parameter-Specific Charge Questions

(A) Urinary Clearance

(A1) Please comment on the appropriateness of the input values selected for maternal urinary clearance during pregnancy and lactation in EPA's analysis. Are the available data and rationale for the values selected transparently and objectively described? Are you aware of other publications or data that could be used to guide these choices or which provide alternative input values that are equally valid or more appropriate? Likewise, are the values selected for urinary clearance for the infant and older child the best estimates, given the available science and data? Are there other data that would provide better (or equally valid alternative) guidance or estimates? Is there any reason to believe that urinary clearance might not be a limiting factor in the elimination of perchlorate for infants?

Janusz Byczkowski	The urinary clearance has been addressed appropriately and discussed as well as presented in a sufficient detail (in Appendix B). Given the limited data, particularly for perchlorate, it seems tat the approach and values presented by EPA are reasonable and this Reviewer is not aware of any data that could contradict this approach.
Brian Cummings	The authors do a good job discussing the choice and values used maternal urinary clearance during and after pregnancy and lactation. This includes are significant discussion of the impact of changes made to these parameters and alternatives. The available data and analysis, for the most part, are rationale, transparently and objectively described, with one exception (see C-2) below. I am not aware of any further publication that could be used for input values that are more appropriate. One question that I did have is what was the rationale for choosing the lower clearance value from Clewell et al., (2007) as opposed to the others (Page 12, 2nd paragraph). Please see my comments above concerning other data that provide alternative guidance with regards to urinary clearance in neonates, infant and children. Several studies support the hypothesis that urinary clearance is a limiting factor of perchlorate elimination. While it's possible that it may not be the only factor involved, it is clearly a major one. In absence of any data to the contrary, which could not be found, the authors are correct in this assumption.
Panos Georgopolous	The analysis presented in the document under review concluded that urinary clearance was a "key" process/parameter; i.e. model predictions were very sensitive with respect to the magnitude of urinary clearance. However, there are a number of issues concerning the parameters employed in modeling urinary clearance that need substantial clarification. Appendix B (pp 39-50) of the document under review provides an extensive discussion of the assumptions and approximations involved in selecting and estimating these parameters. Various inconsistencies in the selection/estimation procedures are in fact recognized explicitly in Appendix B, but in general these inconsistencies are "accepted" on the basis of either a minimal anticipated effect on the calculations of the model, or as a means for avoiding a more complex analysis. For example, the last paragraphs of p. 40 states that because "... renal clearance is largely controlled by glomerular filtration and non-specific fluid resorption, the expectation is that the relative clearance for iodide and perchlorate [....] should be constant across ages, body weights, and lifestages. In EPA's evaluation for the child and "average" (non-pregnant, non-lactating) adult, this

proportionality has been maintained." However, in the model of Clewell et al. (2007) "the maternal urinary clearance value [....] was set at 60% of the value in the non-pregnant human based on observed difference in the pregnant and male rat models [....].These maternal lactation values go against the argument given just above that the proportionality should be maintained, but EPA chose to use the maternal values as so set. It is likely worthwhile to evaluate these maternal values in light of the generally higher urinary excretion seen in pregnant/lactating women, but alteration of these clearance constants would require refitting of other parameters, and so EPA chose not to conduct that specific evaluation." Clearly this is an issue that requires further consideration. It should also be mentioned that this discussion is preceded by the following rather puzzling statement that "The tables in the papers identify the units of [urinary clearance] as L/h/kg, but clearly this should be $L/h/kg^{0.75}$ to be consistent with this mathematical formulation, which is how the CLU values are calculated in the computer code." Such a selection units/dimensions contradict the physics of the problems and in fact it appears that the tables in the original articles (Clewell et al., 2007 and Merrill et al., 2005) state the correct units. (The first paragraph on page 41 of the document under review also employs correct units/dimensions.) The issue of consistent allometric scaling is an important one and there exist various publications that can be helpful in clarifying issues such as the above (e.g. Johnson, 2008; West et al., 1997; Kurz et al., 1998). Issues of inconsistent scaling in fact appear across the entire description of urinary clearance parameters (Appendix B).

Various other inconsistencies are discussed and "accepted" in relation to the calculations of urinary clearance in the neonate (pages 42-43) and in the pregnant/lactating woman. For example, on page 47, it is stated:

> "Keeping with the assumed proportionality between perchlorate and iodide, based on these data the same relationship would be expected to hold: higher clearance rather than reduced. A dilemma occurs in considering the data of Aboul-Khair et al. (1964); however, in that the control iodine clearance as measured by them is 31.05 ± 3.66 mL/min (mean ± SE), while the value determined by Merrill et al. (2005) for non-pregnant adults is 44.3 mL/min. Likewise Aboul-Khair et al. (1964) report thyroid iodide uptake at 2.5 hr postinjection as 21.4 ± 1.4 % of the administered dose, but the amount predicted by the Merrill et al. (Merrill et al., 2005) model (in the absence of perchlorate) is 7.78%. Therefore, the data of Aboul-Khair et al. (1964) was normalized to their own controls for both urinary clearance and iodide uptake, and then use that relative change as a model input (for clearance, multiplying the non-pregnant clearance rate constant by the pregnant:control ratio from Aboul-Khair et al. (1964) or in estimating changes in thyroid NIS (to fit relative increases in thyroid uptake)."

Clearly, the inconsistency in absolute values reported in the above paragraph should be the focus of further study; while the normalization employed by EPA offers a way of circumventing the issue, this "solution" could only be considered qualitative in nature.

In this reviewer's opinion, a consistent treatment of the urinary clearance process for various life stages emerges clearly as a research need, based on the outcomes of the sensitivity testing and the issues presented in Appendix B of the document under review.

References Cited in Answer to Question A-1:

Clewell, R.A., Merrill, E.A., Gearhart, J.M., Robinson, P.J., Sterner, T.R., Mattie, D.R.,

and Clewell, H.J., 3rd. 2007. Perchlorate and radioiodide kinetics across life stages in the human: using PBPK models to predict dosimetry and thyroid inhibition and sensitive subpopulations based on developmental stage. *J Toxicol Environ Health A* 70 (5):408-28.

Johnson, T.N. 2008. The problems in scaling adult drug doses to children. *Arch Dis Child* 93 (3):207-11.

Kurz, H., Sandau, K., Dawson, T.H., Brown, J.H., Enquist, B.J., and West, G.B. 1998. Allometric ccaling in biology. *Science* 281 (5378):751.

Merrill, E.A., Clewell, R.A., Robinson, P.J., Jarabek, A.M., Gearhart, J.M., Sterner, T.R., and Fisher, J.W. 2005. PBPK model for radioactive iodide and perchlorate kinetics and perchlorate-induced inhibition of iodide uptake in humans. *Toxicol Sci* 83 (1):25-43.

West, G.B., Brown, J.H., and Enquist, B.J. 1997. A general model for the origin of allometric scaling laws in biology. *Science* 276 (5309):122-126.

Sean Hays

The available data and rationale are transparently described. However, I disagree with the rationale for the choice of maternal urinary clearance values. This is probably the most sensitive parameter for the most sensitive scenario/receptor and the EPA has chosen to use rodent data over human data. This is inadequate. EPA should choose to use the available human data which indicates there is no measurable or consistent difference in urinary clearance during pregnancy as compared to the non-pregnant state. The EPA chose a reasonable urinary clearance for the lactating mother scenario. I agree with EPA's choice for the urinary clearance among infants and older children.

Frederick Kaskel

The input values selected for maternal urinary clearance during pregnancy and lactation used in EPA's analysis are appropriate, transparent and objectively described. The choice of three alternatives for pregnancy is a rational compromise in lieu of the lack of additional human data. The use of a lower clearance is a safe assumption. On page 10 urinary clearance values for perchlorate and iodide across all lifestages were determined to be sensitive parameters for prediction of NIS thyroidal iodide uptake inhibition by perchlorate. EPA determined that urinary clearance of perchlorate and iodide in neonates is slower than is indicated by scaling based on body weight. Urinary elimination of a number of compounds including drugs and drug metabolites also indicate that renal clearance is slower per unit of body weight in neonates. Modification of the PBPK models to describe slower clearance of perchlorate and iodide in neonates resulted in an increase in predicted levels of NIS inhibition in infants.

The values selected for urinary clearance for infants and older children are the best estimates for the available data. The interpretation of the data that suggested an increase in predicted levels of NIS inhibition in infants at a perchlorate dose-rate of 7 ug/kg-day is a safe assumption. The indices of renal function are based on the literature which indicated that the GFR increases steadily postnatally but does not reach adult values until approximately 2 years of age. I know of no other data that would provide better guidance or estimates and it is unlikely that there are other factors than the urinary clearance in the elimination of perchlorate for infants. However, one should consider that tubular function in this age group is not fully matured and possible developmental changes in transport activity might be important but no data is available for perchlorate elimination during

	development. On page11 EPA chose to estimate perchlorate induced inhibition using scaling of urinary cleareance proportional to body weight for children at 1 year of age and older which results in somewhat higher estimates of iodide uptake inhibition than reported by Clewell although still slightly less than predicted for the average adult exposed at the same dose. EPA's estimates of urinary clearance in infants and children are lower than those used in Clewell but reflects published GFR values.
Kannan Krishnan	The inadequacy of use of urinary clearance values in various human lifestages based on (i) the pregnant:nonpregnant values in rats, and (ii) the scaling of renal function for neonates on the basis of $BW^{0.75}$ – are well justified by EPA. The outcome is consistent with available experimental and/or physiological data. The selection of lower clearance value for pregnancy as well as the option 2 for lactating women, though not the optimal (given the interindivudal variability), would appear to be pragmatic and consistent with the rationale provided by EPA. However this reviewer has the following additional observations: • The R2 value for the fit described in Figure B-6 is poor raising concern about the adequacy of the equation • Did EPA analyze the data in Figure B-5 on the basis of body surface data for the various age groups (of pregnant women)? • On page 42, para 3, Figure B-1 should read Figure B-2? • What does GFR-based scaling mean in Figure B2? Is it body surface scaled?
Chensheng Lu	Considering that perchlorate, as well as iodide, does not further metabolize in human body, the urinary clearance should be a limiting factor in removing perchlorate and iodide from humans at all lifestages, and an important parameter in the perchlorate PBPK model. Unfortunately, data for urinary clearance of perchlorate and iodide by the mother during pregnancy and lactation are not consistent among three sources (Clewell, Aboul-Khair, and Delange) cited by EPA. The choices that EPA made for selection clearance for pregnancy and lactation are quite arbitrary, and the reasoning, if any, are not found. If GFR is corresponding to the cardiac output (meaning higher blood flow rate equal to higher GFR), urinary clearance of any given compound during the pregnancy should be higher than non-pregnancy. Urinary clearance during lactation period might be the opposite to the pregnancy due to the difference of cardiac output. EPA should seek for differences of urinary clearance (mainly via GFR) of compounds during pregnancy and lactation outside the iodide and perchlorate literatures. EPA has clearly documented how they determined the alternative scaling of urinary clearance of perchlorate and iodide by body weight and has provided a thorough explanation of why EPA chose to use $(BW)^1$, instead of commonly used $(BW)^{0.75}$, in neonates. The justification is sound and supported by the data published in the literature. Similar justification of using $(BW)^1$ scaling for perchlorate clearance in older children

(ages 2-12) is also provided, however, the sentence of "EPA's estimates of urinary clearance in infants and children are lower than those used in Clewell et al. (2007), but are values EPA judges to be scientific estimate, not bounds." (on page 11, 1st paragraph) is not clear to the Reviewer. The information to support this sentence may come from Appendix B (pages 44-46), particularly from Figure B-4. However, Figure B-4 itself is difficult to understand (for instance, how is Lower 95% related to the yellow diamonds, and how the line of Data average is constructed?), and therefore renders less convincing remark of using $(BW)^1$ scaling for older children. EPA may want to review this and provide a clearer explanation on the data presented in Figure B-4.

Lauren Zeise

The discussion of maternal urinary clearance values could be somewhat improved. In regard to Figure B-6 motivation is not given for the fitting of the quadratic function to the data for iodide clearance vs gestation week, and it is unclear where the postpartum data set – greater than week 39 data set - on the plot appeared from and why it is included in the modeling of clearance during pregnancy. The highest mean value was measured by Adoul-Khair at the latest pregnancy time point. Inclusion of the extra data set weighs the function down late in pregnancy when the highest value was measured by Aboul-Khair. Further, including a gestation week of 45 on the plot axis is confusing to the reader. There is a large extrapolation to clearance during the early pregnancy time point and renal clearance can be increased fairly early in gestation. The quadratic fit may underpredict clearance during this period. However, given that EPA is declining to estimate early fetal effects, this portion of the extrapolation is not critical. It is unclear why the fit is being presented however. Finally variability among individuals is an important consideration and it would therefore be of interest to see on the plot or otherwise reported an indication of variability in the individuals studied. Some indication of this is given in Table 2 of Aboul-Khair et al., where renal clearance values for iodide have been serially averaged for each pregnant individual studied. In addition, individual measurements for controls are given. For Figure B-7 it would be good to show error bars or confidence bounds.

Minor error, on page 42, data from Guignard et al. are plotted in Figure B-2 not B-1.

Are you aware of other publications or data that could be used to guide these choices or which provide alternative input values that are equally valid or more appropriate?

Increased urinary clearance of iodide during pregnancy is a widely recognized phenomenon and data on the magnitude besides that reported by Aboul-Khair would be useful and important to locate. Further, the inconsistency of PK outcomes and the Aboul-Khair measured values in controls for IV iodide dose uptake 2.5 hours post injection is quite troublesome and calls into question the PK modeling. The approach on page 49 described to deal with the inconsistency is not entirely satisfactory. EPA should look hard for additional data sets to cross check assumptions regarding iodide uptake and renal clearance during pregnancy and early postpartum.

Likewise, are the values selected for urinary clearance for the infant and older child the best estimates, given the available science and data? Are there other data that would provide better (or equally valid alternative) guidance or estimates? Is there any reason to believe that urinary clearance might not be a limiting factor in the elimination of perchlorate for infants?

I am not aware of better values for infants and the older child. The EPA laid out a

reasonable analysis and approach for developing estimates for the infant and older child. There is interindividual variability in clearance and it would be preferable if this were more emphasized and acknowledged in the discussion, and attempts to better describe it quantitatively, for example in terms of varying glomerular filtration rates normalized by body size.

(B) Breast-milk ingestion

(B1) Infants are generally known to consume very little milk or formula on the first day of life, with ingestion quickly increasing over time. However the first time-point for which ingestion data are available for infants is at 7 days, which EPA extrapolated back to 0 ingestion at time=0 in its analysis (birth; Figure 1). The breast-milk ingestion rate was estimated based on mean values reported in Arcus-Arth et al. (2005), while the water ingestion used for the breast-feeding mother was the 90th percentile value from U.S. EPA (2004).

Is EPA's extrapolation and rationale transparently and objectively described? Does this function appropriately characterize the available data and information? Are there other data that could be used to obtain a better or equally valid alternative estimate of mean breast-milk ingestion rate for infants in the first few days of life? Should an estimate other than the mean be used to determine the breast-milk ingestion rate?

Janusz Byczkowski	The Arcus-Arth et al. (2005) data for breast milk intake are, perhaps, the best currently available, and thus the smooth function that fits these data is the right approach. It seems that the exponentially growing intake for the first week postpartum may be an overestimate, but given the inadequate information about intake during the first weeks postpartum, the applied growth function appears to be a reasonable, practical solution, even though seemingly quite a health-protective. The idea of adding on the top of this overestimation the upper bound (e.g., the estimate of 90th percentile intake) would lead to a marked exaggeration of milk consumption, unrealistically high for a typical infant. While this Reviewer cannot suggest any better approach to the milk intake, please see the answers to G-2 and G-3 for a closely related potential problem with the estimated residual breast milk volume.
Brian Cummings	EPA's rationale for ingestion rates for infants at time 0 follows rationale assumptions and the approach appears objective. While its true that infants consume little in the first day of life, the extrapolation of 7 day data to day 1 allows for a margin of safety. I am not aware of any data that EPA has not presented. While other estimates for breast-mile ingestion may exist, the ones used by the EPA are clear, rationale and tractable. Thus, they will be easily validated in future models.
Panos Georgopolous	EPA's selection of point values for the testing analysis appears appropriate and adequately justified as a reasonable conservative assumption. However, in this reviewer's opinion, the uncertainties and variability inherent in the problem at hand would be better addressed by a distributional (probabilistic) rather than point calculation. The large population above the 90th percentile and the potential "spread" of exposure factors above that percentile, would further justify such an analysis.
Sean Hays	The approach and methods are objectively described. However, this is one of the weakest portions of EPA's analysis. The modeling of perchlorate and iodine kinetics in the neonate is highly uncertain. EPA needs to recognize this and make this clear to the reader. If the purpose of this analysis is to determine the relative difference in inhibition of iodide uptake by the thyroid for the various scenarios (e.g., normal adult, child, nursing infant, fetus, and bottle-fed infant), then the mean on all exposure and pharmacokinetic

	parameters should be used and used consistently throughout the analysis. Otherwise, the current approach exhibits bias for one scenario over the other.
Frederick Kaskel	EPA's extrapolation and rationale are transparent and objectively described to assess the breast milk ingestion rate.
Kannan Krishnan	• The fitting of the available data from Kahn and Stralka, with a mathematical function is adequate. However, the extrapolation from day 7 towards day 0 (or at birth) is not warrented given that the newborn is not a sub-group used in the assessment of relative sensitivity of lifestages (Table 3, page 22). • The motivation for choosing 90^{th} pctle for consumption rates needs to be clearly presented, since the expectation is a calculation either based on mean values in the various groups or 95^{th} pctle values. Therefore, the rationale and scientific basis for the choice and use of the 90^{th} pctle values in these calculations should be more clearly presented.
Chensheng Lu	Based on the data presented in Figure 1, the milk ingestion rates, or suckling rate, are quite different between Clewell et al. and Arcus-Arth et al., however, EPA's decision to use Arcus-Arth's data requires further clarification. EPA claimed that Clewell et al.'s data is inadequate to describe the suckling rates in the first couple weeks of life, however, based on the Reviewer's examination on Figure 1, the abrupt increase of milk ingestion during Day 1, and between Day 1 and 7 as presented by Arcus-Arth et al. seems unlikely. The difficulty of collecting breast-milk ingestion rate for infants in the first few days of life is understandable, and the deviation of the mean breast-milk ingestion from the true value might not be as large as we thought. Therefore, the mean breast-milk ingestion rate might be robust enough for use.
Lauren Zeise	EPA's approach is objectively and transparently described, and the Agency was correct that the Clewell et al. description is inconsistent with the currently available peer reviewed literature. It is unclear why a mean value is used for infants and an upper 90^{th} percentile is used for the breast feeding mother. This is not adequately explained. *Does this function appropriately characterize the available data and information?* The function does not characterize the available data and information. It will be quite confusing to anyone but a modeler. The equation on page 15 has milk describes milk ingestion rate as Milk ingestion rate (mL/hr) = KTRANS = 28.3*(BW-3.375)0.175 It then plots milk ingestion as a function of bodyweight and shows values for days 1, 3, 5 and 7 of life as on the bw vs milk ingestion plot. This formulation was used as a convenient way of giving values to KTRANS but is problematic because it works only for the specific circumstances using the mean values for breast milk intake in Arcus-Arth et

al. data and will be confusing for anyone but a modeler.

In using bodyweight as a surrogate for age (3.375 kg as the zero age bodyweight) it builds in an illogical structure that will be hard for the general public to understand and limits the usefulness of the model for using data beyond the mean values in Arth-Arcus et al. For example, there is zero milk ingestion for a bodyweight of 3.375 and milk ingestion rates below that value cannot be defined. Furthermore, the expression has milk ingestion increasing with increasing bodyweight indefinitely. This also is contrary to what occurs – as infants age solid foods and other liquids are introduced and breast feeding reduces. Arth-Arcus et al. show that for the available data sets milk consumption – in terms of volume per bodyweight per day – decreases with age in a linear fashion. Thus there is another inconsistency introduced by the way the model is formulated. At different ages the mean milk ingestion at a given bodyweight will differ.

A more logical approach would be to develop an expression for milk ingestion in terms of volume per bodyweight per day could be expressed as a function of age. A separate expression could then be used to convert this to KTRANS. To deal with the early low consumption rate on days 1-3 the measured values could be used.

Figure 1 notes that the data are from Arcus-Arth et al. but in fact it is entirely inconsistent with Arcus-Arth et al. for the above discussed reasons.

Are there other data that could be used to obtain a better or equally valid alternative estimate of mean breast-milk ingestion rate for infants in the first few days of life?

It is not correct that the first time point for which ingestion data are available is 7 days. There are values in the literature for intake on days 1, 2, 3, 4 and 5. Indeed at days 4 and 5 the intake is quite high and consistent with the linear relationship for volume consumed per kg per day vs age reported in Arth-Arcus et al. See table 8 in that paper.

Should an estimate other than the mean be used to determine the breast-milk ingestion rate?

Whether or not the mean is used depends on later steps in the process, and ways that variability will be taken into account. There are over 4 million births in the US annually. The overall procedure for characterizing intra-species variability and central tendency needs to be designed to be able to address the large number of infants “in the tails” of the distribution. It would be preferable to build a PK approach that would enable fuller description of variability in iodide uptake inhibition. The use of mean values and the formulation used to compute KTRANS precludes this.

(C) Water ingestion

(C1) For pregnancy, EPA used a normalized (90th percentile) water ingestion rate of 33 mL/kg-day (U.S.EPA 2004), which was multiplied by the maternal BW as described by the PBPK model growth-functions during pregnancy to obtain total water ingestion for the mother.

Is EPA's approach and rationale transparently and objectively described? Is this approach appropriate for characterizing the upper-bound for ingestion of pregnant women? Are there other approaches or data that could be used to obtain a better or equally valid alternative estimate for this parameter? (Note that the self-reported body weight values in U.S. EPA (2004) indicate the same average body weight for pregnant women as for non-pregnant; data appears inaccurate, so the PBPK model body-weight description was used instead.)

Janusz Byczkowski	It seems that linking the upper bound estimate of water ingestion to maternal body weight growth function is an appropriate approach, evidently, more realistic than the self-reported data. The water ingestion issue has been mentioned in the reviewed EPA draft document but not extensively discussed. It is not clear what (if any) modification to the water ingestion variable has been made by EPA in comparison to the original pregnancy model reported by Clewell *et al*. (the algorithm for "r*dose_p*" is already marked in the original CSL file as "*modified by PMS"*).
Brian Cummings	EPA's approach is rationale, transparent and objectively described. I agree with using 90% water ingestion for pregnant woman for a upper bound rate, as it adds a safety factor; however clarification is needed as to how this value was derived. A review of the documents used by EPA to determine this value reports 90% bootstrapping levels as apposed to overall ingestions levels? Was the 90% bootstrap value used, or did EPA calculate 90% ingestion from these data. How exactly was the value of 33 mL/kg-day determined?
Panos Georgopolous	The approach taken by EPA appears reasonable. As in the answer to the previous question, this reviewer's opinion is that a distributed zonal analysis (Monte Carlo) can provide more substantial insight on patterns of potential exposure rather than the point calculations presented here.
Sean Hays	Throughout this analysis, EPA was inconsistent in choosing upper bounds or means for various parameters. As such, there is no clear understanding of the objectives of EPA's analysis. If the purpose of this analysis is to determine the relative difference in inhibition of iodide uptake by the thyroid for the various scenarios (e.g., normal adult, child, nursing infant, fetus, and bottle-fed infant), then the mean on all exposure and pharmacokinetic parameters should be used and used consistently throughout the analysis. Otherwise, the current approach exhibits bias for one scenario over the other.
Frederick Kaskel	EPA's approach and rationale for pregnancy water ingestion rate is transparent and objectively described, and is based on the available literature. I know of no additional data that could be used to obtain a better estimate of mean breast-milk ingestion rate for

	infants in the first few days of life. The mean estimate is fine.
Kannan Krishnan	• The cited value of 33 ml/kg-day corresponds to the 90th percentile value for pregnant women ("consumers only") of the direct and indirect community water ingestion (chapter 6, page 16, U.S. EPA 2004). However, the 90th percentile value of total water ingestion for the same group was 39 ml/kg-day. It is unclear then as to why EPA specifes the use of total water ingestion rate but actually uses the value corresponding to another group (i.e,. community water ingestion). Furthermore, the 90th percentile value for pregnant women, reported in US EPA (2004), was associated with a small sample size (n=65, which does not meet the minimum reporting requirements described in the "Third report on Nutrition Monitoring in United States"). This raises the question of why not use (or justify the non-use of) the value from Ershow et al (1991) based on much larger sample size (n=188). These authors reported 90th pctle values for tap and total water ingestion of 34.5 and 48.9 ml/kg-day respectively. • It is also unclear to this reviewer as to why 90th pctle value is chosen for the computations and not either the median or the 95th percentile value. • This reviewer is not concerned about the use of subject-specific or group-specific body weight in the PBPK model to facilitate the calculations for pregnant women, as long as the ingestion rate is expressed in units of ml/kg-day, as done here.
Chensheng Lu	EPA's has objectively described its approach in using water ingestion rate of 33 mL/kg-day. However, the rationale of using the 90th percentile value was not provided by the EPA in this analysis. As for the BW estimates, it is unclear of how accurate it is to use the PBPK model growth-functions during pregnancy for estimating BW of pregnant women. Will NHANES data provide some sort of national average of the water ingestion rates stratified by lifestages and the BW of pregnant women? Or could EPA validate the PBPK model growth-functions for weight estimates using the NHANES data?
Lauren Zeise	The approach is transparently and objectively described, but the rationale is somewhat unclear. Some parameters are based on mean values, others on midpoints and still others on upper 90% bounds. It would be of interest to understand parameter distributions and how this translates to distributions for iodide uptake inhibition. This may be beyond what EPA has resources and time to do, but failing that, it would be desirable to have a clear presentation of the approach. EPA appears to be taking a plausible scenarios approach. But a clearer explanation is needed. The table below is taken from EPA (2004). It shows the 95th percentile upper bound for community water as 43 mL/kg/day, a reasonably higher level than the 90th percentile. In the perchlorate document, the reason for choosing the 90th percentile and not some other value needs to be justified. It is also worth noting that the number of pregnant women captured in the survey is quite small, and raises some concern that the upper bound values may be under estimates. For example, the upper bound estimate on the 90th percentile for pregnant women was 46 mL/kg-day.

Table 6.3.B2. Per Capita Water Consumption—Pregnant Women (mL/kg/day)

Estimate	Total Water 1978 NFCS (Ershow et. al. 1991)	Tap Water 1978 NFCS (Ershow et. al. 1991)	Total Water 1994–96, 98 CSFII	Community Water 1994–96, 98 CSFII
Sample Size	188•	188•	69#	65#
Mean	32.1	18.3	21#	14#
50th %	30.5	16.4	19#	9#
90th %	48.9	34.5	39#	33#
95th %	53.5	39.6	44#	43#

• Women aged 15 to 49 years; # Women aged 15 to 44 years.

Is this approach appropriate for characterizing the upper-bound for ingestion of pregnant women?

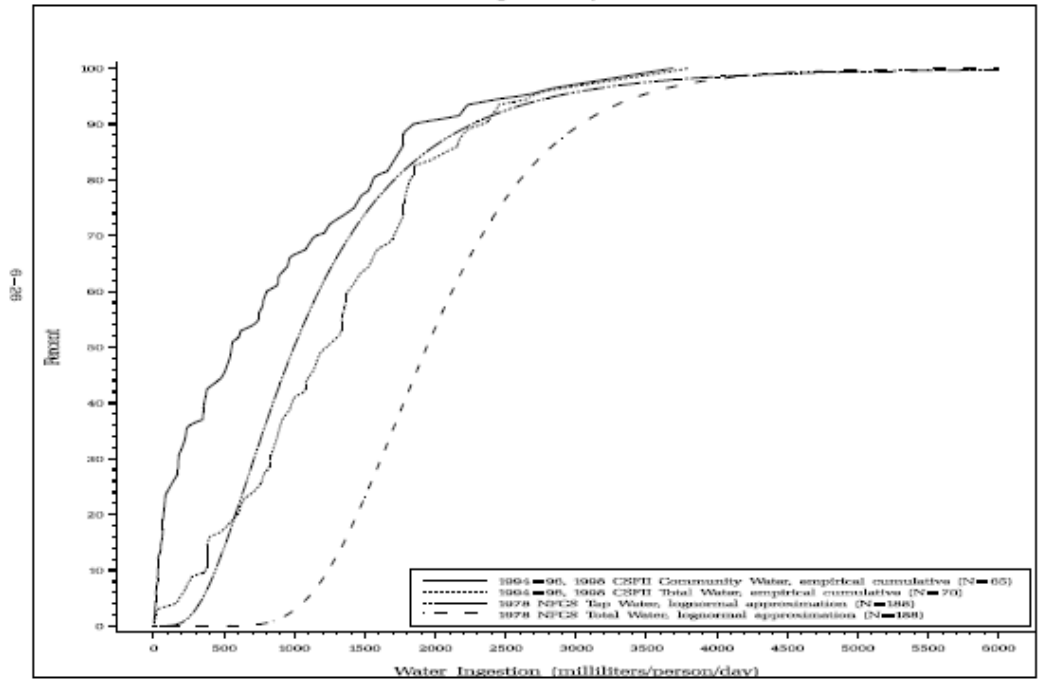
With 4.3 million births in the US each year, above the 90th percentile will be 430,000 women. Thus a very large number of women may consume water above this level, and one is left wondering about the importance of the assumption and how sensitive the results are to it. Following EPA (2004), the upper 95 percentile is 44 mL/kg/day, still representing a rather large number of women - 215,000.

Are there other approaches or data that could be used to obtain a better or equally valid alternative estimate for this parameter? (Note that the self-reported body weight values in U.S. EPA (2004) indicate the same average body weight for pregnant women as for non-pregnant; data appears inaccurate, so the PBPK model body-weight description was used instead.)

There are other approaches that could be used to obtain a better or equally valid alternative estimate. One consideration is the extent to which we may be confident that a pregnant woman may use drinking water in cooking and for her fluid intake without having to be concerned about harming her fetus. For this analysis one might consider the basic water requirments for women living in hot climates. For this one might select a value somewhat above the value of 3.0 L/day considered an “adequate intake” by the Institute of Medicine (2004; Dietary Reference Intakes for Water, Potassium, Sodium Chloride and Sulfate, IOM Food and Nutrition Board).

Another would be to pick a plausible upper bound value from the cumulative distribution observed. For example, from the figure below, taken from EPA (2004), it can be seen that a reasonable plausible upper bound may fall between 3.5 and 4 liters per day.

Figure 6.2.C1a. Cumulative Distributions of Per Capita Direct and Indirect Water Ingestion
Pregnant Consumers Only
ml/person/day



(C2) For lactation, EPA used a *fixed* total (90th percentile) water ingestion rate of 2959 mL/day (U.S. EPA 2004), with the rationale that while the woman's weight and self-water demand are expected to drop after pregnancy (as described by the PBPK model time-dependent weight equations), the demand for milk production would be increasing, and the reported value was not for a specific age-range of child.

Is EPA's approach and rationale transparently and objectively described? Is this an appropriate value to use for the ingestion rate of lactating women? Are there other better or equally valid alternative approaches or values that could be used?

Janusz Byczkowski	The use of 90th percentile of water ingestion by lactating woman apparently covers the extra demand due to breast-feeding, and perhaps, it is health protective. The approach is adequately described in the reviewed EPA draft document. This Reviewer is not aware of any better approach.
Brian Cummings	EPA approach is rational as it assumes that lactating women will still have increased water needs. While the approach is transparently and objectively described I am have some questions about the actual level, which results in ~45.5 ml/kg-day, which is substantially higher than the 90% ingestion rate reported above (assuming that the value was not a bootstrap). Doesn't it seem more likely that water ingestion would equalize? While the overall demand would decrease after pregnancy, this decrease would be countered by lactation? Are the water demands for lactation higher than pregnancy? What data exist on this subject other than models?
Panos Georgopolous	The approach taken by EPA appears reasonable, though a probabilistic (Monte Carlo) analysis would provide additional insight regarding the range of potential exposures. Furthermore, since the simulations for lactating women produce estimates of perchlorate concentration in breast milk, a population/distribution-level analysis with appropriate parameterizations could be used to provide valuable testing of the model in relation to the available data presented in Pearce *et al.* (2007) as well as Kirk *et al.* (2005, 2007) ***References Cited in Answer to Question B-2:*** Kirk, A.B., Martinelango, P.K., Tian, K., Dutta, A., Smith, E.E., and Dasgupta, P.K. 2005. Perchlorate and iodide in dairy and breast milk. *Environ Sci Technol* 39 (7):2011-7. Kirk, A.B., Dyke, J.V., Martin, C.F., and Dasgupta, P.K. 2007. Temporal patterns in perchlorate, thiocyanate, and iodide excretion in human milk. *Environ Health Perspect* 115 (2):182-6. Pearce, E.N., Leung, A.M., Blount, B.C., Bazrafshan, H.R., He, X., Pino, S., Valentin-Blasini, L., and Braverman, L.E. 2007. Breast milk iodine and perchlorate concentrations in lactating Boston-area women. *J Clin Endocrinol Metab* 92 (5):1673-7.
Sean Hays	Throughout this analysis, EPA was inconsistent in choosing upper bounds or means for various parameters. As such, there is no clear understanding of the objectives of EPA's analysis. If the purpose of this analysis is to determine the relative difference in inhibition

	of iodide uptake by the thyroid for the various scenarios (e.g., normal adult, child, nursing infant, fetus, and bottle-fed infant), then the mean on all exposure and pharmacokinetic parameters should be used and used consistently throughout the analysis. Otherwise, the current approach exhibits bias for one scenario over the other.
Frederick Kaskel	EPA's approach and rationale for lactation water ingestion rate is appropriate and transparent and objectively described. I know of no other approaches.
Kannan Krishnan	• The ingestion rate of 2959 ml/day, used by EPA, corresponds to the 90th percentile value of "consumers only" lactating women for direct and indirect community water ingestion. In comparison, the 90th percentile value of total water ingestion in consumers only lactating women is reported to be 3021 ml/ day (chapter 6 page 17). The EPA report (page 24, para 2) states that the intent was to use the "total" consumers-only water intake in the calculations. The source and consequence of this discrepancy should be addressed. • Further, U.S. EPA (2004) indicated that the 90th pctl value (2959 ml/day) is associated with a small sample size (n=41, which does not meet the minimum reporting requirements described in the "Third report on Nutrition Monitoring in United States"), raising a concern of its use rather than the value from Ershow et al. (1991). Additionally, it is unclear as to why the 90th pctl rather than 95th pctl of the water ingestion is used in these calculations. • In light of the fact that the water ingestion rate in lactating women is significantly greater (see chapter 6 pages 16-17, U.S. EPA 2004) , on a ml/kg-day basis, than in pregnant women, the rationale used for using a fixed ingestion rate needs to be more fully articulated.
Chensheng Lu	The rationale of using a fixed total water ingestion rate is justifiable and transparently and objectively described. However, the reasoning of selection of 2,959 mL/day at the 90th percentile is missing in this analysis. It would be assuring if EPA could provide the complete distribution of the estimates of total water ingestion.
Lauren Zeise	The approach is transparently and objectively described, but the rationale is somewhat unclear. As noted in response to C-1, some parameters are based on mean values, others on midpoints and still others on upper 90% bounds. A clearer explanation is needed on why the 90th percentile is chosen here, and not some other higher bound given the number of women-infant pairs affected. The tables below are taken from EPA (2004). They show the 90th percentile upper bound for community water is not substantially smaller than the 95th percentile when expressed as mL/kg/day, but appears more different when expressed as mL/person/day (2959 vs 3588), suggesting the difference may be driven by bodyweight differences at the 90th and 95th percentile. Still the reason for choosing the 90th percentile requires further explanation.

Table 6.3.C2. Per Capita Water Consumption—Lactating Women (mL/kg/day)

Estimate	Total Water 1978 NFCS (Ershow et. al. 1991)	Tap Water 1978 NFCS (Ershow et. al. 1991)	Total Water 1994–96, 98 CSFII	Community Water 1994–96, 98 CSFII
Sample Size	77•	77•	40#	33#
Mean	37.0	21.4	28‡	26‡
50th %ile	35.1	20.5	25‡	20‡
90th %ile	53.7	35.1	53‡	54‡
95th %ile	59.2	37.4	57‡	55‡

• Women aged 15 to 49 years; # Women aged 15 to 44 years.
‡ The sample size does not meet minimum reporting requirements as described in the "Third Report on Nutrition Monitoring in the United States".

Table 6.3.C1. Per Capita Water Consumption—Lactating Women (mL/person/day)

Estimate	Total Water 1978 NFCS (Ershow et. al. (1991))	Tap Water 1978 NFCS (Ershow et. al. (1991))	Total Water 1994–96, 98 CSFII	Community Water 1994–96, 98 CSFII
Sample Size	77•	77•	41#	34#
Mean	2,242	1,310	1,806‡	1665‡
50th %ile	2,164	1,330	1,498‡	1 645‡
90th %ile	3,169	1,945	3,021‡	2959‡
95th %ile	3,353	2,191	3,767‡	3588‡

• Women aged 15 to 49 years; # Women aged 15 to 44 years.

Is this an appropriate value to use for the ingestion rate of lactating women?

Similar to the response given to charge question C-1, use of the 90th percentile raises concerns that a substantial number of mother infant pairs are not sufficiently considered. The majority of newborn infants breast feed, and substantial numbers of infants do so through age 6 months, and there are still large numbers above the 90th percentile.

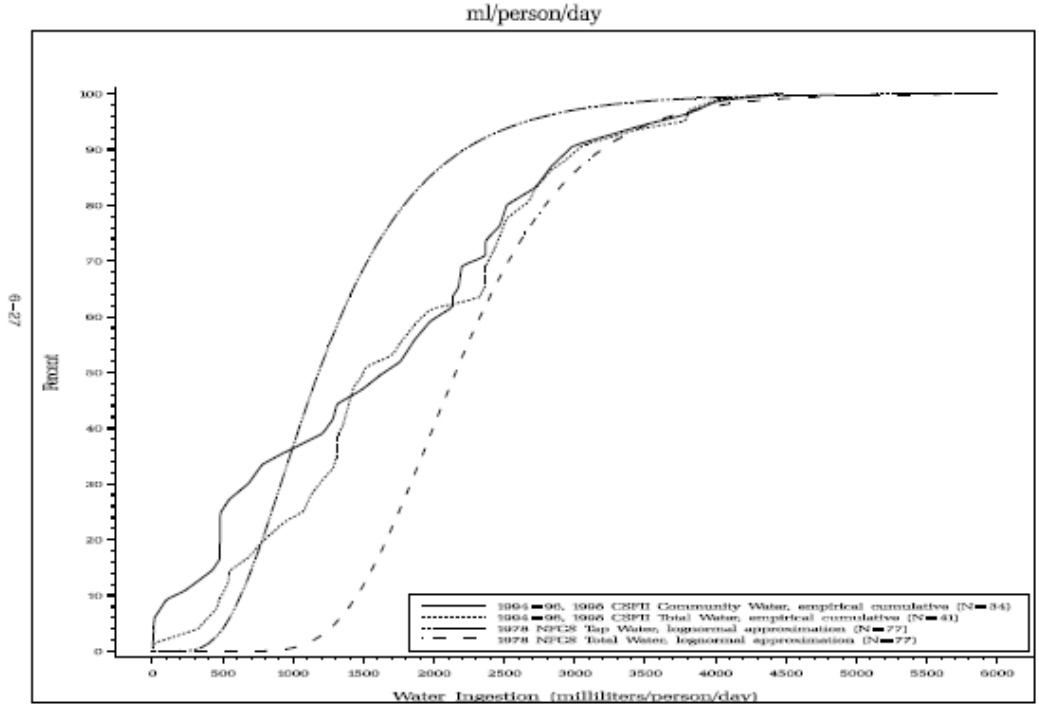
With regard to arguments on water needs of lactating women, there is a paucity of data. One could add the argument that IOM (2004) made that the intake of non-pregnant women added to the fluid output in breastfeeding provides a reality check on water ingestion rate.

Are there other better or equally valid alternative approaches or values that could be used?

There are other approaches that could be used to obtain a better or equally valid alternative estimate. One consideration is the extent to which we may be confident that a lactating woman may use drinking water in cooking and for her fluid intake without having to be concerned about harming her baby. The basic water requirements for women living in hot climates might be considered. For this one might select a value somewhat above the value of 3.8 L/day considered an "adequate intake" for lactating women by the Institute of Medicine (2004).

Looking at the cumulative distribution observed for lactating women, in the figure below taken from EPA (2004), it can be seen that a reasonable plausible upper bound may fall somewhere around 4 liters per day.

Figure 6.2.C1b. Cumulative Distributions of Per Capita Direct and Indirect Water Ingestion
Lactating Consumers Only
ml/person/day



(C3) For bottle-fed infants, EPA made extrapolations of the 90th percentile water-ingestion in early life based on measurements made for the age ranges: 0-30 days, 1-3 months, and 6-12 months (points in Figure 2, upper panel). For the purposes of this analysis, bottle-fed implies feeding with formula requiring the addition of water.

Is EPA's extrapolation and rationale transparently and objectively described? Does the overall function used to represent the changes in ingestion with age (and body weight) appropriately characterize the available data and information? Are there other data that could be used to obtain a better or equally valid alternative estimate of water ingestion rate for infants in the first few days of life (e.g., 7-day old)? The water ingestion rates used by EPA are based on 90th percentile ingestion data and thus are likely to exceed (minimal) physiological needs of infants as defined in nutritional guidelines. Are the water ingestion rates used by EPA reasonable in comparison to the physiological needs of infants at these various life stages? Are there other approaches or data (e.g., see the FDA memo) that could be used to obtain a better or equally valid alternative estimate for this parameter?

Janusz Byczkowski	For bottle-fed infant, analogously to maternal water ingestion, linking water intake to body weight growth function is an appropriate approach (see answer to C-1, above). This issue has been adequately described in the reviewed EPA draft document. The water ingestion rates used by EPA appear to be realistic, and thus, reasonable. This Reviewer is not aware of any better approach.
Brian Cummings	EPA approach is rationale and transparently described. It is standard practice to scale water ingestion to body weight, which does increase with age. Weight gain in the new born scales more rapidly than almost any other time period and it sequestered into specific groups; however, why was the calculation not scaled to 2 and 3 years as it done by WHO? Another point of interest is in regards to the 1st seven days of birth. Most infants either maintain birth weight or slightly lose 10% of their birth weight. This, as pointed out by EPA is directly related to water ingestion. Should this than represent another group or be removed (i.e. 0-7 days, or 7-30 days?).
Panos Georgopolous	EPA's extrapolation and rationale are adequately described. However, it is doubtful that the use of any single-point estimate would provide adequate understanding of the potential range of exposures, and corresponding doses, for bottle-fed infants.
Sean Hays	Same response as last question.
Frederick Kaskel	EPA's extrapolation and rationale for bottle fed infant's water ingestion in early life is transparent and objectively described. The overall function used to represent the changes in ingestion with age (and body weight) appropriately characterize the available data. The water ingestion rate for infants used by the EPA are reasonable in comparison to the physiological needs of infants. The estimated 90% water intake rate used by EPA in PBPK model stimulations is appropriate.

Kannan Krishnan

- Even though the EPA rationale is satisfactory, it is unclear as to why the emphasis is placed on the section of the curve (i.e., first few days after birth) which is neither used in the lifestage analysis nor supported by any data.

- A report published by a Public Health Agency in Québec contains data on water consumption of 393 infants of 8 weeks of age. For bottle-fed only infants (n = 278), mean (IC95%) value for total water ingestion was 122 ± 43 (117-127) ml/kg-day, or 655 ± 233 (627-682) ml/day. The corresponding 90th percentile values were 179 ml/kg-day and 981 ml/day. For more details the following source may be consulted:

- http://www.inspq.qc.ca/publications/default.asp?NumPublication=334

- A copy of the above report in PDF is also attached herewith.

Chensheng Lu

EPA has not informed the rationale of using the 90th percentile total water ingestion rate in early life stage, as well as during the lactaton (as stated earlier in the review), and therefore, the possibility of the estimated numbers are likely exceeding minimal physiological needs of infants raises a concern. If this is the case in which the 90th percentile total water ingestion rate exceeds the norms, this approach of using the 90th percentile is problematic. Since this sub-analysis focuses on bottle-fed infants, EPA could follow the nutritional guidelines to estimate the total water-ingestion rate (such as the frequency of feeding per 24 hours and the quantify of formula and water mixing per feeding).

Lauren Zeise

Yes, although the reason for using a quadratic relationship was not described. The approach of expressing water ingestion in units mL/kg/day and modeling it as a function of age is much preferred over the approach used for breast milk consumption (e.g., in Figure 1).

Does the overall function used to represent the changes in ingestion with age (and body weight) appropriately characterize the available data and information?

This approach is a reasonable way of describing the upper 90th bound given in the Kahn and Stralka (2008) paper.

Are there other data that could be used to obtain a better or equally valid alternative estimate of water ingestion rate for infants in the first few days of life (e.g., 7-day old)?

An alternative for estimating ingestion rate for the first few days of life would be to rely on data sets for breast milk consumption during the first 7 days (e.g., Casey et al. 1986, Am J Dis Child 140:933; Neubauer et al. 1993, Am J Clin Nutr, 58:54), since breast fed infants do not require supplemental water and the results may be more indicative than the assumed relationship used, although sample sizes are relatively small. It is noteworthy that intake in mL/kg/d during this period is not a smooth function of bodyweight. It is quite low during the first two days of life but by age four or five days the intake is essentially the same as at age 7 days. It is possible that the function 1-e^{-day} does a reasonably good job of describing this. EPA could compare the values predicted by this

function at days 1-7 to those seen in the literature for breast milk consumption on those days.

The water ingestion rates used by EPA are based on 90th percentile ingestion data and thus are likely to exceed (minimal) physiological needs of infants as defined in nutritional guidelines. Are the water ingestion rates used by EPA reasonable in comparison to the physiological needs of infants at these various life stages? Are there other approaches or data ***(e.g., see the FDA memo****) that could be used to obtain a better or equally valid alternative estimate for this parameter?*

The available data used by EPA indicate that infant formula consumption varies by individuals, and correspondingly water consumption does as well. It is reasonable to consider the minimal physiological needs of infants, as defined in nutritional guidelines, although a precise understanding energy needs and use in infancy still appears to be a matter of discussion (Reilly et al. 2005, Br J Nutr 94: 56-63). At any particular age bodyweights, growth rate and degree of activity varies, and so consumption can not be precisely calculated based on formula energy content and recipes for making up bottle fed formula. Further, some infants are overfed and others are underfed. Thus although it would be useful to compare water consumption with what one would expect given nutritional guidelines and typical formula recipes, the nutritional guideline would not lead to a reliable upper bound value for water consumption. Assumptions would be needed to go from the water consumption based on the nutritional guideline level to an upper bound estimate. Though as the FDA memo notes, "there is a relationship between the volume of water an infant needs, and his/her caloric requirements for healthy growth" the exact relationship to assume and the interindividual variability in that relationship has not been provided and it is unclear that it would provide a more reliable estimate of water consumption than is given in EPA's perchlorate report.

(D) Perchlorate concentrations in formula

(D1) EPA used 1.42 µg/L as the concentration of perchlorate in formula for bottle-fed infants. This estimate was based on information from FDA's Total Diet Study, supported by Pearce *et al.*'s (2007) findings.

Is EPA's approach and rationale transparently and objectively described? Is 1.42 µg/L an appropriate value to use for the concentration of perchlorate in infant formula? Are there other better or equally valid alternative approaches or values that could be used?

Janusz Byczkowski

The estimate of perchlorate concentration in formula for bottle-fed infants seems to be appropriate and well substantiated, as it was based on the two independent sets of data (FDA TDS and Pearce et al., 2007), which by themselves do not differ significantly from each other. The derivation of average value is clearly described in the reviewed EPA draft document.

Brian Cummings

EPA's approach and rationale are transparent and well described. The approach is logical and based on the most current information. A search for other levels for this values results in similar results. This is obvious an area for which more data is needed (see above).

Panos Georgopolous

The rationale for the selection of 1.42 µg/L as a preset value for the concentration of perchlorate for bottle-fed infants is not adequately discussed. This value is the average of 12 samples (8 of them above detection limit) presented in Murray et al. (2008); it is also close to the average value (1.45 ppb) of the 17 samples analyzed by Pearce et al. (2007). It should be noted that the values of perchlorate concentrations in the samples of Pearce et al. range from 0.2 to 4.1 ppb. It would be useful to examine the sensitivity of uptake for a reasonable concentration range rather than only the average value.

References Cited in Answer to Question D-1:

Murray, C.W., Egan, S.K., Kim, H., Beru, N., and Bolger, P.M. 2008. US Food and Drug Administration's Total Diet Study: Dietary intake of perchlorate and iodine. *J Expos Sci Environ Epidemiol* 18 (6):571-580.

Pearce, E.N., Leung, A.M., Blount, B.C., Bazrafshan, H.R., He, X., Pino, S., Valentin-Blasini, L., and Braverman, L.E. 2007. Breast milk iodine and perchlorate concentrations in lactating Boston-area women. *J Clin Endocrinol Metab* 92 (5):1673-7.

Sean Hays

Based on the stated objectives of this analysis, EPA should adjust the intake of perchlorate from infant formula to result in a daily exposure consistent with the point of departure. This will yield consistent results across all scenarios to assure a fair and impartial comparison of relative differences in inhibition of thyroid iodine uptake can be made.

Frederick Kaskel	EPA's approach and rationale for the concentration of perchlorate in formula for bottle fed infants is appropriated and transparency
Kannan Krishnan	The EPA,s approach is clearly described and appears to be consistent with the current state of knowledge. However, it would be better to clearly identify the basis for the choice of the mean value rather than median, 90th or 95th pctl value (presumably the limited, available data did not permit such a determination).
Chensheng Lu	The Reviewer believes that perchlorate level in formula used by EPA is the best available data; especially this level is consistent to the public numbers from an independent research. The Reviewer is not aware other better or equally valid alternative approaches or values that could be used.
Lauren Zeise	The approach is not entirely transparent and the description could be improved. A sample calculation for Table 4 describing how perchlorate intake for bottle fed infants is estimated would be helpful. *Is 1.42 µg/L an appropriate value to use for the concentration of perchlorate in infant formula? Are there other better or equally valid alternative approaches or values that could be used?* According to the Pearce et al. methodology: "Seventeen brands of infant formulae were also assessed for iodine and perchlorate levels. A single sample of each different type of liquid formula available at a local supermarket was purchased for testing. Nine brands were sold in concentrated form and designed to be diluted by half before use. Iodine and perchlorate levels were measured directly in these samples, and the results were divided by half to reflect the concentration intended for infant use. The other eight brands were sold ready for use." Thus, for the nine formula that were designed to be diluted, Pearce et al. assumed that there was no perchlorate in the diluting water. EPA reports the correct average of 1.45 µg/L calculated from 17 Pearce et al. samples. But for the young bottle fed infant the calculation should reflect the intake of perchlorate from neat formula plus the intake from the water used to dilute it. It is reasonable to assume that the only perchlorate intake in the seven and 60 day infant would be water and formula. Thus the undiluted values for formula perchlorate should be used. The undiluted average from Pearce is 1.97 µg/L, but that includes formula that is ready to use undiluted as well as formula that requires dilution. For use in Table 4, the focus should be on concentrations of formula that would require dilution. The young seven and 60 day infant population drinking ready to use formula with no other consumption is more a concern of the FDA than the EPA; they would not be receiving perchlorate contaminated tap water. In the Pearce et al. study, the perchlorate concentration in the 9 samples of formula that would be diluted was 1.96 µg/L. The two highest of the nine values reported would require dilution correspond to 3 µg/L and 3.2 µg/L, double the

value reported in Pearce et al. Table 1.

The problem with the FDA data is that they represent composite samples, prepared as they would be expected to be consumed. Also, the detection limit used by FDA is 1 µg/L. The composite would be averaged across different formula brands and certain types. Thus they do not provide an indication of what higher end exposures might be. The composite sample results, in units µg/L, are:

202 Infant formula, milk, hi-Fe: ND, 2.5, 2.0, 2.0

203 Infant formula, milk, lo-Fe: 1.2, ND, 3.6, 2.1

309 BF, infant formula, soy: ND, ND, 0.8 *, 0.8 *

* indicates above the limit of detection but below the limit of quantitation and ND indicates not detected.

Each value represents a composite from three cities in a given region. Thus a concentration in particular product may be three times as high as the value reported. Because of consumer loyalty and habit it is far more likely that a consumer will use the same product over an extended period of time. From the values tabulated, value of 1.42 µg/L will be an underestimate of perchlorate concentration in contaminated infant formula. Further, the concentration of perchlorate in water used by FDA to prepare the formula in to-be-eaten form has not been reported, but is likely to be low or not present, given the several NDs in the table. Because FDA uses composite samples, it would be preferable to use the high end value from FDA (3.6 µg/L) or a value of say 3 µg/L from Pearce et al. Clearly better and more extensive measurement of perchlorate in infant formula is desirable.

(E) Radioiodide excretion into breast-milk by NIS

(E1) In the model, EPA included perchlorate inhibition of NIS radioiodide excretion into breast-milk, as well as inhibition of radioiodide transport by perchlorate for all NIS-containing tissues, thereby making the code consistent with the model description in Clewell et al. (2007). Is this inclusion appropriate? Are the impacts of this inclusion transparently and objectively described?

Janusz Byczkowski	Because the mechanism of iodide transfer from blood into the thyroid gland and other tissues, including mammary, is mediated by the sodium-iodide symporter (NIS), it was logical and appropriate for EPA to include the algorithm for competitive inhibition of NIS by perchlorate in these extra-thyroid tissues too. The inclusion of such a mechanistic approach has been briefly mentioned in main text of the EPA draft document (in Section 2), and then, extensively discussed in the Appendix A. The rationale and the consequent improvement in PBPK model predictions have been described objectively and presented clearly (in Appendix A).
Brian Cummings	This inclusion is appropriate because it's unlikely that a situation exists where perchlorate or iodide is absent from the diet. This represents a logical and important refinement in the model. However, a section is needed, towards the end, which clearly discusses the impacts of perchlorate inhibition. Further, the rationale for scaling NIS to body weight and other tissues is not clear. Studies suggest that NIS expression changes over development (see below). At least one study suggests that the expression of NIS is higher per g of tissue in young children (< 12 years) compared to adults. Further, another study suggests that this difference accounts for higher levels of iodide uptake in children than in adults. Thus, scaling NIS levels to body weight (i.e. age) may not be appropriate. Faggino et al., Journal of Nuclear Medicine, 45(2), 232-237.
Panos Georgopolous	The inclusion of perchlorate inhibition of NIS radioiodide excretion into breast-milk, as well as of inhibition of radioiodide transport by perchlorate for all NIS-containing tissues, is appropriate. The impacts of this inclusion are adequately ("transparently and objectively") described in the document.
Sean Hays	Based on the data from Pearce et al. (2007), one would expect there to be no effect of perchlorate on the excretion of iodine in breast milk. As such, this feature of the model that EPA has included may not be accurate with perchlorate kinetics. EPA should investigate the data of Pearce et al. more fully and explore other data sets to see what evidence is available to include such a feature in the model.
Frederick Kaskel	The inclusion of perchlorate inhibition of NIS radioiodide excretion into breast-milk, as well as inhibition of radioiodide transport by perchlorate for all NIS-containing tissues, is appropriate, transparent and objectively described. The EPA added inhibition of radioiodide transport by perchlorate for radioiodide excretion into breast milk by NIS markedly increased the predicted percent inhibition of thyroidal radioiodide uptake in the breast fed infant.

Kannan Krishnan	Yes. The EPA's approach is logical and internally-consistent. The impact of this inclusion is described in sufficient detail.
Chensheng Lu	Yes, this inclusion is not only appropriate but also needed, and the impacts of this inclusion are transparently and objectively described in this analysis. This work reflects EPA's efforts in reviewing the model established by Clewell et al. and seeking for improvement of the PBPK model.
Lauren Zeise	It is reasonable and appropriate to assume that perchlorate inhibits the transport of iodide in NIS containing tissues and iodide excretion into breast-milk. The impact of its inclusion is transparently and objectively described. There is a straightforward layout in Appendix A of changes in model assumptions and their impacts. Further, the effect of decreased iodide levels in breast milk from smoking – with potential inhibition caused by thiocyanate - has also been observed (Laurberg et al. 2004, J Clin Endo Met 89:181-187), consistent with the finding that this should be taken into account in the modeling.

Additional Reviewer Comments

Brian Cummings

Miscellanous Comments;

1. An extra period is present in the first bullet point on page 59.

2. The third paragraph on page 42 refers to Figure B-1. Should this be Figure B-2?

3. Please list the years for the references for Merrill et al. listed on page 39.

Sean Hays

Additional comments:

The approach of using PBPK modeling is admirable and the EPA should be commended. However, the EPA should also have considered easier and more straightforward approaches. The one-compartment PK model developed by EPA (Lorber, 2008), paired with measured perhclorate and iodine levels in breast milk and infant formula would have provided simpler and equally valid approaches for answering the question of the relative difference in steady-state perchlorate levels (this is ultimately the endpoint of interest) in the various receptors/scenarios. While I agree with using PBPK modeling, I also think EPA should think about simpler approaches that are equally or more valid, and sometimes much simpler and more easily embraced by the regulatory and risk assessment community.

Appendix A: I agree with the model modifications made by EPA.

Appendix B: Appendix B is well written and easier to follow than the corresponding text in the main report relating to urinary clearance values (section 3.1).

Kannan Krishnan

Overview:

This EPA report summarizes work conducted to evaluate the PBPK models for perchlorate and radioiodide for quantitating relative sensitivity of different subgroups (lifestages). The two-stage model evaluation process involved verification of model codes and examination of the parameterization approaches. Following the revision of the PBPK models by EPA, they were checked by a contractor who also verified the output of the model by reproducing various figures from original publications. Despite the thoroughness of the work, this life-stage variability analysis (either due to lack of data or due to uncertainty associated with available data) did not account for certain subgroups (e.g., elderly, foetus during early gestation periods, iodine-deficient or hypothyroid status during pregnancy) and did not account for variability of parameter values within subgroups in the simulations (i.e., with the use of Bayesian or Monte Carlo type methods).

ADDITIONAL COMMENTS

In section 4.3. of the report, it is indicated as follows:

"For the 6- to 12-month and 1- to 2-year-old children, the water intake rates of 0.971 L/kg-day and 0.674 L/kg-day, respectively, were set based on 90th percentile values for

direct and indirect water consumers-only intake (Kahn and Stralka, 2008). Additionally, to calculate L/day for these age groups, the corresponding age group mean body weights obtained from NHANES 1999-2006 were used: 9.2 kg for 6- to 12-month and 11.4 kg for 1- to 2-year-old children."

The above statements indicate that the water intake by a 6 - 12 months old child would be about 9 L/day whereas the body weight itself is only about 9 kg. So the above numbers should be verified with the original report of Kahn and Stralka (2008). This reviewer's verification with the original report would indicate 90th percentile values of 120 ml/kg/d and 64 ml/kg/d, respectively, for the 6 – 12 month and 1-2 yr old groups. This is in contrast to EPA's numbers of 971 ml/kg/d and 674 ml/kg/d as indicated above.

Appendix A
Individual Reviewer Comments

PEER REVIEW COMMENTS FROM

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Contract No. EP-C-07-024
Task Order No. 54
October 22, 2008

INHIBITION OF THE SODIUM-IODIDE SYMPORTER BY PERCHLORATE: AN EVALUATION OF LIFESTAGE SENSITIVITY USING PHYSIOLOGICALLY-BASED PHARMACOKINETIC (PBPK) MODELING
(DUE DATE: NO LATER THAN MONDAY, NOVEMBER 10, 2008)

Background and Purpose

The U.S. Environmental Protection Agency (EPA) has prepared a draft document analyzing the inhibition of thyroidal radioiodide uptake (RAIU) by perchlorate (an anion in CASRNs 7790-98-9; 7791-03-9; 7778-74-7 and 7601-89-0) for multiple life-stages, including pregnancy, fetal development, lactation, infancy, childhood, and adulthood, using physiologically based pharmacokinetic modeling (PBPK). The EPA has asked to review the changes applied to modeling parameters and specific changes in the PBPK code, that were intended to make the code consistent with the published description.

Answers to Charge Questions

General Charge Questions:

G-1. Is EPA's analysis logical, clear and appropriate in depth and length? Has EPA accurately, clearly and objectively represented and synthesized the scientific evidence for the changes made to or specification of the model code and input parameters?

The reviewed EPA draft document is logical, clear and concise. In general, reasons for changes in code and input parameters and most of their their consequences are objectively described. However, given a large number of abbreviations and acronyms, a glossary listing these abbreviations and acronyms would be helpful.

G-2. Please identify any additional studies or other data sources that should be considered in the assessment of the specific parameters addressed below, including urinary clearance (of iodide and/or perchlorate) and ingestion rates (breast milk, formula and water), especially in neonates.

It seems, that most of the published relevant data have been already reviewed and/or included in the modeling and analysis.

However, this Reviewer could not identify the reference posted in the EPA Perchlorate human lactation model code (pp. 74 and 76, lines 67 and 131, respectively): *Gentry et al. (2001)*. It is suggested, that for the *VMk* parameter ("Residual milk volume", see the answer to G-3 below), another data source could be used: Dewey K.G., Heinig M.J., Nommsen L.A., and Lonnerdal B.: Maternal versus infant factors related to breast milk intake and residual milk volume: the DARLING study. Pediatrics 1991: 87: 829–837.

G-3. Besides the specific parameters identified below, please comment on any other parameter or model choices described in the document that you think are incorrect or require further explanation or where other available information could provide better estimates.

The numerical value of the *VMk* parameter, identified in the EPA Perchlorate human lactation model code (p. 76), line 131: "*CONSTANT VMk = 0.6320 ! Residual milk volume (L) (Gentry et al 2001)*" seems to be unrealistically high. It is closer to the low daily breast milk intake by infant rather than to the residual milk volume (< 650 g/day *vs* 109 g/day; Dewey et al., 1991).

Since the mammary glands respond to feeding stimuli by secreting breast milk on demand, the "residual milk volume" usually refers only to the small volume of unconsumed milk. Without suckling stimulation, even lower void volume of milk remains in alveoli, lactiferous ducts and sinuses between the feeding sessions, and it stays in equilibrium with blood under near steady-state conditions (Byczkowski, J.Z. in *U.S. EPA (2002): Final Report "EXPLORATION OF PERINATAL PHARMACOKINETIC ISSUES"*, EPA/630/R-01/004, May 10, 2001. On-line: http://oaspub.epa.gov/eims/eimscomm.getfile?p_download_id=120867). While milk intakes vary with caloric demand of the infant (represented by *KTrans* in the EPA model), as reported by Dewey *et al*. (1991), infants with low intakes left as much milk unconsumed as those with higher intakes, which justifies the residual milk volume to remain constant (109 g/day).

On the other hand, the EPA PBPK modeling algorithm suggests that the *VMk* parameter corresponds rather to the initial volume of milk (632 g), further linked to the growth function of infant's body weight by *KTrans,* which infant receives in each "*pulse*". Even though this approach may adequately describe the volume of breast milk actually ingested by growing infant, it is not physiologically accurate and does not allow for any interspecies extrapolation. Since *VMk* affects concentrations of both iodide and perchlorate in breast milk, it is difficult to predict if and how the suggested change in *VMk* description would change the RAIU inhibition in the breast-fed neonate. Before any change, a sensitivity analysis should be performed with varied *VMk* value, to evaluate how *VMk* parameter affects the PBPK model output and to decide if and how this potential problem should be addressed.

G-4. Please discuss research that you think would be likely to increase confidence in these models and their use in predicting RAIU inhibition by perchlorate in different life stages (for an average individual within each life-stage).

If the overall model output is sensitive to *VMk* parameter, the confidence in the PBPK modeling of "*Breast-fed neonate*" could be increased by a better description of "residual milk volume" (see answers to G-2 and G-3, above).

G-5. Does EPA accurately characterize the strengths and limitations of the analysis? Please comment on any particular strengths that may not be mentioned or adequately characterized for the estimates of RAIU for different life stages. Please also comment on any specific sources of uncertainty that you believe have been overlooked in EPA's analysis or that require further discussion, and which might be significant to EPA's estimates of RAIU for different life stages.

The reviewed EPA draft document adequately describes strengths and limitations of the analysis. However, the overall strength of any PBPK modeling used for risk assessment is its ability to simulate adequately the experimental data. While a partial validation of the unmodified iodide PBPK model was performed and reported by Clewell *et al*., in the EPA document, only limited comparisons of some variables to the actual data have been presented (in Appendix C). The strength of the EPA modeling analysis could be better presented by including comparisons of the PBPK model simulations to all significant real-life experimental data.

G-6. As recommended in EPA's 2000 Risk Characterization Handbook, is the analysis transparent in terms of the steps, logic, key assumptions, limitations, and decisions? Specifically, does characterization of the results of EPA's work fully explain: a) the analysis approach employed; b) the use of assumptions and their impact on the analysis; c) the use of extrapolations and their impact on the analysis; d) plausible alternatives and the choices made among those alternatives; e) the impacts of one choice vs. another on the analysis; f) significant data gaps and their implications for the analysis; g) the scientific conclusions identified separately from default assumptions and policy decisions, if any; and h) the major conclusions, and the discussions of EPA's confidence and uncertainties in the conclusions?

It seems that the reviewed EPA draft document complies with guidelines and recommendations of the EPA 200 *Risk Characterization Handbook*. As stated in the answer to G-1 (above), it is logical, clear and concise. The alternatives are presented and the decision points are adequately explained.

Perhaps, the contribution of specific PBPK uncertainties to the overall uncertainty of modeling RAIU inhibition by perchlorate could be summarily discussed and presented in a separate section.

Parameter-Specific Charge Questions:

(A) Urinary clearance

A-1. Please comment on the appropriateness of the input values selected for maternal urinary clearance during pregnancy and lactation in EPA's analysis. Are the available data and rationale for the values selected transparently and objectively described? Are you aware of other publications or data that could be used to guide these choices or which provide alternative input values that are equally valid or more appropriate? Likewise, are the values selected for urinary clearance for the infant and older child the best estimates, given the available science and data? Are there other data that would provide better (or equally valid alternative) guidance or estimates? Is there any reason to believe that urinary clearance might not be a limiting factor in the elimination of perchlorate for infants?

The urinary clearance has been addressed appropriately and discussed as well as presented in a sufficient detail (in Appendix B). Given the limited data, particularly for perchlorate, it seems tat the approach and values presented by EPA are reasonable and this Reviewer is not aware of any data that could contradict this approach.

(B) Breast-milk ingestion

B-1. Infants are generally known to consume very little milk or formula on the first day of life, with ingestion quickly increasing over time. However the first time-point for which ingestion data are available for infants is at 7 days, which EPA extrapolated back to 0 ingestion at time=0 in its analysis (birth; Figure 1). The breast-milk ingestion rate was estimated based on mean values reported in Arcus-Arth et al. (2005), while the water ingestion used for the breast-feeding mother was the 90th percentile value from U.S. EPA (2004).

Is EPA's extrapolation and rationale transparently and objectively described? Does this function appropriately characterize the available data and information? Are there other data that could be used to obtain a better or equally valid alternative estimate of mean breast-milk ingestion rate for infants in the first few days of life? Should an estimate other than the mean be used to determine the breast-milk ingestion rate?

The Arcus-Arth *et al*. (2005) data for breast milk intake are, perhaps, the best currently available, and thus the smooth function that fits these data is the right approach. It seems that the exponentially growing intake for the first week postpartum may be an overestimate, but given the inadequate information about intake during the first weeks postpartum, the applied growth function appears to be a reasonable, practical solution, even though seemingly quite a health-protective. The idea of adding on the top of this overestimation the upper bound (e.g., the estimate of 90th percentile intake) would lead to a marked exaggeration of milk consumption, unrealistically high for a typical infant.

While this Reviewer cannot suggest any better approach to the milk intake, please see the answers to G-2 and G-3 for a closely related potential problem with the estimated residual breast milk volume.

(C) Water ingestion

C-1. For pregnancy, EPA used a normalized (90th percentile) water ingestion rate of 33 mL/kg-day (U.S.EPA 2004), which was multiplied by the maternal BW as described by the PBPK model growth-functions during pregnancy to obtain total water ingestion for the mother.

Is EPA's approach and rationale transparently and objectively described? Is this approach appropriate for characterizing the upper-bound for ingestion of pregnant women? Are there other approaches or data that could be used to obtain a better or equally valid alternative estimate for this parameter? (Note that the self-reported body weight values in U.S. EPA (2004) indicate the same average body weight for

pregnant women as for non-pregnant; data appears inaccurate, so the PBPK model body-weight description was used instead.)

It seems that linking the upper bound estimate of water ingestion to maternal body weight growth function is an appropriate approach, evidently, more realistic than the self-reported data. The water ingestion issue has been mentioned in the reviewed EPA draft document but not extensively discussed. It is not clear what (if any) modification to the water ingestion variable has been made by EPA in comparison to the original pregnancy model reported by Clewell *et al*. (the algorithm for "r*dose_p*" is already marked in the original CSL file as "*modified by PMS"*).

C-2. For lactation, EPA used a fixed total (90th percentile) water ingestion rate of 2959 mL/day (U.S. EPA 2004), with the rationale that while the woman's weight and self-water demand are expected to drop after pregnancy (as described by the PBPK model time-dependent weight equations), the demand for milk production would be increasing, and the reported value was not for a specific age-range of child.

Is EPA's approach and rationale transparently and objectively described? Is this an appropriate value to use for the ingestion rate of lactating women? Are there other better or equally valid alternative approaches or values that could be used?

The use of 90th percentile of water ingestion by lactating woman apparently covers the extra demand due to breast-feeding, and perhaps, it is health protective. The approach is adequately described in the reviewed EPA draft document. This Reviewer is not aware of any better approach.

C-3. For bottle-fed infants, EPA made extrapolations of the 90th percentile water-ingestion in early life based on measurements made for the age ranges: 0-30 days, 1-3 months, and 6-12 months (points in Figure 2, upper panel). For the purposes of this analysis, bottle-fed implies feeding with formula requiring the addition of water.

Is EPA's extrapolation and rationale transparently and objectively described? Does the overall function used to represent the changes in ingestion with age (and body weight) appropriately characterize the available data and information? Are there other data that could be used to obtain a better or equally valid alternative estimate of water ingestion rate for infants in the first few days of life (e.g., 7-day old)? The water ingestion rates used by EPA are based on 90th percentile ingestion data and thus are likely to exceed (minimal) physiological needs of infants as defined in nutritional guidelines. Are the water ingestion rates used by EPA reasonable in comparison to the physiological needs of infants at these various life stages? Are there other approaches or data (e.g., see the FDA memo) that could be used to obtain a better or equally valid alternative estimate for this parameter?

For bottle-fed infant, analogously to maternal water ingestion, linking water intake to body weight growth function is an appropriate approach (see answer to C-1, above). This issue has been adequately described in the reviewed EPA draft document. The water ingestion rates used by EPA appear

to be realistic, and thus, reasonable. This Reviewer is not aware of any better approach.

(D) Perchlorate concentration in formula

D-1. EPA used 1.42 µg/L as the concentration of perchlorate in formula for bottle-fed infants. This estimate was based on information from FDA's Total Diet Study, supported by Pearce et al.'s (2007) findings.

Is EPA's approach and rationale transparently and objectively described? Is 1.42 µg/L an appropriate value to use for the concentration of perchlorate in infant formula? Are there other better or equally valid alternative approaches or values that could be used?

The estimate of perchlorate concentration in formula for bottle-fed infants seems to be appropriate and well substantiated, as it was based on the two independent sets of data (FDA TDS and Pearce et al., 2007), which by themselves do not differ significantly from each other. The derivation of average value is clearly described in the reviewed EPA draft document.

(E) Radioiodide excretion into breast-milk by NIS

E-1. In the model, EPA included perchlorate inhibition of NIS radioiodide excretion into breast-milk, as well as inhibition of radioiodide transport by perchlorate for all NIS-containing tissues, thereby making the code consistent with the model description in Clewell et al. (2007). Is this inclusion appropriate? Are the impacts of this inclusion transparently and objectively described?

Because the mechanism of iodide transfer from blood into the thyroid gland and other tissues, including mammary, is mediated by the sodium-iodide symporter (NIS), it was logical and appropriate for EPA to include the algorithm for competitive inhibition of NIS by perchlorate in these extra-thyroid tissues too. The inclusion of such a mechanistic approach has been briefly mentioned in main text of the EPA draft document (in Section 2), and then, extensively discussed in the Appendix A. The rationale and the consequent improvement in PBPK model predictions have been described objectively and presented clearly (in Appendix A).

PEER REVIEW COMMENTS FROM

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Technical Charge to Peer Reviewers

Contract No. EP-C-07-024

Task Order No. 54

October 22, 2008

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

WRITTEN COMMENTS ARE DUE NO LATER THAN MONDAY, NOVEMBER 10, 2008

CHARGE QUESTIONS: My responses to the charge questions are in italics

General Charge Questions:

G-1. Is EPA's analysis logical, clear and appropriate in depth and length? Has EPA accurately, clearly and objectively represented and synthesized the scientific evidence for the changes made to or specification of the model code and input parameters?

EPA's analysis is logical and clear. The length is appropriate and the appendix aid in understanding their approach while adding to depth. Care is taken to explain what changes were made to the model, why there were made, the scientific evidence and literature used, the implications of these changes. In many cases EPA has simulated these changes and found little, to no effect on the model.

G-2. Please identify any additional studies or other data sources that should be considered in the assessment of the specific parameters addressed below, including urinary clearance (of iodide and/or perchlorate) and ingestion rates (breast milk, formula and water), especially in neonates.

Zuckier et. al., Journal of Nuclear Medicine, 45(3), 500-507, 2004. This study mainly assesses perrhenate, but also studies the interaction of iodide and perchlorate in NIS tissues, both in vivo and in vitro. It specifically studies biodistributions of these compounds in the presence and absence of each other. It takes into account the effect of NIS on this distribution. It may prove helpful.

A search of pubmed did not reveal any references to iodide or perchlorate clearance not already

mentioned by the authors. The most recent article I could find on either subject was DeWoskin and Thompson, 2008, which the authors use.

Why was a compartment analysis figure not shown for this revised model? Such figures are useful to readers in conceptualizing the model. These were included in the literature on which the current model was based.

G-3. Besides the specific parameters identified below, please comment on any other parameter or model choices described in the document that you think are incorrect or require further explanation or where other available information could provide better estimates.

The methods used for scaling of clearance to body weight, age and surface area are appropriate; however, such scaling is most accurate for clearance when the substance in question is not reabsorbed or secreted. Given that fact that both pendrin and NIS are reported to act on perchlorate, and given reports that NIS expression does not scale to bodyweight in some tissues (see below), do the authors feel that their approach is still valid? Do alterations in NIS expression need to be included in this model? If they are, would this increase the risk for children age 10-14, when NIS expression is believed to altered?

GFR in children is typically scaled according to muscle mass, which scales well with the cube of height in boys and girls from 6 months to adult (see Check, DB et al., Am. J. Clin. Nutr, 30:851, 1977). Scaling formulas have even been derived for children based on creatinine levels (See Diseases in the Kidney, Chapter 80, Seventh Edition, Editor = Schrier, Page 2355). Could these formulas be used to more accurately reflect GFR in children when calculating perchlorate and iodide clearance?

G-4. Please discuss research that you think would be likely to increase confidence in these models and their use in predicting RAIU inhibition by perchlorate in different life stages (for an average individual within each life-stage).

Studies directly assessing the effect of perchlorate on the clearance of NIS substrates are needed. Further, data on the mechanisms of perchlorate inhibition of NIS is lacking. Research investigating the toxicity of substrates of NIS, in the presence and absence of perchlorate, is needed. Finally, urinary clearances of environmental pollutants in infants and neonates are needed.

G-5. Does EPA accurately characterize the strengths and limitations of the analysis? Please comment on any particular strengths that may not be mentioned or adequately characterized for the estimates of RAIU for different life stages. Please also comment on any specific sources of uncertainty that you believe have been overlooked in EPA's analysis or that require further discussion, and which might be significant to EPA's estimates of RAIU for different life stages.

EPA does a good job of characterizing the strengths and limitations of the model, but needs a separate paragraph at the end directly addressing these points.

Did EPA take into account any hormone effects on NIS or thyroid function? The previous literature, on which this model is based, devotes some discussion to this subject. This is particularly important when discussing susceptibility during puberty.

G-6. As recommended in EPA's 2000 Risk Characterization Handbook, is the analysis transparent in terms of the steps, logic, key assumptions, limitations, and decisions? Specifically, does characterization of the results of EPA's work fully explain: a) the analysis approach employed; b) the use of assumptions and their impact on the analysis; c) the use of extrapolations and their impact on the analysis; d) plausible alternatives and the choices made among those alternatives; e) the impacts of one choice *vs*. another on the analysis; f) significant data gaps and their implications for the analysis; g) the scientific conclusions identified separately from default assumptions and policy decisions, if any; and h) the major conclusions, and the discussions of EPA's confidence and uncertainties in the conclusions?

The analysis conforms to EPA guidelines on transparency with regards to steps and logic. The key assumptions of the model are clearly listed, as well as the decisions involved in parameters changes and impact of these changes. The limitations of the model could be more clearly listed (see above).

The analysis approach employed is adequately explained as are the basis for assumption used in this model. EPA goes to great lengths to test the impact of these assumptions by determining the sensitivity for each parameter changed.

The extrapolations used are clearly outlined and well as their rationale for them; however, for the most part, only the theoretical impact of these extrapolations are discussed. Some validation is presented, but this mostly uses previously validated data. Was the model applied to any data sets in the literature not previously studied? Are such data available at this time?

The impacts of choice for most of the critical parameters are discussed. This is particular true when discussing choices for BW, clearance and scaling these values.

Plausible alternatives for some of the parameters could be more clearly listed. This is particularly true of the impact on altering the level of water ingestion to 90%. What were the alternatives to this value and how did they affect them model?

Alternative choices for scaling to BW and clearance are clearly listed as well as their impacts.

The major and scientific conclusions for this work are clearly stated and appear to be separated from any grand statements on policy decisions.

The authors do discuss data gaps throughout the manuscript, but a specific section is needed, towards the end, listing these gaps in itemized, or table form. This should be followed by paragraph that list major perceived weaknesses and uncertainty of this model, which need to be more clearly stated.

Parameter-Specific Charge Questions:

(A) Urinary clearance

A-1. Please comment on the appropriateness of the input values selected for maternal urinary clearance during pregnancy and lactation in EPA's analysis. Are the available data and rationale for the values selected transparently and objectively described? Are you aware of other publications or data that could be used to guide these choices or which provide alternative input values that are equally valid or more appropriate? Likewise, are the values selected for urinary clearance for the infant and older child the best estimates, given the available science and data? Are there other data that would provide better (or equally valid alternative) guidance or estimates? Is there any reason to believe that urinary clearance might not be a limiting factor in the elimination of perchlorate for infants?

The authors do a good job discussing the choice and values used maternal urinary clearance during and after pregnancy and lactation. This includes are significant discussion of the impact of changes made to these parameters and alternatives.

The available data and analysis, for the most part, are rationale, transparently and objectively described, with one exception (see C-2) below. I am not aware of any further publication that could be used for input values that are more appropriate. One question that I did have is what was the rationale for choosing the lower clearance value from Clewell et al., (2007) as opposed to the others (Page 12, 2nd

paragraph).

Please see my comments above concerning other data that provide alternative guidance with regards to urinary clearance in neonates, infant and children. Several studies support the hypothesis that urinary clearance is a limiting factor of perchlorate elimination. While it's possible that it may not be the only factor involved, it is clearly a major one. In absence of any data to the contrary, which could not be found, the authors are correct in this assumption.

(B) Breast-milk ingestion

B-1. Infants are generally known to consume very little milk or formula on the first day of life, with ingestion quickly increasing over time. However the first time-point for which ingestion data are available for infants is at 7 days, which EPA extrapolated back to 0 ingestion at time=0 in its analysis (birth; Figure 1). The breast-milk ingestion rate was estimated based on mean values reported in Arcus-Arth et al. (2005), while the water ingestion used for the breast-feeding mother was the 90th percentile value from U.S. EPA (2004).

Is EPA's extrapolation and rationale transparently and objectively described? Does this function appropriately characterize the available data and information? Are there other data that could be used to obtain a better or equally valid alternative estimate of mean breast-milk ingestion rate for infants in the first few days of life? Should an estimate other than the mean be used to determine the breast-milk ingestion rate?

EPA's rationale for ingestion rates for infants at time 0 follows rationale assumptions and the approach appears objective. While its true that infants consume little in the first day of life, the extrapolation of 7 day data to day 1 allows for a margin of safety. I am not aware of any data that EPA has not presented. While other estimates for breast-mile ingestion may exist, the ones used by the EPA are clear, rationale and tractable. Thus, they will be easily validated in future models.

(C) Water ingestion

C-1. For pregnancy, EPA used a normalized (90th percentile) water ingestion rate of 33 mL/kg-day (U.S.EPA 2004), which was multiplied by the maternal BW as described by the PBPK model growth-functions during pregnancy to obtain total water ingestion for the mother.

Is EPA's approach and rationale transparently and objectively described? Is this approach appropriate for characterizing the upper-bound for ingestion of pregnant women? Are there other approaches or data that could be used to obtain a better or equally valid alternative estimate for this parameter? (Note that the self-reported body weight values in U.S. EPA (2004) indicate the same average body weight for pregnant women as for non-pregnant; data appears inaccurate, so the PBPK model body-weight description was used instead.)

EPA's approach is rationale, transparent and objectively described. I agree with using 90% water ingestion for pregnant woman for a upper bound rate, as it adds a safety factor; however clarification is needed as to how this value was derived. A review of the documents used by EPA to determine this value reports 90% bootstrapping levels as apposed to overall ingestions levels? Was the 90% bootstrap value used, or did EPA calculate 90% ingestion from these data. How exactly was the value of 33 mL/kg-day determined?

C-2. For lactation, EPA used a *fixed* total (90th percentile) water ingestion rate of 2959 mL/day (U.S. EPA 2004), with the rationale that while the woman's weight and self-water demand are expected to drop after pregnancy (as described by the PBPK model time-dependent weight equations), the demand for milk production would be increasing, and the reported value was not for a specific age-range of child.

Is EPA's approach and rationale transparently and objectively described? Is this an appropriate value to use for the ingestion rate of lactating women? Are there other better or equally valid alternative approaches or values that could be used?

EPA approach is rational as it assumes that lactating women will still have increased water needs. While the approach is transparently and objectively described I am have some questions about the actual level, which results in ~45.5 ml/kg-day, which is substantially higher than the 90% ingestion rate reported above (assuming that the value was not a bootstrap). Doesn't it seem more likely that water ingestion would equalize? While the overall demand would decrease after pregnancy, this decrease would be countered by lactation? Are the water demands for lactation higher than pregnancy? What data exist on this subject other than models?

C-3. For bottle-fed infants, EPA made extrapolations of the 90th percentile water-ingestion in early life

based on measurements made for the age ranges: 0-30 days, 1-3 months, and 6-12 months (points in Figure 2, upper panel). For the purposes of this analysis, bottle-fed implies feeding with formula requiring the addition of water.

Is EPA's extrapolation and rationale transparently and objectively described? Does the overall function used to represent the changes in ingestion with age (and body weight) appropriately characterize the available data and information? Are there other data that could be used to obtain a better or equally valid alternative estimate of water ingestion rate for infants in the first few days of life (e.g., 7-day old)? The water ingestion rates used by EPA are based on 90th percentile ingestion data and thus are likely to exceed (minimal) physiological needs of infants as defined in nutritional guidelines. Are the water ingestion rates used by EPA reasonable in comparison to the physiological needs of infants at these various life stages? Are there other approaches or data **(e.g., see the FDA memo**) that could be used to obtain a better or equally valid alternative estimate for this parameter?

EPA approach is rationale and transparently described. It is standard practice to scale water ingestion to body weight, which does increase with age. Weight gain in the new born scales more rapidly than almost any other time period and it sequestered into specific groups; however, why was the calculation not scaled to 2 and 3 years as it done by WHO?

Another point of interest is in regards to the 1st seven days of birth. Most infants either maintain birth weight or slightly lose 10% of their birth weight. This, as pointed out by EPA is directly related to water ingestion. Should this than represent another group or be removed (i.e. 0-7 days, or 7-30 days?).

(D) Perchlorate concentration in formula

D-1. EPA used 1.42 µg/L as the concentration of perchlorate in formula for bottle-fed infants. This estimate was based on information from FDA's Total Diet Study, supported by Pearce *et al*.'s (2007) findings.

Is EPA's approach and rationale transparently and objectively described? Is 1.42 µg/L an appropriate value to use for the concentration of perchlorate in infant formula? Are there other better or equally valid alternative approaches or values that could be used?

EPA's approach and rationale are transparent and well described. The approach is logical and based on the most current information. A search for other levels for this values results in similar results. This is obvious an area for which more data is needed (see above).

(E) Radioiodide excretion into breast-milk by NIS

E-1. In the model, EPA included perchlorate inhibition of NIS radioiodide excretion into breast-milk, as well as inhibition of radioiodide transport by perchlorate for all NIS-containing tissues, thereby making the code consistent with the model description in Clewell et al. (2007). Is this inclusion appropriate? Are the impacts of this inclusion transparently and objectively described?

This inclusion is appropriate because it's unlikely that a situation exists where perchlorate or iodide is absent from the diet. This represents a logical and important refinement in the model. However, a section is needed, towards the end, which clearly discusses the impacts of perchlorate inhibition. Further, the rationale for scaling NIS to body weight and other tissues is not clear. Studies suggest that NIS expression changes over development (see below). At least one study suggests that the expression of NIS is higher per g of tissue in young children (< 12 years) compared to adults. Further, another study suggests that this difference accounts for higher levels of iodide uptake in children than in adults. Thus, scaling NIS levels to body weight (i.e. age) may not be appropriate.

Faggino et al., Journal of Nuclear Medicine, 45(2), 232-237.

Miscellanous Comments;

1. *An extra period is present in the first bullet point on page 59.*
2. *The third paragraph on page 42 refers to Figure B-1. Should this be Figure B-2?*
3. *Please list the years for the references for Merrill et al. listed on page 39.*

PEER REVIEW COMMENTS FROM

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CHARGE QUESTIONS

General Charge Questions:

G-1. Is EPA's analysis logical, clear and appropriate in depth and length? Has EPA accurately, clearly and objectively represented and synthesized the scientific evidence for the changes made to or specification of the model code and input parameters?

The document under review, "Inhibition of the Sodium-Iodide Symporter (NIS) by Perchlorate: an Evaluation of Lifestage Sensitivity Using Physiologically-Based Pharmacokinetic (PBPK) Modeling", has a very specific, and deliberately narrow, objective. Indeed it defines (p. 21) sensitivity "as the predicted response in percent RAIU (radioactive iodide uptake) inhibition 24 hours after iodide intravenous injection for an average individual within a specific subgroup (e.g., bottle-fed infants) relative to the predicted response in percent RAIU inhibition for an average, non-pregnant adult, where response is the percent RAIU inhibition 24 hours after iodide IV injection." Though this is rather constrained as a sensitivity metric, it can be reasonably argued that it addresses adequately the biological issue of concern here.

The emphasis of the analysis is on sensitivity with respect to "lifestage". Parameters and processes, related to different lifestages, were modeled based on assumptions that are discussed in rather extensive detail in the document under review. The lifestages evaluated in the document correspond to "average" adult, non-pregnant woman of child-bearing age, pregnant woman, lactating woman, fetus, breast-fed infant, bottle-fed infant, 1 year old child, and and 2 year old child. The tools employed for the analysis were the PBPK models of Clewell *et al.* (2007) for the pregnant woman/fetus and for the lactating woman/breastfed infant. Results for the "bottle-fed" neonate were obtained by altering the dose specification in the model for the breast-fed infant. The PBPK model for the average adult was that of Merrill *et al.* (2005), while the model for the non-pregnant woman of childbearing age was a direct modification of the model for the pregnant woman, obtained by removing the placental and fetal compartments, but retaining the mammary compartment.

The above PBPK models, with various corrections and adjustments (that are discussed in detail in the appendices of the document) were used to estimate the predicted percent RAIU inhibition for the average adult and different specific ("average") individuals representing potentially sensitive subgroups. It should be mentioned here that the actual text of the document under review states that the calculations were made for "subgroups, including potentially sensitive subgroups"; however population-based modeling (with considerations of inter-individual and intra-individual variability) was not actually pursued.

"Base" calculations were made assuming a dose equal to the point of departure (POD) of 7 μg/kg-day, (consistent with the recommendations of the National Research Council - NRC, 2005) and were summarized in Table 3 of the document under review. The relative sensitivity of different subgroups was determined by comparing the percent RAIU inhibition of each subgroup to the percent RAIU inhibition for an average adult at a dose equal to the POD.

The document states that the "model predictions may generally be considered central estimates for each subgroup (at the consumption levels modeled) that account for PK (pharmacokinetic) differences, and do not take into account within-group variability in pharmacokinetics, uncertainty in model parameters and predictions, or population differences in PD." It should be noted that fetal simulations were reported for only the end of gestation (Gestation Week 40).

The analysis presented in the document concluded that urinary clearance was a "key" parameter (i.e., model predictions were highly sensitive to the values of this variable). Though for modeling pregnancy and early infancy a conservative parameterization was adopted, the document emphasizes that "a full population analysis of urinary clearance was not conducted, and given that variability in other PK parameters was not addressed, these estimates should not be considered a true upper confidence bound on RAIU inhibition" (p. 23). The document also identified the fetus as the most sensitive subgroup with respect to percent RAIU inhibition at a dose equal to the POD, in general agreement with earlier PBPK modeling (Clewell *et al.*, 2007) and estimating approximately 5-fold higher percent RAIU inhibition for the fetus at gestational week 40 than for the average adult. In fact it is also stated that "simulations at earlier gestation weeks indicate that the fetus is more sensitive than the adult throughout pregnancy, but are considered too quantitatively uncertain to assign exact relative sensitivities" (p. 23).

Overall it can be stated that EPA's analysis is clear and with sufficient discussion of assumptions involving model and parameter specification (including adjustments and corrections to the original models and their codes). It should be noted, however, that in multiple instances (discussed further in the answers to the following questions) the rationale behind specific assumptions and parameterizations relates more to "convenience" rather than to scientific defensibility. Although this may not necessarily affect the general conclusions, it is nevertheless a weakness of the analysis presented in the document under review.

References Cited in Answer to Question G-1:

Clewell, R.A., Merrill, E.A., Gearhart, J.M., Robinson, P.J., Sterner, T.R., Mattie, D.R., and Clewell, H.J., 3rd. 2007. Perchlorate and radioiodide kinetics across life stages in the human: using PBPK

models to predict dosimetry and thyroid inhibition and sensitive subpopulations based on developmental stage. *J Toxicol Environ Health A* 70 (5):408-28.

Merrill, E.A., Clewell, R.A., Robinson, P.J., Jarabek, A.M., Gearhart, J.M., Sterner, T.R., and Fisher, J.W. 2005. PBPK model for radioactive iodide and perchlorate kinetics and perchlorate-induced inhibition of iodide uptake in humans. *Toxicol Sci* 83 (1):25-43.

NRC. 2005. Health Implications of Perchlorate Ingestion. National Research Council of the National Academies, National Academies Press. Washington, D.C. http://www.nap.edu/catalog/11202.html

G-2. Please identify any additional studies or other data sources that should be considered in the assessment of the specific parameters addressed below, including urinary clearance (of iodide and/or perchlorate) and ingestion rates (breast milk, formula and water), especially in neonates.

The scientific literature relevant to NIS inhibition by perchlorate is currently growing fast; the same holds true for related literature areas covering fields such as demographics and exposure informatics and modeling, Physiologically-Based Pharmacokinetic and Pharmacodynamic modeling methods, etc. Though the document under review is not expected to provide a thorough literature review of the subject of perchlorate inhibition of NIS and of related exposure and risk issues, it could certainly provide a more complete picture to its readers, by incorporating some of the references suggested below.

These suggestions are grouped in three categories: (a) "general references," that cover various aspects of perchlorate exposure and effect, (b) references that focus on studies of human exposure to perchlorate, and (c) references that focus on biological (physiological and biochemical) issues, either directly specific to perchlorate and NIS inhibition or indirectly related, such as e.g. references on information for urinary clearance related parameters or on information for PBPK modeling specific to infants.

It should be noted in particular that USFDA (The US Food and Drug Administration) has developed PBPK modeling recommendations, as well as computer software that implements them, for early life stages (Luecke *et al.,* 2007, 2008); at a minimum, it would be useful to examine how these parameterizations compare to the ones adopted in the analysis presented in the document under review. (Similarly, it would be useful to compare exposure-related parameter selections used in the reviewed work to corresponding relevant recommendations in USEPA's Child-Specific Exposure Factors Handbook).

General:

ATSDR. 2008. Toxicological Profile for Perclorates. Agency for Toxic Substances and Disease Registry. Atlanta, GA. http://www.atsdr.cdc.gov/toxprofiles/tp162.pdf

Charnley, G. 2008. Perchlorate: Overview of risks and regulation. *Food and Chemical Toxicology* 46 (7):2307-2315.

De Groef, B., Decallonne, B.R., Van der Geyten, S., Darras, V.M., and Bouillon, R. 2006. Perchlorate versus other environmental sodium/iodide symporter inhibitors: potential thyroid-related health effects. *Eur J Endocrinol* 155 (1):17-25.

Gu, B., and Coates, J.D. 2006. *Perchlorate: Environmental Occurrence, Interactions and Treatment*. New York: Springer.

Kirk, A.B. 2006. Environmental perchlorate: why it matters. *Anal Chim Acta* 567 (1):4-12.

Kirk, A.B., Dyke, J.V., Martin, C.F., and Dasgupta, P.K. 2007. Temporal patterns in perchlorate, thiocyanate, and iodide excretion in human milk. *Environ Health Perspect* 115 (2):182-6.

Kirk, A.B., Martinelango, P.K., Tian, K., Dutta, A., Smith, E.E., and Dasgupta, P.K. 2005. Perchlorate and iodide in dairy and breast milk. *Environ Sci Technol* 39 (7):2011-7.

Wang, R.Y., and Needham, L.L. 2007. Environmental chemicals: from the environment to food, to breast milk, to the infant. *J Toxicol Environ Health B Crit Rev* 10 (8):597-609.

Exposure:

Baier-Anderson, C., Blount, B.C., Lakind, J.S., Naiman, D.Q., Wilbur, S.B., and Tan, S. 2006. Estimates of exposures to perchlorate from consumption of human milk, dairy milk, and water, and comparison to current reference dose. *J Toxicol Environ Health A* 69 (3-4):319-30.

Blount, B.C., Valentin-Blasini, L., Osterloh, J.D., Mauldin, J.P., and Pirkle, J.L. 2007. Perchlorate exposure of the US Population, 2001-2002. *J Expo Sci Environ Epidemiol* 17 (4):400-7.

Ginsberg, G.L., Hattis, D.B., Zoeller, R.T., and Rice, D.C. 2007. Evaluation of the U.S. EPA/OSWER preliminary remediation goal for perchlorate in groundwater: focus on exposure to nursing infants. *Environ Health Perspect* 115 (3):361-9.

Zender, R., Bachand, A.M., and Reif, J.S. 2001. Exposure to tap water during pregnancy. *J Expo Anal Environ Epidemiol* 11 (3):224-30.

Physiological/Biochemical:

Brandt, J.R., Wong, C.S., Hanrahan, J.D., Qualls, C., McAfee, N., and Watkins, S.L. 2006. Estimating absolute glomerular filtration rate in children. *Pediatr Nephrol* 21 (12):1865-72.

Clewell, R.A., and Gearhart, J.M. 2002. Pharmacokinetics of toxic chemicals in breast milk: use of PBPK models to predict infant exposure. *Environ Health Perspect* 110 (6):A333-7.

Dohan, O., De la Vieja, A., Paroder, V., Riedel, C., Artani, M., Reed, M., Ginter, C.S., and Carrasco, N. 2003. The Sodium/Iodide Symporter (NIS): Characterization, Regulation, and Medical Significance. *Endocrine Reviews* 24 (1):48-77.

Hawcutt, D.B., and Smyth, R.L. 2008. One size does not fit all: getting drug doses right for children. *Archives of Disease in Childhood* 93 (3):190-191.

Ito, S., and Alcorn, J. 2003. Xenobiotic transporter expression and function in the human mammary gland. *Adv Drug Deliv Rev* 55 (5):653-65.

Johnson, T.N. 2008. The problems in scaling adult drug doses to children. *Arch Dis Child* 93 (3):207-11.

Kurz, H., Sandau, K., Dawson, T.H., Brown, J.H., Enquist, B.J., and West, G.B. 1998. Allometric ccaling in biology. *Science* 281 (5378):751a-.

Lewandowski, T.A., Seeley, M.R., and Beck, B.D. 2004. Interspecies differences in susceptibility to perturbation of thyroid homeostasis: a case study with perchlorate. *Regul Toxicol Pharmacol* 39 (3):348-62.

Luecke, R.H., Pearce, B.A., Wosilait, W.D., Slikker, W., Jr., and Young, J.F. 2007. Postnatal growth considerations for PBPK modeling. *J Toxicol Environ Health A* 70 (12):1027-37.

Luecke, R.H., Pearce, B.A., Wosilait, W.D., Doerge, D.R., Slikker, W., Jr., and Young, J.F. 2008. Windows based general PBPK/PD modeling software. *Comput Biol Med* 38 (9):962-78.

McManaman, J.L., and Neville, M.C. 2003. Mammary physiology and milk secretion. *Adv Drug Deliv Rev* 55 (5):629-41.

Packard, G.C., and Birchard, G.F. 2008. Traditional allometric analysis fails to provide a valid predictive model for mammalian metabolic rates. *J Exp Biol* 211 (Pt 22):3581-7.

Spitzweg, C., Dutton, C.M., Castro, M.R., Bergert, E.R., Goellner, J.R., Heufelder, A.E., and Morris, J.C. 2001. Expression of the sodium iodide symporter in human kidney. *Kidney Int* 59 (3):1013-23.

Strawson, J., Zhao, Q., and Dourson, M. 2004. Reference dose for perchlorate based on thyroid hormone change in pregnant women as the critical effect. *Regulatory Toxicology and Pharmacology* 39 (1):44-65.

West, G.B., Brown, J.H., and Enquist, B.J. 1997. A general model for the origin of allometric scaling laws in biology. *Science* 276 (5309):122-126.

G-3. Besides the specific parameters identified below, please comment on any other parameter or model choices described in the document that you think are incorrect or require further explanation or where other available information could provide better estimates.

As discussed in more detail in the answer to the questions regarding the characterization of urinary clearance processes, there is a need to develop and thoroughly test a consistent framework for modeling

these processes for different lifestages. "Correcting" the inconsistencies, that are in fact identified in Appendix B of the document under review, would be a first step towards the implementation of such a framework.

G-4. Please discuss research that you think would be likely to increase confidence in these models and their use in predicting RAIU inhibition by perchlorate in different life stages (for an average individual within each life-stage).

The main challenge related to this question is "defining" an "average individual within each lifestage." Equally challenging would be the identification of an "average exposure" within each lifestage. EPA should consider the merits of a probabilistic sensitivity/uncertainty analysis and eventually the feasibility of population-based PBPK modeling (with explicitly defined sensitive subpopulations). Such an approach will provide a more realistic assessment of the actual ranges of the outcomes considered in the analysis, will eventually improve risk characterization efforts, and help explain both inter-individual and intra-individual variability within a (sub) population.

G-5. Does EPA accurately characterize the strengths and limitations of the analysis? Please comment on any particular strengths that may not be mentioned or adequately characterized for the estimates of RAIU for different life stages. Please also comment on any specific sources of uncertainty that you believe have been overlooked in EPA's analysis or that require further discussion, and which might be significant to EPA's estimates of RAIU for different life stages.

In this reviewer's opinion the main strength of the analysis is the explicit listing of unresolved issues (and inconsistencies) in the modeling described in the document under review.

The main limitations involve:

- the emphasis on point estimates rather than on pursuing a distributional (probabilistic) approach for characterizing exposures (with explicit variability and uncertainty of activities for each individual and across a sub-population,
- the emphasis on "average" individuals for each lifestage rather than on pursuing population-based modeling with explicit characterization of inter-individual and intra-individual pharmacokinetic (physiological and biochemical) variabilities, and
- the consideration of iodide and perchlorate exposure "in isolation" and not in a context of "total" exposure that would consider other NIS inhibitors (thiocyanate, nitrates).

G-6. As recommended in EPA's 2000 Risk Characterization Handbook, is the analysis transparent in terms of the steps, logic, key assumptions, limitations, and decisions? Specifically, does characterization of the results of EPA's work fully explain: a) the analysis approach employed; b) the use of assumptions and their impact on the analysis; c) the use of extrapolations and their impact on the analysis; d) plausible alternatives and the choices made among those alternatives; e) the impacts of one choice vs. another on the analysis; f) significant data gaps and their implications for the analysis; g) the scientific conclusions identified separately from default assumptions and policy decisions, if any; and h) the major conclusions, and the discussions of EPA's confidence and uncertainties in the conclusions?

The document and - in particular the appendices – are quite explicit ("transparent") in listing and discussing all the assumptions and approximations involved in the analysis. This takes place at a level of detail that exceeds what is typically expected in the peer reviewed literature and the authors of the document under review should be commended for this. However, the justifications of what can be called "emergency solutions" to various problems discussed in the document, especially those related to inconsistencies in modeling urinary clearance processes (including inconsistencies in scaling factors as well as "ad hoc" adjustments to achieve agreement for predicted values) are often weak. It can, probably reasonably, be argued that, for the ranges of concentrations and exposures considered, the effect of correcting the above inconsistencies will not have a substantive impact on calculated outcomes; however, it is still very important to develop a "fully defensible" model that incorporates up-to-date scientific information and assumptions that are consistent "across lifestages". Clearly, resolving the inconsistencies that have been identified through the efforts presented in the document under review will be useful in numerous other applications involving exposures to chemicals *in utero* and during infancy.

Parameter-Specific Charge Questions:

(A) Urinary clearance

A-1. Please comment on the appropriateness of the input values selected for maternal urinary clearance during pregnancy and lactation in EPA's analysis. Are the available data and rationale for the values selected transparently and objectively described? Are you aware of other publications or data that could be used to guide these choices or which provide alternative input values that are equally valid or more appropriate? Likewise, are the values selected for urinary clearance for the infant and older child the best estimates, given the available science and data? Are there other data that would provide better (or equally valid alternative) guidance or estimates? Is there any reason to believe that urinary clearance might not be a limiting factor in the elimination of perchlorate for infants?

The analysis presented in the document under review concluded that urinary clearance was a "key" process/parameter; i.e. model predictions were very sensitive with respect to the magnitude of urinary clearance. However, there are a number of issues concerning the parameters employed in modeling urinary clearance that need substantial clarification. Appendix B (pp 39-50) of the document under review

provides an extensive discussion of the assumptions and approximations involved in selecting and estimating these parameters. Various inconsistencies in the selection/estimation procedures are in fact recognized explicitly in Appendix B, but in general these inconsistencies are "accepted" on the basis of either a minimal anticipated effect on the calculations of the model, or as a means for avoiding a more complex analysis. For example, the last paragraphs of p. 40 states that because "... renal clearance is largely controlled by glomerular filtration and non-specific fluid resorption, the expectation is that the relative clearance for iodide and perchlorate [....] should be constant across ages, body weights, and lifestages. In EPA's evaluation for the child and "average" (non-pregnant, non-lactating) adult, this proportionality has been maintained." However, in the model of Clewell et al. (2007) "the maternal urinary clearance value [....] was set at 60% of the value in the non-pregnant human based on observed difference in the pregnant and male rat models [....].These maternal lactation values go against the argument given just above that the proportionality should be maintained, but EPA chose to use the maternal values as so set. It is likely worthwhile to evaluate these maternal values in light of the generally higher urinary excretion seen in pregnant/lactating women, but alteration of these clearance constants would require refitting of other parameters, and so EPA chose not to conduct that specific evaluation." Clearly this is an issue that requires further consideration. It should also be mentioned that this discussion is preceded by the following rather puzzling statement that "The tables in the papers identify the units of [urinary clearance] as L/h/kg, but clearly this should be $L/h/kg^{0.75}$ to be consistent with this mathematical formulation, which is how the CLU values are calculated in the computer code." Such a selection units/dimensions contradict the physics of the problems and in fact it appears that the tables in the original articles (Clewell et al., 2007 and Merrill et al., 2005) state the correct units. (The first paragraph on page 41 of the document under review also employs correct units/dimensions.) The issue of consistent allometric scaling is an important one and there exist various publications that can be helpful in clarifying issues such as the above (e.g. Johnson, 2008; West et al., 1997; Kurz et al., 1998). Issues of inconsistent scaling in fact appear across the entire description of urinary clearance parameters (Appendix B).

Various other inconsistencies are discussed and "accepted" in relation to the calculations of urinary clearance in the neonate (pages 42-43) and in the pregnant/lactating woman. For example, on page 47, it is stated:

> "Keeping with the assumed proportionality between perchlorate and iodide, based on these data the same relationship would be expected to hold: higher clearance rather than reduced. A dilemma occurs in considering the data of Aboul-Khair et al. (1964); however, in that the control iodine clearance as measured by them is 31.05 ± 3.66 mL/min (mean ± SE), while the value determined by Merrill et al. (2005) for non-pregnant adults is 44.3 mL/min. Likewise Aboul-

> Khair et al. (1964) report thyroid iodide uptake at 2.5 hr postinjection as 21.4 ± 1.4 % of the administered dose, but the amount predicted by the Merrill et al. (Merrill et al., 2005) model (in the absence of perchlorate) is 7.78%. Therefore, the data of Aboul-Khair et al. (1964) was normalized to their own controls for both urinary clearance and iodide uptake, and then use that relative change as a model input (for clearance, multiplying the non-pregnant clearance rate constant by the pregnant:control ratio from Aboul-Khair et al. (1964) or in estimating changes in thyroid NIS (to fit relative increases in thyroid uptake)."

Clearly, the inconsistency in absolute values reported in the above paragraph should be the focus of further study; while the normalization employed by EPA offers a way of circumventing the issue, this "solution" could only be considered qualitative in nature.

In this reviewer's opinion, a consistent treatment of the urinary clearance process for various life stages emerges clearly as a research need, based on the outcomes of the sensitivity testing and the issues presented in Appendix B of the document under review.

References Cited in Answer to Question A-1:

Clewell, R.A., Merrill, E.A., Gearhart, J.M., Robinson, P.J., Sterner, T.R., Mattie, D.R., and Clewell, H.J., 3rd. 2007. Perchlorate and radioiodide kinetics across life stages in the human: using PBPK models to predict dosimetry and thyroid inhibition and sensitive subpopulations based on developmental stage. *J Toxicol Environ Health A* 70 (5):408-28.

Johnson, T.N. 2008. The problems in scaling adult drug doses to children. *Arch Dis Child* 93 (3):207-11.

Kurz, H., Sandau, K., Dawson, T.H., Brown, J.H., Enquist, B.J., and West, G.B. 1998. Allometric ccaling in biology. *Science* 281 (5378):751.

Merrill, E.A., Clewell, R.A., Robinson, P.J., Jarabek, A.M., Gearhart, J.M., Sterner, T.R., and Fisher, J.W. 2005. PBPK model for radioactive iodide and perchlorate kinetics and perchlorate-induced inhibition of iodide uptake in humans. *Toxicol Sci* 83 (1):25-43.

West, G.B., Brown, J.H., and Enquist, B.J. 1997. A general model for the origin of allometric scaling laws in biology. *Science* 276 (5309):122-126.

(B) Breast-milk ingestion

B-1. Infants are generally known to consume very little milk or formula on the first day of life, with ingestion quickly increasing over time. However the first time-point for which ingestion data are available for infants is at 7 days, which EPA extrapolated back to 0 ingestion at time=0 in its analysis (birth; Figure 1). The breast-milk ingestion rate was estimated based on mean values reported in Arcus-Arth et al. (2005), while the water ingestion used for the breast-feeding mother was the 90th percentile value from U.S. EPA (2004).

Is EPA's extrapolation and rationale transparently and objectively described? Does this function appropriately characterize the available data and information? Are there other data that could be used to obtain a better or equally valid alternative estimate of mean breast-milk ingestion rate for infants in the first few days of life? Should an estimate other than the mean be used to determine the breast-milk ingestion rate?

EPA's selection of point values for the testing analysis appears appropriate and adequately justified as a reasonable conservative assumption. However, in this reviewer's opinion, the uncertainties and variability inherent in the problem at hand would be better addressed by a distributional (probabilistic) rather than point calculation. The large population above the 90th percentile and the potential "spread" of exposure factors above that percentile, would further justify such an analysis.

(C) Water ingestion

C-1. For pregnancy, EPA used a normalized (90th percentile) water ingestion rate of 33 mL/kg-day (U.S.EPA 2004), which was multiplied by the maternal BW as described by the PBPK model growth-functions during pregnancy to obtain total water ingestion for the mother.

Is EPA's approach and rationale transparently and objectively described? Is this approach appropriate for characterizing the upper-bound for ingestion of pregnant women? Are there other approaches or data that could be used to obtain a better or equally valid alternative estimate for this parameter? (Note that the self-reported body weight values in U.S. EPA (2004) indicate the same average body weight for pregnant women as for non-pregnant; data appears inaccurate, so the PBPK model body-weight description was used instead.)

The approach taken by EPA appears reasonable. As in the answer to the previous question, this reviewer's opinion is that a distributed zonal analysis (Monte Carlo) can provide more substantial insight on patterns of potential exposure rather than the point calculations presented here.

C-2. For lactation, EPA used a fixed total (90th percentile) water ingestion rate of 2959 mL/day (U.S. EPA 2004), with the rationale that while the woman's weight and self-water demand are expected to drop after pregnancy (as described by the PBPK model time-dependent weight equations), the demand for milk production would be increasing, and the reported value was not for a specific age-range of child.

Is EPA's approach and rationale transparently and objectively described? Is this an appropriate value to use for the ingestion rate of lactating women? Are there other better or equally valid alternative approaches or values that could be used?

The approach taken by EPA appears reasonable, though a probabilistic (Monte Carlo) analysis would provide additional insight regarding the range of potential exposures. Furthermore, since the simulations for lactating women produce estimates of perchlorate concentration in breast milk, a population/distribution-level analysis with appropriate parameterizations could be used to provide valuable testing of the model in relation to the available data presented in Pearce *et al.* (2007) as well as Kirk *et al.* (2005, 2007)

References Cited in Answer to Question B-2:

Kirk, A.B., Martinelango, P.K., Tian, K., Dutta, A., Smith, E.E., and Dasgupta, P.K. 2005. Perchlorate and iodide in dairy and breast milk. *Environ Sci Technol* 39 (7):2011-7.

Kirk, A.B., Dyke, J.V., Martin, C.F., and Dasgupta, P.K. 2007. Temporal patterns in perchlorate, thiocyanate, and iodide excretion in human milk. *Environ Health Perspect* 115 (2):182-6.

Pearce, E.N., Leung, A.M., Blount, B.C., Bazrafshan, H.R., He, X., Pino, S., Valentin-Blasini, L., and Braverman, L.E. 2007. Breast milk iodine and perchlorate concentrations in lactating Boston-area women. *J Clin Endocrinol Metab* 92 (5):1673-7.

C-3. For bottle-fed infants, EPA made extrapolations of the 90th percentile water-ingestion in early life based on measurements made for the age ranges: 0-30 days, 1-3 months, and 6-12 months (points in Figure 2, upper panel). For the purposes of this analysis, bottle-fed implies feeding with formula requiring the addition of water.

Is EPA's extrapolation and rationale transparently and objectively described? Does the overall function used to represent the changes in ingestion with age (and body weight) appropriately characterize the available data and information? Are there other data that could be used to obtain a better or equally valid alternative estimate of water ingestion rate for infants in the first few days of life (e.g., 7-day old)? The water ingestion rates used by EPA are based on 90th percentile ingestion data and thus are likely to exceed (minimal) physiological needs of infants as defined in nutritional guidelines. Are the water ingestion rates used by EPA reasonable in comparison to the physiological needs of infants at these various life stages? Are there other approaches or data (e.g., see the FDA memo) that could be used to obtain a better or equally valid alternative estimate for this parameter?

EPA's extrapolation and rationale are adequately described. However, it is doubtful that the use of any single-point estimate would provide adequate understanding of the potential range of exposures, and corresponding doses, for bottle-fed infants.

(D) Perchlorate concentration in formula

D-1. PA used 1.42 µg/L as the concentration of perchlorate in formula for bottle-fed infants. This estimate was based on information from FDA's Total Diet Study, supported by Pearce et al.'s (2007) findings.

Is EPA's approach and rationale transparently and objectively described? Is 1.42 µg/L an appropriate value to use for the concentration of perchlorate in infant formula? Are there other better or equally valid alternative approaches or values that could be used?

The rationale for the selection of 1.42 µg/L as a preset value for the concentration of perchlorate for bottle-fed infants is not adequately discussed. This value is the average of 12 samples (8 of them above detection limit) presented in Murray *et al.* (2008); it is also close to the average value (1.45 ppb) of the 17 samples analyzed by Pearce *et al.* (2007). It should be noted that the values of perchlorate concentrations in the samples of Pearce et al. range from 0.2 to 4.1 ppb. It would be useful to examine the sensitivity of uptake for a reasonable concentration range rather than only the average value.

References Cited in Answer to Question D-1:

Murray, C.W., Egan, S.K., Kim, H., Beru, N., and Bolger, P.M. 2008. US Food and Drug Administration's Total Diet Study: Dietary intake of perchlorate and iodine. *J Expos Sci Environ Epidemiol* 18 (6):571-580.

Pearce, E.N., Leung, A.M., Blount, B.C., Bazrafshan, H.R., He, X., Pino, S., Valentin-Blasini, L., and Braverman, L.E. 2007. Breast milk iodine and perchlorate concentrations in lactating Boston-area women. *J Clin Endocrinol Metab* 92 (5):1673-7.

(E) Radioiodide excretion into breast-milk by NIS

E-1. In the model, EPA included perchlorate inhibition of NIS radioiodide excretion into breast-milk, as well as inhibition of radioiodide transport by perchlorate for all NIS-containing tissues, thereby making the code consistent with the model description in Clewell et al. (2007). Is this inclusion appropriate? Are the impacts of this inclusion transparently and objectively described?

The inclusion of perchlorate inhibition of NIS radioiodide excretion into breast-milk, as well as of inhibition of radioiodide transport by perchlorate for all NIS-containing tissues, is appropriate. The impacts of this inclusion are adequately ("transparently and objectively") described in the document.

PEER REVIEW COMMENTS FROM

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General Charge Questions:

G-1. Is EPA's analysis logical, clear and appropriate in depth and length? Has EPA accurately, clearly and objectively represented and synthesized the scientific evidence for the changes made to or specification of the model code and input parameters?

YES.

G-2. Please identify any additional studies or other data sources that should be considered in the assessment of the specific parameters addressed below, including urinary clearance (of iodide and/or perchlorate) and ingestion rates (breast milk, formula and water), especially in neonates.

NA

G-3. Besides the specific parameters identified below, please comment on any other parameter or model choices described in the document that you think are incorrect or require further explanation or where other available information could provide better estimates.

NA

G-4. Please discuss research that you think would be likely to increase confidence in these models and their use in predicting RAIU inhibition by perchlorate in different life stages (for an average individual within each life-stage).

The results of this modeling effort should be classified as theoretical since no validation exercises have been performed and EPA should be clear to state this. There are existing data which could help to validate the model predictions, especially related to the most sensitive scenario (e.g., the nursing infant of the exposed mother). In particular, Pearce et al. (2007) provides matched data on perchlorate and iodine in breast milk and urine samples from nursing mothers. EPA should obtain this data from the study authors. This will greatly help to test the model predictions. Furthermore, the authors found no correlation (either positive or negative) between perchlorate and iodine in breast milk samples. The authors of this study indicate this is consistent with other researchers. This may raise questions about the results of EPA's modeling efforts. Since no results for the concentrations of perchlorate and iodine in milk as a function of perchlorate dose are provided in EPA's report, it is impossible to determine the validity of this issue.

G-5. Does EPA accurately characterize the strengths and limitations of the analysis? Please comment on any particular strengths that may not be mentioned or adequately characterized for the estimates of RAIU for different life stages. Please also comment on any specific sources of uncertainty that you believe have been overlooked in EPA's analysis or that require further discussion, and which might be significant to EPA's estimates of RAIU for different life stages.

In places, EPA adequately highlights the strengths and weaknesses of their analysis. However, there are other areas where the limitations have not been adequately addressed (e.g., lack of validation).

G-6. As recommended in EPA's 2000 Risk Characterization Handbook, is the analysis transparent in terms of the steps, logic, key assumptions, limitations, and decisions? Specifically, does characterization of the results of EPA's work fully explain: a) the analysis approach employed; b) the use of assumptions and their impact on the analysis; c) the use of extrapolations and their impact on the analysis; d) plausible alternatives and the choices made among those alternatives; e) the impacts of one choice *vs*. another on the analysis; f) significant data gaps and their implications for the analysis; g) the scientific conclusions identified separately from default assumptions and policy decisions, if any; and h) the major conclusions, and the discussions of EPA's confidence and uncertainties in the conclusions?

Yes, the analysis is transparent.

Parameter-Specific Charge Questions:

(A) Urinary clearance

A-1. Please comment on the appropriateness of the input values selected for maternal urinary clearance during pregnancy and lactation in EPA's analysis. Are the available data and rationale for the values selected transparently and objectively described? Are you aware of other publications or data that could be used to guide these choices or which provide alternative input values that are equally valid or more appropriate? Likewise, are the values selected for urinary clearance for the infant and older child the best estimates, given the available science and data? Are there other data that would provide better (or equally valid alternative) guidance or estimates? Is there any reason to believe that urinary clearance might not be a limiting factor in the elimination of perchlorate for infants?

The available data and rationale are transparently described. However, I disagree with the rationale for the choice of maternal urinary clearance values. This is probably the most sensitive parameter for the most sensitive scenario/receptor and the EPA has chosen to use rodent data over human data. This is inadequate. EPA should choose to use the available human data which indicates there is no measurable or consistent difference in urinary clearance during pregnancy as compared to the non-pregnant state. The EPA chose a

reasonable urinary clearance for the lactating mother scenario. I agree with EPA's choice for the urinary clearance among infants and older children.

(B) Breast-milk ingestion

B-1. Infants are generally known to consume very little milk or formula on the first day of life, with ingestion quickly increasing over time. However the first time-point for which ingestion data are available for infants is at 7 days, which EPA extrapolated back to 0 ingestion at time=0 in its analysis (birth; Figure 1). The breast-milk ingestion rate was estimated based on mean values reported in Arcus-Arth et al. (2005), while the water ingestion used for the breast-feeding mother was the 90th percentile value from U.S. EPA (2004).

Is EPA's extrapolation and rationale transparently and objectively described? Does this function appropriately characterize the available data and information? Are there other data that could be used to obtain a better or equally valid alternative estimate of mean breast-milk ingestion rate for infants in the first few days of life? Should an estimate other than the mean be used to determine the breast-milk ingestion rate?

The approach and methods are objectively described. However, this is one of the weakest portions of EPA's analysis. The modeling of perchlorate and iodine kinetics in the neonate is highly uncertain. EPA needs to recognize this and make this clear to the reader. If the purpose of this analysis is to determine the relative difference in inhibition of iodide uptake by the thyroid for the various scenarios (e.g., normal adult, child, nursing infant, fetus, and bottle-fed infant), then the mean on all exposure and pharmacokinetic parameters should be used and used consistently throughout the analysis. Otherwise, the current approach exhibits bias for one scenario over the other.

(C) Water ingestion

C-1. For pregnancy, EPA used a normalized (90^{th} percentile) water ingestion rate of 33 mL/kg-day (U.S.EPA 2004), which was multiplied by the maternal BW as described by the PBPK model growth-functions during pregnancy to obtain total water ingestion for the mother.

Is EPA's approach and rationale transparently and objectively described? Is this approach appropriate for characterizing the upper-bound for ingestion of pregnant women? Are there other approaches or data that could be used to obtain a better or equally valid alternative estimate for this parameter? (Note that the self-reported body weight values in U.S. EPA (2004) indicate the same average body weight for pregnant women as for non-pregnant; data appears inaccurate, so the PBPK model body-weight description was used instead.)

Throughout this analysis, EPA was inconsistent in choosing upper bounds or means for various parameters. As such, there is no clear understanding of the objectives of EPA's

analysis. If the purpose of this analysis is to determine the relative difference in inhibition of iodide uptake by the thyroid for the various scenarios (e.g., normal adult, child, nursing infant, fetus, and bottle-fed infant), then the mean on all exposure and pharmacokinetic parameters should be used and used consistently throughout the analysis. Otherwise, the current approach exhibits bias for one scenario over the other.

C-2. For lactation, EPA used a *fixed* total (90^{th} percentile) water ingestion rate of 2959 mL/day (U.S. EPA 2004), with the rationale that while the woman's weight and self-water demand are expected to drop after pregnancy (as described by the PBPK model time-dependent weight equations), the demand for milk production would be increasing, and the reported value was not for a specific age-range of child.

Is EPA's approach and rationale transparently and objectively described? Is this an appropriate value to use for the ingestion rate of lactating women? Are there other better or equally valid alternative approaches or values that could be used?

Throughout this analysis, EPA was inconsistent in choosing upper bounds or means for various parameters. As such, there is no clear understanding of the objectives of EPA's analysis. If the purpose of this analysis is to determine the relative difference in inhibition of iodide uptake by the thyroid for the various scenarios (e.g., normal adult, child, nursing infant, fetus, and bottle-fed infant), then the mean on all exposure and pharmacokinetic parameters should be used and used consistently throughout the analysis. Otherwise, the current approach exhibits bias for one scenario over the other.

C-3. For bottle-fed infants, EPA made extrapolations of the 90^{th} percentile water-ingestion in early life based on measurements made for the age ranges: 0-30 days, 1-3 months, and 6-12 months (points in Figure 2, upper panel). For the purposes of this analysis, bottle-fed implies feeding with formula requiring the addition of water.

Is EPA's extrapolation and rationale transparently and objectively described? Does the overall function used to represent the changes in ingestion with age (and body weight) appropriately characterize the available data and information? Are there other data that could be used to obtain a better or equally valid alternative estimate of water ingestion rate for infants in the first few days of life (e.g., 7-day old)? The water ingestion rates used by EPA are based on 90th percentile ingestion data and thus are likely to exceed (minimal) physiological needs of infants as defined in nutritional guidelines. Are the water ingestion rates used by EPA reasonable in comparison to the physiological needs of infants at these various life stages? Are there other approaches or data **(e.g., see the FDA memo**) that could be used to obtain a better or equally valid alternative estimate for this parameter?

Same response as last question.

(D) Perchlorate concentration in formula

D-1. EPA used 1.42 µg/L as the concentration of perchlorate in formula for bottle-fed infants. This estimate was based on information from FDA's Total Diet Study, supported by Pearce *et al*.'s (2007) findings.

Is EPA's approach and rationale transparently and objectively described? Is 1.42 µg/L an appropriate value to use for the concentration of perchlorate in infant formula? Are there other better or equally valid alternative approaches or values that could be used?

Based on the stated objectives of this analysis, EPA should adjust the intake of perchlorate from infant formula to result in a daily exposure consistent with the point of departure. This will yield consistent results across all scenarios to assure a fair and impartial comparison of relative differences in inhibition of thyroid iodine uptake can be made.

(E) Radioiodide excretion into breast-milk by NIS

E-1. In the model, EPA included perchlorate inhibition of NIS radioiodide excretion into breast-milk, as well as inhibition of radioiodide transport by perchlorate for all NIS-containing tissues, thereby making the code consistent with the model description in Clewell et al. (2007). Is this inclusion appropriate? Are the impacts of this inclusion transparently and objectively described?

Based on the data from Pearce et al. (2007), one would expect there to be no effect of perchlorate on the excretion of iodine in breast milk. As such, this feature of the model that EPA has included may not be accurate with perchlorate kinetics. EPA should investigate the data of Pearce et al. more fully and explore other data sets to see what evidence is available to include such a feature in the model.

Additional comments:

The approach of using PBPK modeling is admirable and the EPA should be commended. However, the EPA should also have considered easier and more straightforward approaches. The one-compartment PK model developed by EPA (Lorber, 2008), paired with measured perhclorate and iodine levels in breast milk and infant formula would have provided simpler and equally valid approaches for answering the question of the relative difference in steady-state perchlorate levels (this is ultimately the endpoint of interest) in the various receptors/scenarios. While I agree with using PBPK modeling, I also think EPA should think about simpler approaches that are equally or more valid, and sometimes much simpler and more easily embraced by the regulatory and risk assessment community.

Appendix A: I agree with the model modifications made by EPA.

Appendix B: Appendix B is well written and easier to follow than the corresponding text in the main report relating to urinary clearance values (section 3.1).

PEER REVIEW COMMENTS FROM

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General Charge Questions:

G-1. Physiologically based pharmacokinetic (PBPK) models were modified to predict inhibition of the sodium-iodide symporter (NIS)47 for pregnant and lactating women, nursing infants, and for the subsequent stages of childhood. The published models were modified by EPA to fix errors and incorporate new data, particularly data on lifestage variability in the urinary clearance of pechlorate, to which NIS inhibition is sensitive. The models are suitable to provide quantitative predictions to the Agency on the lifestyle variability of perchlorate NIS inhibition of thyroidal iodide uptake. EPA's analysis is logical, clear and appropriate in depth and length and EPA has accurately, clearly and objectively represented and synthesized the scientific evidence for the changes made to or specification of the model code and input parameters.

G-2. Additional studies that should be considered in the assessment of the specific parameters such as urinary clearance of iodide and/or perchlorate and ingestion rates (breast milk, formula and water) in neonates and these include data on maturation of tubular transport rates. On page 39 of the report, the issue of the role of pendrin transporter for iodide during development is addressed. One cannot assume that perchlorate and iodide are handled similarly by the developing kidney based on their similar charge and diameter; more data is needed in the investigation of tubular maturation of the transporters that regulate the clearance of iodide and perchlorate. This is also addressed on page 40, second paragraph in the report where it is stated that one cannot assume that the relative clearance for iodide and perchlorate should be constant across all ages and life stages. Additionally on page 42 the EPA states that there is no data on renal transporters during infancy to suggest the level and pattern of expression changes required to change clearance/GFR. Thus, the report used DeWoskin and Thompson's published data for scaling of renal excretion for infants by body weight and on page 44 the EPA extended its extrapolation to a 60-day-old, 5 kg child is sound. These assumptions are reasonable but indicate the importance of additional investigations in newborn models and in humans.

G-3. There are no other parameters or model choices described in the document that are incorrect or require further explanation or provide better estimates.

G-4. Newer estimates of renal function have been provided by Schwartz which should be evaluated

G-5. EPA accurately characterized the strengths and limitations of the analysis. However, as indicated in the report, additional information on the possible effects of maturation of glomerular filtration, tubular

reabsorption and secretion, and changes in body composition during the neonatal period is indicated in order to more confidently apply EPA's estimates of RAIU for different life stages.

G-6. The analysis is transparent in terms of the steps, logic, key assumptions, limitations, and decision. The characterization of the results of EPA's work fully explains: a) the analysis approach employed; b) the use of assumptions and their impact on the analysis; c) the use of extrapolations and their impact on the analysis; d) plausible alternatives and the choices made among those alternatives; e) the impacts of one choice vs. another on the analysis; f) significant data gaps and their implications for the analysis; g) the scientific conclusions identified separately from default assumptions and policy decisions, and h) the major conclusions and the discussions of EPA's confidence and uncertainties in the conclusions.

Parameter-Specific Charge Questions:

(A) Urinary clearance

A-1. The input values selected for maternal urinary clearance during pregnancy and lactation used in EPA's analysis are appropriate, transparent and objectively described. The choice of three alternatives for pregnancy is a rational compromise in lieu of the lack of additional human data. The use of a lower clearance is a safe assumption. On page 10 urinary clearance values for perchlorate and iodide across all lifestages were determined to be sensitive parameters for prediction of NIS thyroidal iodide uptake inhibition by perchlorate. EPA determined that urinary clearance of perchlorate and iodide in neonates is slower than is indicated by scaling based on body weight. Urinary elimination of a number of compounds including drugs and drug metabolites also indicate that renal clearance is slower per unit of body weight in neonates. Modification of the PBPK models to describe slower clearance of perchlorate and iodide in neonates resulted in an increase in predicted levels of NIS inhibition in infants.

The values selected for urinary clearance for infants and older children are the best estimates for the available data. The interpretation of the data that suggested an increase in predicted levels of NIS inhibition in infants at a perchlorate dose-rate of 7 ug/kg-day is a safe assumption. The indices of renal function are based on the literature which indicated that the GFR increases steadily postnatally but does not reach adult values until approximately 2 years of age. I know of no other data that would provide better guidance or estimates and it is unlikely that there are other factors than the urinary clearance in the elimination of perchlorate for infants. However, one should consider that tubular function in this age group is not fully matured and possible developmental changes in transport activity might be important but no data is available for perchlorate elimination during development. On page11 EPA chose to

estimate perchlorate induced inhibition using scaling of urinary cleareance proportional to body weight for children at 1 year of age and older which results in somewhat higher estimates of iodide uptake inhibition than reported by Clewell although still slightly less than predicted for the average adult exposed at the same dose. EPA's estimates of urinary clearance in infants and children are lower than those used in Clewell but reflects published GFR values.

(B) Breast-milk ingestion

B-1. EPA's extrapolation and rationale are transparent and objectively described to assess the breast milk ingestion rate.

(C) Water ingestion

C-1 EPA's approach and rationale for pregnancy water ingestion rate is transparent and objectively described, and is based on the available literature. I know of no additional data that could be used to obtain a better estimate of mean breast-milk ingestion rate for infants in the first few days of life. The mean estimate is fine.

C-2 EPA's approach and rationale for lactation water ingestion rate is appropriate and transparent and objectively described. I know of no other approaches.

C-3 EPA's extrapolation and rationale for bottle fed infant's water ingestion in early life is transparent and objectively described. The overall function used to represent the changes in ingestion with age (and body weight) appropriately characterize the available data. The water ingestion rate for infants used by the EPA are reasonable in comparison to the physiological needs of infants. The estimated 90% water intake rate used by EPA in PBPK model stimulations is appropriate.

(D) Perchlorate concentrations in formula

D-1. EPA's approach and rationale for the concentration of perchlorate in formula for bottle fed infants is appropriated and transparency.

(E) Radioiodide excretion in breast milk by NIS

(E-1) The inclusion of perchlorate inhibition of NIS radioiodide excretion into breast-milk, as well as inhibition of radioiodide transport by perchlorate for all NIS-containing tissues, is appropriate, transparent and objectively described. The EPA added inhibition of radioiodide transport by perchlorate for

radioiodide excretion into breast milk by NIS markedly increased the predicted percent inhibition of thyroidal radioiodide uptake in the breast fed infant.

PEER REVIEW COMMENTS FROM

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Dr. Kannan Krishnan's Review of the Document entitled
"INHIBITION OF THE SODIUM-IODIDE SYMPORTER BY PERCHLORATE: AN EVALUATION OF LIFESTAGE SENSITIVITY USING PHYSIOLOGICALLY-BASED PHARMACOKINETIC (PBPK) MODELING"

Overview:

This EPA report summarizes work conducted to evaluate the PBPK models for perchlorate and radioiodide for quantitating relative sensitivity of different subgroups (lifestages). The two-stage model evaluation process involved verification of model codes and examination of the parameterization approaches. Following the revision of the PBPK models by EPA, they were checked by a contractor who also verified the output of the model by reproducing various figures from original publications. Despite the thoroughness of the work, this life-stage variability analysis (either due to lack of data or due to uncertainty associated with available data) did not account for certain subgroups (e.g., elderly, foetus during early gestation periods, iodine-deficient or hypothyroid status during pregnancy) and did not account for variability of parameter values within subgroups in the simulations (i.e., with the use of Bayesian or Monte Carlo type methods).

General Charge Questions:

G-1. Is EPA's analysis logical, clear and appropriate in depth and length? Has EPA accurately, clearly and objectively represented and synthesized the scientific evidence for the changes made to or specification of the model code and input parameters?

RE:

- The EPA analysis of perchlorate-mediated inhibition of the NIS in humans is based on Merrill et al. (2005) and Clewell et al. (2007) PBPK models, and specifically addresses the variability of NIS inhibition as a function of lifestage. The document is clear and concise. The depth and legnth of presentation are appropriate, given the objective.

- The structure of the PBPK models published by Merrill/Clewell has not been altered; rather some of the input parameters as well as equations have been modified either to correct an error or to reflect current state of knowledge more appropriately.

G-2. Please identify any additional studies or other data sources that should be considered in the assessment of the specific parameters addressed below, including urinary clearance (of iodide and/or perchlorate) and ingestion rates (breast milk, formula and water), especially in neonates.

RE:

This reviewer is not aware of any studies in neonates that would provide better estimates of urinary clearance of perchlorate and iodide. Even though isolated studies reporting ingestion rates (breast milk, formula and water) in infants in other parts of the world could be obtained from the literature, such studies probably would only introduce further uncertainty. However, the study of Kirk et al. (2005). Perchlorate and iodine in dairy and breast milk. Environ Sci technol 39: 2011-17 may used to corroborate the findings of the present study – as it relates to the relationships between drinking water concentration and breast milk concentration.

G-3. Besides the specific parameters identified below, please comment on any other parameter or model choices described in the document that you think are incorrect or require further explanation or where other available information could provide better estimates.

RE:

The parameters of this model consist of:

- Physiological parameters
- Intake/contact rates
- Partition coefficients
- Permeability-area cross product
- Urinary clearance
- Binding parameters
- Maximal velocity and affinity constants

The parameter values found in the original reports and refined following EPA's evaluation would appear to be supported by available literature. However, focused data collection might facilitate the improvement of the partition coefficient values used in the model as well as the urinary clearance values for perchlorate and iodide in the various lifestages.

G-4. Please discuss research that you think would be likely to increase confidence in these models and their use in predicting RAIU inhibition by perchlorate in different life stages (for an average individual within each life-stage).

RE:

- In the iodide/perchlorate models, the chemical concentration entering the tissues corresponds to the arterial PLASMA concentration whereas the flow rate to tissues corresponds to BLOOD (RBC + Plasma) FLOW rates. Either the influx in all mass balance equations should correspond to whole blood concentration or the flow rate should correspond to plasma flows – since the RBC:plasma partition coefficient (PRBC_p) is not always equal to 1 (see for example lines 112 on page 38, or line 115 on page 4 of the EPA model code file), and the chemical movement between plasma and RBC is diffusion-limited and no flow-limited. The consequence of this modeling assumption may be verified to ensure confidence in the use of these models. For example, if the simulations indicate that the concentration profile of perchlorate is identical in RBC and plasma compartments, qualitatively and quantitatively, then the above observation has no consequence.

- Further, consideration should be given to the possibility of being able to simulate iodine-deficient (or hypothyroid) situation in pregnant women by modulating specific parameters of the model.

- Both the response to the previous question and the comments under general overview are all applicable here.

G-5. Does EPA accurately characterize the strengths and limitations of the analysis? Please comment on any particular strengths that may not be mentioned or adequately characterized for the estimates of RAIU for different life stages. Please also comment on any specific sources of uncertainty that you believe have been overlooked in EPA's analysis or that require further discussion, and which might be significant to EPA's estimates of RAIU for different life stages.

RE:

- The strength relates to the use of PBPK model to assess the lifestage sensitivity to inhibition of the sodium-iodide symporter by perchlorate; the use of fetus as a subgroup to evaluate the relative sensitivity to adults; consideration of relevant route/source of exposure (drinking water);

- The weaknesses are related to the fact that the analysis did not include certain subgroups (e.g., elderly, foetus during early gestation periods, iodine-deficient or hypothyroid status during pregnancy) and did not address variability of parameter values within subgroups in the

simulations (i.e., with the use of Bayesian or Monte Carlo type methods).

G-6. As recommended in EPA's 2000 Risk Characterization Handbook, is the analysis transparent in terms of the steps, logic, key assumptions, limitations, and decisions? Specifically, does characterization of the results of EPA's work fully explain: a) the analysis approach employed; b) the use of assumptions and their impact on the analysis; c) the use of extrapolations and their impact on the analysis; d) plausible alternatives and the choices made among those alternatives; e) the impacts of one choice *vs*. another on the analysis; f) significant data gaps and their implications for the analysis; g) the scientific conclusions identified separately from default assumptions and policy decisions, if any; and h) the major conclusions, and the discussions of EPA's confidence and uncertainties in the conclusions?

The analysis is transparent and the assumptions as well as alternative approaches are generally described in sufficient detail. The conclusions are essentially scientific in nature, based on data obtained from PBPK model simulations. The following improvements are suggested:

1. The reason for limiting the present analysis to eight sub-groups (i.e., pregnant woman, fetus, lactating woman, breast-fed infant, bottle-fed infant, 1 year old and 2 year old child, "average" adult, and non-pregnant woman of child-bearing age) may be specified at the outset. In this regard, it may be useful to clarify as to why the elderly and teens were not part of the sub-groups analysed in this study.

2. Clarify as to why the results of this analysis are also applicable to chronic exposure exposure situations (compared to typically acute (short-term) simulations)

3. The justification of the choice of 24-hr RAIU as the endpoint should be included. Was 24-hr AUC considered as an alternative measure ? What was the scientific basis for basing the analysis on a single RAIU value in infants and adults obtained at one specific time point (i.e,. 24 hr). Some consideration/discussion of the sensitivity of that time point to the key input parameters as a function of age might be useful.

Parameter-Specific Charge Questions:

(A) Urinary clearance

A-1. Please comment on the appropriateness of the input values selected for maternal urinary clearance during pregnancy and lactation in EPA's analysis. Are the available data and rationale for the values selected transparently and objectively described? Are you aware of other publications or data that could be used to guide these choices or which provide alternative input values that are equally valid or more appropriate? Likewise, are the values selected for urinary clearance for the infant and older child the best estimates, given the available science and data? Are there other data that would provide better (or equally valid alternative) guidance or estimates? Is there any reason to believe that urinary clearance might not be a limiting factor in the elimination of perchlorate for infants?

RE:

The inadequacy of use of urinary clearance values in various human lifestages based on

(i) the pregnant:nonpregnant values in rats, and
(ii) the scaling of renal function for neonates on the basis of $BW^{0.75}$

– are well justified by EPA. The outcome is consistent with available experimental and/or physiological data. The selection of lower clearance value for pregnancy as well as the option 2 for lactating women, though not the optimal (given the interindivudal variability), would appear to be pragmatic and consistent with the rationale provided by EPA. However this reviewer has the following additional observations:

- The R2 value for the fit described in Figure B-6 is poor raising concern about the adequacy of the equation
- Did EPA analyze the data in Figure B-5 on the basis of body surface data for the various age groups (of pregnant women)?
- On page 42, para 3, Figure B-1 should read Figure B-2?
- What does GFR-based scaling mean in Figure B2? Is it body surface scaled?

(B) Breast-milk ingestion

B-1. Infants are generally known to consume very little milk or formula on the first day of life, with ingestion quickly increasing over time. However the first time-point for which ingestion data are available for infants is at 7 days, which EPA extrapolated back to 0 ingestion at time=0 in its analysis (birth; Figure 1). The breast-milk ingestion rate was estimated based on mean values reported in Arcus-Arth et al. (2005), while the water ingestion used for the breast-feeding mother was the 90th percentile value from U.S. EPA (2004).

Is EPA's extrapolation and rationale transparently and objectively described? Does this function appropriately characterize the available data and information? Are there other data that could be used to obtain a better or equally valid alternative estimate of mean breast-milk ingestion rate for infants in the first few days of life? Should an estimate other than the mean be used to determine the breast-milk ingestion rate?

RE:

- The fitting of the available data from Kahn and Stralka, with a mathematical function is adequate. However, the extrapolation from day 7 towards day 0 (or at birth) is not warrented given that the newborn is not a sub-group used in the assessment of relative sensitivity of lifestages (Table 3, page 22).

- The motivation for choosing 90^{th} pctle for consumption rates needs to be clearly presented, since the expectation is a calculation either based on mean values in the various groups or 95^{th} pctle values. Therefore, the rationale and scientific basis for the choice and use of the 90^{th} pctle values in these calculations should be more clearly presented.

(C) Water ingestion

C-1. For pregnancy, EPA used a normalized (90^{th} percentile) water ingestion rate of 33 mL/kg-day (U.S.EPA 2004), which was multiplied by the maternal BW as described by the PBPK model growth-functions during pregnancy to obtain total water ingestion for the mother.

Is EPA's approach and rationale transparently and objectively described? Is this approach appropriate for characterizing the upper-bound for ingestion of pregnant women? Are there other approaches or data that could be used to obtain a better or equally valid alternative estimate for this parameter? (Note that the self-reported body weight values in U.S. EPA (2004) indicate the same average body weight for pregnant women as for non-pregnant; data appears inaccurate, so the PBPK model body-weight description was used instead.)

RE:

- The cited value of 33 ml/kg-day corresponds to the 90^{th} percentile value for pregnant women ("consumers only") of the direct and indirect community water ingestion (chapter 6, page 16,

U.S. EPA 2004). However, the 90th percentile value of total water ingestion for the same group was 39 ml/kg-day. It is unclear then as to why EPA specifes the use of total water ingestion rate but actually uses the value corresponding to another group (i.e,. community water ingestion). Furthermore, the 90th percentile value for pregnant women, reported in US EPA (2004), was associated with a small sample size (n=65, which does not meet the minimum reporting requirements described in the "Third report on Nutrition Monitoring in United States"). This raises the question of why not use (or justify the non-use of) the value from Ershow et al (1991) based on much larger sample size (n=188). These authors reported 90th pctle values for tap and total water ingestion of 34.5 and 48.9 ml/kg-day respectively.

- It is also unclear to this reviewer as to why 90th pctle value is chosen for the computations and not either the median or the 95th percentile value.

- This reviewer is not concerned about the use of subject-specific or group-specific body weight in the PBPK model to facilitate the calculations for pregnant women, as long as the ingestion rate is expressed in units of ml/kg-day, as done here.

C-2. For lactation, EPA used a *fixed* total (90th percentile) water ingestion rate of 2959 mL/day (U.S. EPA 2004), with the rationale that while the woman's weight and self-water demand are expected to drop after pregnancy (as described by the PBPK model time-dependent weight equations), the demand for milk production would be increasing, and the reported value was not for a specific age-range of child.

Is EPA's approach and rationale transparently and objectively described? Is this an appropriate value to use for the ingestion rate of lactating women? Are there other better or equally valid alternative approaches or values that could be used?

RE:

- The ingestion rate of 2959 ml/day, used by EPA, corresponds to the 90th percentile value of "consumers only" lactating women for direct and indirect community water ingestion. In comparison, the 90th percentile value of total water ingestion in consumers only lactating women is reported to be 3021 ml/ day (chapter 6 page 17). The EPA report (page 24, para 2) states that the intent was to use the "total" consumers-only water intake in the calculations. The source and consequence of this discrepancy should be addressed.

- Further, U.S. EPA (2004) indicated that the 90th pctl value (2959 ml/day) is associated with a small sample size (n=41, which does not meet the minimum reporting requirements described in

the "Third report on Nutrition Monitoring in United States"), raising a concern of its use rather than the value from Ershow et al. (1991). Additionally, it is unclear as to why the 90th pctl rather than 95th pctl of the water ingestion is used in these calculations.

- In light of the fact that the water ingestion rate in lactating women is significantly greater (see chapter 6 pages 16-17, U.S. EPA 2004) , on a ml/kg-day basis, than in pregnant women, the rationale used for using a fixed ingestion rate needs to be more fully articulated.

C-3. For bottle-fed infants, EPA made extrapolations of the 90th percentile water-ingestion in early life based on measurements made for the age ranges: 0-30 days, 1-3 months, and 6-12 months (points in Figure 2, upper panel). For the purposes of this analysis, bottle-fed implies feeding with formula requiring the addition of water.

Is EPA's extrapolation and rationale transparently and objectively described? Does the overall function used to represent the changes in ingestion with age (and body weight) appropriately characterize the available data and information? Are there other data that could be used to obtain a better or equally valid alternative estimate of water ingestion rate for infants in the first few days of life (e.g., 7-day old)? The water ingestion rates used by EPA are based on 90th percentile ingestion data and thus are likely to exceed (minimal) physiological needs of infants as defined in nutritional guidelines. Are the water ingestion rates used by EPA reasonable in comparison to the physiological needs of infants at these various life stages? Are there other approaches or data **(e.g., see the FDA memo**) that could be used to obtain a better or equally valid alternative estimate for this parameter?

- Even though the EPA rationale is satisfactory, it is unclear as to why the emphasis is placed on the section of the curve (i.e., first few days after birth) which is neither used in the lifestage analysis nor supported by any data.

- A report published by a Public Health Agency in Québec contains data on water consumption of 393 infants of 8 weeks of age. For bottle-fed only infants (n = 278), mean (IC95%) value for total water ingestion was 122 ± 43 (117-127) ml/kg-day, or 655 ± 233 (627-682) ml/day. The corresponding 90th percentile values were 179 ml/kg-day and 981 ml/day. For more details the following source may be consulted:
- http://www.inspq.qc.ca/publications/default.asp?NumPublication=334
- A copy of the above report in PDF is also attached herewith.

(D) Perchlorate concentration in formula

D-1. EPA used 1.42 µg/L as the concentration of perchlorate in formula for bottle-fed infants. This estimate was based on information from FDA's Total Diet Study, supported by Pearce *et al*.'s (2007) findings.

Is EPA's approach and rationale transparently and objectively described? Is 1.42 µg/L an appropriate value to use for the concentration of perchlorate in infant formula? Are there other better or equally valid alternative approaches or values that could be used?

RE:

The EPA,s approach is clearly described and appears to be consistent with the current state of knowledge. However, it would be better to clearly identify the basis for the choice of the mean value rather than median, 90^{th} or 95^{th} pctl value (presumably the limited, available data did not permit such a determination).

(E) Radioiodide excretion into breast-milk by NIS

E-1. In the model, EPA included perchlorate inhibition of NIS radioiodide excretion into breast-milk, as well as inhibition of radioiodide transport by perchlorate for all NIS-containing tissues, thereby making the code consistent with the model description in Clewell et al. (2007). Is this inclusion appropriate? Are the impacts of this inclusion transparently and objectively described?

RE:

Yes. The EPA's approach is logical and internally-consistent. The impact of this inclusion is described in sufficient detail.

PEER REVIEW COMMENTS FROM

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Technical Charge to External Peer Reviewers

Contract No. EP-C-07-024
Task Order No. 54
October 22, 2008

INHIBITION OF THE SODIUM-IODIDE SYMPORTER BY PERCHLORATE: AN EVALUATION OF LIFESTAGE SENSITIVITY USING

PHYSIOLOGICALLY-BASED PHARMACOKINETIC (PBPK) MODELING

WRITTEN COMMENTS ARE DUE NO LATER THAN MONDAY, NOVEMBER 10, 2008

BACKGROUND

The U.S. Environmental Protection Agency (EPA) is seeking an external peer review of its analysis of the inhibition of thyroidal radioiodide uptake (RAIU) by perchlorate for multiple life-stages including pregnancy, fetal development, lactation, infancy, childhood, and adulthood. This analysis involves modifications to the computational implementation (code) for a set of existing PBPK models that describe the kinetics of radioiodide and perchlorate in humans during these different life-stages. Since the models themselves are published in the peer-reviewed literature, EPA is not seeking review of the models per se, but of changes in a small number of parameters, and of specific changes in the code intended to make the code consistent with the published description. EPA's analysis and modifications, including identification of model code errors and examination of data and assumptions used for specific input parameters, are described in the attached document.

REVIEW MATERIALS AND INSTRUCTIONS

Review Document

EPA's Draft Report, *Inhibition of the Sodium-Iodide Symporter by Perchlorate: An Evaluation of Lifestage Sensitivity Using Physiologically-based Pharmacokinetic (PBPK) Modeling* (Including a report of a contractor-led detailed review and quality assurance check of the model code in Appendix C)

Background Materials Provided on CD

1. Model Code in PDF and zipped acslXtreme workspace files
2. FDA memo on "Volume of Feeds for Infants" (for Parameter-Specific Charge Question C-3)
3. EPA's 2000 Risk Characterization Handbook (for General Charge Question G-6)
4. Supporting References

Peer review of this analysis is being sought to ensure that EPA's analysis, modifications to existing PBPK models, and data inputs and assumptions are clearly and transparently described and are scientifically sound and supported by the available data. Please provide detailed explanations of your responses to the charge questions.

CHARGE QUESTIONS

General Charge Questions:

G-1. Is EPA's analysis logical, clear and appropriate in depth and length? Has EPA accurately, clearly and objectively represented and synthesized the scientific evidence for the changes made to or specification of the model code and input parameters?

The Reviewer is convinced that EPA has performed an outstanding job in improving the PBPK model so the codes written in the model are consistent to the physiology of iodide uptake in the thyroid glands and the uptake inhibition by perchlorate. It is also clear that EPA has tried to perfect the input parameters to increase the predictability of the model.

G-2. Please identify any additional studies or other data sources that should be considered in the assessment of the specific parameters addressed below, including urinary clearance (of iodide and/or perchlorate) and ingestion rates (breast milk, formula and water), especially in neonates.

Considering the elevated inhibition of RAIU in bottle-fed infants, EPA should seek for additional data to re-affirm the water ingestion rates that are used by EPA, particularly the use of the 90^{th} percentile values in which the situations exceed the expectation of the fundamental knowledge. The Reviewer has no knowledge of whether there are studies or data sources that EPA could use.

G-3. Besides the specific parameters identified below, please comment on any other parameter or model choices described in the document that you think are incorrect or require further explanation or where other available information could provide better estimates.

EPA should explain the rationale of using the 90^{th} percentile values in the analysis. It seems to the Reviewer that such choice is deemed to create an upper bound limit, however, throughout the document, EPA has stated that this is not the purpose due to the uncertainties involved in the model simulation and other reasons.

G-4. Please discuss research that you think would be likely to increase confidence in these models and their use in predicting RAIU inhibition by perchlorate in different life stages (for an average individual within each life-stage).

What will significantly increase the confidence in these PBPK model is to use the real-world data (such as perchlorate in drinking water and the level of iodide in blood or thyroidal functions in population)

liking perchlorate exposure and iodide inhibition. The article published by Blount et al. (EHP 2006 114(12) 1865-1871) would be an ideal application for these PBPK models. Unfortunately, data used in Blount et al. study (NHANES) do not include children ages 6 and below.

G-5. Does EPA accurately characterize the strengths and limitations of the analysis? Please comment on any particular strengths that may not be mentioned or adequately characterized for the estimates of RAIU for different life stages. Please also comment on any specific sources of uncertainty that you believe have been overlooked in EPA's analysis or that require further discussion, and which might be significant to EPA's estimates of RAIU for different life stages.

This document is well written and has highlighted what EPA has accomplished in assessing RAIU inhibition at different life stages resulting from perchlorate exposure. The Reviewer thought EPA has thoroughly discussed the strengths and limitation of this analysis, including the uncertainty analysis.

One uncertainty, however, has not been addressed by EPA is the use of direct IV dose of radioiodide to the bottle-fed infants in order to determining iodide uptake inhibition caused by perchlorate in formula. Although this approach seems intuitive, it may not reflect the real-world scenario in which iodide intake is usually taking place by oral ingestion. Pharmacokinetically speaking, the absorption of chemicals in humans could vary significantly between oral ingestion and bolus *iv* injection. EPA needs to conduct an uncertainty analysis to assure that such approach would not impact the outcomes significantly.

G-6. As recommended in EPA's 2000 Risk Characterization Handbook, is the analysis transparent in terms of the steps, logic, key assumptions, limitations, and decisions? Specifically, does characterization of the results of EPA's work fully explain: a) the analysis approach employed; b) the use of assumptions and their impact on the analysis; c) the use of extrapolations and their impact on the analysis; d) plausible alternatives and the choices made among those alternatives; e) the impacts of one choice *vs*. another on the analysis; f) significant data gaps and their implications for the analysis; g) the scientific conclusions identified separately from default assumptions and policy decisions, if any; and h) the major conclusions, and the discussions of EPA's confidence and uncertainties in the conclusions?

EPA has clearly explained their approaches employed including the assumptions, alternatives, and the use of extrapolations and their impacts on the analyses. Apparently, there are significant data gaps, particularly for newborn infants that lead to some limitations of using this revised PBPK model. However, it is rather common for many PBPK modeling work, and therefore should NOT be considered a major limitation of this analysis.

It is apparent that the outcome of the PBPK model prediction is dictated by the use of urinary clearance of

perchlorate and iodide. Other parameters have somewhat less impacts on the results. EPA has taken the right approach focusing on the parameters related to perchlorate exposure and iodide intakes. The revised PBPK model that EPA modified has demonstrated the importance of those parameters, and the Reviewer agrees with the EPA's scientific conclusion in which the modified Clewell et al. model is acceptable to calculate the lifestage differences in the degree of NIS inhibition of thyroidal radioiodide uptake at a given level of perchlorate exposure.

Parameter-Specific Charge Questions:

(A) Urinary clearance

A-1. Please comment on the appropriateness of the input values selected for maternal urinary clearance during pregnancy and lactation in EPA's analysis. Are the available data and rationale for the values selected transparently and objectively described? Are you aware of other publications or data that could be used to guide these choices or which provide alternative input values that are equally valid or more appropriate? Likewise, are the values selected for urinary clearance for the infant and older child the best estimates, given the available science and data? Are there other data that would provide better (or equally valid alternative) guidance or estimates? Is there any reason to believe that urinary clearance might not be a limiting factor in the elimination of perchlorate for infants?

Considering that perchlorate, as well as iodide, does not further metabolize in human body, the urinary clearance should be a limiting factor in removing perchlorate and iodide from humans at all lifestages, and an important parameter in the perchlorate PBPK model.

Unfortunately, data for urinary clearance of perchlorate and iodide by the mother during pregnancy and lactation are not consistent among three sources (Clewell, Aboul-Khair, and Delange) cited by EPA. The choices that EPA made for selection clearance for pregnancy and lactation are quite arbitrary, and the reasoning, if any, are not found. If GFR is corresponding to the cardiac output (meaning higher blood flow rate equal to higher GFR), urinary clearance of any given compound during the pregnancy should be higher than non-pregnancy. Urinary clearance during lactation period might be the opposite to the pregnancy due to the difference of cardiac output. EPA should seek for differences of urinary clearance (mainly via GFR) of compounds during pregnancy and lactation outside the iodide and perchlorate literatures.

EPA has clearly documented how they determined the alternative scaling of urinary clearance of

perchlorate and iodide by body weight and has provided a thorough explanation of why EPA chose to use $(BW)^1$, instead of commonly used $(BW)^{0.75}$, in neonates. The justification is sound and supported by the data published in the literature. Similar justification of using $(BW)^1$ scaling for perchlorate clearance in older children (ages 2-12) is also provided, however, the sentence of "EPA's estimates of urinary clearance in infants and children are lower than those used in Clewell et al. (2007), but are values EPA judges to be scientific estimate, not bounds." (on page 11, 1st paragraph) is not clear to the Reviewer. The information to support this sentence may come from Appendix B (pages 44-46), particularly from Figure B-4. However, Figure B-4 itself is difficult to understand (for instance, how is Lower 95% related to the yellow diamonds, and how the line of Data average is constructed?), and therefore renders less convincing remark of using $(BW)^1$ scaling for older children. EPA may want to review this and provide a clearer explanation on the data presented in Figure B-4.

(B) Breast-milk ingestion

B-1. Infants are generally known to consume very little milk or formula on the first day of life, with ingestion quickly increasing over time. However the first time-point for which ingestion data are available for infants is at 7 days, which EPA extrapolated back to 0 ingestion at time=0 in its analysis (birth; Figure 1). The breast-milk ingestion rate was estimated based on mean values reported in Arcus-Arth et al. (2005), while the water ingestion used for the breast-feeding mother was the 90th percentile value from U.S. EPA (2004).

Is EPA's extrapolation and rationale transparently and objectively described? Does this function appropriately characterize the available data and information? Are there other data that could be used to obtain a better or equally valid alternative estimate of mean breast-milk ingestion rate for infants in the first few days of life? Should an estimate other than the mean be used to determine the breast-milk ingestion rate?

Based on the data presented in Figure 1, the milk ingestion rates, or suckling rate, are quite different between Clewell et al. and Arcus-Arth et al., however, EPA's decision to use Arcus-Arth's data requires further clarification. EPA claimed that Clewell et al.'s data is inadequate to describe the suckling rates in the first couple weeks of life, however, based on the Reviewer's examination on Figure 1, the abrupt increase of milk ingestion during Day 1, and between Day 1 and 7 as presented by Arcus-Arth et al. seems unlikely. The difficulty of collecting breast-milk ingestion rate for infants in the first few days of life is understandable, and the deviation of the mean breast-milk ingestion from the true value might not be as large as we thought. Therefore, the mean breast-milk ingestion rate might be robust enough for use.

(C) Water ingestion

C-1. For pregnancy, EPA used a normalized (90th percentile) water ingestion rate of 33 mL/kg-day (U.S.EPA 2004), which was multiplied by the maternal BW as described by the PBPK model growth-functions during pregnancy to obtain total water ingestion for the mother.

Is EPA's approach and rationale transparently and objectively described? Is this approach appropriate for characterizing the upper-bound for ingestion of pregnant women? Are there other approaches or data that could be used to obtain a better or equally valid alternative estimate for this parameter? (Note that the self-reported body weight values in U.S. EPA (2004) indicate the same average body weight for pregnant women as for non-pregnant; data appears inaccurate, so the PBPK model body-weight description was used instead.)

EPA's has objectively described its approach in using water ingestion rate of 33 mL/kg-day. However, the rationale of using the 90th percentile value was not provided by the EPA in this analysis. As for the BW estimates, it is unclear of how accurate it is to use the PBPK model growth-functions during pregnancy for estimating BW of pregnant women. Will NHANES data provide some sort of national average of the water ingestion rates stratified by lifestages and the BW of pregnant women? Or could EPA validate the PBPK model growth-functions for weight estimates using the NHANES data?

C-2. For lactation, EPA used a *fixed* total (90th percentile) water ingestion rate of 2959 mL/day (U.S. EPA 2004), with the rationale that while the woman's weight and self-water demand are expected to drop after pregnancy (as described by the PBPK model time-dependent weight equations), the demand for milk production would be increasing, and the reported value was not for a specific age-range of child.

Is EPA's approach and rationale transparently and objectively described? Is this an appropriate value to use for the ingestion rate of lactating women? Are there other better or equally valid alternative approaches or values that could be used?

The rationale of using a fixed total water ingestion rate is justifiable and transparently and objectively described. However, the reasoning of selection of 2,959 mL/day at the 90th percentile is missing in this analysis. It would be assuring if EPA could provide the complete distribution of the estimates of total water ingestion.

C-3. For bottle-fed infants, EPA made extrapolations of the 90th percentile water-ingestion in early life based on measurements made for the age ranges: 0-30 days, 1-3 months, and 6-12 months (points in Figure 2, upper panel). For the purposes of this analysis, bottle-fed implies feeding with formula requiring the addition of water.

Is EPA's extrapolation and rationale transparently and objectively described? Does the overall function used to represent the changes in ingestion with age (and body weight) appropriately characterize the available data and information? Are there other data that could be used to obtain a better or equally valid alternative estimate of water ingestion rate for infants in the first few days of life (e.g., 7-day old)? The water ingestion rates used by EPA are based on 90th percentile ingestion data and thus are likely to exceed (minimal) physiological needs of infants as defined in nutritional guidelines. Are the water ingestion rates used by EPA reasonable in comparison to the physiological needs of infants at these various life stages? Are there other approaches or data **(e.g., see the FDA memo**) that could be used to obtain a better or equally valid alternative estimate for this parameter?

EPA has not informed the rationale of using the 90th percentile total water ingestion rate in early life stage, as well as during the lactaton (as stated earlier in the review), and therefore, the possibility of the estimated numbers are likely exceeding minimal physiological needs of infants raises a concern. If this is the case in which the 90th percentile total water ingestion rate exceeds the norms, this approach of using the 90th percentile is problematic. Since this sub-analysis focuses on bottle-fed infants, EPA could follow the nutritional guidelines to estimate the total water-ingestion rate (such as the frequency of feeding per 24 hours and the quantify of formula and water mixing per feeding).

(D) Perchlorate concentration in formula

D-1. EPA used 1.42 µg/L as the concentration of perchlorate in formula for bottle-fed infants. This estimate was based on information from FDA's Total Diet Study, supported by Pearce *et al*.'s (2007) findings.

Is EPA's approach and rationale transparently and objectively described? Is 1.42 µg/L an appropriate value to use for the concentration of perchlorate in infant formula? Are there other better or equally valid alternative approaches or values that could be used?

The Reviewer believes that perchlorate level in formula used by EPA is the best available data, especially this level is consistent to the public numbers from an independent research. The Reviewer is not aware other better or equally valid alternative approaches or values that could be used.

(E) Radioiodide excretion into breast-milk by NIS

E-1. In the model, EPA included perchlorate inhibition of NIS radioiodide excretion into breast-milk, as well as inhibition of radioiodide transport by perchlorate for all NIS-containing tissues, thereby making the code consistent with the model description in Clewell et al. (2007). Is this inclusion appropriate? Are the impacts of this inclusion transparently and objectively described?

Yes, this inclusion is not only appropriate but also needed, and the impacts of this inclusion are transparently and objectively described in this analysis. This work reflects EPA's efforts in reviewing the model established by Clewell et al. and seeking for improvement of the PBPK model.

PEER REVIEW COMMENTS FROM

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Note: Dr. Zeise performed this review as a private consultant and not as an agent of the California Environmental Protection Agency

Technical Charge to External Peer Reviewers

Contract No. EP-C-07-024
Task Order No. 54
October 22, 2008

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

WRITTEN COMMENTS ARE DUE NO LATER THAN MONDAY, NOVEMBER 10, 2008

CHARGE QUESTIONS

General Charge Questions:

G-1. Is EPA's analysis logical, clear and appropriate in depth and length? Has EPA accurately, clearly and objectively represented and synthesized the scientific evidence for the changes made to or specification of the model code and input parameters?

In general EPA's analysis is clear and logical, and succinct, although there are several suggestions for improvement in the comments below. With regard to depth, the limited treatment of variability and uncertainty is problematic. Pharmacokinetic models can provide a structure for exploring and integrating variability, but this was not done in this analysis. This major limitation is recognized by EPA (page 25). EPA points out the model predictions apply to "a subgroup average for typical, healthy individuals, and effectively describe the RAIU inhibition relative to that same individual as his/her own control." EPA further points out that "These models were not designed to account for whether the pregnant women is hypothyroid or iodine deficient." Analysis of such large, susceptible populations is a critical aspect of understanding the potential health impact of perchlorate drinking water exposure. A more rigorous and explicit treatment of variability is needed to get a better handle on intra-human variability in response to perchlorate exposure. The analysis would also be improved by more rigorous statistical and quantitative treatment of uncertainty. The degree to which the analysis for the GW 40 fetus may or may not represent the first and second trimester fetus needs explicit and careful treatment.

On a smaller point, it would help if greater motivation was provided for some of the statistical fits to data. Some statistical fits provided an expedient and practical way forward in the analysis but appeared to introduce logical inconsistency. It would be preferable for a more expanded discussion

to provide a context for the approach taken.

G-2. Please identify any additional studies or other data sources that should be considered in the assessment of the specific parameters addressed below, including urinary clearance (of iodide and/or perchlorate) and ingestion rates (breast milk, formula and water), especially in neonates.

Additional possible studies and data sources are identified in response to specific charge questions below.

G-3. Besides the specific parameters identified below, please comment on any other parameter or model choices described in the document that you think are incorrect or require further explanation or where other available information could provide better estimates.

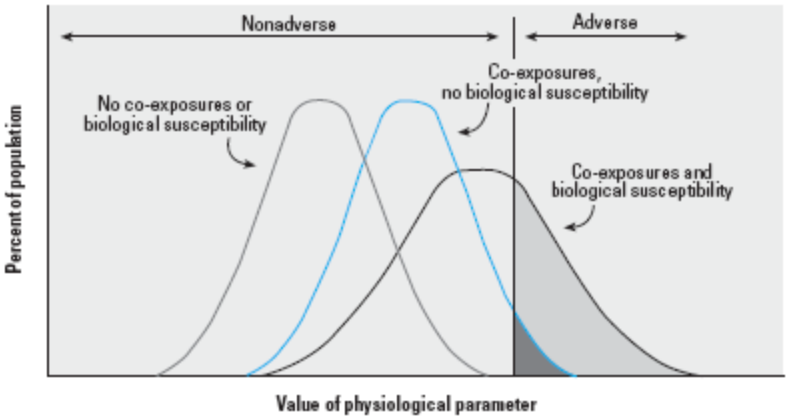


Figure 2. Distribution of a typical physiological parameter within the population and how that may vary depending on the influence of chemical and biologic background.

The above figure, taken from Woodruff et al. (2008; EHP 116:1568), illustrates the main limitation in the analysis and approach to modeling. The question in evaluating the potential risks from perchlorate in drinking water is about the extent to which the incremental exposure from water results in adverse effects to the mom, her baby, her developing fetus or others. In the above figure it is above the extent to which the perchlorate drinking water exposure, in the presence of coexposure and biological sensitivity, is creating adverse outcomes in the population. The fidelity of the analysis depends on whether individuals with biological susceptibility have been adequately addressed and also whether coexposures that affect iodide inhibition have been adequately considered.

EPA analysis enables biological susceptibility and coexposures to be partially addressed in the

assessment, but it needs to move further to enable a fuller treatment. With regard to biological susceptibility EPA considers susceptible subgroups – the infant, fetus, mom – and an important factor that increases susceptibility in these groups – low renal clearance. But the analysis does not enable the agency to consider the extent of impact on other sensitive subgroups in these populations, such as those with clinical and subclinical hypothyrodism, those that may be genetically predisposed (see e.g., Scinicariello, EHP 113(11):1479-84), and those that are iodine deficient. The EPA analysis also considers an important coexposure – perchlorate intake via food. However, the analysis does not consider the combined impact with thiocyanate, which also affects iodide uptake at the NIS. Thiocyanate is also found in breast milk (see e.g., Kirk et al. 2007, EHP, 115:182-186), cigarette smoke, and common foods. The recent finding in women who smoked, that those with low urinary iodine levels had decreasing T_4 with increasing perchlorate (Steinmaus et al. 2007, EHP, 115:1333-1338) as well as reduced content of iodine in breast milk and the urine of breast feeding infants of smokers (Laurberg et al. 2004, J Clin Endo Met 89:181-187) indicates the importance of considering coexposures to thiocyanate. Nitrate, ubiquitous though far less potent than perchlorate, should also be considered (see e.g., DeGroef et al., 2006, Eur J Endocrin 155: 17-25).

G-4. Please discuss research that you think would be likely to increase confidence in these models and their use in predicting RAIU inhibition by perchlorate in different life stages (for an average individual within each life-stage).

Research to get a better handle on renal clearance of iodine and perchlorate during pregnancy and postpartum; biomonitoring of perchlorate, iodide, thiocyanate and thyroid hormone during and after pregnancy during lactation in smoking and non-smoking women. Measurements of perchlorate in baby formula – in non-composited samples.

G-5. Does EPA accurately characterize the strengths and limitations of the analysis? Please comment on any particular strengths that may not be mentioned or adequately characterized for the estimates of RAIU for different life stages. Please also comment on any specific sources of uncertainty that you believe have been overlooked in EPA's analysis or that require further discussion, and which might be significant to EPA's estimates of RAIU for different life stages.

EPA does not sufficiently elaborate on the limitation of focusing on "healthy" individuals, and the lack of consideration of the large susceptible populations.

Some parts of the analysis are scenario based, using 90th percentile values, while other parts use

mean values. With over 4 million infants born in the US each year, scenario analyses should be added. These would be directed at ascertaining the inhibition levels for the some plausible higher susceptibility cases, such as infant and fetus exposures associated with a mom with relatively high thiocyanate exposure (e.g., from broccoli consumption or smoking), low renal clearance, who got all her fluids directly or indirectly from tap water.

G-6. As recommended in EPA's 2000 Risk Characterization Handbook, is the analysis transparent in terms of the steps, logic, key assumptions, limitations, and decisions? Specifically, does characterization of the results of EPA's work fully explain: a) the analysis approach employed; b) the use of assumptions and their impact on the analysis; c) the use of extrapolations and their impact on the analysis; d) plausible alternatives and the choices made among those alternatives; e) the impacts of one choice vs. another on the analysis; f) significant data gaps and their implications for the analysis; g) the scientific conclusions identified separately from default assumptions and policy decisions, if any; and h) the major conclusions, and the discussions of EPA's confidence and uncertainties in the conclusions?

EPA does a reasonably good job laying out the logic, key assumptions, limitations and decisions. But for the most part, it is done in a manner that will be understandable to someone with a modeling background. It will be difficult to follow and very accessible to a more general reader. More motivation of the forms for the statistical fits is needed, and a more quantitative and rigorous treatment of uncertainty. EPA reasoning for using 90th percentile values for some parameters and mean values for others is not explained well. Failure to address certain large susceptible populations and the possible sizes of these populations should be discussed. The degree to which the analysis for the GW 40 fetus may or may not represent the first and second trimester fetus needs explicit and careful treatment.

Parameter-Specific Charge Questions:

(A) Urinary clearance

A-1. Please comment on the appropriateness of the input values selected for maternal urinary clearance during pregnancy and lactation in EPA's analysis. Are the available data and rationale for the values selected transparently and objectively described?

The discussion of maternal urinary clearance values could be somewhat improved. In regard to Figure B-6 motivation is not given for the fitting of the quadratic function to the data for iodide

clearance vs gestation week, and it is unclear where the postpartum data set – greater than week 39 data set - on the plot appeared from and why it is included in the modeling of clearance during pregnancy. The highest mean value was measured by Adoul-Khair at the latest pregnancy time point. Inclusion of the extra data set weighs the function down late in pregnancy when the highest value was measured by Aboul-Khair. Further, including a gestation week of 45 on the plot axis is confusing to the reader. There is a large extrapolation to clearance during the early pregnancy time point and renal clearance can be increased fairly early in gestation. The quadratic fit may underpredict clearance during this period. However, given that EPA is declining to estimate early fetal effects, this portion of the extrapolation is not critical. It is unclear why the fit is being presented however. Finally variability among individuals is an important consideration and it would therefore be of interest to see on the plot or otherwise reported an indication of variability in the individuals studied. Some indication of this is given in Table 2 of Aboul-Khair et al., where renal clearance values for iodide have been serially averaged for each pregnant individual studied. In addition, individual measurements for controls are given. For Figure B-7 it would be good to show error bars or confidence bounds.

Minor error, on page 42, data from Guignard et al. are plotted in Figure B-2 not B-1.

Are you aware of other publications or data that could be used to guide these choices or which provide alternative input values that are equally valid or more appropriate?

Increased urinary clearance of iodide during pregnancy is a widely recognized phenomenon and data on the magnitude besides that reported by Aboul-Khair would be useful and important to locate. Further, the inconsistency of PK outcomes and the Aboul-Khair measured values in controls for IV iodide dose uptake 2.5 hours post injection is quite troublesome and calls into question the PK modeling. The approach on page 49 described to deal with the inconsistency is not entirely satisfactory. EPA should look hard for additional data sets to cross check assumptions regarding iodide uptake and renal clearance during pregnancy and early postpartum.

Likewise, are the values selected for urinary clearance for the infant and older child the best estimates, given the available science and data? Are there other data that would provide better (or equally valid alternative) guidance or estimates? Is there any reason to believe that urinary clearance might not be a limiting factor in the elimination of perchlorate for infants?

I am not aware of better values for infants and the older child. The EPA laid out a reasonable analysis and approach for developing estimates for the infant and older child. There is interindividual variability in clearance and it would be preferable if this were more emphasized and acknowledged in the discussion, and attempts to better describe it quantitatively, for example in terms of varying glomerular filtration rates normalized by body size.

(B) Breast-milk ingestion

B-1. Infants are generally known to consume very little milk or formula on the first day of life, with ingestion quickly increasing over time. However the first time-point for which ingestion data are available for infants is at 7 days, which EPA extrapolated back to 0 ingestion at time=0 in its analysis (birth; Figure 1). The breast-milk ingestion rate was estimated based on mean values reported in Arcus-Arth et al. (2005), while the water ingestion used for the breast-feeding mother was the 90th percentile value from U.S. EPA (2004).

Is EPA's extrapolation and rationale transparently and objectively described?

EPA's approach is objectively and transparently described, and the Agency was correct that the Clewell et al. description is inconsistent with the currently available peer reviewed literature. It is unclear why a mean value is used for infants and an upper 90th percentile is used for the breast feeding mother. This is not adequately explained.

Does this function appropriately characterize the available data and information?

The function does not characterize the available data and information. It will be quite confusing to anyone but a modeler.

The equation on page 15 has milk describes milk ingestion rate as

Milk ingestion rate (mL/hr) = KTRANS = 28.3*(BW-3.375)0.175

It then plots milk ingestion as a function of bodyweight and shows values for days 1, 3, 5 and 7 of life as on the bw vs milk ingestion plot. This formulation was used as a convenient way of giving values to KTRANS but is problematic because it works only for the specific circumstances using the mean values for breast milk intake in Arcus-Arth et al. data and will be confusing for anyone but a modeler.

In using bodyweight as a surrogate for age (3.375 kg as the zero age bodyweight) it builds in an illogical structure that will be hard for the general public to understand and limits the usefulness of the model for using data beyond the mean values in Arth-Arcus et al. For example, there is zero milk ingestion for a bodyweight of 3.375 and milk ingestion rates below that value cannot be defined. Furthermore, the expression has milk ingestion increasing with increasing bodyweight indefinitely. This also is contrary to what occurs – as infants age solid foods and other liquids are introduced and breast feeding reduces. Arth-Arcus et al. show that for the available data sets milk consumption – in terms of volume per bodyweight per day – decreases with age in a linear fashion. Thus there is another inconsistency introduced by the way the model is formulated. At different ages the mean milk ingestion at a given bodyweight will differ.

A more logical approach would be to develop an expression for milk ingestion in terms of volume per bodyweight per day could be expressed as a function of age. A separate expression could then be used to convert this to KTRANS. To deal with the early low consumption rate on days 1-3 the measured values could be used.

Figure 1 notes that the data are from Arcus-Arth et al. but in fact it is entirely inconsistent with Arcus-Arth et al. for the above discussed reasons.

Are there other data that could be used to obtain a better or equally valid alternative estimate of mean breast-milk ingestion rate for infants in the first few days of life?

It is not correct that the first time point for which ingestion data are available is 7 days. There are values in the literature for intake on days 1, 2, 3, 4 and 5. Indeed at days 4 and 5 the intake is quite high and consistent with the linear relationship for volume consumed per kg per day vs age reported in Arth-Arcus et al. See table 8 in that paper.

Should an estimate other than the mean be used to determine the breast-milk ingestion rate?

Whether or not the mean is used depends on later steps in the process, and ways that variability will be taken into account. There are over 4 million births in the US annually. The overall procedure for characterizing intra-species variability and central tendency needs to be designed to be able to address the large number of infants "in the tails" of the distribution. It would be preferable to build a PK approach that would enable fuller description of variability in iodide uptake inhibition. The use of mean values and the formulation used to compute KTRANS precludes this.

(C) Water ingestion

C-1. For pregnancy, EPA used a normalized (90th percentile) water ingestion rate of 33 mL/kg-day (U.S.EPA 2004), which was multiplied by the maternal BW as described by the PBPK model growth-functions during pregnancy to obtain total water ingestion for the mother.

Is EPA's approach and rationale transparently and objectively described?

The approach is transparently and objectively described, but the rationale is somewhat unclear. Some parameters are based on mean values, others on midpoints and still others on upper 90% bounds. It would be of interest to understand parameter distributions and how this translates to distributions for iodide uptake inhibition. This may be beyond what EPA has resources and time to do, but failing that, it would be desirable to have a clear presentation of the approach. EPA appears to be taking a plausible scenarios approach. But a clearer explanation is needed.

The table below is taken from EPA (2004). It shows the 95th percentile upper bound for community water as 43 mL/kg/day, a reasonably higher level than the 90th percentile. In the perchlorate document, the reason for choosing the 90th percentile and not some other value needs to be justified. It is also worth noting that the number of pregnant women captured in the survey is quite small, and raises some concern that the upper bound values may be under

estimates. For example, the upper bound estimate on the 90th percentile for pregnant women was 46 mL/kg-day.

Table 6.3.B2. Per Capita Water Consumption—Pregnant Women (mL/kg/day)

Estimate	Total Water 1978 NFCS (Ershow et. al. 1991)	Tap Water 1978 NFCS (Ershow et. al. 1991)	Total Water 1994–96, 98 CSFII	Community Water 1994–96, 98 CSFII
Sample Size	188•	188•	69#	65#
Mean	32.1	18.3	21#	14#
50th %	30.5	16.4	19#	9#
90th %	48.9	34.5	39#	33#
95th %	53.5	39.6	44#	43#

• Women aged 15 to 49 years; # Women aged 15 to 44 years.

Is this approach appropriate for characterizing the upper-bound for ingestion of pregnant women?

With 4.3 million births in the US each year, above the 90th percentile will be 430,000 women. Thus a very large number of women may consume water above this level, and one is left wondering about the importance of the assumption and how sensitive the results are to it. Following EPA (2004), the upper 95 percentile is 44 mL/kg/day, still representing a rather large number of women - 215,000.

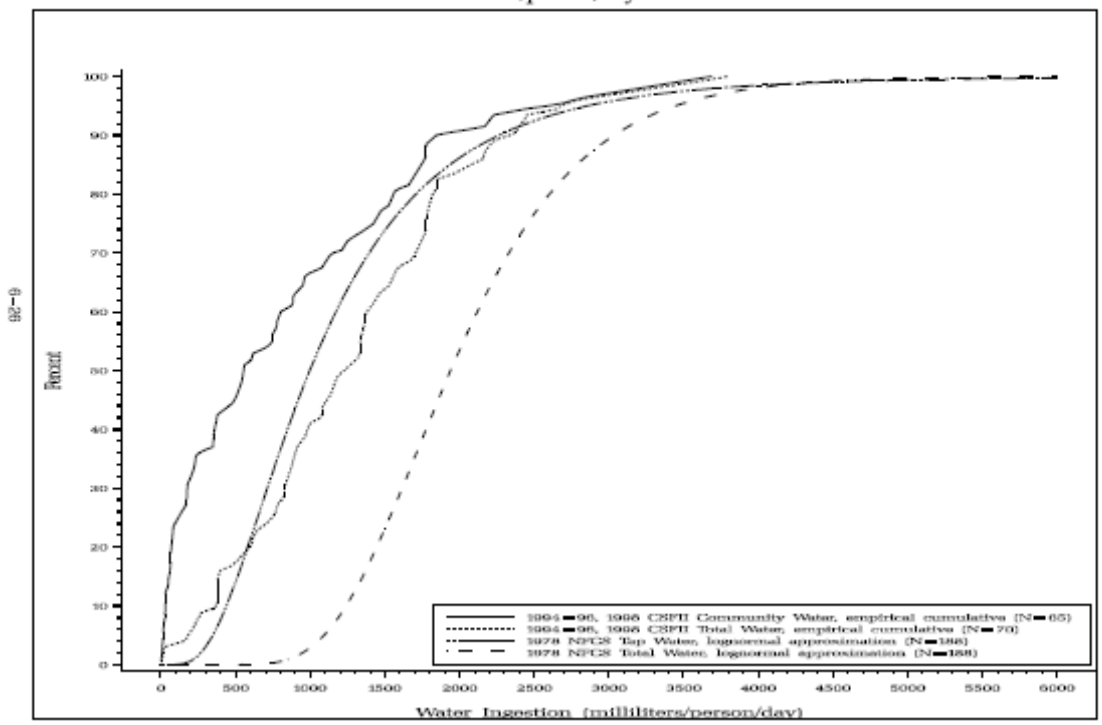
Are there other approaches or data that could be used to obtain a better or equally valid alternative estimate for this parameter? (Note that the self-reported body weight values in U.S. EPA (2004) indicate the same average body weight for pregnant women as for non-pregnant; data appears inaccurate, so the PBPK model body-weight description was used instead.)

There are other approaches that could be used to obtain a better or equally valid alternative estimate. One consideration is the extent to which we may be confident that a pregnant woman may use drinking water in cooking and for her fluid intake without having to be

concerned about harming her fetus. For this analysis one might consider the basic water requirments for women living in hot climates. For this one might select a value somewhat above the value of 3.0 L/day considered an "adequate intake" by the Institute of Medicine (2004; Dietary Reference Intakes for Water, Potassium, Sodium Chloride and Sulfate, IOM Food and Nutrition Board).

Another would be to pick a plausible upper bound value from the cumulative distribution observed. For example, from the figure below, taken from EPA (2004), it can be seen that a reasonable plausible upper bound may fall between 3.5 and 4 liters per day.

Figure 6.2.C1a. Cumulative Distributions of Per Capita Direct and Indirect Water Ingestion
Pregnant Consumers Only
ml/person/day



C-2. For lactation, EPA used a fixed total (90th percentile) water ingestion rate of 2959 mL/day (U.S. EPA 2004), with the rationale that while the woman's weight and self-water demand are expected to drop after pregnancy (as described by the PBPK model time-dependent weight equations), the demand for milk production would be increasing, and the reported value was not for a specific age-range of child.

Is EPA's approach and rationale transparently and objectively described?

The approach is transparently and objectively described, but the rationale is somewhat unclear. As noted in response to C-1, some parameters are based on mean values, others on midpoints and still others on upper 90% bounds. A clearer explanation is needed on why the 90th percentile is chosen here, and not some other higher bound given the number of women-infant pairs affected.

The tables below are taken from EPA (2004). They show the 90th percentile upper bound for community water is not substantially smaller than the 95th percentile when expressed as mL/kg/day, but appears more different when expressed as mL/person/day (2959 vs 3588), suggesting the difference may be driven by bodyweight differences at the 90th and 95th percentile. Still the reason for choosing the 90th percentile requires further explanation.

Table 6.3.C2. Per Capita Water Consumption—Lactating Women (mL/kg/day)

Estimate	Total Water 1978 NFCS (Ershow et. al. 1991)	Tap Water 1978 NFCS (Ershow et. al. 1991)	Total Water 1994–96, 98 CSFII	Community Water 1994–96, 98 CSFII
Sample Size	77*	77*	40#	33#
Mean	37.0	21.4	28‡	20‡
50th %ile	35.1	20.5	25‡	20‡
90th %ile	53.7	35.1	53‡	54‡
95th %ile	59.2	37.4	57‡	55‡

* Women aged 15 to 49 years; # Women aged 15 to 44 years.
‡ The sample size does not meet minimum reporting requirements as described in the "Third Report on Nutrition Monitoring in the United States".

Table 6.3.C1. Per Capita Water Consumption—Lactating Women (mL/person/day)

Estimate	Total Water 1978 NFCS (Ershow et. al. (1991))	Tap Water 1978 NFCS (Ershow et. al. (1991))	Total Water 1994–96, 98 CSFII	Community Water 1994–96, 98 CSFII
Sample Size	77*	77*	41#	34#
Mean	2,242	1,310	1,806#	1665#
50th %ile	2,164	1,330	1,498#	1 645#
90th %ile	3,169	1,945	3,021#	2959#
95th %ile	3,333	2,191	3,767#	3588#

* Women aged 15 to 49 years; # Women aged 15 to 44 years.

Is this an appropriate value to use for the ingestion rate of lactating women?

Similar to the response given to charge question C-1, use of the 90th percentile raises concerns that a substantial number of mother infant pairs are not sufficiently considered. The majority of newborn infants breast feed, and substantial numbers of infants do so through age 6 months, and there are still large numbers above the 90th percentile.

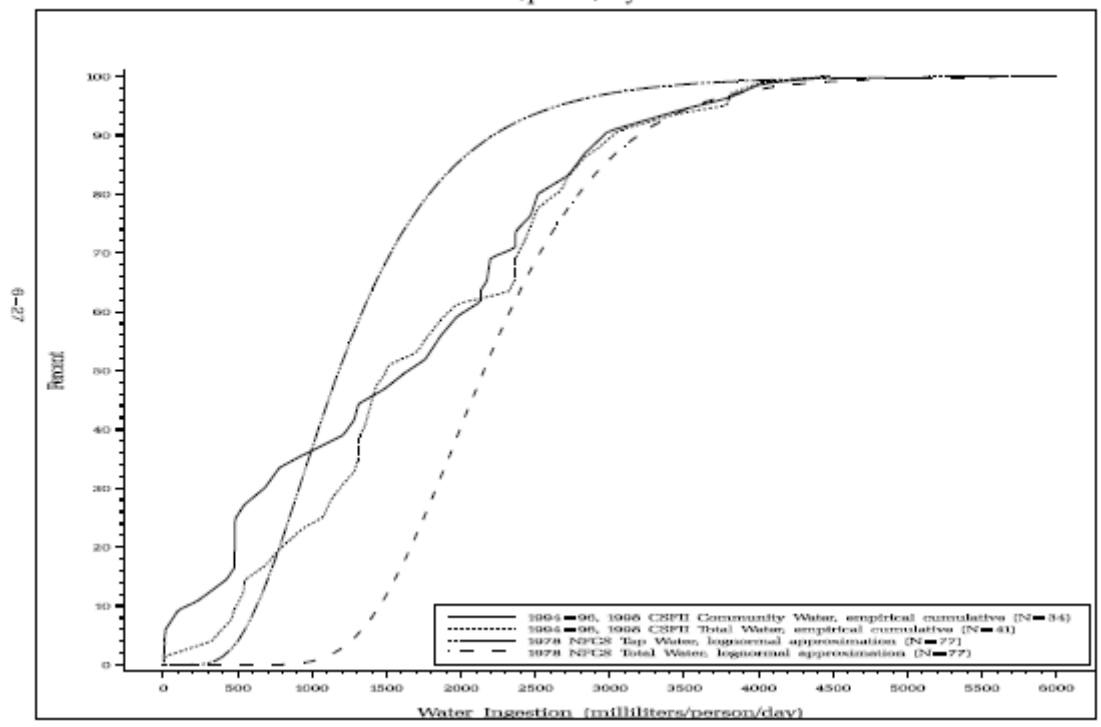
With regard to arguments on water needs of lactating women, there is a paucity of data. One could add the argument that IOM (2004) made that the intake of non-pregnant women added to the fluid output in breastfeeding provides a reality check on water ingestion rate.

Are there other better or equally valid alternative approaches or values that could be used?

There are other approaches that could be used to obtain a better or equally valid alternative estimate. One consideration is the extent to which we may be confident that a lactating woman may use drinking water in cooking and for her fluid intake without having to be concerned about harming her baby. The basic water requirements for women living in hot climates might be considered. For this one might select a value somewhat above the value of 3.8 L/day considered an “adequate intake” for lactating women by the Institute of Medicine (2004).

Looking at the cumulative distribution observed for lactating women, in the figure below taken from EPA (2004), it can be seen that a reasonable plausible upper bound may fall somewhere around 4 liters per day.

Figure 6.2.C1b. Cumulative Distributions of Per Capita Direct and Indirect Water Ingestion
Lactating Consumers Only
ml/person/day



C-3. For bottle-fed infants, EPA made extrapolations of the 90th percentile water-ingestion in early life based on measurements made for the age ranges: 0-30 days, 1-3 months, and 6-12 months (points in Figure 2, upper panel). For the purposes of this analysis, bottle-fed implies feeding with formula requiring the addition of water.

Is EPA's extrapolation and rationale transparently and objectively described?

Yes, although the reason for using a quadratic relationship was not described. The approach of expressing water ingestion in units mL/kg/day and modeling it as a function of age is much preferred over the approach used for breast milk consumption (e.g., in Figure 1).

Does the overall function used to represent the changes in ingestion with age (and body weight) appropriately characterize the available data and information?

This approach is a reasonable way of describing the upper 90th bound given in the Kahn and Stralka (2008) paper.

Are there other data that could be used to obtain a better or equally valid alternative estimate of water ingestion rate for infants in the first few days of life (e.g., 7-day old)?

An alternative for estimating ingestion rate for the first few days of life would be to rely on data sets for breast milk consumption during the first 7 days (e.g., Casey et al. 1986, Am J Dis Child 140:933; Neubauer et al. 1993, Am J Clin Nutr, 58:54), since breast fed infants do not require supplemental water and the results may be more indicative than the assumed relationship used, although sample sizes are relatively small. It is noteworthy that intake in mL/kg/d during this period is not a smooth function of bodyweight. It is quite low during the first two days of life but by age four or five days the intake is essentially the same as at age 7 days. It is possible that the function $1\text{-}e^{\text{-day}}$ does a reasonably good job of describing this. EPA could compare the values predicted by this function at days 1-7 to those seen in the literature for breast milk consumption on those days.

The water ingestion rates used by EPA are based on 90th percentile ingestion data and thus are likely to exceed (minimal) physiological needs of infants as defined in nutritional guidelines. Are the water ingestion rates used by EPA reasonable in comparison to the physiological needs of infants at these various life stages? Are there other approaches or data ***(e.g., see the FDA memo)*** *that could be used to obtain a better or equally valid alternative estimate for this parameter?*

The available data used by EPA indicate that infant formula consumption varies by individuals, and correspondingly water consumption does as well. It is reasonable to consider the minimal physiological needs of infants, as defined in nutritional guidelines, although a precise understanding energy needs and use in infancy still appears to be a matter of discussion (Reilly et al. 2005, Br J Nutr 94: 56-63). At any particular age bodyweights, growth rate and degree of activity varies, and so consumption can not be precisely calculated based on formula energy content and recipes for making up bottle fed formula. Further, some infants are overfed and others are underfed. Thus although it would be useful to compare water consumption with what one would expect given nutritional guidelines and typical formula recipes, the nutritional guideline would not lead to a reliable upper bound value for water consumption. Assumptions would be needed to go from the water consumption based on the nutritional guideline level to an upper bound estimate. Though as the FDA memo notes, "there is a relationship between the volume of water an infant needs, and

his/her caloric requirements for healthy growth" the exact relationship to assume and the interindividual variability in that relationship has not been provided and it is unclear that it would provide a more reliable estimate of water consumption than is given in EPA's perchlorate report.

(D) Perchlorate concentration in formula

D-1. EPA used 1.42 µg/L as the concentration of perchlorate in formula for bottle-fed infants. This estimate was based on information from FDA's Total Diet Study, supported by Pearce et al.'s (2007) findings.

Is EPA's approach and rationale transparently and objectively described?

The approach is not entirely transparent and the description could be improved. A sample calculation for Table 4 describing how perchlorate intake for bottle fed infants is estimated would be helpful.

Is 1.42 µg/L an appropriate value to use for the concentration of perchlorate in infant formula? Are there other better or equally valid alternative approaches or values that could be used?

According to the Pearce et al. methodology:

> "Seventeen brands of infant formulae were also assessed for iodine and perchlorate levels. A single sample of each different type of liquid formula available at a local supermarket was purchased for testing. Nine brands were sold in concentrated form and designed to be diluted by half before use. Iodine and perchlorate levels were measured directly in these samples, and the results were divided by half to reflect the concentration intended for infant use. The other eight brands were sold ready for use."

Thus, for the nine formula that were designed to be diluted, Pearce et al. assumed that there was no perchlorate in the diluting water. EPA reports the correct average of 1.45 µg/L calculated from 17 Pearce et al. samples. But for the young bottle fed infant the calculation should reflect the intake of perchlorate from neat formula plus the intake from the water used to dilute it. It is reasonable to assume that the only perchlorate intake in the seven and 60 day infant would be water and formula. Thus the undiluted values for formula perchlorate should be used.

The undiluted average from Pearce is 1.97 µg/L, but that includes formula that is ready to use undiluted as well as formula that requires dilution. For use in Table 4, the focus should be on

concentrations of formula that would require dilution. The young seven and 60 day infant population drinking ready to use formula with no other consumption is more a concern of the FDA than the EPA; they would not be receiving perchlorate contaminated tap water. In the Pearce et al. study, the perchlorate concentration in the 9 samples of formula that would be diluted was 1.96 µg/L. The two highest of the nine values reported would require dilution correspond to 3 µg/L and 3.2 µg/L, double the value reported in Pearce et al. Table 1.

The problem with the FDA data is that they represent composite samples, prepared as they would be expected to be consumed. Also, the detection limit used by FDA is 1 µg/L. The composite would be averaged across different formula brands and certain types. Thus they do not provide an indication of what higher end exposures might be. The composite sample results, in units µg/L, are:

202 Infant formula, milk, hi-Fe: ND, 2.5, 2.0, 2.0
203 Infant formula, milk, lo-Fe: 1.2, ND, 3.6, 2.1
309 BF, infant formula, soy: ND, ND, 0.8 *, 0.8 *
* indicates above the limit of detection but below the limit of quantitation and ND indicates not detected.

Each value represents a composite from three cities in a given region. Thus a concentration in particular product may be three times as high as the value reported. Because of consumer loyalty and habit it is far more likely that a consumer will use the same product over an extended period of time. From the values tabulated, value of 1.42 µg/L will be an underestimate of perchlorate concentration in contaminated infant formula. Further, the concentration of perchlorate in water used by FDA to prepare the formula in to-be-eaten form has not been reported, but is likely to be low or not present, given the several NDs in the table. Because FDA uses composite samples, it would be preferable to use the high end value from FDA (3.6 µg/L) or a value of say 3 µg/L from Pearce et al. Clearly better and more extensive measurement of perchlorate in infant formula is desirable.

(E) Radioiodide excretion into breast-milk by NIS

E-1. In the model, EPA included perchlorate inhibition of NIS radioiodide excretion into breast-milk, as well as inhibition of radioiodide transport by perchlorate for all NIS-containing tissues, thereby making the code consistent with the model description in Clewell et al. (2007). Is this inclusion appropriate? Are the impacts of this inclusion transparently and objectively described?

It is reasonable and appropriate to assume that perchlorate inhibits the transport of iodide in NIS containing tissues and iodide excretion into breast-milk. The impact of its inclusion is transparently and objectively described. There is a straightforward layout in Appendix A of changes in model assumptions and their impacts. Further, the effect of decreased iodide levels in breast milk from smoking – with potential inhibition caused by thiocyanate - has also been observed (Laurberg et al. 2004, J Clin Endo Met 89:181-187), consistent with the finding that this should be taken into account in the modeling.