

Preliminary Comments from Members of the Perchlorate Advisory Panel on the EPA White Paper: Life Stage Considerations and Interpretation of Recent Epidemiological Evidence to Develop a Maximum Contaminant Level Goal For Perchlorate (May 18, 2012).

Comments Received as of July 16, 2012

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Comments from Dr. Anderson

The epidemiologic human data reported to date suggest that the levels of perchlorate present in the US drinking water are unlikely to exert deleterious effects on the thyroid gland in adults and perhaps neonates. No studies have assessed the thyroidal status of the fetus in context of maternal perchlorate ingestion, which is not unexpected due to the difficulty of conducting such a study. Thus, studies of perchlorate effects on the pregnant female are of exceptional importance. In this light the maternal TH status during pregnancy has been shown in published studies to be not linked to perchlorate ingestion suggesting a lack of effect of environmental perchlorate ingestion on the maternal thyroid. Nonetheless, given the importance of assessing this life stage for the development of drinking water standards, additional statistically powered studies are necessary. The designs of the published studies were too different, in some the perchlorate levels were very low, and some were underpowered to confidently state that one study is of greater relevance than others. Given that maternal thyroid status has been shown to predict intellectual development of offspring it is reasonable that assessments of maternal thyroid status during pregnancy can provide appropriate insight into the status of the fetal thyroidal state. In addition, the possible linkage between adult female thyroidal status and perchlorate ingestion remains somewhat equivocal with the highly powered Blount study showing an association of perchlorate with T4 and TSH in women, however other subsequent studies have not detected such a linkage.

Additional animal studies may be necessary as well to better guide risk analysis. A recent study by Gilbert and colleagues (*Environmental Health Perspectives* 2008. 116(6): 752-760) assessed dose response effects of perchlorate on neurodevelopment in rats. This is the most complete study of this nature published to date and importantly showed altered neurodevelopment at even the lowest perchlorate doses assessed. These findings should be confirmed and extended to even lower doses and provide a good basis for assessing developmental perchlorate exposure at specific doses and neurodevelopment. Future animal studies should necessarily assess the known environmental perchlorate drinking water levels and effects, if any, on neurodevelopment. This has not been done to my knowledge.

The epidemiological focus on combined iodine deficiency and perchlorate exposure are extremely important and really should be extended to other possible thyroidal toxicants and dietary conditions that could adversely impact the thyroid such as other micronutrient deficiencies. Equally importantly, there has been no attention paid to possible genetic influences and perchlorate exposure. While the population level assessments of combined deficiencies are equivocal, at an individual level perchlorate exposure in the face of moderate to severe micronutrient deficiencies are likely to result in exacerbated thyroidal impact. Again targeted animal studies are likely necessary to provide necessary guidance to this question as human population studies may not be powered adequately to reveal the effects of these putative interactions. What is “adequate” iodine intake at various life stages in the face of perchlorate exposure? No clear answer is available in the literature.

Importantly, there are no data in humans or animals of the effects of perchlorate dosing on brain thyroid hormone levels. This is not a trivial issue to address experimentally but is extremely important as the brain hormone levels do not necessarily follow blood levels of hormone due to the requirement for thyroid hormone transport across the BBB and impact of local brain deiodination reactions on the levels of brain T3 achieved.

Comments from Dr. Barton

How should EPA consider PBPK modeling to derive an MCLG for perchlorate?

The analyses that EPA presented in the White Paper using PBPK modeling were appropriate and useful and such analyses should continue to be part of deriving an MCLG for perchlorate. From this point of view, it was disturbing that Table 1 (MCLGs derived using a modified default approach without the model) was in the main text, while model-based analyses were relegated to the appendix, and Figure 1 was in the main text suggesting that the predicted doses were more important than the predicted inhibition of iodide intake.

One challenge of deriving an MCLG is how to consider the RfD. One interpretation is that it is necessary to avoid exposure to more than 0.7 ug/kg/day (the RfD), the approach in Table 1. EPA can take this approach and then have no idea to what degree it is being protective and for what proportion of the population as is clearly evident in the materials they have provided.

The other interpretation is that one is trying to prevent neurological effects that would arise from inhibiting iodide uptake into the thyroid and thus thyroid hormone production, so the key value of the RfD was basing it on a perchlorate dose that resulted in a decrease in radioactive iodide uptake (RAIU) of approximately 2%. This level of inhibition in the Greer et al., 2002 study was felt to be within the individual intraday variability of iodide uptake as well as being approximately the detection limit of the assay. The analysis in Table A-4 is a more appropriate analysis asking what the predicted RAIU inhibition would be for different life stages assuming different drinking water concentrations. This analysis shows that, using the upper 90% percentile for drinking water intake, the inhibition at the different life stages is 2% or less for a “biologically average” individual for drinking water concentrations of 15 ug/L.

Many of the questions for developing a health protective MCLG (e.g., charge questions for integration of information) reflect a lack of information that directly link perchlorate in drinking water with adverse outcomes. Absent that, an alternative approach would be to use a mode of action analysis, defining the steps involved in the process to the extent possible and asking about linkages among these steps. In this context, the PBPK model provides a relationship (for the average individual at each lifestage) between drinking water intake and perchlorate pharmacokinetics. The model also predicts the key first pharmacodynamic step, that is inhibition of the target, the sodium-iodide symporter, by perchlorate. The steps after that, alterations in thyroid hormones and neurodevelopmental impacts are not included in the model but there is some literature cited that addresses these aspects. If EPA wants to begin to estimate the extent of effects and the size of the populations involved, focusing more on a mechanistic mode of action analysis might begin to provide useful estimates in a way that focusing on 0.7 ug/kg/day does not.

Since the various epidemiological papers sometimes report water concentrations, but often report other measures, notably urinary perchlorate, it also would be beneficial to use the PBPK model to input the water concentrations that would give rise to the measured urinary perchlorate so that there was a common metric for comparing all the studies. This is a bit tricky since the model does not yet address population variability in perchlorate pharmacokinetics and the pharmacodynamic step of symporter inhibition, but it still could be done for the average individual in the studies. This is unlikely to resolve the mix of negative and positive findings, likely arising from a variety of factors including (as clearly noted in specific studies) varying baseline iodide intake or exposure to other inhibitors, for example via smoking, having major

impacts. However, it would be useful to be able to place all the studies on an estimated common scale.

What are the strengths and limitations of the two PBPK model results described in this effort?

The evaluation of life stage-dependent variations in RAUI for a fixed dose (the POD/NOEL of 7 ug/kg/day for healthy adults) is a useful assessment of the sensitivity of the different life stages. It indicates that the recommendation to apply an uncertainty factor to the POD to account for interindividual variability when obtaining the RfD was appropriate. The previous peer reviews of the PBPK model have supported its use, although there are limitations on how well one can know that the parameter values at the different life stages capture the average behavior at each stage. EPA made a number of modest changes and improvements to the model following its peer review supporting its continued use.

One issue raised with the relative life stage sensitivity to inhibition is whether the differences predicted for specific life stages should be no more than half the uncertainty factor of 10 reflecting PK variability, and if greater than that an adjustment might be needed. Since the endpoint analyzed is inhibition of iodide uptake by the symporter, the endpoint is the first key pharmacodynamic step so it is not clear that one should focus only on the part of the uncertainty factor related to PK. While it would be ideal to have models that addressed the full range of biological variability, it would be necessary to model normal iodide intake, the production of thyroid hormones, and the response of neurological development to those levels; this is far beyond the scope of the current model. On the other hand, since iodide uptake is essentially the first step in this cascade, limiting changes in this step should limit the potential for perchlorate to have significant health effects though this cannot overcome the deficits that may arise from low iodide intake or the possible need for relatively higher thyroid hormone levels to support superior neurodevelopment. As noted above, this also provides a key step in a mode of action based analysis that might begin to answer EPA's questions about protecting human health in a way that their default analysis does not and cannot.

The analyses of RAUI inhibition predicted for drinking water concentrations at different life stages is particularly valuable. It reinforces that inhibition is the combined result of differences in drinking water consumption, iodide (in the case of breast fed infants), and life stage-dependent biological parameters defining perchlorate pharmacokinetics. The analysis uses the upper 90th percentile on drinking water consumption; one could do the analyses for different percentiles but obviously the inhibition will be predicted to be less for lower percentiles and greater for the higher percentiles so the approach EPA selected seems reasonable. While the model can predict RAUI inhibition below 2% (e.g., adults are predicted to have approximately 0.2% inhibition), these values are unmeasurable so their significance is particularly unclear (e.g., they may simply be in the biological noise). Overall, this analysis provide some confidence that measurable perchlorate levels in drinking water will have minimal effects on iodide uptake and thus thyroid hormone levels for the vast majority of the population, including sensitive early life stages.

Note: On page 17 of the white paper, EPA states "It is important to note the PBPK model predictions address the pharmacokinetic characteristics, but the pharmacodynamic factors are not addressed in the PBPK modeling (e.g., receptor binding, thyroid axis regulations)." This statement is incorrect because the model predicts inhibition of the symporter (expressed as RAUI inhibition), so it does include the first and key step in the pharmacodynamics. Given the

widespread use of PK and PD in the scientific literature, particularly for pharmaceuticals, it really isn't appropriate for EPA to be redefining what is PK and what is PD. Based upon EPA's definition, much of what is published as PKPD modeling would be incorrectly described. Modulation of the target, whether measured as target occupancy or changes in target activity, is a critical step for defining the PD relationships. This model is appropriately described as a PBPK-PD model. This nomenclature does not imply that it describes every step in the pharmacodynamic processes between symporter inhibition and developmental neurological impacts.

Comments from Dr. Carrasco

Although some of the studies reported are admittedly complex, it is clear that many of them were carried out in a rather incomplete fashion. Values for some of the anions (perchlorate, iodide, thiocyanate) were obtained for some bodily fluids, such as maternal milk and urine, but not for blood, which would have been extremely informative for the purpose of determining the concentration gradients of the anions. Similarly, in some of the studies, only some thyroid function parameters were measured. Without a complete panel of thyroid function indicators, it is difficult to draw solid conclusions. A few examples are given below.

In the paper by Amitai *et al*, serum perchlorate levels were determined in blood donors rather than in the mothers. Furthermore, the sample size was extremely small (4-5); by contrast, T₄ levels were determined for 97-843 newborns. It is unclear how many samples were used to quantitate the concentrations of nitrate, thiocyanate, and iodide and conclude that there was a statistically significant difference in serum iodide concentrations between the exposed and control populations.

In the paper by Dasgupta *et al*, the authors based their estimations of excretion amounts and their calculation of selectivity on the premise that A (NIS) reacts independently with B, C, and D (the different anions). This assumption is incorrect, given that, for instance, perchlorate competitively inhibits the transport of iodide: perchlorate is transported using the same NIS binding site as iodide. Moreover, the apparent affinity of NIS for perchlorate is over 10 times higher than that for iodide.

In the paper by Téllez *et al* (supported by the Perchlorate Study Group), there are many results that are hard to explain. The lowest urinary iodide excretion was found in Taltal, the city with the second highest concentration of iodide in the water out of the three cities that were tested; in the postpartum samples, maternal thyroid function was analyzed in ~40 women per group, but urinary perchlorate levels were measured in far fewer. The reported concentrations of iodide and perchlorate in the milk do not correlate with the amounts of iodide and perchlorate in the water. Inexplicably, serum perchlorate levels (provided in the data from the first and second prenatal visits) are only given in the postpartum data for the inhabitants of Antofagasta (below detection levels), and not for the other two groups. The statements based on potency of inhibition do not take into account the critical importance of the relative concentrations of the anions, or the apparent affinity of NIS for the anions.

It should be clearly stated that perchlorate not only significantly inhibits NIS but also is an actively transported substrate of NIS (Dohán *et al* (2007) *PNAS*, Tran *et al* (2008) *Am J Physiol Endocrinol Metab*, Paroder-Belenitsky *et al* (2011) *PNAS*).

The paper by Laurberg *et al* (2004) in *JCEM* should be discussed. It shows an association between smoking and reduced iodide concentration in the milk and in the neonate's urine.

There is a mistake in Table 1. The three rows labeled "breast-fed infants" actually contain data from lactating mothers. In addition, given that perchlorate is actively transported by NIS in the placenta and lactating breast, the MCLG values presented in rows 2-5 seem high.

Page 17, last paragraph: it should say "perchlorate-inhibited" rather than "perchlorate-mediated."

Page 20: although the ultimate target tissue is indeed the thyroid, the expression of NIS in both placenta and lactating breast should not be overlooked.

In summary, because some of the studies report inconsistent data and/or results that are overinterpreted, and because more complete studies are badly needed, efforts should be made to design and carry out new studies to overcome the limitations of those that have already been done.

Comments from Dr. Emond

How should EPA consider PBPK modeling to derive an MCLG for perchlorate?

- According to Table 3, the Radioactive Iodide Uptake (RAIU) at the point of departure (POD) was 7 micrograms per kilogram per day ($\mu\text{g}/\text{kg}/\text{day}$), and this showed an important inhibition during the neonatal life stage until 60 days post-delivery. At this stage, the RAIU represents a maximum of 12.5% at Day 7 of exposure for the newborn to 7.9% at Day 60. A little increase occurred from Day 0 to Day 7; the mother probably did not nurse the newborn that much at Day 0. However, sometime between Day 0 and Day 7, something different occurred because the RAIU reached a maximum, and then the inhibition decreased linearly.
- For the maximum contaminant level goal (MCLG) calculation, we should consider the newborn to be in the sensitive life stage; therefore, the neonate should be protected if this prediction is relevant to the observation for this group age (most important if data exist).
- A better characterization of this life stage between Day 0 and Day 7 should be investigated using the model to understand why this stage is so sensitive.
- If a sensitivity analysis has not yet been performed, this should be conducted to gain an understanding of the sensitive parameters that have driven this observation.
- We need to be able to understand how this prediction is correlated with epidemiological data.
- Considering that life stage, we may also characterize the feeding of newborns with bottles.
- With the physiologically based pharmacokinetic (PBPK) model, we have a way to describe the mode of action; however, we still have some gaps concerning kinetic or dynamic behavior. Despite these gaps, the PBPK model represents a gold standard that need to be encouraged.
- According to the results presented in Table 3, the PBPK model used on a sensitive population seems to be enough to protect the general population. However, we could introduce a supplementary uncertainty factor of 3.16 (≈ 3) depending of the availability of the data.
- The most important question is whether we have enough data for this subpopulation to support this PBPK modeling prediction.

• What are the strengths and limitations of the two PBPK model results described in this effort?

Strengths

- The gestational model written by the R. Clewell group published a different version of its PBPK model with an improvement or description pathway for different life styles.
- According to the scientific literature, the science converges to say that the sodium iodide symporter (NIS) seems to be the limiting step or a least the major factor that contributes to the toxicity.
- The PBPK model coding has previously been reviewed (please see reference document EPA/600/R-08/106A, dated October 2, 2008). Even with some minor corrections that were made to the models, it appears that the reviewers believed that the models were adequate enough to predict the kinetic distribution of perchlorate, including the iodine competition on the NIS, which is one of the major modes of action driving the RAIU distribution.

Limitations

- The multiple models are a limitation because these dilute the strengths of this risk assessment.

- We cannot tell whether the same person is represented by these two models (pregnant and not pregnant). However, if a woman aged 20 years becomes pregnant, then we can tell this is the same woman because we can observe all of the physiological modifications that come with pregnancy, but in this case, we have two models of women—three models total if we count the one as an older child. Another limitation is that we do not know whether the models predict exactly the same profile? A better way and more adequate way to do that is to develop one framework with a switch for inactivate pregnancy or another mode of action.
- It is difficult to follow the mindset behind the approach taken because EPA used two different models to predict different life stages, and I have not seen the rationale to do this.

Comments from Dr. Fisher

Comments on “Factors influencing the sensitivity of fetuses, neonates, infants and children”, page 10.

- 1) Bullet 5, ‘slower urinary clearance in neonates’. This statement is incorrect. Studies have evaluated urinary clearance of iodide from infancy (few weeks) to the first years of life. Urinary clearance of iodide in the young is more rapid than in adults (Malvaux et al., 1965, Oddie et al., 1966, and Ponchon et al., 1966). By analogy, this probably applies to perchlorate.
- 2) Bullet 3, ‘thyroidal turnover of iodide’, The HPT axis in the fetus, neonates and young children are accelerated in terms of thyroidal uptake of iodide and the formation and secretion of thyroid hormones from the thyroid gland and urinary excretion of iodide (after birth). The turnover is naturally greater in iodide deficiency because the thyroid stores (mass of iodide and thyroid hormones) is less, so the fraction of the stores that is replaced, on a per day basis, goes up for the adult and child. Also TSH stimulates the HPT axis causing an increased throughput in iodide deficient conditions. Increased turnover does not necessarily equate to vulnerability (eg., increased blocking effects of perchlorate on thyroidal uptake of iodide).
- 3) During fetal development the mother contributes her T4 to the fetus. On page 8, Vulmsa et al. (1989) is cited with a value of 30%. That is, 30% of the fetal T4 is derived from the mother. Please state where in the paper, the 30% was derived from. It seems that a range of values may be more appropriate? This is important because the PBPK model probably was not calibrated to reflect the iodide pool in the fetus derived from fetal metabolism of maternal T4 and also does not account for maternal T4.

Charge Questions.

1) How should the EPA consider PBPK modeling to derive an MCLG for perchlorate? The PBPK modeling should be used to help reduce uncertainty in deriving an MCLG. Although based on many scientific studies, the predictions remain theoretical for the most sensitive populations. Thus, the PBPK models should be viewed as computational evidence (support) to aid in decision making, in a similar fashion as key research studies do, which are also limited, in some fashion, for the task at hand.

2) What are the strengths and limitations of the two PBPK model results described in this effort?

Using a PBPK model to calculate RAIU estimates for life stages, on one hand is daunting because of the lack of data to verify the estimates in the fetus and young, or a pregnant mother, but on the other hand, the modeling provides a theoretical construct, with uncertainty, to evaluate life stages, going beyond the limits of data. Thus the computational evaluation has merit and value because the model is focused on the primary mode of action for perchlorate. By far, the most sensitive elements in the RAIU model are the model predicted radioiodide and perchlorate concentrations in serum because the K_m or K_i for the NIS protein is so much greater than the model predicted serum levels (V_{max} not sensitive). In this regard, assumptions about systemic clearance of perchlorate or radioiodide (see comment earlier) may affect model predictions for % inhibition of RAIU for the young. An evaluation of this aspect of the model is worthwhile to ensure similar outcomes (or not).

The evaluation of RAIU inhibition at the lowest dose of perchlorate in the Greer study seems interesting, but the second PBPK RAIU simulation series is most interesting because it reflects human dietary and environmental exposure to perchlorate, not an experimental dose of perchlorate.

As a modeler I am curious about which model parameters most influenced the % inhibition calculations in the young? For example, serum levels of perchlorate increased because intake or assumptions about excretion in urine, both, or perhaps other model parameters?

GOING BEYOND RAIU

Another aspect of the modeling work is that it only reflects uptake (and binding) of radioactive iodide in the thyroid gland, and as such, represents only a precursor event in terms of the potential disturbances of the HPT axis. The relationships between iodide intake, perchlorate exposure, disturbances in the HPT axis (RAIU and thyroid hormone changes) and adverse outcomes are complex. A computational effort is ongoing to evaluate serum thyroid hormones changes (eg., free serum T4) in the pregnant mom and fetus for a range of dietary iodide intakes (70 to 250 µg/day) and exposures to perchlorate (0.001 – 1000 µg/kg/day). A portion of this work was presented at SOT 2012 (Lumen et al., 2012).

A biologically based dose response (BBDR) model for the HPT axis in pregnant woman and fetus at near term is comprised of 4 maternal and fetal sub-model compartments (iodide, perchlorate, thyroxine (T4) and triiodothyronine (T3) sub-models). PBPK sub-models were integrated based on the primary mode of action of perchlorate to competitively inhibit the sodium iodide symporter mediated uptake of iodide. Using the model, the effects of perchlorate on the maternal and fetal HPT-axis were evaluated for a range of iodide intake conditions focusing on maternal hypothyroxinemia, where the fT4 levels drop to the lower regions of normality with no expected change in the serum TSH concentrations.

Annie Lumen simulated the perchlorate exposure conditions shown in the white paper and looked at model predicted changes in serum fT4 levels in the mom and fetus (see Table below). The maternal and fetal fT4 levels at steady state were evaluated for three drinking water concentrations for perchlorate (15, 20, 24.5 µg/L) without and with contribution from food and from ingestion of the POD dose of perchlorate (7 µg/kg/day). A 90th percentile drinking water intake rate of 2.57 L/day and a food intake dose of 0.198 µg/kg/day (Huber et al., 2011) were used for a euthyroid pregnant term mother ingesting 200 µg of iodide per day.

Our modeling focus to date is on predicting conditions of hypothyroxinemia or hypothyroidism (onset of increased TSH production) caused by the interactions of iodide intake and perchlorate exposures. The model calibrated value of maternal fT4 for an intake of 200 µg of iodide/day is 13.8 pmol/L, in reference to which a value of 10pmol/L (a 27.5% decrease) was chosen to represent the lower limit of for a euthyroid state, below which, hypothyroxinemia is assumed to ensue (Moleti et al., 2011). Using this value as a guideline, model simulations suggested that for an iodide diet of 200 µg/day, the maternal exposure to perchlorate would need to be 32.3 µg/kg/day to decrease the maternal fT4 levels to 10pmol/L.

Predicted percent decreases in the fT4 serum concentrations for different perchlorate exposure scenarios								
	Body Weight (kg)	15ug/L		20ug/L		24.5ug/L		POD
		0.494 µg/kg/day	0.692 µg/kg/day	0.659 µg/kg/day	0.857 µg/kg/day	0.807 µg/kg/day	1.005 µg/kg/day	7 µg/kg/day
		Water Only	Water+ Food	Water Only	Water+ Food	Water Only	Water+ Food	

Maternal	72.3	0.58%	0.81%	0.77%	1.01%	0.95%	1.18%	7.61%
Fetal	-	0.98%	1.36%	1.3%	1.68%	1.58%	1.96%	11.85%

References

Huber, DR, Blount, BC, Mage, DT, Letkiewicz, FJ, Kumar, A, and Allen, RH. 2011. Estimating perchlorate exposure from food and tap water based on US biomonitoring and occurrence data. *Journal of Exposure Science and Environmental Epidemiology*, 21, 395-407.

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Malvaux, P, Beckers, C, and De Visscher, M. 1965. Dynamic studies on the inorganic iodine compartment and its exchanges during adolescence. 25, *Journal of Clinical Endocrinology and Metabolism*, 817-822.

Moleti, M, Trimarchi, F, and Vermiglio, F. 2011. Doubts and Concerns about Isolated Maternal Hypothyroxinemia. *Journal of Thyroid Research*, 2011: 463029.

Oddie, TH, Meade, JH, Jr., Myhill, J., and Fisher, DA. 1966. Dependence of renal clearance of radioiodide on sex, age and thyroid status. *Journal of Clinical Endocrinology and Metabolism*, 26, 1293-1296.

Ponchon, G, Beckers, C, and De Visscher, M. 1966. Iodide kinetics studies in newborn and infants. *Journal of Clinical Endocrinology and Metabolism*, 12, 1392-1394.

Comments from Dr. Fox,

Overarching issues not specifically included in the charge questions (developed during Issue IV group call)

Is any perturbation an adverse effect?

Incorporate knowledge of mode-of-action (MOA) as we address each Issue and charge question.

Issue IV: Devising a framework for analysis

Following advice of NRC 2011 (Review of EPA's Draft IRIS Assessment of Formaldehyde) – recommend that we devise a consistent framework or approach to analysis that will allow us to clearly present our review of the scientific information and justify answers to the charge questions (and subsequent recommendations to the Agency).

A Draft Framework for Considering Each Type of Information

- 1) Critically evaluate the quality and content of each type of information in a transparent manner (may need to address each study or component of the larger 'dataset', e.g., life-stage specific intake estimates). Take advantage of available quality evaluation tools (STROBE¹, GRADE², others?) and synthesis methods (systematic review, meta-analysis) for epidemiological literature. Document:
 - a. Strengths
 - b. Limitations
 - c. Information on variability
 - d. Key uncertainties of the information
- 2) What is the contribution of the information to understanding perchlorate exposure, sensitivity, variability, risk? How do specific characteristics limit or support the contribution?
- 3) How do 1 and 2 relate to the charge questions?
 - a. Is the information adequate to answer the questions? What are the nuances?
- 4) Articulate recommendations for each Issue on the basis of this analysis

To inform this analytical framework, can EPA staff please advise the panel on Agency "current thinking/practice/guidance" on integration of information or weight of evidence? Are there key guidance or reference documents?

Other Comments on Issue IV

IV.a. How can EPA best use the total body of information to derive a health protective MCLG while considering the results of epidemiology and biomonitoring data in establishing bounds on potential values?

Implementing a common framework for each Issue may assist the panel and the Agency in extracting and applying the best quality, most relevant data for the task of deriving a MCLG for perchlorate. Under this question we can synthesize and discuss recommended methods/approaches and conclusions for Issues I – III.

¹ Strengthening the Reporting of Observational Studies in Epidemiology (STROBE)

<http://www.strobe-statement.org/index.php?id=available-checklists>

² Grading of Recommendations Assessment, Development and Evaluation (GRADE)

<http://www.gradeworkinggroup.org/index.htm>

IV.b. How can EPA use the available data to estimate reductions in adverse health effects that are likely to result from reducing perchlorate in drinking water?

Need discussion of the adverse health effect and evaluation of the epidemiological data. Refer to Science and Decisions (NRC 2009) recommendations on a unified approach to dose-response. Also, consider re-visiting benchmark dose modeling to inform this question.

Comments from Dr. Heiger-Bernays

LIFE STAGE CONSIDERATIONS AND INTERPRETATION OF RECENT EPIDEMIOLOGICAL EVIDENCE TO DEVELOP AN MAXIMUM CONTAMINANT LEVEL GOAL (MCLG) FOR PERCHLORATE

Issue IV: Pertaining to Integration of Information

1. How can EPA best use the total body of information to derive a health protective MCLG, while considering the results of epidemiology and biomonitoring data in establishing bounds on potential values?

The MCLG is intended to be “the level at which no known or anticipated adverse effects on the health of persons occur and which allows an adequate margin of safety.” Using this rationale, the MCLG should reflect both the potential adverse effects and adequate margins of safety.

The NRC (2005)³ recommended the RfD of 0.7 µg/kg-day, based on a small group of healthy, iodine sufficient adults and includes an uncertainty factor meant to be protective of the most sensitive subpopulation – fetuses of pregnant women who have hypothyroidism or iodide deficiency. Since 2005, a much better appreciation for the numbers of women who are iodide insufficient has emerged. To this point, the science demonstrates that there are populations (that can be estimated) for whom additional inhibition of iodide uptake would be detrimental to the health of their fetus and infants. While the NRC recommended 7 µg/kg-day as the POD, this appears to reflect a LOAEL rather than a NOEL. This is a measurable endpoint, one that is part of the mode of action, and clearly necessary for the decrease in thyroid hormone synthesis and function.

In its White Paper, in Table A-3, EPA presents some data from the pharmacokinetic modeling that demonstrate that at the POD of 7 µg/kg-day, the range in RAIU is 1.6-12.5 – the greatest in the breast-fed infants. These data suggest that at the POD, >10% inhibition occurs. EPA notes that it believes that “this RfD is the most scientifically defensible endpoint available at this time for assessing risk from perchlorate exposure.” In addition, at the RfD, RAIU inhibition is 1.1-6.7 times greater than adults and 7.8 times greater in breast-fed infants. The POD is assigned an uncertainty factor of 10 to derive the RfD.

With a large dataset available, EPA has the opportunity to recognize and evaluate the risks posed to a portion of the population. The difficulty is to establish a defensible, data-driven protocol that can be applied to other agents that may (potentially) perturb the physiology at critical windows of development and also recognize the “natural” variation in these systems across the population.

- a. The NRC in its document entitled: Science and Decisions: Advancing Risk Assessment (NRC 2009)⁴ provide recommendations on a unified approach to dose-response, the necessity to

³ NRC. 2005. Health Implications of Perchlorate Ingestion. National Research Council of the National Academies, Washington, DC: Natl Acad Press.

⁴ NRC. 2009. Science and Decisions. National Research Council of the National Academies, Washington, DC: Natl Acad Press.

estimate and document the uncertainties in the doses, exposures and outcomes. In addition, the necessity to recognize and begin to address the issue of cumulative risk should be integral to EPA's approach to perchlorate.

- b. A priori approaches for inclusion or exclusion or weighting of studies is warranted. Using the recommendations of NRC 2011⁵ (Review of EPA's Draft IRIS Assessment of Formaldehyde), clear descriptions of the studies is possible.
 - c. Perchlorate is a chemical for which the molecular events preceding measured effects are identified. How will EPA treat the effects of the binding since these precursor events have shown to lower T4/TSH (in some studies)? Will EPA retain the recommendation by NRC that this type of hormonal "regulation" is not an adverse effect at all?
 - d. The MCLG is not required to be based on a threshold effect (see carcinogens). In fact, Blount et al. (2006) found associations between urinary perchlorate and serum T4 and TSH in women. There may be a threshold, but perhaps not. If a threshold is appropriate, what level of perturbation constitutes an adverse effect (see a) above) and how will the distribution around this perturbation (or effect) be bounded?
 - e. The EPA has provided data in the White Paper that documents distributions in responses, in exposures and hence in RSC. Using the range of the data, rather than point estimates, allows a more accurate picture of the US population's exposures and outcomes.
 - f. Uncertainty, like the distributions should be explicitly addressed and quantified for at least the most relevant populations (breast-fed and bottle-fed infants of thyroid insufficient mothers).
2. How can EPA use the available data to estimate reductions in adverse health effects (i.e., dose response) that are likely to result from reducing perchlorate levels in drinking water?

In its recent analysis, "The Partial Lead Service Line Replacement Report" (2011)⁶, the SAB found that, "based on the current scientific data, PLSLRs have not been shown to reliably reduce drinking water lead levels in the short term, ranging from days to months, and potentially even longer". A similar finding may be relevant for perchlorate and impact on estimating reductions due to reducing perchlorate concentrations in drinking water, although there may be statistical methods available to estimate the relative contribution from the decrease in perchlorate in drinking water to RAIU inhibition and to estimates of thyroid function in the sensitive population (infants born to mothers who thyroid insufficient).

⁵ NRC (2011). Review of EPA's Draft IRIS Assessment of Formaldehyde. National Research Council of the National Academies, Washington, DC: Natl Acad Press.

⁶ SAB. (2011). Drinking Water Committee Augmented for the Review of the Effectiveness of Partial Lead Service Line Replacements

By examining a shift in the distribution curve, it might be possible to determine the “risk reduction.” However, EPA might present both the risk reduction demonstration along with the need to address cumulative risks.

Dose-response and Pharmacokinetic Modeling:

- a. Is the POD (as NOEL) derived from a small study in healthy women reflective of either our current understanding of the MOA of perchlorate or the sensitive population? Based on newer science and better understanding of thyroid-mediated effects (beyond perchlorate), does EPA still consider the POD recognized by NRC as a NOEL?

Epidemiology & Exposure Data

- a. EPA might use the most robust data on the most relevant populations. Since the examination by NRC (2007), additional studies and data on two populations have accumulated: those of women of child-bearing age who are thyroid insufficient and larger US studies that may have more relevance for US populations.
- b. In addition to the environmental perchlorate data collected in support of this effort, has the EPA reviewed the perchlorate data collected by the states of California, Massachusetts and queried all of the sources of data? This would provide more information on the range of the perchlorate exposures.

Comments from Dr. Herbstman

Issue III: Epidemiological Evidence

Key Question: How should EPA consider the post-NRC epidemiology data in deriving an MCLG?

Ten studies published since the National Research Council's (NRC) 2005 report were identified by the Office of Water. These studies can be roughly categorized into 2 groups: 1) studies that characterize the distribution of perchlorate exposure and iodine (as well as other compounds that interact with the sodium (Na)-iodide (I) symporter (NIS) including thiocyanate and nitrate); and 2) studies that address the association between perchlorate exposure and thyroid disruption, which is a downstream effect of NIS-mediated iodide uptake. No studies directly address neurodevelopmental consequences of perchlorate exposure, which is a downstream effect of thyroid disruption. In addition to the ten identified by the Office of Water, I have identified three additional peer-reviewed studies (Cao et al. 2010; Leung et al. 2012; Pearce et al. 2012) that should also be included in Group 2.

1) Perchlorate exposure and iodide

(Dasgupta et al. 2008; Huber et al. 2011; Kirk et al. 2005; Valentin-Blasini et al. 2011)

Huber et al. assess the relative contribution of food and drinking water to the daily intakes of perchlorate in the U.S. (using NHANES data). This study found that relative to drinking water, food contributes substantially more to human perchlorate exposure (approximately 4:1); this ratio is higher among young children. This is concerning because it is not clear that regulation targeting only water sources will have an adequate impact on overall perchlorate exposure. Looking specifically at perchlorate exposure among young children and infants, exposure to perchlorate from milk (human, cow, or formula) is of concern, as it may displace iodide, which is critically necessary during this developmental window. Valentin-Blasini et al. report that breast-fed infants have the highest levels of perchlorate; however, compared to soy or formula-fed infants, in this sample, their iodide levels were also the highest. In the population studied by Dasgupta et al., only 1 of 13 children examined had adequate iodide intake (based on the IOM recommendations) and 9 of 13 children ingested perchlorate via breast milk at levels exceeding the RfD identified by the NRC. This RfD is based on adult response to perchlorate; the actual dose at which infants could be affected is unknown. While none of the women in the study had both "high" perchlorate and "high" iodide suggesting that there is some competitive transport between perchlorate and iodine, the selective transport of perchlorate was lower than previously estimated. Kirk et al. compared perchlorate and iodide levels in grocery store cow's milk and human milk samples, finding that perchlorate in breast milk is much higher than in cow's milk and low iodide levels in breast milk are "of concern".

Taken together, breast-fed infants should be considered a sensitive sub-population, as the evidence indicates that perchlorate transfer via breast milk is high and in some populations, iodine transfer is low. This combination could make these infants particularly susceptible to thyroid disruption and subsequent neurodevelopmental effects.

2) Perchlorate exposure and thyroid hormones

(Cao et al. 2010; Leung et al. 2012; Pearce et al. 2012; Amitai et al. 2007; Blount et al. 2006; Pearce et al. 2010; Pearce et al. 2011; Steinmaus et al. 2010; Tellez Tellez et al. 2005) The most

influential study in this group is by Blount et al. This study, conducted in NHANES, a nationally representative sample, found that among women who were iodine insufficient (<100ug/L, based on WHO definition), perchlorate was a significant predictor of lower T4 and higher TSH (perchlorate also predicted higher TSH among women who were iodine sufficient). It is of note that in the NHANES samples from 2005---2006, 38% of non---pregnant women of childbearing age and 57% of pregnant women were iodine insufficient (Caldwell et al. 2011).

Other studies attempted to replicate the findings by Blount et al. among pregnant women at various geographical locations, with varying levels of overall iodine sufficiency (in some but not all cases, individual iodine levels were measured). Steinmaus et al. observed an association between perchlorate (dichotomized based on water delivery source) and TSH from the California newborn screening database. This very large study (n=497,458) found that in areas with high perchlorate, there was a significant increased odds of having high TSH levels. It should be noted that iodide was not measured. Cao et al. /observed a significant association between urinary perchlorate and higher (urinary) TSH; the effects were most evident among those with <100 ug/L iodide. The study also found that perchlorate was associated with increased T4 (which is in the opposite direction as hypothesized). All other studies cited above found no association between perchlorate and thyroid hormones. The reason for this discrepancy is not clear. One theory suggests that it may be due to the inability to evaluate the effects of perchlorate on thyroid hormones in an iodine sufficient population. In some studies, iodine was not measured or was measured in only a small subset (e.g., Tellez et al.). In other studies (e.g., Pearce et al. 2011), the sample size is too small to stratify on iodide sufficiency. However, even in circumstances where a relatively large proportion of the study sample is iodide insufficient, allowing for reasonable statistical power (e.g., Turin population from Pearce et al. 2010, n=433), no association between perchlorate and thyroid hormones was observed.

It has also been hypothesized that women with detectable thyroperoxidase antibodies (TPO---Ab ≥ 0.5 U/ml) might be more susceptible to perchlorate---related thyroid dysfunction. The prevalence of positive TPO---Ab among U.S. women was estimated to be 17% in the NHANES III sample (Hollowell et al. 2002). Some studies measured TPO---Ab (e.g., Pearce et al. 2011) and treated TPO--- Ab status as a confounder, which would not allow the effect of perchlorate on thyroid hormones to vary depending on TPO---Ab status.

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Comments from Dr. LaKind

Issue IV: Pertaining to Integration of Information

- How can EPA best use the total body of information to derive a health protective MCLG, while considering the results of epidemiology and biomonitoring data in establishing bounds on potential values?
- How can EPA use the available data to estimate reductions in adverse health effects (i.e., dose response) that are likely to result from reducing perchlorate levels in drinking water?

Major Topics/Questions for Consideration

A. Dose-response

1. Is there evidence in the research published since the time of the NRC (2005) report suggesting that a change in the RfD is needed? Does the PBPK modeling indicate that an uncertainty factor of 10 is insufficiently protective of sensitive life stages?

The NRC (2005) report recommended the RfD of 0.7 $\mu\text{g}/\text{kg}\text{-day}$ which included an uncertainty factor meant to be protective of the most sensitive subpopulation – fetuses of pregnant women who have hypothyroidism or iodide deficiency. In its White Paper, EPA noted that it believes that “this RfD is the most scientifically defensible endpoint available at this time for assessing risk from perchlorate exposure.” However, it also noted (White paper, pg 5) that at the RfD, RAIU inhibition is 1.1-6.7 times greater than adults. This is less than the uncertainty factor of 10 used to derive the RfD. Is there any evidence from human or modeling research indicating that 0.7 $\mu\text{g}/\text{kg}\text{-dy}$ is not protective of the sensitive subpopulations?

2. As EPA states in its White Paper, “there are currently no data available to link perchlorate to neurobehavioral effects in infants in children.” Given the disparate results in the exposure and epidemiology research, is there a “best” proxy for effect (i.e., is one thyroid hormone more reliable than the others for predicting effect? Is one less prone to analytical issues? Less variable based on time of day or other sample collection variables?)? Or is it better to use PBPK modeling and rely on RAIU inhibition for determining an MCLG? Are the models sufficiently validated for this type of reliance?

3. Blount et al. (2006) found associations between urinary perchlorate and serum T4 and TSH in women. Is *any* perturbation an adverse effect? Would this imply that a threshold approach is not relevant to perchlorate? If a threshold is appropriate, what level of perturbation constitutes an adverse effect?

4. The RfD considers iodide-insufficient women as a sensitive sub-population. For this sub-population, even in the absence of perchlorate exposure, thyroid hormone deficiencies may already be impacting the fetuses. For these women, will *any* exposure to perchlorate worsen the outcome? Can an MCLG protect against effects of perchlorate for women whose low iodide levels may be causing adverse effects (i.e., is there sufficient research to disentangle the relative contributions of low iodide and perchlorate to adverse health outcomes?)?

To some extent, the tension between environmental and clinical approaches to this issue has been described (Renner R, 2010 More Iodine or Less Perchlorate?. Environ Health Perspect 118(7): doi:10.1289/ehp.118-a289).

B. Exposure

5. While the RfD is designed to be protective against adverse effects during a lifetime, for perchlorate some of the literature suggests that short-term excursions during sensitive life-stages can result in adverse effects. Does the literature published since the publication of the NRC report suggest that exposures to sensitive life stages are resulting in exceedances of the RfD?

6. Valentin-Blasini et al. reported on perchlorate intake for infants in approximately the first year of life. They found breastfed infants to have higher exposures than formula-fed infants. This appears to contradict EPA's White Paper (pg 20) in which EPA states that the MCLG for breastfed infant should be higher because formula-feeding results in greater exposures.

C. Weight of Evidence/Study Quality

7. If EPA elects to place most weight on epidemiology studies of mothers and infants, can a meta-analysis using weighting for quality of study be conducted? Do any of the results from the cross-sectional epidemiology studies suggest that an MCLG based on life-stage specific body weights, water consumption and RSC and the current RfD would not be sufficiently protective of sensitive life stages? Should any of the studies be excluded from consideration either on the basis of quality or inapplicability to the US?

8. If EPA chooses to place most weight on exposure studies (e.g., NHANES) examining association between urinary perchlorate and changes in thyroid hormone can a meta-analysis using weighting for quality of study be conducted? Do any of these studies suggest that an MCLG using life-stage specific body weights, water consumption and RSC and the current RfD would not be sufficiently protective of sensitive life stages? Should any of the studies be excluded from consideration on the basis of quality?

D. Risk Reduction

9. The estimates of Huber et al. suggest that population subgroups including pregnant women and children 6 years and older would not exceed the RfD (with water intake at the 90th percentile and 15 µg/L perchlorate in water). The results of Purnendu et al. (2008) suggest that some breastfed infants may have intakes exceeding the RfD. For risk reduction, the focus would likely have to be on infants and very young children, recognizing that at water distribution entry points only 1.9% of water samples had detectable perchlorate and for many water utilities, perchlorate was detected one time only (Huber et al). Is EPA confident in the estimates given in the available literature on exposure, including those pertaining to perchlorate in breastmilk? Are the data from the most recent literature in agreement with past estimates of exposure? How can variability in exposures be evaluated?

10. According to the footnote for Table 1, White Paper (pg 13), the RSC values were adopted from Tables 5 and 6 of EPA's Federal Register notice of 2008. However, Table 5 of the Federal Register notice includes RSC information for age groups only as young as 6 months, and Table 6 only as young as 6-11 years. Where do the RSC values for infants in Table 1 of the White Paper come from? These values are obviously important both for deriving an MCLG and for estimating reductions in adverse health effects.

11. Can Valentin-Blasini et al. be used as the basis for estimating risk reductions? Issues: For a study of this kind, the N is a relatively large. However, can we draw national conclusions from a study of 92 infants? Further, as Kirk et al. (albeit with a self-described limited number of samples) did not find a good correlation between breast milk and drinking water perchlorate levels, how would this be accomplished? Can EPA use PBPK models to estimate MCLGs that will correspond to breastfeeding women's intake at the RfD? If such models exist, are they sufficiently validated for this purpose?

Preliminary Recommendations to EPA

Once the issues above have been resolved, the following is recommended:

1. Conduct a meta-analysis using a transparent methodology that scores studies based on quality and has clear inclusion/exclusion criteria.
2. Resolve inconsistencies between White Paper and literature.
3. Explore the use of PBPK modeling for better understanding of relationship between perchlorate in drinking water and breast milk.
4. Directly address the issue of hormone perturbations and "adverse effect."

Comments from Dr. Lipkin

As a pediatrician with expertise in neurodevelopmental disabilities and developmental and behavioral pediatrics, and my inclusion in Issue Area 1 (Sensitive Life Stages), my review of the materials has been particularly focused on articles and discussions in the White Paper centered around perchlorate exposure in fetus, infants, and children and the known and unknown consequences of such exposures.

The White Paper prepared by EPA staff clearly reviews the published data available on exposure to perchlorate in US and other communities, particularly with its presence in the water supply as well as in biological samples in pregnant women and infants. It similarly clearly reports on the limitations in our knowledge of the consequences of perchlorate exposure in fetuses, infants, and children. This limitation challenges the EPA and SAB in drawing conclusions and recommendations that will safeguard the health of these vulnerable populations. While more data is available since the NRC report of 2005, data remains unavailable regarding neurodevelopmental outcome of exposed fetuses, infants, and children. As a result, recommendations will require making assumptions and drawing conclusions based on these assumptions. For example, it will need to be assumed that effects on measures of thyroid function may result in later cognitive or behavioral dysfunction, despite the absence of such evidence.

In addition to the citations provided by the EPA Office of Water, the following additional articles cited in the materials provided may be of additional use to the SAB, particularly those in the Issue Area 1 group:

1. Chang, Crothers, Lai, and Lamm; Birth Defects Research (Part A) 2003- This appears to be the only study examining the neurodevelopment of children exposed to perchlorate;
2. Cao, Blount, Valentin-Blasini, et. al. Environ. Health Perspect. 2010- Recent publication of thyroid function in exposed infants. In my review of the article, I noted that this is an earlier publication by the same group in the provided Valent-Blasini 2011 article, using the same samples, but it offers different data for our consideration.
3. NRC 2005 report- Given the frequent reference to this report and its serving as the starting point for the White Paper, its availability for review or reference during the proceedings may be helpful.

Comments from Dr. Peck

Summary

Of the 10 studies selected for review since the 2005 NRC report, six are epidemiologic studies of the association between perchlorate exposure and thyroid function in human populations and four are exposure assessment/biomonitoring studies. The epidemiologic studies predominantly address maternal thyroid function during early pregnancy (Tellez et al. 2005; Pearce et al. 2010; Pearce et al. 2011) or neonatal thyroid hormone concentration within days of delivery (Tellez et al. 2005; Steinmaus et al. 2010; Amitai et al. 2007). Blount et al. (2006) evaluated the association between perchlorate exposure and thyroid function in a nationally representative sample of men and (mostly non-pregnant) women using data and urine specimens collected from NHANES 2001-2002. This was the first study to separately evaluate associations among women with insufficient iodine intake (urinary iodine <100 µg/L). Thus, all studies have targeted populations that may be potentially vulnerable to the effects of perchlorate on competitive inhibition of thyroid iodine uptake.

All but one of the six studies are cross-sectional study designs, while the study by Tellez et al. (2005) prospectively assessed thyroid hormone measurements in a cohort of 184 women at two points in pregnancy (early and late) and in the mother and neonate following delivery. This study, however, and two others (Amitai et al. 2007; Steinmaus et al. 2010) utilized ecologic measures of perchlorate exposure based on maternal residence in proximity to water supplies with recorded perchlorate concentrations. Amitai et al. (2007) and Tellez et al. (2005) failed to observe an association between high or very high perchlorate in the community water supply and neonatal T4 (Amitai et al. 2005), FT4 (Tellez et al. 2005) or TSH (Tellez et al. 2005) or maternal FT4 or TSH in early or late pregnancy or postpartum (Tellez et al. 2005). Whereas, in a large study of 497,458 newborns, Steinmaus et al. (2010) reported modest increased odds of neonatal TSH greater than the 99.9th or 95th percentiles among those whose mothers resided in California communities with water perchlorate concentrations >5 µg/L. Given the concern for exposure misclassification in studies relying on ecologic exposure assessment, three studies have attempted to improve upon this limitation by using individual-level biomarkers of urinary perchlorate concentrations. Of these, two failed to observe associations between perchlorate concentrations and thyroid function during early pregnancy (Pearce et al. 2010, Pearce et al. 2011). One was a relatively small study of pregnant women in California and Argentina (Pearce et al. 2011) who were characterized as iodine sufficient with high perchlorate exposure averaging two to three times higher than reports for the general U.S. population. The other was a larger study of euthyroid and hypothyroid/hypothyroxinemic pregnant women in Italy and Wales who had relatively low mean iodine levels as a population and were less highly exposed, with urinary perchlorate concentrations averaging between 2 to 5 µg/L (Pearce et al. 2010). In the analysis of NHANES 2001-2002 data by Blount et al. (2006), no associations were observed in men. However, in women with urinary iodine <100 µg/L, urinary perchlorate concentrations were positively associated with TSH concentration and negatively associated with T4 concentrations. In women with urinary iodine ≥ 100 µg/L, perchlorate remained positively associated with TSH, but was not statistically association with T4 concentrations. The geometric mean perchlorate exposure for women in this NHANES 2001-2002 sample was 2.84 (2.54-3.18).

In summary, the current evidence for a potential effect of perchlorate exposure on maternal and neonatal thyroid function is limited and insufficient to draw definitive conclusions regarding the drinking water exposure range of concern. The guidance offered from existing studies of thyroid function during pregnancy indicates that alterations in maternal thyroid hormone concentrations

have not been observed in populations with average urinary perchlorate concentrations as low as 2.1 µg/L (Pearce et al. 2010) or as high as 13.5 µg/L (Pearce et al. 2011). Limiting analyses in these studies to women with urinary iodine < 100 µg/L did not change results. The small size of the study by Pearce et al. (2010), however, limited statistical power to detect existing associations, which reduces confidence in the negative results. Studies of neonatal thyroid function have not incorporated individual measures of perchlorate exposure (Steinmaus et al. 2010; Amitai et al. 2007; Tellez et al. 2005) and are susceptible to exposure misclassification bias. By limiting exposure definitions to level of community water contamination, other predominant sources of perchlorate exposure such as food sources are not considered. Amitai et al. (2007) did not observe associations with neonatal T4 when water sources contained perchlorate concentrations in the range of 42-92 µg/L or as high as 340 µg/L when compared to <3 µg/L. Tellez et al. (2005) observed no statistical association with neonatal FT4 or TSH when water sources contained perchlorate concentrations averaging 5.2 µg/L or 113.9 µg/L compared to <4 µg/L. Steinmaus et al. (2010), however, observed statistically significant associations with high neonatal TSH concentrations when water sources exceeded perchlorate concentration >5 µg/L compared to ≤5 µg/L.

It is notable that the two positive studies of associations with thyroid hormone alterations in women of reproductive age and neonates were also the largest studies (Blount et al. 2006; Steinmaus et al. 2010). General considerations for the interpretation of the existing epidemiologic evidence would include lack of individual exposure assessment, appropriate model specification, adequate evaluation of potential confounders, and in some instances limited statistical power to evaluate vulnerable subgroups. Additionally, all studies are based on the assumption that the perchlorate exposure measurements represent stable, long-term environmental exposures.

REVIEW OF INDIVIDUAL EPIDEMIOLOGIC STUDIES

A. Study of Thyroid Function in General U.S. Population

Blount et al. 2006

Urinary perchlorate and thyroid hormone levels in adolescent and adult men and women living in the United States. EHP 2006;114:1865-1871.

Blount et al. examined the association between urinary perchlorate concentrations and serum TSH and total T4 levels among NHANES 2001-2002 participants, which included 2299 men and women ≥12 years of age. Blood samples collected over a two year period were analyzed for serum total T4 and TSH levels by two different laboratories using two different laboratory methods (microparticle enzyme immunoassay for 2001 samples and chemiluminescent immunoassay for 2002 samples). The two methods were subsequently determined by the NCHS to produce comparable values over the two year time period. Geometric mean urinary perchlorate concentration in women were 2.84 µg/L (95% CI 2.54-3.18). Geometric mean urinary iodine concentrations for women in this population were 126 µg/L. (95% CI 115-138).

Multiple regression models were estimated using population weights to adjust for the complex sampling design. An extensive list of covariates were selected for evaluation based on known or suspected associations with T4 or TSH and extensively examined for proper variable specification. The authors aimed to assess effects of perchlorate that were independent of other factors known to alter thyroid function : age, race/ethnicity, bmi, estrogen use, menopausal

status, pregnancy status, premenarche status, serum C-reactive protein, serum albumin, serum cotinine, hours of fasting, urinary thiocyanate, urinary nitrate and selected medication groups. Models were also controlled for log creatinine by adding the variable as an independent variable in the model to adjust for variability in urinary dilution

Analyses stratified by gender revealed no observed associations between perchlorate and T4 or TSH levels in men. A stratified analysis was conducted for women based on the World Health Organization's definition of sufficient iodine intake as $\geq 100 \mu\text{g/L}$. In women with urinary iodine $< 100 \mu\text{g/L}$ ($n=348$), log transformed perchlorate concentrations were negatively associated with T4 and positively associated with TSH. These findings are considered consistent with competitive inhibition of iodide uptake. Among women with urinary iodine $\geq 100 \mu\text{g/L}$, log transformed perchlorate remained positively associated with TSH but was not statistically significantly associated with T4.

This nationally representative cross-sectional study is advantageous in its use of urinary biomarkers to quantify individual-level perchlorate exposure. The limits of the study, however, are its cross-sectional design and the use of a single spot urine sample to estimate iodine and perchlorate measures that tend to vary over time due to variable sources of exposure (i.e. from food) and its relatively short half-life. The cross-sectional design is an informative and important building block for generating hypotheses, conducting preliminary evaluations and completing descriptive analyses. Causal inference, however, is limited by the reliance on prevalent exposure and outcome measures and the inability to establish temporality. The study has also been criticized for examining total T4 concentrations instead of FT4 concentrations, which are less influenced by fluctuations in binding proteins.

B. Studies of Maternal and Neonatal Thyroid Function

Tellez et al. 2005

Long-term environmental exposure to perchlorate through drinking water and thyroid function during pregnancy and the neonatal period. *Thyroid* 2005;15:963-977.

This study prospectively followed a cohort of 184 pregnant women throughout pregnancy to measure maternal and neonatal thyroid function in relation to perchlorate exposure. Perchlorate exposure assignment was based on residence in one of three cities in northern Chile. Pregnant women from three cities were enrolled during the first prenatal care visit (if < 24 weeks gestation and lived in city for > 6 months and not on thyroid medication or iodine-containing medications within last 3 months). Thyroid function was assessed at the first (mean 16.1 weeks (sd 4.1)) prenatal visit during early pregnancy, the second prenatal visit during late pregnancy (mean 32.4 weeks, sd 3.0) and postpartum by measuring serum T3, FT4, TSH, thyroglobulin, thyroid peroxidase antibodies (TPO Ab), and thyroglobulin antibodies (Tg Ab). Neonatal thyroid hormones were also assessed in cord blood samples. The women in this study population had median iodine levels of $269 \mu\text{g/L}$, consistent with WHO recommendations for sufficient iodine levels.

Perchlorate exposure was ecologically defined as residence in one of three cities characterized by low (Antofagasta), medium (Chanaral) or high (Taltal) levels of perchlorate concentrations in the municipal water supplies. Perchlorate was also measured in samples of home tap water from each subject. These measures were used to describe the mean perchlorate concentrations in the different geographic areas (below the limit of detection ($< 4.0 \mu\text{g/L}$), $5.2 \mu\text{g/L}$ (sd 0.63) and $113.9 \mu\text{g/L}$ (sd 13.3), but the tap water samples were not evaluated in association with thyroid hormone

function. Individual-level urinary perchlorate concentrations were also measured, but analyses of the individual-level exposure data was secondary and not described in detail. The only description of these analyses indicated that regression analysis of 281 observations with both urine perchlorate values and serum thyroid hormone data resulted in “no significant correlation between perchlorate excretion and T3, FT4, TSH or Tg.” Since only 184 pregnant women were recruited, these analyses must have included the measures repeated at first and second pregnant visit, but details such as point estimate, precision of confidence intervals, control for confounding variables were omitted.

Regression analyses controlling for maternal age, parity, weeks gestation (or weeks post-partum) and antibodies (TPO Ab and Tg Ab) provided no evidence of associations between residence in areas with high perchlorate concentrations and serum TSH or FT4 levels in early pregnancy, late pregnancy, the post-partum period or in neonates (in serum cord blood). Maternal T3 levels at the first and second prenatal visits were statistically significantly elevated among residents of Chanaral, the city with moderate perchlorate concentrations in their municipal water supplies, as compared to the cities with the lowest and highest water concentrations of perchlorate. However, T3 levels did not differ between those residing in the city with the lowest and the highest average perchlorate concentrations in their drinking water. Neonates in Chanaral (the same city with moderate perchlorate concentrations in their drinking water) were found to have lower T3 and lower thyroglobulin (Tg) concentrations compared to those residing in the other cities, while these values for neonates in the other two cities did not differ from one another.

Other outcomes assessed in this study included birth weight, birth length and head circumference and these did not differ between cities (controlling for maternal age, parity, gender, gestational age and antibodies). Breast milk iodine levels also did not decrease with increasing perchlorate in the municipal water supplies.

Women in the city with the highest water perchlorate concentrations (Taltal) had lower urinary iodine levels during late pregnancy and post-partum, but first trimester iodine levels were only significantly lower after concentrations were corrected for creatinine.

The primary limitation of this study is the potential for exposure misclassification resulting from the use of city residence to define perchlorate exposure. Although the median urinary perchlorate concentrations characterized a range of population perchlorate exposures from lower to higher values (20.5 µg/g creatinine in Antofagasta, 37 µg/g creatinine in Chanaral and highest at 110 µg/g creatinine in Taltal), the distribution of individual-level urinary perchlorate concentrations in the 3 cities overlapped, indicating the presence of misclassification of individual exposure levels when measures were based only on city of residence.

C. Studies of Thyroid Function During Pregnancy

Pearce et al. 2011

The effect of environmental perchlorate on thyroid function in pregnant women from Cordoba, Argentina and Los Angeles, California.

This cross-sectional study evaluated first trimester thyroid function and urinary perchlorate concentrations in 134 pregnant women from Los Angeles (mean gestational age 9.1 weeks +/- 2.2) and 107 pregnant women from Cordoba, Argentina (mean gestational age 10.0 weeks +/- 2.0). Median iodine concentrations in the two groups were 144 µg/L for the California subjects and of 130 µg/L for the subjects from Argentina, although one-third of the values were reported to be less than 100 µg/L. Median urinary perchlorate concentrations measured in spot urine samples were 7.8 µg/L (range 0.4-284) and 13.5 µg/L (range 1.1-676), respectively. Thus,

exposure in this sample was two to three times greater than previous reports for the U.S. population.

Using linear regression models, no associations between log transformed perchlorate and (log)serum TSH, free T4 index or total T3 were observed overall or when the subset of women with iodine values <100 µg/L was assessed separately (among 40 California women and 43 women from Argentina). Models were adjusted for urinary iodine, creatinine, gestational age and thyroperoxidase antibodies; however, adjustment for iodine and TPO Ab status is questionable. Since the mechanism by which perchlorate may alter thyroid hormone status is by competitively inhibiting iodide uptake, iodide concentrations could be considered to lie on the causal pathway between perchlorate and thyroid hormone alterations. Inappropriately controlling for a causal intermediate can distort results, presumably by underestimating the true perchlorate effect due to controlling for effects that occur through this pathway. Furthermore, the rationale provided for controlling for both iodine and thyroperoxidase antibody titers is that women with low iodine or TPO antibodies may be susceptible to perchlorate exposure due to some underlying compromise in thyroid function. If the effect is expected to differ across defined subgroups, it is appropriate to examine the factor as a potential effect measure modifier (via stratification or interactions) rather than as a control variable.

Crude linear regression model results were not provided for comparison of results with and without control for these factors. Spearman correlations were reported and also failed to detect associations, although a direct comparison with the linear regression results cannot be made. A Pearson correlation using the same transformed variables would have been more directly comparable to the crude regression model and allowed for some assessment of the impact of controlling for iodine.

According to power analyses provided by the authors, the study was powered to detect much larger correlations than those observed; thus, the sample size is too small to confirm the absence of associations between perchlorate and thyroid hormone function with confidence.

Pearce et al. 2010

Perchlorate and thiocyanate exposure and thyroid function in first-trimester pregnant women. *Journal of Clinical Endocrinology and Metabolism* 201;95:3207-3215.

This cross-sectional study was conducted among samples of euthyroid and hypothyroid/hypothyroxinemic women from health centers in Wales (n= 480 euthyroid and 374 hypothyroid/hypothyroxinemic) and Italy (n=526 and 261, respectively). The participants received thyroid screening before 16 weeks gestation (mean 87.6 days at enrollment). Serum levels of FT4, TSH and thyroperoxidase antibody were measured along with urinary perchlore, iodine and thiocyanate.

Urinary iodine was low in subgroups from both locations (medians of 98 µg/L and 117 µg/L for hypothyroid/hypothyroxinemic and euthyroid women in Wales and 55µg/L and 50µg/L for the women in Italy). Median urinary perchlorate for the respective subgroups was 5.04 µg/L (range 0.04-108)and 5.2 µg/L (range 0.02-168) in Italy and 2.1 µg/L (range 0.03-368) and 2.6 µg/L (range 0.3-49) in Wales.

Women were selected from a parent study (a randomized trial) based on availability of stored urine samples and thyroid function data. Since only some women in the original study were randomized to receive immediate thyroid function assay and the remainder had blood specimens stored for measurement after pregnancy completion, it is not clear if the study sample was

restricted to those receiving immediate evaluation or if it included a mix of thyroid assays occurring on fresh and stored samples. If the analysis of thyroid hormones was delayed for some samples until after delivery, it is important to consider whether thyroid hormone levels are stable after approximately 7 or more months of freezer storage. Since specimens were collected over a 5 year period from 2002-2006, it is also relevant to question whether samples were collected and stored at each site for similar time periods and under similar conditions. According to a study by Panesar and Lit (2010), blood specimens analyzed after 8 to 11 years of freezer storage resulted in significantly lower TSH and increased FT4 and T3 concentrations. (Panesar NS and Lit LCW. Stability of serum thyroid hormones following 8-11 years of cold storage. Clin Chem Lab Med 2010;48:409-412.) While thyroid hormones are reported to remain stable in whole blood stored at room temperature for 7 days (Diver et al. 1994), reports of stability across intermediate to long-term freezer storage periods ranging from months to years are limited (Diver MH, Hughes JG, Hutton JL, West CR, Hipkin LJ. The long-term stability in whole blood of 14 commonly requested hormone analytes. Ann Clin Biochem 1994;31:561-5.) If the long-term stability of thyroid hormone concentrations is compromised and the timing of laboratory analyses were related in some manner to perchlorate exposures, results could be biased.

Multiples of the median (MoM) were calculated for TSH and FT4 separately for each center in order to account for systematic differences in thyroid hormone measurements that may have resulted from the use of two different laboratory methods at the two locations.

Spearman correlation coefficients for the bivariate associations between perchlorate and FT4 and TSH concentrations were estimated separately for the euthyroid and hypothyroid/hypothyroxinemic groups at each site and for the combination of the euthyroid groups from both sites. Statistically significant correlations were not observed for either FT4 or TSH, nor when analyses were restricted to those with urinary iodine < 100 µg/L .

In multivariate regression models predicting the reciprocal of the square root of FT4 MoMs among euthyroid women from both sites, no associations with log transformed perchlorate concentrations were observed. In addition to controlling for either cotinine > 500 ng/ml or thiocyanate concentrations, these models were adjusted for urine iodide and TPO Ab positivity, which could be intermediates on the causal pathway or modifiers of the perchlorate-thyroid hormone associations and the appropriateness of controlling for such factors should be carefully considered . Control for an intermediate on the causal pathway or combining etiologically distinct subgroups would be expected to attenuate the observed association between perchlorate and FT4. In similar multivariate analyses, no associations were observed for the square root of TSH MoMs, although iodine was removed as a covariate from the model since it was not statistically significantly associated with the TSH outcome.

It is noteworthy that the selected cotinine cutpoint of >500 ng.ml would represent relatively heavy smoking and would not successfully control for more modest levels of active smoking commonly indicated by urinary creatinine concentration of 15 ng/ml or 50 ng/ml. Other potential confounders such as age, race, bmi, or creatinine concentrations were not considered in these models and of particular note there was no evaluation of confounding or effect measure modification by gestational age to consider the potential impact of changes in increasing FT4 and decreasing TSH concentrations that occur during the first trimester due to increased circulating concentrations of human chorionic gonadotropin and estrogen (Morreale de Escobar G, Ares S, Berberl P, Obregon MJ, Escobar dely Rey F. The changing role of maternal thyroid hormone in fetal brain development. Seminars in Perinatology. 2008;32:380-386.)

Overall, the study incorporated individual urinary perchlorate measurements to examine associations in potentially vulnerable subgroups of pregnant women with hypothyroidism or insufficient iodine status. All samples were collected prior to initiation of L-T4 therapy. The study did not observe associations between perchlorate exposure and thyroid function in samples of euthyroid or hypothyroid/hypothyroxinemic subgroups of pregnant women in early pregnancy or in subgroups within these groupings with urinary iodine concentrations $< 100 \mu\text{g/L}$. The outcome assessment differed from other studies in that FT4 and TSH levels were assessed as multiples of the median. Considerations for interpreting these results including appropriate model specifications, the question of outcome measurement error due to the potential influence of non-uniform specimen storage factors, and adequate control for additional factors that may be reasonably strongly associated with both perchlorate exposure and thyroid hormone function.

D. Studies of Neonatal Thyroid Function

Steinmaus et al. 2010

Perchlorate in drinking water during pregnancy and neonatal thyroid hormone levels in California. JOEM 52;1217-1224.

This large cross-sectional study linked 1998 data from the California newborn screening program with perchlorate measurements from 1997-1998 drinking water program (800 measurements from 200 different water sources) to evaluate drinking water exposure during pregnancy in relation to TSH levels in neonates in 497,458 mother-infant dyads.

Perchlorate exposure was ecologically defined as residence in a community with average water perchlorate concentrations $> 5 \mu\text{g/L}$ or $\leq 5 \mu\text{g/L}$. Communities without perchlorate measurements were classified as unexposed, although analyses removing these observations from the unexposed group did not change the results.

High TSH concentrations were defined using age-specific cutpoints representing the 99.9th and 95th percentiles for ages less than or equal to 24 hours and greater than 24 hours. (TSH levels $\geq 25 \text{ uU/mL}$ (99.9th percentile) or 15 uU/mL (95th percentile) for ages ≤ 24 hours; TSH levels $\geq 25 \text{ uU/mL}$ (99.9th percentile) or $\geq 8 \text{ uU/mL}$ (95th percentile) for ages > 24 hours.) Logistic regression analyses were stratified by age at blood collection after birth (≤ 24 hours or > 24 hours) due to the surge in neonatal TSH levels that occurs within hours after delivery. Potential residual confounding by collection age was addressed by adding indicator variables to the model for five age categories defined by hours of age.

Using the 95th percentile cutpoint for high TSH, the authors observed a positive association between perchlorate exposure and high TSH levels (logistic regression, OR =1.23 (1.16-1.31) for ≤ 24 hours and OR=1.27 (1.22-1.33) for > 24 hours. Using the 99.9th percentile to define high TSH, a statistically significant OR of 1.53 (95% CI 1.24-1.89) was observed among neonates tested at 24 hours or less, but not for neonates tested after 24 hours (OR=0.79. 95% CI 0.41-1.27). The estimate for > 24 hours, however, was based on only 13 subjects. All models were adjusted for gender, race/ethnicity, birth weight, feeding type, mother's age, per capita income and collection age.

The authors provided a thorough evaluation of potential effect measure modification by gender, race/ethnicity, and type of feeding (breast-fed or formula-fed infants) and thorough consideration of impact of confounding by age at time of sample collection by examining alternative variable specifications.

The primary limitation is the potential for misclassification error due to lack of individual measurements of perchlorate exposure. The ecologic nature of the exposure variables may not reflect perchlorate concentrations in the mother's actual water source. The community water measurements are limited to municipal water supplies and would not capture exposure (or lack of exposure) via private wells. Furthermore, without individual level exposure data, the investigation cannot take into account frequency or quantity of drinking water consumed that may be from tap water, bottled or filtered water or from other locations such as work and school. Time at residence in the community was also not addressed. Assigned water concentrations based on residential zip code obtained from the screening record may not represent location or source of water exposure during the etiologic relevant window of pregnancy if mothers moved during pregnancy. Studies of residential mobility patterns have reported that 22% of women in an Atlanta study population moved during pregnancy, with approximately half of those moving remaining within the same county (Miller A, Siffel C, Correa A. Residential mobility during pregnancy: patterns and correlations. *Maternal and Child Health Journal* 2010; 14:625-634.) The

authors presume the direction of this potential bias would be toward the null given the exposure is a dichotomous variable and measurement error expected to be unrelated to TSH levels. However, it remains possible that unmeasured factors such as maternal smoking or unplanned pregnancy could be associated with neonatal TSH levels while also being related to characteristics that influence perchlorate measurement error such as residential mobility or tendency to have well-water in rural areas. Thus, potential for differential misclassification which could influence results in either direction should not be ruled out .

Amitai et al, 2007

Gestational exposure to high perchlorate concentrations in drinking water and neonatal thyroxine levels. *Thyroid* 2007; 17:843-850.

This paper examined associations with between perchlorate exposure during pregnancy and neonatal T4 (thyroxine) levels measured as part of the newborn screening program in Israel. Blood sampling by heel stick was conducted within 36-48 hours of delivery (unless the infant was preterm, where assessment occurred at one week of age). Perchlorate exposure was assigned according to maternal residence in geographic areas in Israel with very high ($\leq 340 \mu\text{g/L}$) , high (42-92 $\mu\text{g/L}$) and low ($<3 \mu\text{g/L}$) perchlorate concentrations in the local wells used for drinking water. Subset analyses were conducted among mother's reporting typical use of tap water in an effort to consider potential exposure misclassification due to lack of individual measurement of exposure.

The sample size was comprised of 97 newborns with very high exposure, 216 with high exposure and 843 with low exposure (total n=1156). Although this study did not assess maternal iodine concentrations, the authors report that a previous study of pregnant women in the same region characterized the population as iodine sufficient (median=143 $\mu\text{g/L}$). (Benbassat C, Tsvestov G, Schindel B, Hod M, Blonder Y, Ben AS. Assessment of iodine intake in the coastal area. *Isr Med Assoc J* 2004;6:75-77.)

No differences in mean T4 levels were observed across the 3 areas defined by levels of water perchlorate concentrations(ANOVA $p=0.95$). The results were unchanged when restricted to mothers who reported tap water as their usual source of drinking water. The authors state that they considered the effects of potential confounders on T4 levels (maternal age, birth weight, gestational age, or gender) but it appears that they only assessed statistical associations between these factors and T4 levels rather than inspecting multivariate models or stratified analyses with and without adjustment for the factors that may potentially distort the T4-perchlorate association .In a subanalysis among mothers who reported tap water consumption, 25 newborns from the very high exposure area were matched to 25 newborn from the low exposure group by gestational age, gender and maternal age. No associations with neonatal T4 levels were observed in the matched analysis.

A separate analysis was conducted to examine serum perchlorate concentrations from samples of blood donors residing in the three study areas. These analyses were based on small numbers (and marginally statistically significant associations) but indicated that mean serum perchlorate levels were highest among those residing in the geographic area with the highest perchlorate concentrations in the drinking water and the average serum concentrations for the high and low exposure group followed in the expected rank order. However, individual serum concentration in the three regions overlapped as would be expected when exposure misclassification of individual exposure status occurs due to the use of aggregate measures of perchlorate exposure based on residential proximity to selected water supplies. Based on the mean serum perchlorate concentration among the 4 blood donors in the very high exposure group ($\sim 6 \mu\text{g/L}$) and default

assumptions about drinking water consumption (2 L/day) and body weight (70kg), the authors estimate that women in this group had a perchlorate intake of 9.7 $\mu\text{g}/\text{kg}/\text{d}$, which was 38.6% greater than the NOEL of 7 $\mu\text{g}/\text{kg}/\text{day}$. Similar estimates for women in the high exposure group resulted in perchlorate doses of 1.2-2.7 $\mu\text{g}/\text{kg}/\text{day}$, still 1.7-3.8 greater than the RfD of 0.07 $\mu\text{g}/\text{kg}/\text{day}$.

It is unclear how or when mother's residence was determined. If residence refers to residential location at the time of delivery (according to birth records) then residential mobility during pregnancy may introduce an additional source of exposure misclassification. The study's primary limitation is lack of perchlorate exposure assessment at the individual level, inability to examine associations among the potentially vulnerable subgroup with urinary iodine < 100 $\mu\text{g}/\text{L}$, and limited assessment of potential confounders. Keeping these limitations in mind, it is notable that no differences in mean neonatal T4 levels were observed in this highly exposed population.

REVIEW OF BIOMONITORING STUDIES

Dasgupta et al. 2008

Intake of Iodine and Perchlorate and Excretion in Human Milk. *Environmental Science and Technology* 2008; 42: 8115-8121.

This study examined 402 breast milk samples and 103 24-hour urine samples from 13 breastfeeding women to evaluate whether perchlorate inhibits iodide transport in breast milk. The volunteers were ages 24-34, non-smokers and mostly Caucasian with infants ranging in age from 55 to 253 days at the time of first sample collection. The number of samples varied across subjects with 3 to 10 24-hour urine samples and 9 to 37 breast milk samples provided over a period of 3 to 10 sequential days. Median perchlorate concentrations in these samples were 3.2 $\mu\text{g}/\text{L}$ (range 0.6-80) in urine samples and 7.3 $\mu\text{g}/\text{L}$ (0.01-48) in breast milk. Median total iodine concentrations were 110 $\mu\text{g}/\text{L}$ (range 26-630) in urine and 43 $\mu\text{g}/\text{L}$ (range 1-1200) in breast milk.

The authors estimated a model of parallel/competitive transport of perchlorate, thiocyanate, and iodine by the sodium iodide symporter, assuming homeostasis (i.e., total input and output are equal). For each subject, an estimate was calculated for the daily fraction of the total perchlorate excretion (in urine and milk) that is excreted into breast milk. The average of the mean daily excretion fraction for perchlorate in milk was 0.562 (sd=0.116, range 0.394-0.781). The excretion fractions for iodine were lower (mean 0.210, sd=0.108, range 0.086-0.4541). The ratio of the excretion fractions for perchlorate and iodine provided the estimate of selectivity of perchlorate transport over iodide in breast milk. The mean selectivity factor for perchlorate was 3.14 (sd 1.20).

Although a previous study by Tonacchera et al. indicated that selectivity for perchlorate transport by the NIS was 30-fold greater than for iodide (in an in vitro study of Chinese hamster cells expressing NIS), Dasgupta et al. (in vivo study) concluded that transport selectivity for perchlorate was 3.14 times greater than iodine, a value that is 10 times lower than the previous report. Since the previous experiment was based on high perchlorate concentrations in an in vitro study of hamster cells, the authors conclude the differences in results may be due to limited NIS availability or because of differences in different mammalian NIS systems.

When the average iodine intake was calculated for the infants of the 13 lactating subjects, only one of the 13 infants was classified as having adequate iodine intake (range 13.8 $\mu\text{g}/\text{d}$ to 320 $\mu\text{g}/\text{d}$) according to the IOM age-specific recommendations for infants of 110 $\mu\text{g}/\text{d}$ for 0-6 months and 130 $\mu\text{g}/\text{d}$ for 6-12 months of age (Institute of Medicine, Food and Nutrition Board. Dietary Reference Intakes. National Academy Press: Washington, D.C., 2001, p 258.) Estimated

perchlorate dose for each infant was calculated based on age and gender-specific infant weights according to the 50th percentile value reported in the U.S. EPA's Child-Specific Exposures Handbook (EPA-600-P-002B, 2002, Table 11-1). According to these estimates, 9 of the 13 infants exceeded the reference dose of 0.7 µg/kg/d (range 0.3-2.1 µg/kg/d). Thus, the small sample of maternal-infant dyads in this study was mostly composed of mothers with sufficient iodine intake (urinary iodine median 110 µg/L, range 26-630) with breastfed infants who had insufficient iodine intake accompanied by high estimated perchlorate intake.

The use of 24-hour urine samples across sequential days, rather than reliance on selected spot urine samples, may have reduced potential measurement error due to within-individual variability in iodine and perchlorate excretion patterns over time. Although multiple samples were measured and averaged for each subject, the estimates of excretion fractions and selectivity were based on only 13 subjects limiting the precision of reported estimates.

Valentin-Blasini et al. 2011

Perchlorate Exposure and Dose Estimates in Infants. *Environmental Science & Technology* 2011. [dx.doi.org/10.1021es103160](https://doi.org/10.1021/es103160)

The authors used data and urine samples from the Study of Estrogen Activity and Development to calculate perchlorate intake dose for infants using 205 urine samples from 92 infants of age 1-377 days. The data are cross-sectional, but collection of up to four samples over time incorporates a semi-longitudinal component. Infants in this study were healthy, full-term infants with birth weights between 2500-4500 grams. This sample of infants was characterized as iodine-sufficient, with a median iodide concentration of 125 µg/L (range 18.8-5210). Participants were restricted to infants who were exclusively fed either breast milk (n=92), cow milk-based formula (n=51) or soy-based formula (n=63) in order to compare perchlorate exposure by feeding method. Urine samples were collected from infants using cloth diapers or urine bags. Because the cloth diapers were found to have measurable concentrations of perchlorate, measures obtained from diaper collection were adjusted by subtracting 1.24 µg/L. Daily perchlorate dose was estimated as creatinine-adjusted perchlorate concentrations from the spot urine sample (µg/g creatinine) multiplied by daily creatinine excretion for age (g/day) and multiplied by the inverse of the infant's weight (kg).

Perchlorate was detected in a higher proportion of urine samples from breast-fed infants (95%) than from infants consuming cow milk-based formula (86%) or soy-based formula (68%). Higher concentrations of perchlorate were also observed in the urine of breast-fed infants (median 3.9; range <0.05-25.8) compared to those consuming cow milk-based formula (median 2.2; range <0.05-13.1) or soy-based formula (median 0.58; range <0.05-5.49). Among formula-fed infants, urinary iodide levels were higher among those consuming cow-based formula (median 151 vs 108), consistent with published reports of higher iodide levels in cow milk-based formula compared to soy-based formula. Positive correlations were observed between perchlorate and iodide levels in all subjects (r=0.55) and separately in breast-fed and formula-fed infants (coefficients not provided). Correlations between iodide and other NIS inhibitors assessed (nitrate and thiocyanate) were not observed.

Mixed linear models were used to account for the lack of independence of multiple measures (up to 4 measurements) for the same infant. The models controlled for age, body mass index, feeding method and sex of the infant. Of note, the intraclass correlation coefficients (ICC), which provide a measure of the variability of the measurements across multiple samples within the same subject, were low for all analytes except iodide (e.g., rho=0.07 for perchlorate). Several potential sources of variability were speculated by the authors including differences in the timing

of sample collection relative to feeding time, amount of diaper contamination, changing brands of formula or different consumption patterns prior to sampling, occasional perchlorate exposure from older infants consuming solid food, differences in urinary dilution (addressed by creatinine adjustment) or changing exposures over the different sampling periods possibly due to changing diet among breast-feeding mothers.

On average, the estimated daily perchlorate exposure dose for all infants (mean 0.255 $\mu\text{g}/\text{kg}/\text{day}$; median 0.160 $\mu\text{g}/\text{kg}/\text{day}$) was below the EPA reference dose of 0.7 $\mu\text{g}/\text{kg}/\text{day}$, but 2.4 times higher than the median estimated dose reported for U.S. adults. Higher estimated perchlorate dose was observed among breast fed infants (0.420 $\mu\text{g}/\text{kg}/\text{day}$) compared to 0.208 $\mu\text{g}/\text{kg}/\text{day}$ for cow-milk formula-fed infants and 0.065 $\mu\text{g}/\text{kg}/\text{day}$ for soy-milk formula-fed infants. Although average for all groups were below the RfD, a total of 9% of the 205 samples (16% of all infants) exceeded the RfD and 6% of those with more than one sample exceeded the RfD multiple times. Furthermore, a disproportionate number infants 2 months or age or younger were among those with the highest estimated perchlorate dose, suggesting that analyses of potentially vulnerable subgroups should consider differences across infant age.

Associations between urinary perchlorate and T4 and TSH in infants were evaluated in this dataset and published separately. Cao et al. (2010) reported increased perchlorate was associated with higher urinary TSH in infants with iodide < 100 $\mu\text{g}/\text{L}$, but associations with increased T4 concentrations in this subgroup were not statistically significant when the models included nitrate and thiocyanate (Cao Y, Blount BC, Vaelntin-Blasini L, Bernbaum JC, Phillips TM, Rogan WJ. Genotoxic anions, thyroid-stimulating hormone, and thyroid hormones in infants. *Environmental Health Perspectives* 2010;118:1332-1337.)

Huber et al. 2011

Estimating perchlorate exposure from food and tap water based on US biomonitoring and occurrence data. *Journal of Exposure Science and Environmental Epidemiology* 2011; 21:395-407.

The authors merged nationally representative NHANES biomonitoring data with the EPA's Unregulated Contaminant Monitoring Regulation (UCMR) public drinking water database to quantify the relative contributions of food and drinking water as sources of perchlorate exposure for 2700 NHANES subjects.

Urinary perchlorate concentrations for NHANES 2001-2002 participants were matched to the public water system data by county location. Individuals were categorized into 3 groups according to perchlorate occurrence in drinking water (drinking water contribution to perchlorate dose [Bin 1], water source unknown [Bin 2], and no drinking water contribution to perchlorate dose [Bin 3]). Individuals were defined as NOT having a drinking water contribution to urinary perchlorate concentrations [Bin 3] if 1) they resided in counties with drinking water systems that measured no detectable concentrations of perchlorate ($\leq 4 \mu\text{g}/\text{L}$) in samples collected at the entry point of their distribution systems; 2) subjects reported not consuming tap water the day before providing the urine sample or 3) if reverse osmosis was used to treat water in the home.

By assuming that the urinary perchlorate concentrations among those without drinking water contribution reflected the daily dose of perchlorate from food sources only, daily food doses of perchlorate were subtracted from the reference dose to estimate allowable drinking water intake. Perchlorate doses ($\mu\text{g}/\text{kg}/\text{day}$), relative food and water intake of perchlorate and estimated water allowance dose were reported by sex, age group and pregnancy status.

Because only single spot urine samples were available for NHANES study participants, the authors used a creatinine adjustment calculation corrected for adiposity to estimate 24-hour urinary excretion and daily perchlorate intake. The mean daily perchlorate intake for all subjects was 0.061 $\mu\text{g}/\text{kg}/\text{day}$, with higher mean intake in the younger age groups, males and pregnant women. Of note, the increased perchlorate intake in pregnant women was proportional to the 9.5% increase in caloric intake compared to non-pregnant women. Lactating women, however, had a lower mean perchlorate dose compared to reproductive-aged women who are not lactating (0.79 $\mu\text{g}/\text{kg}/\text{day}$ compared to 0.083 $\mu\text{g}/\text{kg}/\text{day}$), which is consistent with the excretion of perchlorate into breast milk resulting in lower urinary excretion. The results indicate that for most groups except the youngest ages of 6-11, drinking water represents 15 to 25% of the total perchlorate intake for mean intake levels and perchlorate from drinking water reaches 2-5% of the RfD. At the highest levels of exposures (90th and 95th percentiles), the drinking water contribution increases to 10-30%. For 6-11 year olds, drinking water represents only 2% of total perchlorate intake at mean levels and contributes <1% of the RfD while food contributes more than 20%. Data were not available for younger children or infants.

The linking of these two national data sources provided an innovative effort to address food and water contributions to perchlorate dose in a large population-based sample. There are, however, several limitations of the data that may have introduced errors. Because participant addresses were not available, water source could only be matched at the county level and the subjects' actual perchlorate concentrations in their true water supply could not be accurately assigned. Exposure misclassification would have occurred for individuals who did not live in the service area of the affected entry point at the specific time that perchlorate was detected, or for those who used private wells or bottled water. Water sampling occurred between 2000-2005 with the majority of samples collected between 2001-2003. While the time periods for urine collection (2001-2001) and water sampling may have substantially overlapped, exposure to the measured perchlorate levels in the presumed water source may not have occurred prior to the assessment of urinary perchlorate concentrations. Thus, drinking water contribution to perchlorate is subject to some degree of measurement error, which would influence the accuracy of the estimates of relative perchlorate intake from food and water and the calculation of the water allowance doses. The direction and impact of such errors, however, is difficult to predict. The authors consider the impact of a scenario where 2% of private wells could have detectable perchlorate (false negatives) and conclude that correcting for this error produced no meaningful changes in the results. However, it does not appear that the authors considered the impact of false positives that would simultaneously occur whereby those with uncontaminated well-water may be falsely contributing to the dose estimate from food and water [Bin 1], potentially underestimating food only estimates which could produce an overestimation of the water contribution to perchlorate dose.

Kirk et al. 2005

Perchlorate and Iodide in Dairy and Breast Milk

Environmental Science and Technology 2005; 39:2011-2017.

In this evaluation of perchlorate and iodine in samples of dairy milk and breast milk from multiple states, the authors examined 47 milk samples from grocers in 11 states and 36 samples of breast milk from lactating women in 18 states.

Perchlorate was detectable in 46 of 47 dairy milk samples with a mean concentration of 2.0 $\mu\text{g}/\text{L}$ (range of non-detectable to 11.0). Perchlorate was detectable in all breast milk samples,

with mean levels approximately five times higher than dairy milk samples (mean 10.5 µg/L, range 1.4-92.5 µg/L).

Iodide concentrations were also examined with a mean of 89 µg/L (9.6-388 µg/L) in dairy milk and a mean of 12.3 µg/L (0.2-54 µg/L) in breast milk.

When examining all 24 breast milk samples with available perchlorate and iodide measures, no correlation between perchlorate and iodide concentrations was observed. However, when the data were restricted to those with breast milk perchlorate concentrations > 10 µg/L, an association was observed with 90% ($R^2=0.9042$) of the variation in iodide concentrations explained by the reciprocal of perchlorate concentrations. These results, however, were based on only 6 observations. The authors did not describe the rationale for selecting the 10 µg/L cutpoint for the subanalysis or the reciprocal transformation of the perchlorate concentrations in their regression model. In a critique by Lamm et al. (2005), replication of the analysis after restriction to those with perchlorate concentrations greater than 20 µg/L, which was the cutpoint used earlier in the same paper to categorize high perchlorate concentrations, reduced the R^2 to 0.016. (Lamm SH, Feinleib M, Engel A, Gibbs JP. Comment on "Perchlorate and iodide in dairy and breast milk. *Environmental Science and Technology* 2005;39:5900-5901.) The reanalysis was based on only 4 observations; thus the results are unstable and not in a consistent direction when evaluating increasingly stringent definitions of high exposure. Furthermore, efforts to evaluate associations between perchlorate and iodide measures do not consider potential confounders. Although some details concerning the study design and statistical methods were not clearly described, it appears that the analysis was conducted using a linear regression model based on the average values computed from 4 to 6 breast milk samples obtained for each individual. The timeline for sample collection was not described, but it appears that a repeated measures analysis that takes clustering into account could have improved the precision of the estimated associations.

In a subsample of 17 women followed up in Spring 2004 for additional breast milk and water samples (tap and bottled), no correlations were observed between perchlorate concentrations in breast milk samples from spring or fall and perchlorate concentrations in tap or bottled water. Correlation coefficients were not reported by the authors.

These data are most useful for providing a descriptive analysis of the extent of perchlorate contamination of dairy milk across numerous states. Due to the small sample size and cross-sectional nature of the evaluation of breast milk samples, the study suggests widespread detection of perchlorate in breast milk samples but offers limited statistical power or precision to evaluate associations between perchlorate concentrations and iodide in breast milk. Overall, no correlation was observed between perchlorate and iodide concentrations in 24 breast milk samples. The evidence for association among the most highly exposed subgroup remains unclear.

Comments from Dr. Rovet

PREAMBLE

Thyroid hormone (TH) is essential for the developing brain and is involved in a number of fundamental neurobiological processes such as neurogenesis, neuronal migration, process growth, synaptogenesis and myelination. TH's specific mode of action is to up- or down-regulate major brain genes that underlie these processes. Timing of need for TH within the brain varies among the different processes with some (e.g., neurogenesis, neuronal migration) needing TH earlier than others (e.g., myelination). Additionally different brain regions also vary in when they need TH both *in utero* and after birth. Since most aspects of brain development are complete by the second year of life, normal TH levels must be maintained from conception through to at least the age of two years. Also, as TH also plays a role in later brain functioning by regulating key neurotransmitters, normal levels of TH are also required throughout life. Furthermore because iodine is a key component of TH and it cannot be manufactured within the body, adequate dietary iodine is essential, particularly during pregnancy when the need for iodine and TH increase by as much as 40-50%.

Human autopsy evidence including from abortuses shows that TH receptors are present as early as the first trimester in human brain and that TH of maternal in origin is measurable in brain tissue. Because the fetal thyroid system has a protracted development, the fetus early in gestation has to rely totally on the maternal supply of TH transferred in a regulated fashion via the placenta. Further since the fetal thyroid does not secrete its own centrally regulated TH until the third trimester, an adequate maternal TH supply is therefore essential in the first half of pregnancy and also serves a supplementary role later until the fetal thyroid matures fully by term. In fact, by term as much as 40% of fetal TH still may be from the mother. After birth, small amounts of TH continue to be transferred to the infant in breast milk. Conditions resulting in TH insufficiencies during critical stages of early brain development include clinical or subclinical hypothyroidism in the mother, whereby effects are presumably worse in early gestation; iodine deficiency, which affects both maternal and fetal/infant supplies of TH; congenital hypothyroidism, which typically begins at the end of gestation and continues postnatally for one to two months; and exposure to natural goitrogens or other chemicals that have thyroid disrupting effects on both the mother's and the fetal/child thyroid gland. Conditions that disturb TH production later childhood when most TH-dependent brain development has occurred include juvenile acquired hypothyroidism as well as exposure to TH-disrupting environmental toxicants.

One such toxicant of current interest, and the topic for this review panel, is perchlorate that is present in drinking water and food. Perchlorate inhibits the transport of iodide into the thyroid, as well as the breast. Notably, perchlorate crosses the placenta readily and so can disrupt fetal thyroid production. Thus perchlorate exposure at sensitive life stages will reduce availability of TH for both the developing and more mature brain. Exposure to perchlorate exposure leads to reduced maternal TH availability in early pregnancy, less fetal and maternal TH in later pregnancy, and less TH from the child's own thyroid postnatally. The perchlorate-exposed breast-fed infant is doubly affected because less maternal iodide is received concurrent with its own thyroid production being limited by perchlorate in mother's milk. Thyroid inhibition can also occur in formula-fed infants, if their formula or the water added to powdered mixtures contains perchlorate; likewise, older infants and young children will be affected by the presence of perchlorate in dairy milk and certain foods.

Thus perchlorate can have a significant impact on the human brain throughout development and in the following sensitive life stages: the pregnant woman, the fetus, the breast-fed or formula-fed infant, and the developing child.

ISSUE 1: SENSITIVE LIFE STAGES

The current charge by the EPA to the SAB is to establish the specific vulnerability of individuals at each of the above life stages as assessed in evidence provided in the 10 supplied recent papers (as well as 1 new submission, Mendez & Eftim, in press). These 10 + 1 studies deal with the early pregnant woman (Pearce et al., 2010; Pearce et al., 2012; Tellez et al., 2005), later pregnancy (Tellez et al., 2005), the neonate (Amitai et al., 2007; Steinmaus et al., 2010), infancy (Valentín-Blasini, et al., 2012), the breast-feeding woman (Dasgupta et al., 2008), and the reproductive age woman (Blount et al., 2006; Mendez & Eftim, in press). Two studies also examined perchlorate concentrations in food (Huber et al., 2011), and in dairy and breast milk specifically (Kirk et al., 2005). As no data currently exist linking perchlorate to neurobehavioral effects in infants and children, any inferences on neurobehavior must be drawn from studies of early thyroid insufficiencies and iodine deficiency, for which a considerable literature exists..

Given the current evidence on perchlorate, the EPA has asked four questions on how to consider sensitive life stage factors in deriving an MCLG:

1. What is the effect of life-stage specific differences in body weight and food and drinking water intake?

Huber et al (2011) merged two large data sets [NHANES 2001-2002 and the EPA Unregulated Contaminant Monitoring Regulation (UCMR) of drinking water] to derive values for the mean food intake perchlorate dose and to use this to determine the allowable dose of perchlorate in drinking water for children aged 6-11 years, 12-19 years, females aged 15-44 years, and pregnant females. The authors stratified their sample into three subgroups (termed “bins”): those in counties with reported detectable perchlorate level, those from counties not sampled in the UCMR, and those with samples having perchlorate levels below minimum limit. The approach of this study had several advantages: it used a national sample and involved a large sample size; it conducted separate analyses for males and females; it used creatinine-adjustment equations and made body-weight adjustments; the subgroup comparison into bins allowed for specific analyses; and the analytic approach was strong. A major limitation, however, was that perchlorate was not directly measured in water but rather estimated from values for the counties in which the NHANES sample lived and measures from samples ascertaining their water from wells were not obtained, thus creating a degree of measurement error. From this study, the amount of tolerable exposure of pregnant women and children was calculated. Another limitation was that children below age 6 representing the most vulnerable population were not studied. The authors estimated that drinking water contributes to about 20% of perchlorate intake relative to food; this was seen at all ages, except for children under age 11 in whom drinking water makes a smaller contribution to the total intake.

Overall, I found this a difficult paper to read. While it contributed information for deriving an MCLG, the information provided was not specific for this question. None of the other papers dealt with this issue.

According to the EPA Exposure Factors Handbook (cited in the white paper), the intake per body weight is greater for infants and children than adults, despite lower absolute intakes.

2. Are there differences in severity and permanence of potential adverse effects in neonates, infants, and young children compared with adults?

None of the studies provided dealt directly with the issue of severity and permanence of adverse effects at different life stages. While severity can be estimated from the strength of correlation, permanence requires research on neurobehavioral outcome as well as studies of long-term thyroid functioning following perchlorate exposure and this has not been done. A critical review paper combining research at the different life stages has not (to my knowledge) been done, although several of the studies provided contained charts (Dasgupta et al., 2008) or figures (Tellez et al., 2005) comparing their findings with others at the same life stage.

Effects on Neonates

Three studies examined the effect of maternal perchlorate exposure during pregnancy on newborn thyroid screening values: Tellez et al. (2005), Amitai et al. (2007), and Steinmus et al. (2010). The first took place in Chile, the second in Israel, and the third in California.

Tellez et al. (2005) Study: This study was conducted on 184 pregnant women who resided in three different cities in northern Chile having different perchlorate levels in drinking water. The levels were 114 mg/L for city 1 (Taltal), 7 mg/L for city 2 (Chanaral), and 0.5mg/L for city 3 (Antofagasta). However, two of the cities (Taltal and Chanaral) were small and some of the women from Taltal reportedly chose to deliver in the birthing hospital of the larger city, Antofagasta. Thus it is possible that they drank the lower perchlorate containing water in Antofagasta at the time of delivery. Information about the women is described below (question # 4). At time of delivery, neonatal weight, length, and head circumference were measured as per routine practices by the attending physician and gestational age was recorded based on mother's recall of her last menstrual period. Neonatal cord blood samples were measured for FT4 or TSH, T3, thyroglobulin, and serum perchlorate.

No differences were observed among the three cities in any of the fetal indicators. A city effect was observed for perchlorate, which was highest in Taltal, and T3, which was highest in Chanaral, the city with moderate perchlorate concentrations in drinking water. However, the fact that TSH and free T4 were not different among the cities suggests that the effects of perchlorate were not producing a hypothyroidism in the neonate. The implications of these city differences was not examined in terms of later outcome, although an earlier report by this group indicated no difference in incidence of autism or attention deficit disorders among cities (Crump et al, 2000).

Amitai et al. (2007) Study: This study examined neonatal T4 levels from the Israeli newborn screening program ascertained within 36-48 hours of delivery in relation to known perchlorate exposure levels based on maternal residence in geographic areas with very high, high, and low perchlorate levels in the local wells. The area of interest was Ramat Hasharon, a city in central Israel near Israeli military industries where wells were contaminated to different degrees (with perchlorate) depending on their proximity to the facility. A comparison city, Hertzlia, with low perchlorate levels was included. Water samples were ascertained from wells within each of the three regions. The sample of interest included 96 newborns from the very high perchlorate exposure region (≤ 340 mg/L), 216 from the high exposure region (42-92 mg/L), and 843 from the low (<3 mg/L), all of which had sufficient iodine levels based on previous report (Benbassat

et al, 2004). To estimate exposure levels, researchers analyzed serum concentrations of perchlorate, thiocyanate, nitrate, and iodide from samples of blood bank donors in the respective regions as a proxy.

Results indicated no differences in neonatal T4 levels, which were normally distributed in each of the three regional groups. A subsequent analysis based on maternal tap water exposure levels (via telephone interview) and adjustment for this in the analysis as well as a matched control analysis of selective cases providing the most complete data still showed no significance for neonatal T4 values. However, the proxy analysis did show differences in perchlorate levels among resident blood donors, but the numbers were very small, signifying an effect on adults but not newborns.

Although the Amitai study is promising in indicating a lack of effect of high-level perchlorate intake in drinking water in pregnancy on the fetal thyroid, a number of problems exist with this study. Specifically, because perchlorate has a short half-life and samples were ascertained 2-4 days after birth while area of the birthing hospital was not noted, it is not clear what exactly were the mothers' doses at time of delivery. Also lack of exposure level at an individual level and the use of proxy values limits the value of the information provided by this study.

Steinmaus et al. (2010) Study: This study took place in California and involved merging two databases: the California newborn screening program in 1998, which assayed for TSH, and perchlorate measurements from the 1997-1998 California Drinking Water Program (DWP), which included more than 800 perchlorate measurements from 200 separate water sources. The data set also included extensive information on each newborn and excluded subjects for a number of criteria. The final sample consisted of ~500,000 newborns. The DWP information was used to classify cases as "exposed" or "non-exposed" based on the California regulatory standard of 6 mg/L perchlorate and the statistical analysis used logistic regression to calculate odds-ratios (ORs) for having a high TSH defined either as 25 mU/mL or the 95th percentile (15 mU/mL at <24 hours and 8 mU/mL at > 24 hours). A large number of confounders were entered into the analyses, which were separately conducted for samples collected at different 6-hour hour intervals from birth.

The authors reported significantly elevated ORs for all levels of TSH-analysis signifying that perchlorate in drinking water is associated with increased neonatal TSH.

I found this study to be of superior quality to all others provided and the best of the three neonatal studies in terms of its scope and level of analysis. The use of TSH is also a superior marker of thyroid gland susceptibility than T4-only in the other two studies. *On this basis, I would conclude that perchlorate does affect the fetal thyroid at the time of birth.*

Effects on Infants

Two of the studies dealt with young children: Valentín-Blasini et al. (2012) and Dasgupta et al. (2008).

Valentín-Blasini (2012) Study: This study capitalized on the SEAD Study of estrogen activity to measure urine samples in diapers from 92 infants aged 1-377 days. 206 urine samples were collected and analyzed for daily perchlorate dose in full term normal birthweight infants residing in an iodine-sufficient region. The dose-estimate was creatinine-adjusted and infants were stratified as being breast-fed, formula-fed with cows milk, or formula-fed with soy milk.

Perchlorate was detected in 95% of breast-fed infants versus 86% for cow milk formula and 68% for soy formula and the average perchlorate levels were also higher in the breast-fed group. Urinary iodide levels were higher in the cow-based versus soy-based formula group. Positive correlations were observed between perchlorate and iodide levels across the groups and within individual breast- and formula-fed stratifications. Correlations between iodide and other NIS inhibitors were not observed. Notably, the estimated daily perchlorate dose for all infants was below the EPA reference dose (0.7 mg/kg/day) but 2.4 times higher than the median dose for US adults, especially in the breast fed group. Also, 9% of all samples (16% of all infants) exceeded the RfD with 6% of those providing more than one sample exceeding this multiple times, especially among those 2 months of age or younger, which is a critical time of thyroid hormone need for the developing brain.

Associations between urinary perchlorate and T4 and TSH in infants were reported in a separate study by Cao et al. (2010) showing effects only for infants with iodide < 100 mg/L. However, the effect for T4 vanished if models included nitrate and thiocyanate. This pattern suggests that the infants with low iodide when exposed to perchlorate show a compensatory HPT reaction to maintain normal T4 levels. The long-term effects of this compensation for later thyroid functioning need to be determined.

Dasgupta et al. (2008) Study: This study differed in scope from Valentín-Blasini by conducting multiple breast-milk and urine measurements from a small sample of breastfeeding women donors living in an iodine sufficient area. The objective of the study was to determine excretion levels of perchlorate, thiocyanate, and iodine in milk and urine and relate these to parallel/competitive transport by NIS.

Breast-milk samples were collected in tubes from 13 women who were at varying stages of lactation and provided samples on consecutive feedings, ideally without major gaps. Urine was collected on the second morning following the first micturition collection. All samples were frozen in home freezers, collected as soon as possible after series completion, transported on dry ice to the research facility, and stored at -10 degrees C until processed and analyzed. Thus 402 breast milk samples and 103 24-hour urine samples were provided. A sophisticated series of sampling procedures and analyses were conducted, given the specialization of the senior author.

Results showed the median perchlorate sample in breast milk was above the RfD and the median iodine concentration was low. Levels in urine were slightly more appropriate. Calculation of average iodine intake revealed that only one of the 13 infants had a normal intake.

This study, based on a small sample size thus limiting the generalizability of its findings, indicates that perchlorate exposure does limit the amount of iodine excreted in breast milk. However, as infant thyroid hormone levels were not directly measured, we do not know to what degree these infants TH-deficient and the impact of this on their developing brains.

Effects on Young Children

The only study with any data on children was the recent submission by Mendez & Eftim (in press). This study is based on analyses of the 2007-2008 NHANES data set, which includes data from subjects 6-12 years of age. The current study examined phthalate levels in addition to total and free T4, TSH, thyroglobulin and thyroid peroxidase antibodies and perchlorate, iodine, thiocyanate, and nitrate. However as phthalates were only measured in a subset of the 2007-2008

NHANES, the size of the sample was reduced. Analyses were conducted by sex but unfortunately separate data for the children are not shown in this paper nor is a comparison made of these youth with older subjects. Creatinine-adjusted perchlorate levels were found to be associated with reductions in T4; however, the effect was modest. The effects of this exposure on stressing the thyroid system leading to mild or moderate hypothyroxinemia, which can impact on the fetus during pregnancy was discussed.

Effects on Older Children and Adults

The Blount (2006) study used the NHANES 2001-2002 data-set to see whether perchlorate predicted T4 or TSH levels in individuals >12 years. Multiple regression models with an extensive list of covariates found an effect on TSH, but not T4, for women only if they had iodine levels < 100. Children and adults were not examined separately. This study has implications for fetuses if women are exposed to perchlorate in pregnancy and live in a region of low iodine. While a strength of this study is its large nationally representative sample, it is limited by the cross-sectional design, use of a spot urine, and measuring only total T4.

Effects on Pregnant Women

Three of the studies dealt with pregnancy: Téllez et al. (2005), Pearce et al. (2010), Pearce et al. (2011).

Téllez et al. (2005) Study: This study from northern Chile conducted extensive studies on the women from the three cities over the course of their pregnancy and postnatally, as well as on the neonates described above. The women were first seen when presenting for prenatal care at ~16 weeks and then at ~33 weeks gestation and ~12 weeks postpartum, at which time their serum T3, FT4, TSH, Thyroglobulin and TPO and thyroglobulin antibody levels were measured. Home tap water samples were estimated from samples collected from each of the cities but were not specific to the women in the study.

Regression analyses indicated “no significant correlation between perchlorate excretion and T3, FT4, TSH, or TG” and no evidence of an association between specific residence and thyroid function test results in the women. Breast milk iodine levels also did not decrease in relation to increasing perchlorate in municipal water supplies. However, this study was limited by using municipal water levels as a proxy for perchlorate concentrations in the mothers.

Pearce et al. (2010) Study: Pearce and colleagues capitalized on the large randomized trial of thyroxine therapy for hypothyroxinemic and/or hyperthyrotropinemic women in Wales and Turin, Italy by John Lazarus. In this study, serum levels of FT4, TSH, and thyroperoxidase antibody were measured along with urinary perchlorate, iodine, and thiocyanate at ~16 weeks gestation. Women were stratified to have their data analyzed during or after pregnancy. Those analyzed during pregnancy having low FT4 or high TSH (or both) received thyroxine therapy in their pregnancy; the others were analyzed after pregnancy and if their thyroid levels were atypical, were retested and treated as necessary.

The biochemical data from the two primary sites were analyzed separately in the Pearce study because different laboratory methods were used at the different sites and at both sites, women were grouped as being euthyroid or hypothyroid/ hypothyroxinemic. The thyroid function values were transformed into “multiples of the median (MoM) values” and results were analyzed using a series of multiple linear regression analyses.

Results revealed low median urinary iodine values and detectable but low average perchlorate levels in all groups. However, a large range was reported, especially among hypothyroid/hypothyroxinemic women from the Cardiff site. Multiple regression analyses controlling for thiocyanate and adjusting for urinary iodide and TPO antibodies (as modifiers) revealed no associations between perchlorate levels and FT4 or TSH MoMs in either the euthyroid or hypothyroid/hypothyroxinemic groups from the two sites.

This study, however, was limited by measurement error in non-uniform specimen storage times and additional factors may have been associated with both perchlorate and thyroid hormone concentrations, as well as its cross-sectional design. Moreover, given the broad range of perchlorate levels, I wonder about the statistical analytic approach based on linear analyses. Perhaps a better approach would have been using logistical odds ratios determining the impact on thyroid values of having above-cutoff perchlorate levels with and without iodine insufficiency. Above all, the authors call for further studies “to examine the effects of environmental perchlorate exposure in vulnerable populations”.

It should be noted that the Lazarus study published in NEJM in 2012 was reported no benefit of treatment on the offspring at age three. Nevertheless, it will be important to correlate maternal perchlorate and thyroid levels with the measures of child neurobehavioral outcome obtained later.

Pearce et al. (2011) Study: This study was based on samples ascertained from first-trimester pregnant women attending prenatal clinics in Los Angeles and Cordoba Argentina. Women with known thyroid dysfunction and ingesting L-T4 or other medications containing iodine were excluded. Spot urines were obtained at the first prenatal clinic visit and sent in frozen batches to either Boston Medical center or Keck School of Medicine. Measured were TSH, TT4, TT3, a thyroid hormone-binding ratio estimate of TBG, free hormone indices, thyroid peroxidase antibodies, and perchlorate content. Results were compared by site and separate and combined (by site) multiple linear regression analyses were conducted.

Results revealed the Cordoba women had higher values of all thyroid parameters but did not differ in urinary iodine or perchlorate. Both groups were iodine sufficient, although a broad range of values was reported. Perchlorate values also showed a broad range but the proportion with elevations in each group was not provided. Results showed no association between urinary perchlorate concentrations with any of the thyroid parameters including in the subset of women with low iodine values.

While this study again supports the notion that perchlorate exposure does not affect thyroid function, *including in women with low iodine excretion*, I wonder whether a linear regression technique is optimal for the data and again support a logistical regression approach. Until this is conducted, I cannot agree with the authors’ assertion of “no effect of environmental perchlorate on thyroid function in first trimester women”.

3. What is the effect of shorter half-life and lower reserves for thyroid hormone in infants compared to adults?

The implications of this question for infants versus adults is that infants will be additionally more vulnerable to perchlorate effects than adults. To date, no study has directly compared these two age groups for having different thyroid hormone levels in relation to comparable perchlorate

levels in drinking water and food or the levels of perchlorate that contribute to thyroid dysfunction. In the infant, hypothyroidism will have adverse effects on selective brain functions, abilities, and behavior. The cumulative effect of sustained hypothyroidism in infancy from continuous high perchlorate exposure levels will have numerous manifestations including risk of ADHD and autism spectrum disorder as well as learning disabilities.

4. What is the impact of intrauterine exposure to perchlorate on thyroid status in fetuses?

None of the studies dealt directly with this issue. The three studies that examined perchlorate levels on thyroid parameters in pregnant women failed to show effects. However these studies each contained a number of methodological limitations, as described above. Presumably, if perchlorate exposure reduces the maternal iodine and thyroid hormone, this will adversely affect fetal brain development, as shown in the literature on these two substrates. My research (in preparation and in submission, many abstracts) shows a definite effect of elevated maternal TSH levels in the different trimesters on specific child abilities and aspects of teenage brain development.

Comments from Dr. Stein

Issue III: Pertaining to Epidemiological and Biomonitoring Studies

How should EPA consider the post-NRC epidemiology data in deriving and MCLG?

While several new epidemiological and biomonitoring studies have been published since the 2005 NRC report, there are still no studies examining the association between prenatal and/or early life perchlorate exposure and child cognitive and behavioral development. The data on the association between perchlorate exposure and thyroid function in humans remain inconclusive, most likely because of the divergent study populations and methodological differences among the studies. Some studies did observe changes in thyroid function among subpopulations of perchlorate exposed individuals, but there does not appear to be information on when thyroid perturbation in these groups becomes sufficient to result in neurological or other impairments, especially among fetuses or neonates. The largest studies of newborn thyroid function relied on ecological assessment of perchlorate levels in drinking water. Drinking water represents only a portion of total perchlorate exposure, so fetal exposures based on average maternal drinking water concentrations would likely underestimate total perchlorate dose. In the absence of more relevant epidemiological studies, the MCLG must rely on numerous uncertainty factors, especially since the reference dose is based on a study of just 37 healthy adults. A plan to monitor the state of the science with respect to adverse health effects of perchlorate exposure, and adjust the MCLG if needed, could be implemented.

- Is there an additive or multiplicative effect of perchlorate and other chemicals that block iodide transport into the thyroid? For instance, several studies comment on iodide-deficient pregnant women as an especially vulnerable subpopulation. But what about the group of iodide-deficient pregnant women who also smoke and have a diet high in nitrate? Among this group, is a lower level of perchlorate sufficient for impaired thyroid function because the other anions are also inhibiting iodide uptake? Or are thiocyanate and nitrate levels superfluous because perchlorate has a higher affinity for NIS? Do the uncertainty factors account for these types of mixed exposures? Steinmaus et al 2007 shows an enhanced adverse association between perchlorate and T4 as levels of urinary thiocyanate increases.
- Is it known at what point iodide inhibition causes thyroid function to become so perturbed that the feedback loop stops functioning and neurological impairment begins? One study reported that iodine intake less than 20 $\mu\text{g}/\text{day}$ in pregnant women resulted in major neurological impairment in offspring. Is comparable information available for infants and children? How does this value relate to the 1.8% no effect iodide inhibition level from the Greer study? Has the value from Greer been replicated?
- Several studies examine maternal iodine deficiency and/or maternal thyroid impairment in relation to neonatal and early childhood behavior and development, and report adverse effects. Only one study, however, appears to have followed the offspring beyond age 2.5. This study (Vermiglio 2004) reports dramatic decreases in IQ, but the study was small ($n=27$) and iodide sufficiency was determined ecologically. Existing birth cohorts with longitudinal follow-up may help identify limits for iodide sufficiency and thyroid function with respect to cognitive and behavioral development.

- The relative source contribution of perchlorate in drinking water varies across studies. Using NHANES data, Huber (2011) reports that for the overall population and most age/sex subgroups the food to water exposure ratio is about 4:1. The White Paper Table 1, however, lists much higher relative source contributions. Why are there such disparate RSC estimates? What is the rationale for using the more liberal RSC?
- The second PBPK application (fixed drinking water concentration) seems to be a more realistic rubric than the first PBPK application (fixed dose).
- Figure 1 indicates that with the second PBPK application infants and children up to 12 months of age may be exposed to as much as five times the perchlorate reference dose of 0.7 $\mu\text{g}/\text{kg}/\text{day}$. Has this potential been accounted for in the MCLG? It sounds like the uncertainty factor of 10 may not sufficiently account for the range of differences in perchlorate pharmacokinetics across sensitive life stages.
- Is basing a reference dose on a single study of 37 healthy adults typical?
- Of the identified studies, only one is a prospective study with measures of prenatal urinary perchlorate concentration and neonatal thyroid function.
 - Pearce et al (2011): This medium sized prospective study found no association between measured, prenatal perchlorate levels and neonatal thyroid function, even among women with low urinary iodine levels.
- There are 5 cross sectional studies examining measures of urinary perchlorate concentration and thyroid function. Three of these studies use NHANES data.
 - Cao et al (2010): This cross-sectional study examined urinary perchlorate levels and thyroid function in infants at multiple time points up to 12 months of age. Thyroid hormones were measured in urine, not blood. In the parent study the correlation between T4 and TSH in blood and urine was moderate. In the overall analyses there is an association between thiocyanate and thyroid hormones, but not between perchlorate and thyroid hormones. When stratified by urinary iodide status, among infants with urinary iodide less than 100 $\mu\text{g}/\text{L}$ there is a small association between perchlorate and thyroid hormones, although T4 is increased, not decreased. The associations did not vary by infant sex.
 - Pearce et al (2010): This large cross-sectional study found no association between measured urinary perchlorate exposure and thyroid function among pregnant women, despite the fact that the median urinary iodine levels were low. The composition of the cohort (hypothyroid and euthyroid; Wales and Italy) is odd and necessitates some separate analyses, but the overall methodology seems sound.
 - Blount et al (2006): This cross-sectional study uses NHANES 2001 – 2002 data to show biologically plausible and consistent directions of association between urinary perchlorate levels and T4/TSH, especially among women with low urinary iodine levels. It is unclear why these findings have not be replicated in

other studies. Are the hypotheses for the lack of an effect among men (women have increased susceptibility to autoimmune thyroid disease; estradiol may block TSH-induced NIS expression; estrogens increase T4-binding globulin) sufficient to truly explain this null findings among males?

- Steinmaus et al (2010): This cross-sectional study uses NHANES 2001 – 2002 data to examine the impact of smoking and thiocyanate on the association between urinary perchlorate and serum T4 and TSH among females at least 12 years old with urinary iodide less than 100 ug/L. There appears to be a pattern of decreasing T4 with increasing perchlorate that is heightened as urinary thiocyanate levels increase.
- Schreinemachers (2011): This cross-sectional study uses NHANES 2001 – 2002 data to examine the association between urinary perchlorate and indirect measures of thyroid function: HDL cholesterol, hemoglobin, and hematocrit. It is not clear what to make of the small variation within the normal range that these biomarkers have in relation to urinary perchlorate concentration. This study may be more relevant for metabolic changes in relation to thyroid function than for thyroid function and neurodevelopment.
- The remaining epidemiologic studies all use ecological measures of perchlorate in drinking water.
- Tellez Tellez et al (2005): It is not clear why the study measured perchlorate in urine but then used city of residence as a proxy for exposure, especially when the “perchlorate gap” appears to differ quite a bit across cities.
- Kirk et al (2005) note that iodine deficiency may be a particular problem among children concurrently exposed to high levels of lead because lead reduces the production of transthyretin, the protein that transports T4. Is this a mechanism that needs to be further explored for identifying additional susceptible subpopulations?
- There is a comment to Kirk et al (2005) from the original reviewers of the manuscript questioning the validity of the article’s conclusions. Additionally there is a 2nd comment also questioning the presentation and interpretation of the data. The arguments presented in these two letters need to be evaluated when incorporating the Kirk study into the body of literature.
- Amitai et al (2007): This study observes no effect between perchlorate and neonatal thyroxine levels, but exposure was determined based on city of residence and maternal iodine status was not assessed. The telephone interview determining drinking water habits was not blinded as to residential location, which was the proxy for exposure.
- Steinmaus et al (2010): This is an extremely large study using neonatal thyroid hormone screening results from California, but exposure is an ecological, dichotomous, measure of perchlorate in drinking water. The study indicates a small effect of perchlorate on TSH. A letter to the editor argues that these findings are due to nitrate in drinking water rather than perchlorate in drinking water, and disagrees with the methodology for selecting

water districts into the exposed and unexposed groups.

- Buffler et al (2006): This is a previous iteration of the Steinmaus 2010 study. In this version there was no association between an ecological, dichotomous measure of perchlorate exposure based on water district and either TSH or primary congenital hypothyroidism. The authors note that the proportion of Colorado River water, which is known to be contaminated with perchlorate, varied considerably depending on the city and time of year. Consequently, water district averages may not reflect perchlorate exposure during the relevant period of pregnancy.