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Review of the “Public Health Goal for Perchlorate in Drinking Water”

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I have reviewed the draft document dated January 11, 2011 as well as the online posted comments of external interested parties. I am an environmental epidemiologist with a primary research area outside of perchlorate and related health effects, but have done some work with perchlorate. I am pleased to have been given the opportunity to review this document and to offer some technical suggestions. In general, I will focus my comments on **“4. The accuracy of the information regarding the new studies published since the 2004 perchlorate PHG “**. However, in doing so, I will provide some suggestions regarding the “Developmental and Reproductive Toxicology” section.

Overall this is a well written and reasonably clear document. The Introductory paragraph of the “Developmental and Reproductive Toxicology” section is used to make the argument for looking at the TSH level in the 1st 24 hours after birth. Further, it attempts to describe different perchlorate exposures of the fetus/infant and how they may bias effect estimates of studies described in the section. However, at the beginning of the section, it should be stated clearly what the exposure of interest is for these studies. I suspect you want total perchlorate exposure during pregnancy, for which drinking water perchlorate exposure is a proxy. If so, this is an imperfect proxy as there may be other sources of perchlorate exposure during pregnancy, and thus bias may result from the use of this proxy as well. This should be discussed. The text that then explains how non-differential exposure misclassification may occur when other sources of perchlorate exposure (e.g. use of formula) are more prominent in the first few days of live, adequately describes the limitations in the use of this exposure at this time in the newborn’s life.

Second, there is no discussion of outcome misclassification, although all of these samples are measurements of a newborn’s TSH or T4 level soon after birth, taken to a laboratory for analysis. How could outcome misclassification and any resulting bias or loss of statistical power affect the results presented?

Third, a discussion summarizing what is known about potential confounders of the “during pregnancy” perchlorate exposure and TSH/T4 levels within the first few days is needed. Many of the studies on which more emphasis is given (Crump et al. 2000; Kelsh et al. 2003) control minimally for potential confounders between cities. In order for the reader to properly consider the role of confounding in the effect estimates given, a discussion of these confounders (both related to city of residence as the exposure and related to actual perchlorate level in subject’s drinking water) should be provided. I suggest introducing it in the introductory section and then

expanding the confounder discussion at the end of this section to quantify by how much specific confounders could change the effect estimate of exposed city in these models (similar to Table 22).

Table 13 attempts to provide descriptive features of selected studies that were most heavily weighted when making summary inference across these studies. This could be a useful table, but a few pieces of additional information are missing and should be added. First, in both this table and throughout the text, confidence intervals should be provided for each study wherever possible so as to provide the precision of the estimates presented (e.g. odds ratio). The p-value does not provide the precision of an effect estimate.

Second, for the Brechner (2000) study, the Li (2000a) study, and the Crump (2000) study, the statement of results is not informative (e.g. “mean TSH = 27% higher” does not say that subjects from the exposed city had a higher TSH level than subjects from the unexposed study. Make it clear). Third, a column with the statistical method used in the study (e.g. logistic regression, linear regression, etc.) should be added. Third, a column listing the potential confounders adjusted for in the analysis should also be presented. Since this is a potential explanation for some of these findings, this table should allow a reader to compare confounder control across the studies. Fourth, some of the effect estimates presented are not the main results of the paper, but instead were calculated using data in the tables of the paper by the authors of this draft document. Therefore, these are unadjusted estimates. This should be clearly marked in this table as well. Last, I assume the main result of the Steinmaus et al 2010 paper (OR=1.57 for high TSH in exposed communities compared to low TSH in unexposed communities) uses the same data as the estimated quantity just above it in the Kelsh et al. (2003) study (i.e. both of these OR's=1.57 are the same effect estimate on the same data). If so, both of these effect estimates should not be included in this table, as they are not independent data or independent analyses. If not, then make it clear how they are different.

Related to the above request for confidence intervals is the use of the p-value in the text. In the Steinmaus et al (2010) paper and throughout this draft, a p-value is interpreted as reflecting the probability the findings were due to random chance. This is incorrect and should be removed. The p-value, by definition, combines both the strength of the association observed as well as the precision of that estimate (i.e. sample size). It is used to make a qualitative judgment on whether an effect is different from a hypothesized value, not a quantitative estimation of effect. By definition, the p-value is the probability, under the assumption that the null hypothesis is true (e.g. OR = 1), that you would observed this result or one more extreme. The p-value is not the probability the null hypothesis is true, and thus is not the probability the result observed was due to chance. It should just be viewed as a relative measure of consistency of the study data with a null hypothesized OR=1 (Rothman 2002). The confidence interval should be provided for each study and used to assess the role of random chance in the study. I suggest removing all of these statements from the document.

The document appropriately reviews relevant studies that either examined the change in TSH /T4 levels in the first 24 hours of life associated with perchlorate in the drinking water directly,

or whose main analysis was outside the first 24 hours, but provide data to allow a crude assessment of the association between TSH/T4 levels measured in the first 24 hours of life and perchlorate. Overall, this section is well written, providing the reader with most important information on each study, and potential limitations. However, I have a few comments on these studies.

- 1. Kelsh et al. 2003.** I am a bit concerned about the emphasis placed on effect estimates for the increased relative odds of an 'elevated' TSH associated with living in Redlands compared to San Bernardino/Riverside counties, for those infants <18 hours of age. Although this result is suggestive of an effect, this is just an unadjusted estimate from a study with several limitations (imperfect outcome assessment, not complete TSH screening). Similarly, the relative odds of having a low T4 level (all ages combined) associated with living in Redlands versus San Bernardino/Riverside was also calculated. This again is an unadjusted estimate. The statement that confounding by age at collection, ethnicity, sex, birth weight, or birth year would not likely explain the effect observed, does not address the role of other potential confounders (e.g. other factors more closely linked to socioeconomic status, other environmental toxicant exposures, etc.). I agree with the authors that it is useful to abstract this information from the paper and present these effect estimates here. However, I would suggest you do not make such strong inference from them.
- 2. Lamm and Doemland 1999.** The document states "Results were not adjusted for several important variables". If they did not adjust for several important variables (I assume confounders), state what you think those variables were and whether not adjusting for these factors could explain the null finding. You should be able to evaluate this based on the assumed direction of association/correlation between the variables. Second, the statement about significant misclassification of exposure here needs to be clarified. There are two sources of exposure misclassification: 1) errors in the county to which subjects were assigned (were subjects incorrectly assigned to a county of residence?), and 2) using a county as a proxy for subject's perchlorate exposure instead of measuring it directly. Clarify which is the source of the bias. Discuss whether it's differential versus non-differential, and then whether it could explain the result they observed.
- 3. Li et al, 2000a.** This is clearly a null study, but this lack of any adverse effect may be due to exposure and outcome misclassification, and/or confounding. However, none of these are discussed as potential reasons for this null finding. Further, the authors use Figure 3 of the paper to conclude that the mean T4 level in Las Vegas was 22 µg/mL lower than in Reno. I suggest you add a cautionary statement here that again, this is just observation from a figure and is not adjusted for potential confounders.
- 4. Li et al 2000b.** It would be useful to provide the mean and standard deviation TSH levels in Reno and Las Vegas, rather than just the p-value for the t-test. Further, although I agree weaknesses of the study are residual confounding by age, and uncontrolled confounding by birth weight and ethnic origin, it is not clear if these factors could

completely explain the lack of an effect. For example if a confounder is negatively correlated with living in Las Vegas but is associated with increased TSH level, than not controlling for it would like lead to an underestimate of effect (i.e. the estimated change in TSH associated with living in Las Vegas is lower than the true estimate), and thus would not explain why they found what they found. This is just an example but would be useful to include something like this in the text so the reader understands what variables should have been included in the analysis.

5. **Brechner et al. 2000.** The authors do a reasonable job of documenting the deficiencies of the study. However, they present Table 10 that provides median TSH levels in Flagstaff and Yuma for each day of measurement, and the difference in them. The authors then place a statement in Table 13 that the Yuma values are 27% higher than those in Flagstaff (6.4/24.0) as evidence that perchlorate is associated with increased TSH. However, this is an unadjusted effect estimate that may be due to confounding. Too much causal interpretation is put on a difference in TSH levels associated with living in one city versus another, which is not adjusted for potential confounders by factors such as city differences in SES, other environmental toxicants that may affect TSH levels (e.g. nitrate and thiocyanate), etc. This limitation needs to be made clearer in Table 13.
6. **Buffler et al. 2006.** This is a study with improved TSH assessment (taking advantage of the revised TSH screening program – i.e. all newborns provide a TSH sample), and a revised metric to estimate the perchlorate concentration in each community’s drinking water. Buffler et al report an adjusted relative odds of having a TSH > 25 $\mu\text{U}/\text{mL}$ associated with a high perchlorate community versus a low perchlorate community of 0.73 (95% CI = 0.40, 1.23), for only those subjects with TSH measurements after 24 hours of age. The authors of this document, using data in the paper, calculated an unadjusted odds ratio of having a TSH > 25 $\mu\text{U}/\text{mL}$ regardless of age (both those <24 hours of age and those \geq 24 hours) and present it in Table 11 (OR=1.59; 95% CI = 1.33-1.91). They then conclude that the effect does not appear to be due to confounding by age at sample collection. However, too much emphasis is placed on this simple unadjusted calculation that is likely confounded somewhat by age at sample collection (if in fact there is a difference in age at sample collection by high versus slow perchlorate level) and potentially other confounders. This should be considered suggestive evidence at best, and this limitation should be noted in Table 13.
7. **Steinmaus et al. 2010.** This study attempts to confirm the estimated concentration in Kelsh et al. (2003) and was done by an author of the draft document. Although the authors had the continuous TSH data, they chose to still dichotomize it into High vs. Low. A more convincing argument would have been to estimate the change in TSH level associated with an incremental increase in a community’s perchlorate concentration. Further, use of this continuous outcome would likely result in more statistical power than the dichotomous outcome. Also of note is that this analysis adjusted for many more factors (potential confounders: age at sample collection, gender, mother’s age, income, race/ethnicity, birth weight, and feeding type) than the studies reviewed

previously calling into question whether previous study findings were due to residual confounding. Last, when providing an OR, please provide the precision of that OR (95% confidence interval) rather than just the p-value. Please see my earlier comment on the use of p-values to quantify chance.

This study provides evidence that perchlorate in the drinking water is associated with a high TSH level in California newborns. It also provides a very nice summary of the possibility that nitrate, thiocyanate, and iodine could confound the observed association. The relationship of nitrate, thiocyanate, and iodine with perchlorate and how they may confound and/or interact with perchlorate in affecting fetal TSH levels needs to be discussed in this document. The conclusion of this paper even states *“Further research is needed on this issue, and needed to evaluate the possible role that iodine, thiocyanate, nitrate, and other thyroid-active agents may have played in these findings.”* Therefore, it seems necessary to at minimum include a discussion of these other toxicants and if and how they may interact or confound the associations and inference described in the document.

It is clear that many of the arguments and second hand calculations presented in this document about previous studies were first done as part of this Steinmaus et al. (2000) paper. This discussion section very precisely describes the many limitation of the previous work. This is the first study, and in fact the only one of the five presented in Table 13, that provides an effect estimate that I am not overly concerned may be due to confounding or other study limitation.

8. **Chang et al, 2003.** Too few details are given for this study. To be consistent with the other studies presented, please describe the study population, how the exposure was defined and measured, how the outcome(s) was/were defined and measured, what statistical analyses were used, what confounders were and were not included in the analysis, and how the briefly mentioned study limitations could explain the results observed. Also, the reference was not provided in the reference section.
9. **Tèllez Tèllez et al. 2005 and Amatai et al. 2007.** These studies are adequately described and limitations noted. The Amatai et al. (2007) study particularly points out that mothers with adequate intake of iodine may not be susceptible to the effects of perchlorate, again suggesting the need for a discussion of this relationship in the document.
10. I suggest you include the Cao et al (2010) study in this review, as it assessed the association of urinary perchlorate, nitrate, thiocyanate, and iodide with urinary T4 and TSH levels in infants. I realize it does not assess this association within the first 24 hours of life, but provides evidence that perchlorate affects TSH and T4 levels. Specifically, perchlorate effects on TSH levels were only seen in those with low iodide levels. Further, elevated nitrate and thiocyanate were associated with higher TSH levels. Inconsistent

with the a priori hypothesized direction, increases in nitrate, thiocyanate, and perchlorate were associated with increased T4 levels.

Cao Y, Blount BC, Valentin-Blasini L, Bernbaum JC, Phillips TM, Rogan WJ. Goitrogenic anions, thyroid-stimulating hormone, and thyroid hormone in infants. *Environ Health Perspect* 2010;118:1332-1337.

The title of the next section should perhaps be changed. As I understand it, this document is attempting to summarize the information on whether fetal exposure to perchlorate (drinking water perchlorate) is associated with fetal TSH and T4 levels (first 24 hour TSH or T4 levels used as a proxy). If correct, the title should be changed to state this. The summary inference to be made from this section appears to be based on Table 13, which is based on only five studies. I agree with the authors that the Tellez Tellez et al (2005), Li et al (2000b), and Amatai et al (2007) studies should not be included as they do not assess this association in the time period the authors argue is most important. However, of these 5, only one uses an exposure measure other than 'city with high perchlorate levels in drinking water' versus 'city with low or no perchlorate in drinking water' (Steinmaus et al 2010). Also, it is not clear if the TSH effect estimates from Steinmaus (2010) and Kelsh (2003) are based on the same data. If so, only one should be presented. Further, of these 5 studies, Given the limitations in the studies noted, and the lack of confounder control present in most of these studies, I am not convinced that these studies, by themselves, suggest a clear effect of maternal perchlorate levels in drinking water on adverse changes in fetal TSH and T4 levels. They are clearly suggestive of such an effect, but too many limitations in them could be argued to explain the effects. Ideally, a prospective study based on this summary document could be designed and done to more properly assess this association.

Appropriately, the authors attempt to summarize the limitations of the studies used in this section. The argument for why many of these potential confounders could not likely explain the results observed is not convincing. I would suggest you provide quantitative examples of how much a confounding variable could alter an effect estimate (similar to Table 22 in the document). Perhaps data from the existing Steinmaus et al. (2010) study could provide this. Without it, the argument to use the second-hand, unadjusted effect estimates calculated for several studies using descriptive data summaries in paper tables, in Table 13, is not a strong one. Second, a discussion of how outcome misclassification could have biased the effect estimates presented is warranted.

COMMENTS ON OTHER STUDIES IDENTIFIED AS BEING NEW TO THE DOCUMENT

1. **Braverman et al. 2006.** This study was adequately described and limitations noted
2. **Braverman et al. 2005.** This study was adequately described and limitations noted. However, in line 15, I suggest you present both the mean pre- and post-shift levels of the workers, rather than just the pre-shift level.
3. **Gibbs and Ledingham, 2008.** The actual effect estimates described in the text should be presented, not just that which is statistically significant. Further, do not just present a

“b” which I assume is the parameter estimate. If the parameter estimate and its standard error are provided in the paper, take the parameter estimate and convert it to a meaningful effect estimate and 95% confidence interval, even if the authors of the paper do not.

4. **Blount et al. 2006** and **Steinmaus et al. 2007** are described together as they both used data from 2001-2002 NHANES survey. The study design features of Blount et al. (2006) are adequately described. However, the statements where associations are described as “statistically significant” without providing the direction of the effects observed is misleading. Also, how clinically important are effects these size?

Please see my comment above concerning the p-value and concluding that it reflects the probability that these findings are due to chance. This is incorrect and this statement should be removed.

Table 22 is an appropriate assessment of how much change in the perchlorate parameter estimate occurs when potential confounders are either removed one at a time from a full model or added one at a time to an unadjusted model.

5. **Kirk et al. 2005** and **Pearce et al. 2010**. These two studies measured iodine and perchlorate in breast milk, and using correlation coefficients estimated whether perchlorate exposure to affects breast milk iodine levels. They provide conflicting results, however. These studies are adequately described.

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