

**Comments on the U.S. Environmental Protection Agency's Draft Perchlorate  
Environmental Contamination: Toxicological Review and Risk Characterization  
(NCEA-1-0503, 16 January 2002)**

**The DOD Perchlorate Working Group**

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Although the DOD contributed data used in the risk assessment, the DOD does not support all of the conclusions in the document or the proposed RfD value reported in this document.

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## Executive Summary

The US Environmental Protection Agency (EPA) released an external review draft of the agency's toxicological review and risk characterization regarding perchlorate environmental contamination (EPA. 2002. Perchlorate Environmental Contamination: Toxicological Review and Risk Characterization. External Review Draft. Office of Research and Development. NCEA-1-0503) on 18 January 2002. This response has been prepared in reply to EPA's request for public comment.

The Department of Defense (DOD), EPA, and other stakeholders have worked closely together since January 1998 under the auspices of the Interagency Perchlorate Steering Committee (IPSC) to develop and implement a process to define the potential risk of perchlorate contamination in the environment. Consistent with recommendations of the Expert Panel on the Role of Science at EPA, the IPSC process was based upon the assumption that credible science would be used to generate credible decisions. In several ways, the EPA risk characterization for perchlorate does represent a significant advance over previous agency risk characterizations for other chemicals, and in some areas has coherence and clarity that has been lacking in previous risk characterizations for other chemicals. However, although DOD contributed much of the data used by EPA in the risk assessment, the Department does not: support all of the conclusions as stated in the document; support EPA's proposed revised RfD; and feel that all credible science has been used to generate a truly credible decision regarding perchlorate. Several of the major areas in which DOD disagrees with EPA are identified below. We assert that:

- Human studies data (including epidemiological, occupational, and clinical information) were presented and discussed in Chapter 4, giving the perception that the data were factored into EPA's decision on a proposed perchlorate reference dose (RfD). The data in the studies were in fact not used to derive the proposed RfD. In addition, it is apparent that the agency has provided an unbalanced discussion of available epidemiological studies, dismissing the results of several published studies while giving great credence to a single, unpublished study that is not even available to the public. By choosing not to use available human studies data for perchlorate, EPA is failing to act in the national interest by not basing its decision on all available credible science.
- Benchmark analyses data of thyroid histopathology are consistent with the characterization of 0.01 mg/kg-day as a NOAEL, rather than a LOAEL, while dose/response analyses of the motor endpoints resulted in NOAELs on the order of 1 mg/kg-day. Only the brain morphometry and hormone analyses demonstrated effects at 0.01 mg/kg-day. Results for both of these classes of endpoints are highly inconsistent, suggesting that the identification of 0.01 mg/kg-day as a LOAEL or NOAEL is at the very least problematic.
- An overall increase in the uncertainty factor from 100 to 300 is unwarranted and appears excessive to say the least. The agency has chosen to increase the uncertainty factor that it applies to derive the RfD despite the completion and analysis of numerous studies that were recommended by the first Peer Review panel in 1999 specifically to reduce the degree of uncertainty. Areas of contention include: the use of an uncertainty factor of 10 for a LOAEL; the use of an uncertainty factor of 3 for duration; the use of an uncertainty factor of

3 for database insufficiency; and the use of uncertainty factors for cancer risk and immunotoxicity that appear inconsistent with the biochemical mechanism of the mode of action.

- The selection of the point of departure in this case appears to have been based on a qualitative judgment using BMDLs together with the results of other analyses, primarily the questionable NOAELs and LOAELs.
- The point of departure in the human used by the EPA in the calculation of a reference dose (RfD) for perchlorate is 0.01 mg/kg-day, which is the human equivalent exposure (HEE) given in Table 7-3 for the 0.01 mg/kg-day dose at which hormonal effects were observed in the dams in the developmental effects study (Argus, 2001). The HEE for the brain morphometry effects in the pups in the same study is given as 0.02 mg/kg-day. However, in the absence of human models for pregnancy and lactation, it would seem more appropriate to assume that the differences in dosimetry observed in the rat across lifestages would also be expected in the human. Thus the appropriate HEE would be that for the adult male rat dosed at 0.01 mg/kg-day, which is 0.02 mg/kg-day. For more detailed discussion of the relevance of point of departure in determining HEEs, please refer to Appendix A.
- Even though EPA used rat and human pharmacokinetic models to determine HEE levels, the agency's consideration of interspecies variability and the pharmacokinetic and pharmacodynamic differences between humans and laboratory animals is inadequate.
- The agency failed to demonstrate sufficient weight of evidence to quantify the association between a transient, short term physiological response with questionable health consequence, and long-term, low-level perchlorate environmental exposure.
- The assumption that the same methodologies used to develop a human health RfD are applicable to ecological risk is incorrect. The approach used by EPA NCEA is inconsistent with, and differs from, EPA's 1998 Guidelines for Ecological Risk Assessment (US Environmental Protection Agency. Guidelines for Ecological Risk Assessment. EPA/630/R-95/002F, April 1998) and 2000 Soil Screening Level (SSL) draft guidance.
- Deriving the dietary screening benchmark for herbivores based on the same results that the RfD is based on (i.e., perturbations in thyroid and pituitary hormones, thyroid histopathology and changes in brain morphometry in dams and pups) is incorrect. The screening benchmark for herbivores should be derived using toxicity values associated with endpoints known to be ecologically relevant (development, reproduction).
- It is fairly common practice to consider the relative proportion of compound of interest to test compound when deriving benchmark values such as toxicity reference values. This was done for the RfD, but not for ecological receptors. The agency should include the proportion perchlorate/test compound in the derivation of ecological screening benchmarks, or provide justification for why the approach was excluded.

More detailed discussion of these points, as well as additional DOD comments on the agency's risk assessment methodology and conclusions, is provided in the supporting text that follows this summary.

## Comments on the EPA Risk Characterization for Perchlorate

### General Comments on the Risk Assessment Process

In January of 2002, the US Environmental Protection Agency (EPA) released the external review draft of their toxicological review and risk characterization regarding perchlorate environmental contamination (EPA. 2002. Perchlorate Environmental Contamination: Toxicological Review and Risk Characterization. External Review Draft. Office of Research and Development. NCEA-1-0503). Although the Department of Defense (DOD) contributed data used by EPA in the risk assessment, DOD does not support all of the conclusions as stated in the document, nor does DOD support EPA's proposed revised RfD value.

In several ways, the EPA risk characterization for perchlorate (the document) represents a significant advance over previous agency chemical risk characterizations, and in some areas has coherence and clarity that often was lacking in previous risk characterizations for other chemicals. An important feature of the document is its use of mode-of-action considerations to drive the structure and assumptions of the risk assessment approach, in the spirit of the agency's proposed cancer risk assessment guidelines (EPA, 1996). In particular, the perchlorate risk assessment uses mode-of-action information relevant to perchlorate to provide a harmonized assessment for both cancer and noncancer effects. ~~We believe this harmonized assessment~~ represents a first for a major EPA chemical risk characterization.

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Another strong-point of the EPA risk characterization is the careful evaluation and use of the available dosimetry modeling, both to perform cross-species dosimetry in lieu of defaults, as well as to evaluate the potential for age-dependent sensitivity differences. The agency risk characterization makes use of a variety of state-of-the-art dose-response modeling and statistical analyses in support of the weight-of-evidence evaluation of the appropriate point of departure. Perhaps most striking, however, is the way in which the document integrates the qualitative toxicological and mechanistic information with the quantitative dosimetry and dose/response calculations to provide a solid evidentiary support for the elements and conclusions of the risk characterization.

### General Comments on Risk Characterization

The perchlorate risk characterization involves a number of elements: selection of the critical effect, selection of point of departure, dosimetry, and application of uncertainty factors. Each of these elements will be discussed in turn.

**Critical Effect:** The EPA toxicological review makes a solid case for the fact that disruption of thyroid hormone homeostasis by perchlorate's inhibition of iodine uptake is a common factor that serves as the key event leading to a variety of observed effects, both cancer and noncancer. The decision to provide a harmonized cancer and noncancer assessment based on this mode of action is well supported and reasonable.

**Point of Departure:** The use of a weight-of-the-evidence approach for the selection of a point of departure is more appropriate in the case of perchlorate than might be the case for other chemicals, due to the number of related endpoints evaluated in different studies that can be considered to be reflections of the same underlying critical precursor (inhibition of iodine uptake). However, implementation of a weight-of-the-evidence approach is necessarily more complicated, and potentially more subjective, than the traditional approach of carrying out separate calculations on each candidate critical study/endpoint. These difficulties will be discussed further with regard to the selection of uncertainty factors.

The agency selects a point of departure in the rat of 0.01 mg/kg-day, based on a large number of study endpoints related to thyroid function. The basis for this determination is provided by multiple analyses over various diverse studies and endpoints. These analyses include conventional significance testing, benchmark analysis involving a number of different dose-response models, Bayesian statistical analysis, ANOVA and profile analysis. These endpoints reflect exposures for periods ranging from a few weeks to a large fraction of a lifetime, over life-stages varying from gestation to adulthood. Clearly, any attempt to characterize all of these results with a single point of departure represents a difficult challenge.

The difficulty of implementing the weight of the evidence approach is illustrated by the rationale given by the agency for calling the 0.01 mg/kg-day point of departure a LOAEL. The EPA risk characterization suggests that this point of departure was selected on the basis of four classes of endpoints: profile analysis of brain morphometry effects in neonatal rats, increased motor activity in neonatal rats, thyroid histopathology and thyroid hormone changes in a number of studies. However, only two of these classes of endpoints actually demonstrate effects at the selected point of departure. Benchmark analyses of thyroid histopathology are consistent with the characterization of 0.01 mg/kg-day as a NOAEL, rather than a LOAEL, while dose/response analyses of the motor activity endpoints resulted in NOAELs on the order of 1 mg/kg-day. Only the brain morphometry and hormone analyses demonstrated effects at 0.01 mg/kg-day. Moreover, as will be discussed further below, results for both of these classes of endpoints are highly inconsistent, suggesting that the identification of 0.01 mg/kg-day as a LOAEL or NOAEL is problematic.

**Dosimetry:** One of the key strengths of the EPA's risk characterization for perchlorate is the use of chemical-specific dosimetry information to support cross-species extrapolation (calculation of human equivalent exposures), as well as to inform concerns regarding sensitivity to the effects of perchlorate during development. A suite of physiologically-based pharmacokinetic (PBPK) models is described that demonstrate the capability to accurately simulate the kinetics of perchlorate and the resulting inhibition of iodine uptake in the adult rat and human as well as in the pregnant rat / developing fetus and in the lactating rat / neonate. The models are remarkably comprehensive, providing reasonably accurate simulations of a variety of data sets. One of the most compelling aspects of the suite of models is the high degree of consistency between the model structures and parameters. For the most part, only physiological parameters vary across the models. Of course, it is just these differences in physiology that are responsible for much of the observed differences in kinetics. In cases where different values are used for chemical-specific parameters in the models, the differences are supported by evidence from the literature for perchlorate.

Because the PBPK models are grounded in experimental data collected under the conditions of concern for the risk assessment (drinking water exposures in rats and humans), and because of their thorough validation with separate experimental data, there can be high confidence in the dosimetry estimates calculated with the models for the dose metrics used in the risk characterization: blood concentrations of perchlorate and inhibition of iodine uptake.<sup>1</sup> Although it might be possible to obtain similar dosimetry estimates through a combination of classical pharmacokinetic calculations, there would be a much lower level of confidence in such an approach since the assumptions made would not be subject to the same level of validation as the PBPK models. Overall, the dosimetry modeling represents one of the least uncertain aspects of the perchlorate risk characterization.

The point of departure in the human used by the EPA in the calculation of a reference dose (RfD) for perchlorate is 0.01 mg/kg-day, which is the human equivalent exposure (HEE) given in Table 7-3 for the 0.01 mg/kg-day dose at which hormonal effects were observed in the dams in the developmental effects study (Argus, 2001). The HEE for the brain morphometry effects in the pups in the same study is given as 0.02 mg/kg-day. However, in the absence of human models for pregnancy and lactation, it would seem more appropriate to assume that the differences in dosimetry observed in the rat across lifestages would also be expected in the human. Thus the appropriate HEE would be that for the adult male rat dosed at 0.01 mg/kg-day, which is 0.02 mg/kg-day. For more detailed discussion of the relevance of point of departure in determining HEEs, please refer to Appendix A.

**Application of Uncertainty Factors:** A total uncertainty factor of 300 is used to obtain the RfD for perchlorate, resulting in a value of 0.03 micrograms/kg-day. The composite 300 uncertainty factor is three times higher than previously used agency uncertainty factors, despite the completion and analysis of numerous studies that were recommended by the first Peer Review panel in 1999 specifically to reduce the degree of uncertainty. This total uncertainty factor is composed of a factor of 3 for human interindividual variability, 1 for animal to human extrapolation, 10 for use of a LOAEL, 3 for duration, and 3 for the inadequacy of data on immunotoxicity.. Each of these factors will be discussed in turn.

**Human Interindividual Variability:** The default value for this uncertainty factor when the risk assessment is based on an animal study is 10: 3 for pharmacokinetic (dosimetric) variability and 3 for pharmacodynamic (response) variability. The justification for the use of a reduced factor of 3 in the case of perchlorate is not clearly stated, but appears to reflect the evidence that the variability of perchlorate kinetics in euthyroid adults is small, and the belief that, to some extent, the developmental endpoint used in the assessment represents a sensitive subpopulation (the neonate). However, the recent risk assessment for methylmercury used a full factor of 10 for human interindividual variability, even though the assessment was based on neurodevelopmental effects observed in a human study. There is no clear basis for reducing the pharmacokinetic subfactor of 3, since the

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<sup>1</sup> A third possible dose metric, concentration in the thyroid is also presented. However, these model predictions are not subject to the same level of validation as the other two metrics. Moreover, it is not clear why the concentration of perchlorate in the thyroid is relevant to the assumed mode of action, which identifies inhibition of the active uptake of iodide into the thyroid as the critical precursor event. The most appropriate measures for this effect would be the blood concentration of perchlorate or the decrement in iodide uptake.

HEE is based on data for non-pregnant human adults, and there are no data on perchlorate kinetics in pregnant women. The comparison of dosimetry in the rat provides evidence that the dosimetry in the pregnant and neonatal rat is similar (within about a factor of 2) to the adult male rat, but the relevance of this comparison to the human neonate is uncertain in the absence of a human neonatal model. Thus the use of a reduced factor of 3 represents a reasonable, but somewhat non-conservative, aspect of the EPA risk characterization. Further development of PBPK models for perchlorate kinetics in the human during pregnancy and lactation would provide a quantitative basis for evaluating the appropriate pharmacokinetic intrahuman subfactor.

***Interspecies Extrapolation:*** The default value for this uncertainty factor when the risk assessment is based on an animal study is 10: 3 for potential pharmacokinetic (dosimetric) differences and 3 for potential pharmacodynamic (response) differences. The elimination of the pharmacokinetic subfactor in this case is justified due to the use of validated dosimetry models to calculate the HEE. The elimination of the pharmacodynamic subfactor is also well justified, due to the evidence that the rat is particularly sensitive to the kinds of effects that serve as the basis for the perchlorate assessment. In fact, the available evidence suggests that an interspecies factor of less than one would be justified. Thus the use of an interspecies factor of one is a reasonable, but somewhat conservative, aspect of the EPA risk characterization.

The effects of perchlorate on thyroid hormone economy in rats have been well characterized. The mode of action data indicate that perchlorate alters thyroid function by competing with iodide for uptake into the follicular cell via the sodium iodide symporter (NIS), thereby resulting in decreased iodide uptake and a decrease in thyroid hormone synthesis and secretion. The end result is that in the rat, blood levels of thyroid hormone ( $T_3$  and  $T_4$ ) are decreased. Observed developmental effects, such as changes in brain morphometry, are considered to be a consequence of these variations in hormone levels.

There is no evidence to suggest that humans may be less sensitive to the inhibition of iodide uptake by perchlorate; to the contrary, the dosimetry modeling suggests that humans are roughly equally as sensitive as the rat to NIS inhibition. However, the downstream consequences of this inhibition, and more importantly the potential biological outcomes, are certainly different between the two species. It has been well established that humans are able to maintain thyroid hormone levels much more easily than rats due to the presence of thyroxine binding globulin (TBG). Rats do not have this protein and as a result, a greater proportion of blood  $T_4$  and  $T_3$  is “free” (i.e., are not protein bound). Consequently, thyroid hormones are cleared much faster in the rat, when compared with the clearance in humans (the half-life of  $T_4$  in the rat is less than 1 day compared to 5-9 days in humans). Therefore, a compound such as perchlorate that results in decreased secretion of  $T_4$  from the thyroid gland would result in dramatic decreases in blood  $T_3$  and  $T_4$  levels in the rat. In contrast, humans have a much slower clearance of  $T_4$ . Thus, chemicals which inhibit thyroid hormone synthesis would not result in the dramatic changes in blood  $T_4$  and  $T_3$  levels that have been reported in the rat. This conclusion is supported by the results of the analyses of occupational exposures to

perchlorate, where there were no changes in thyroid hormone levels in workers with relatively high exposures.

The ability of humans to more easily maintain blood thyroid hormone levels is important with regard to the developmental toxicity of perchlorate, where decreases in thyroid hormone in fetal and neonatal rats are believed to influence brain development and perhaps induce changes in brain morphometry. However, the doses that result in alterations in blood thyroid hormone levels in rats and consequently produce the developmental effects observed in the brain of rats, would not be expected to produce similar disruption in humans due to the presence of TBG, which is also present in the developing human fetus and neonate (Thorpe-Beeston JG, Nicolaides KH, and McGregor AM. 1992. Fetal thyroid function. *Thyroid*. 2(3):207-217). Therefore, humans would be less sensitive to the effects of perchlorate on thyroid function at the doses of concern for environmental exposure. Thus, an uncertainty factor of less than one for interspecies extrapolation would be justified.

Evidence for the magnitude of rat sensitivity to the inhibition of iodine uptake by perchlorate can be seen by comparing rat and rabbit developmental studies. In the rabbit developmental toxicity study (Argus, 1998c), potential effects of perchlorate on thyroid hormone levels were evaluated. There were no statistically significant differences in the levels of T<sub>3</sub> or TSH in does that received up to 100 mg/kg-day from gestation day (GD) 6 to GD28, when compared with the controls. At the lowest dose tested (0.1 mg/kg-day), T<sub>4</sub> levels were not significantly different. However, T<sub>4</sub> levels were significantly decreased at doses of  $\geq 1$  mg/kg-day, when compared with the controls. There were no treatment-related developmental effects reported at doses up to 100 mg/kg-day. In contrast, T<sub>3</sub> and T<sub>4</sub> were significantly decreased and TSH was significantly increased in female rats that received  $\geq 0.1$  mg/kg-day for 14 days (Caldwell *et al.*, 1995). The EPA pays little attention to the dissimilar results between rats and rabbits in the perchlorate document. However, given the abundant evidence regarding the sensitivity of the rat thyroid hormone system to disruption, the rabbit may actually be a better animal model for human toxicity than the rat for effects that occur as a result of thyroid hormone concentration fluctuations (e.g., changes in brain morphometry).

One possible adjustment that could be made to the perchlorate risk assessment in order to account for the greater sensitivity of the rat would be to use an interspecies uncertainty factor of less than unity. The difficulty is in providing a basis for selecting the specific value to be used. The approach that holds the greatest prospect of providing a quantitative basis for an intraspecies factor of less than unity is the extension of the existing PBPK models to include a description of iodine organification and hormone kinetics/control. A description of this nature has been published for thyroid hormone disruption by dioxin (Kohn, 2000). In the case of dioxin, the disruption appears to be consequent to the induction of hormone metabolic clearance (increased loss), as opposed to inhibition of iodine uptake (decreased production), but the dynamics of the response may be similar.

In fact, the possibility should be considered that the binding of perchlorate to serum albumin in the rat is a second source of stress to the thyroid hormone system. That is, perchlorate may compete with the thyroid hormones for binding to albumin, resulting in an increase in free hormone levels with a resulting increase in their clearance (Yamada, T. 1967. Effects of perchlorate and other anions on thyroxine metabolism in the rat. *Endocrinology* 81:1285-1290). This secondary effect would be restricted to the rat, since perchlorate does not bind to TBG in the human. Development of such a model would require additional experimental data on the interactions of perchlorate with thyroid hormone control, but has the potential to significantly improve the risk assessment for perchlorate.

***Use of a LOAEL Rather Than a NOAEL:*** In the EPA risk assessment for perchlorate, a dose of 0.01 mg/kg-day was identified as a LOAEL in the rat based to a large extent on changes in brain morphometry as reported by Geller (2001). In this study, brain morphometry of one male and one female pup from each litter from the Argus (2001) study were included in the analysis for each postnatal day evaluated. The results of this analysis indicated that there were significant differences in size (thickness) for certain areas of the brain. However, there are findings in the report that are inconsistent, suggesting that the dose of 0.01 mg/kg-day should be considered a minimal, or equivocal, LOAEL, and that a full uncertainty factor of 10 for the use of a LOAEL is not justified.

The morphometric analysis used data from a study conducted by Argus (2001) where one pup/sex/litter was measured. Thus, the sample size for this analysis was relatively small, compared to the sample size that would be obtained if morphometric analyses had been conducted on all of the pups from each litter. Consequently, due to the small sample size, biological variation could potentially influence the appearance of statistical significance. A larger sample size would decrease the probability that this would occur. Further, the majority of the measurements for the different brain regions were within the 99% confidence interval around the mean at both postnatal day (PND) 9 and PND21.

Geller (2001) reported that there were statistically significant dose-related changes in the size of the frontal lobe at PND9. However, frontal lobe size was not significantly different at PND21, suggesting that the change in frontal lobe size had resolved. Similar findings were reported for the parietal lobe. However, it is unclear how this recovery could occur if the change in size is related to treatment, suggesting that these findings may have been the result of something other than treatment (e.g., biological variation).

The area of the brain with the largest variation in size at both PND9 and 21 was the corpus callosum. At PND9, the variation in size was much less than the variation reported at PND21. At both time points, the changes in size in the corpus callosum did not consistently increase or decrease with increasing dose. This is illustrated in Figure 2 of the Geller (2001) report where brain region thickness normalized by the control mean were plotted for each dose group (0, 0.01, 0.1, 1 or 30 mg/kg-day) for the PND21 time point. Increases in corpus callosum thickness were reported for all dose groups, relative to the controls. However, the largest increase from the control mean was for the 0.1 mg/kg-day group, the next to the lowest dose. The smallest increase was for the highest

dose (30 mg/kg-day), with the other two dose groups (0.01 mg/kg-day and 1 mg/kg-day) resulting in virtually the same intermediate increase in size. No rationale can be provided for these findings. The author suggests that one possibility is that at higher doses, compensatory mechanisms are triggered such that the response is smaller than at lower doses. However, no data or analyses are presented in support of this speculation.

EPA's policy on changes in brain morphometry is that, in the absence of data that would prove otherwise, changes in the size of a particular brain region are considered adverse. However, in the absence of a consistent dose-response or data that would support the assertion that the observed responses could be the result of compensatory mechanisms, there is no conclusive evidence that demonstrates that the changes in brain region size were exposure-related. Given the uncertainty associated with the small sample size, considering these changes a LOAEL may not be warranted. Consequently, the use of a full factor of 10 for the use of a LOAEL does not appear to be justified, and a factor of 3 would appear to be sufficient.

The other basis for describing the 0.01 mg/kg-day point of departure as a LOAEL is the results of hormonal analyses in several studies indicating changes in T<sub>4</sub>, T<sub>3</sub> and/or TSH at doses as low as 0.01 mg/kg-day. However, these changes are not consistent and, as with the brain morphometry data, should be considered an equivocal LOAEL/NOAEL, justifying an uncertainty factor of at most 3.

**Duration:** The EPA applies an uncertainty factor of 3 due to concerns for the appearance of increased thyroid tumors at 19 weeks in the F1 generation in the rat multi-generation study, as well as due to the decrease in the NOAELs and LOAELs in the 90-day study as compared to the 14-day study. While this factor may be justified in the case of the rat, per se, it is not clear that such a factor is necessary in developing an RfD for human exposures. It seems possible that *in utero* programming could occur at a dose of 30 mg/kg-day, at which the tumors were observed. Moreover, cumulative damage may be expected for the severe disruption of thyroid homeostasis evident in the rat at the doses used in the 14- and 90-day studies. Maintenance of doses of 0.1 mg/kg-day or greater in the rat clearly produce a highly stressed system which could be expected to deteriorate over time. However, it is not clear how this evidence for a duration component in a highly disrupted rodent hormonal system is relevant to human exposure at much lower doses, where there is no evidence of severe disruption. Thus this factor represents an additional level of conservatism added to the perchlorate assessment with questionable justification.

**Database Insufficiency:** An additional factor of 3 was included to address the continuing EPA concern that perchlorate could cause immunotoxicity. The initial concern for immunotoxic effects was prompted by the observation of hematological effects in some Graves disease patients treated with perchlorate at doses greater than 6 mg/kg-day. The appearance of these effects at such high doses in an impaired population does not, of course, provide a basis for expecting immunotoxicity to occur at the several order of magnitude lower doses at which the developmental effects that serve as the basis for the perchlorate assessment were observed; however, the observation of these effects did

serve as an impetus for including a number of studies of immunological endpoints in the toxicity testing conducted with perchlorate to support the present assessment. The results of the selected evaluations of the potential immunotoxicity of perchlorate are included in the EPA document. The lowest NOAEL is at 0.02 mg/kg-day for the endpoint of increased contact sensitivity. Thus this concern was evaluated and there is no evidence to support the belief that immunotoxicity could be a more sensitive critical effect than the developmental endpoints used as the basis for this assessment.

It is the nature of toxicology that investigators always feel the need for additional studies. The fact that some potential area of toxicity has not been completely explored is not an adequate basis for including an uncertainty factor for database insufficiency. In order to justify such a factor, there should be some expectation that effects of a particular nature may be caused by the compound of interest that the studies available on the compound do not adequately address this concern, and that there is reason to believe that the anticipated effects could occur at lower doses than the critical effects used as the basis for the assessment. Thus this factor represents an additional level of conservatism added to the perchlorate assessment with questionable justification.

**Evaluation of Resulting RfD:** The RfD of 0.03 µg/kg-day, which is equivalent to a drinking water concentration of 1 ppb could be considered overly conservative by more than an order of magnitude, primarily reflecting the application of multiple uncertainty factors to address several different qualitative concerns (use of a LOAEL, progressive toxicity, and immunotoxicity). The total factor of 3 for intrahuman plus interspecies appears to be reasonable. However, the use of a factor of 100 to address other concerns appears excessive. If instead factors of 3 were applied for use of an equivocal LOAEL, as well as 3 for other concerns (i.e., inadequate database), and keeping the same factors for intrahuman and interspecies, the resulting RfD, using the adult male rat HEE of 0.02 mg/kg-day as discussed above, would be 0.6 µg/kg-day, equivalent to 20 ppb in drinking water. The belief that chronic human exposure to perchlorate in drinking water at this concentration is without effect is supported by the results of several epidemiological studies (Lamm *et al.*, 1999; Brechner *et al.*, 2000; Crump *et al.*, 2000; Li *et al.*, 2000a, 2000b, 2001). Only one study (Schwartz, 2001), an unpublished Master's thesis, provides any evidence of the potential for effects at these exposure levels, specifically transient perinatal effects on thyroid hormone levels.

The EPA provides two additional RfD calculations for comparison, one based on the human NOAEL of 7 µg/kg-day in the Greer study, and one based on the tumors in the F1 generation of the multi-generation study. In the case of the Greer study NOAEL, they suggest the use of a total uncertainty factor of 100 (3 each for intrahuman, minimal LOAEL, duration and database), resulting in an RfD of 0.07 µg/kg-day. (If instead factors of 3 were applied for intrahuman, LOAEL and database, the resulting RfD would be 0.2 µg/kg-day or 7 ppb.)

The EPA also obtains a similar result using potential precursors to the thyroid tumors with a margin of exposure of 100. However, the precursors they consider are colloid depletion and thyroid hypertrophy, which are not unequivocal obligatory precursors to thyroid tumors. In fact, one could justify a range of results over many orders of magnitude depending on the "precursor" and margin of exposure selected.

## Specific Comments

**Evaluation of Epidemiological Studies:** The analysis of the epidemiological studies is not very balanced, dismissing several well-conducted, published studies that were negative, while giving great credence to one unpublished study that was positive. The analysis of the studies of occupational cohorts is openly biased, providing extensive criticisms of minor inadequacies of the studies (uncertainties in the exposure assessments and the potential for “survivor bias”) without acknowledging the fact that the conclusions of the studies are not likely to be altered by either of the asserted deficiencies. The fact that two studies have demonstrated that workers exposed to very high concentrations of perchlorate do not display alterations of thyroid function is an important piece of information for evaluating the risks from perchlorate exposure, but the agency appears only to be interested in trying to discredit these findings.

**Benchmark Dose Analyses of Animal Studies:** Part of the justification for the selection of the point of departure in the rat is based on the results of a large number of benchmark dose analyses reported in the document. The draft EPA guidelines for benchmark dose modeling discuss quantitative approaches for dealing with the problem of selecting a point of departure based on multiple benchmark results. In particular, it is recommended in general to make comparisons across endpoints on the basis of the BMDs (best estimates) rather than the BMDLs (lower confidence limits), regardless of whether the BMDL will be used as the actual point of departure. However, the selection of the point of departure in this case appears to have been based on a qualitative judgment using BMDLs together with the results of other analyses (primarily NOAELs and LOAELs).

Given the large number of endpoints evaluated, it is difficult to review each of the decisions involved regarding the dose/response modeling used to perform the benchmark calculations. However, the decision (in the analyses of thyroid histopathology reported in Table 5-1) not to constrain the exponent in the Weibull model to avoid supralinear dose-responses is a departure from common practice and is particularly troubling. Constraining the value of the exponent to be at least unity is generally recommended to avoid unstable and artificially low BMDL estimates. At the least, the results for the constrained Weibull model should also be provided for comparison with the unconstrained results in Table 5-1.

There are also problems with the benchmark modeling of hormone endpoints reported in Appendix 7B, although these results do not play heavily in the selection of the point of departure. Specifically, most of the benchmark risk (BMR) definitions are based on a specified change (e.g., 10%) from the mean control value. Unfortunately, this definition of a BMR for a continuous endpoint (such as a hormone concentration) produces BMDs and BMDLs that are not comparable to those obtained from quantal endpoints (such as the thyroid histopathology results in Table 5-1). As discussed in the draft EPA technical guidance document for benchmark dose analysis, the comparable definition of a BMR would be a change in the mean equal to a specified fraction of the control standard deviation (e.g., 0.61 for a 10% quantal BMR, assuming 5% of the control population is “abnormal”).

**Description of Pharmacokinetic Modeling:** Overall, the description of the PBPK models is very thorough and the validation is compelling. The ability of the models to reproduce a large number of different experimental data sets with a consistent parameterization is quite impressive. It is particularly laudatory that the basis for every parameter in each model is clearly described and that, to the extent possible, parameter values were kept constant across models.<sup>2</sup> There are, of course, some aspects of the models that should be considered provisional. For example, while the descriptions of iodine kinetics are able to coherently reproduce multiple data sets for the administration of iodine radioisotopes, including the interaction with perchlorate, the model would be greatly improved by including a description of the organification of the iodine radioisotope, as well as the endogenous iodine stores. A description of this nature would be necessary to examine, for instance, the effect of iodine deficiency on the kinetics of perchlorate inhibition. However, this enhancement is not necessary for conducting dosimetry in support of the perchlorate RfD. As mentioned earlier, the predictions of the models for perchlorate in the blood and for inhibition of iodine uptake are adequately validated for this purpose.

The approach for calculating the HEEs with the models is well thought out and, in general, appears to be appropriate. With regard to Table 7-1, it should be made clear that the predictions of the models for the HEE based on thyroid AUC are dependent on the assumptions made by the investigators regarding the cross-species scaling of the kinetic parameters for perchlorate in the thyroid, since there are no human data that can be used to estimate these parameters. Given this significant uncertainty, these HEEs based on thyroid AUC should probably not have been included in the table.

Technically, the HEEs based on iodine uptake inhibition should be calculated using the same exposure paradigm in both rat and human. During the course of this review, AFRL/HEST investigators recalculated these HEEs assuming a non-upregulated thyroid, a 12-hour daily exposure and an 8-hour inhibition time point in both species. The results, which are included in the appendix, are similar to those presented in the EPA document, but it is noteworthy that the revised HEE for the adult human corresponding to a dose of 0.01 mg/kg-day in the adult male rat is 0.01 mg/kg-day, indicating equivalent inhibition at low doses.

There appears to be a conflict between the dose metric ratios in Table 7-2 and the HEEs in Table 7-3. For example, the ratio of the perchlorate serum AUC in the male rat to that in the pregnant rat at 0.01 mg/kg-day is given in Table 7-2 as 0.63, indicating that perchlorate concentrations are higher in the pregnant rat. Table 7-3, however, shows an HEE based on the pregnant rat that is half of the HEE based on the male rat, suggesting that perchlorate concentrations are lower in the pregnant rat. The attached appendix includes a revised version of Table 7-3 that is consistent with the AUC ratios in Table 7-2. However, as discussed above, for the purposes of the risk assessment, it would seem more appropriate to use only the HEEs based on the adult male rat, assuming that the relationships of perchlorate AUCs across life stages are similar in the rat and human. If this is done, then Table 7-3 is not needed. This same problem was noted for Table 7-

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<sup>2</sup> It should be noted of course, that the notion of parameters being kept constant across models depends to some extent on the assumptions being made regarding the allometric scaling of the various parameters. For example, partitions and affinities are generally unscaled, while clearances and maximum capacities are generally scaled by body weight to the  $\frac{3}{4}$  power. I have assumed that the latter is what is meant by unit notations such as ng/hr-kg, but this needs to be more explicitly defined in the tables.

7; however, no update is provided in the appendix, because it is anticipated that the entries in Tables 7-5 and 7-6 will need to be recalculated in similar fashion to the HEEs based on inhibition (Table 7-4) discussed above.

**Comments on the EPA Risk Characterization for Perchlorate,  
Human Health Aspects  
The Air Force Research Laboratory, Operational Toxicology Branch (AFRL/HEST) and  
The Naval Health Research Center Detachment-Toxicology (NHRC/TD)**

The following are comments on specific chapters and sections of the draft document.

**4. Human Health Effects Data**

Since January of 1998, DOD, EPA, PSG and other public stakeholders have worked together under the auspices of the Inter Agency Perchlorate Steering Committee to develop and implement a process to define and clarify the potential risk of perchlorate contamination in the environment. In February 1999, the first EPA external peer review panel asked DOD to consider funding additional work including the potential for use of human data in the assessment. In December 2001, however, EPA formally requested that the National Academy of Sciences conduct an expeditious review of the complex scientific and ethical issues posed by EPA's possible use of third-party studies, which intentionally dose human subjects with toxicants to identify or quantify their effects. According to the EPA news release, "during the Academy's consideration of the issues and until a policy is in place, the Agency will not consider or rely on any such human studies in its regulatory decision-making, whether previously or newly submitted. Should EPA be legally required to consider or rely on any such human study during this interim period, the Agency will assemble a Science Advisory Board sub panel to review and comment on scientific appropriateness and ethical acceptability of the study in question, and the Agency will provide an opportunity for public involvement. This external review would occur prior to consideration of the study and would allow the Science Advisory Board to review all available information on the study." Since there is currently no plan to call a Science Advisory Board, the human data currently available on perchlorate will not be fully considered. Therefore the peer review and subsequent policy decisions will not be based on all the available science.

**5. Toxicological Effects in Laboratory Animal Studies**

On page 5-3, U.S. Environmental Protection Agency's Draft Perchlorate Environmental Contamination: Toxicological Review and Risk Characterization (NCEA-1-0503, January 16, 2002) (the document) states that, "The results [of Thyroid Hormone and TSH Co-Laboratory Study Report] suggest that the variability in the RIA determination should be considered when determining effect levels." What this report demonstrated was that if a contract laboratory conducted the hormone analysis, two replicates were sufficient as they run RIA assays continually. If a laboratory is not a contract laboratory and is performing RIAs less frequently, three replicates should be used to measure hormones. The variability is potentially in the source of the laboratory doing the analysis, not in every analysis as implied in the above statement from the document.

The document then indicates how the EPA complied with a request for different approaches for statistical analysis of hormone data by developing two new approaches. This is misleading. The EPA found inconsistencies in how the data were analyzed in certain studies and reanalyzed all

data the same way, ensuring that statistical significant subfactors (gender) were not combined. The following paragraphs discuss these points in greater detail.

**Response to Re-Analysis of Perchlorate Hormone Data from the 1998 ERD:** According to Crofton and Marcus (2001), there were eight studies re-analyzed statistically. The major problem cited was that the original analyses disregarded a significant factor (gender) and pooled the gender and gender\*dose sum of squares with the individual rat variability to form an error term for the test of dose. This pooling affects the appropriateness of the error term and should not be done. The error term for all tests should be the pooled variability of rats in each combination of factor levels.

The usual approach to analyzing data such as those in these eight studies is to start with an analysis of variance where the pooled variability of rats from each combination of factor levels is the error term for all F-tests. Based on ANOVA test results, other tests such as paired comparisons or simple main effect tests may be performed. Paired comparison techniques vary widely. A two-tailed two-sample t-test comparing the control group and each dose group at  $\alpha=0.05$  is preferred in studies where the primary goal is finding the LOAEL because the p-value is easy to interpret. At times, the researcher may decide to use a Bonferroni correction so that  $\alpha=0.05/\#$  tests. Whether rat variability is pooled across all dose groups for the error term of paired comparisons depends on the distribution of the data. The p-values from these t-tests should be made available to the reader so the reader can make his/her own error level adjustments based on the number of tests performed. When tables or figures of dose group means separate males and females, paired comparison results with control for each gender are given regardless of a significant interaction between gender and dose.

Crofton and Marcus (2001) suggest a 'Conservative' and a 'Liberal' approach to analysis of variance. The conservative approach is now used by AFRL/HEST. With the liberal approach, the appropriateness of the denominator becomes questionable since different sources of variability are thrown in with the pooled variability of rats. The t-test is preferred to the Duncan's test since the t-test produces p-values that are easy to interpret.

The list from Table 1 of Crofton and Marcus (2001) indicates problems of pooling in the error term along with other peer review comments. It is assumed the re-analyses corrected these problems and obtained reasonable NOAEL and LOAEL values.

## **5.1 Chronic Studies and Genotoxicity Assays**

**5.1.1 Cancer Studies:** Literature supports thyroid tumors at high doses such as greater than 1% (greater than 1000 mg/kg-day). Even the tumors found in the two-generation study were at a dose higher than typically used for perchlorate animal studies (10 mg/kg-day).

## 5.2 General Toxicity: Short-Term and Subchronic Testing

### 5.2.3 The 90-Day Testing Strategy Bioassay in Rats

#### 5.2.3.1 General Toxicity: Thyroid Histopathology Results, and Satellite

**Reproductive Assay:** Although the document states that the NOEL is 1.0 mg/kg-day for thyroid histopathology at the end, the description of thyroid effects is misleading due to generalized reference to rats instead of always specifically identifying the dose group being discussed. Also there is disagreement with the PWG's description of hyperplasia and the following: study sponsor, the study director and two pathologists involved in the study. Since this study was published in Toxicological Sciences, it was critiqued and accepted by two external peer reviewers selected by the journal (Siglin *et al.*, 2000).

## 5.3 Developmental Neurotoxicity Studies

**5.3.1 The 1998 Developmental Neurotoxicity Study:** The stability of the perchlorate stock solutions and verification of dosing solutions should also be cited as the following technical report which does appear in the reference section of the draft: Tsui, D. T.; Mattie, D. R.; Narayanan, L. (1998) Stability and concentration verification of ammonium perchlorate dosing solutions. Wright-Patterson Air Force Base, OH: Air Force Research Laboratory, Human Effectiveness Directorate; AFRL-HE-WP-TR-1998-0068.

The sentence on lines 27-29 of page 5-34 is not correct. Pups retained for continued observation were given R.O. deionized water with chlorine (added at a maximum of 1.2 ppm as a bacteriostat). No pup was dosed with perchlorate in this study. All dosing stopped at PND10.

The discussion of changes in brain morphometry ignores the actual histology results, which were normal. Brain weights were also similar for control and exposed rats. The document also ignores the possibility that an increase is not adverse since iodine deficiency leads to decreases in brain size and weight. Another point not discussed is the fact that the different doses for the morphometry endpoints were not analyzed at the same time. This raises the possibility of processing variability. And most importantly, EPA chose to ignore the fact that the data were not dose response; the researchers did not see increased effect with increased dose.

The rat is more sensitive to colloid depletion than humans. The emphasis on iodide inhibition across species in the document appears to overshadow this fact. Pups are still developing at PND5 so they are expected to be more sensitive. However, the PND5 pups in this study are culled pups that were used to examine thyroid hormones and TSH as well as thyroid histology and morphometry. There was no standardization of litters at birth to eliminate physiological differences due to difference in litter sizes both within and between groups.

**5.3.1.4 Behavioral Evaluations and 5.3.2 Motor Activity Study:** Two similar studies were conducted to assess the effects of pre- and neonatal ammonium perchlorate on the neurobehavioral development of spontaneous locomotor activity of rat pups placed in an open field. The first study was conducted at Argus Laboratories and was repeated at the Naval Health Research Center Detachment-Toxicology (NHRC/TD) neurobehavioral laboratory. The EPA compared results from both of the studies to establish a NOAEL for the effect. The EPA's primary conclusion from the motor activity data is that there is a reduced habituation in the treated animals such that in the later periods of the test session, the activity in the treated animals does not decrease to the level that it does in the untreated animals. This effect, while not statistically significant with traditional tests, is evident with the EPA's reanalysis using a Bayesian hierarchical model. Based on the model, a NOAEL of 1.0 mg/kg-day was selected for the measure of spontaneous motor activity. A visual analysis of the data from the NHRC/TD study, as presented in the attached graphs, leads to a conclusion of agreement with the NOAEL standard from the EPA Draft Document. Graphs from the Argus study are not available in electronic form so are not attached to this document. Even so, the data are evaluated here in order to compare and contrast the results from both studies.

Although the proposed NOAEL of 1.0 mg/kg-day is supported with the open field motor activity data, there are issues to be considered when evaluating the data from the Argus and NHRC/TD studies. First, there are some methodological differences between the two studies that should be noted. They are described and highlighted in Table 1 below. For the most part, the differences are minor; however, there are two conditions that should be considered. Although it is difficult to determine what, if any, effect the following variables had on the final results, it is noteworthy that the open field used by NHRC/TD was larger than that used by Argus and dosing started two weeks later in the prenatal cycle in the Argus than in the NHRC/TD study. A second issue to be considered while evaluating the data is that the effect of reduced habituation is dependent on age and gender, but the gender and age differences were not the same across the two studies (Table 2). Specifically, the NHRC/TD data show habituation most affected in 22-day-old females and 18-day-old males whereas the most apparent effect was in 14-day-old males in the Argus study. In these cases, the data suggest reduced habituation in the treated animals. A third issue for consideration is the results in the two studies that directly counter one another (Table 3). In particular, in the Argus study, the 14-day-old males show reduced habituation, but in the NHRC/TD study they demonstrate increased habituation.

In conclusion, the differences in methodology and absence of replication of results in the two motor activity studies should be considered in a thorough evaluation of the data. Even so, the EPA's identified NOAEL for spontaneous motor activity in the developing rat pup, as based on the NHRC/TD study, is consistent with the data.

**Table 1: An Outline of the Methods and Differences for the Two Motor Activity Studies.**

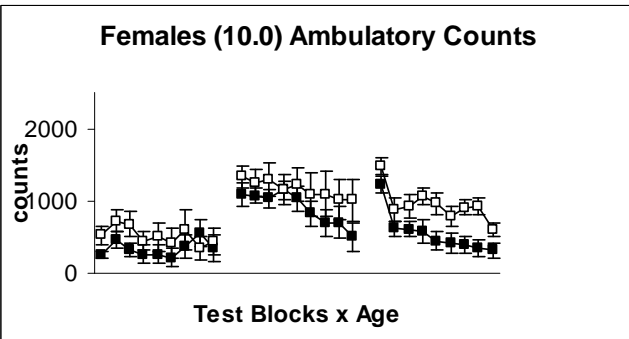
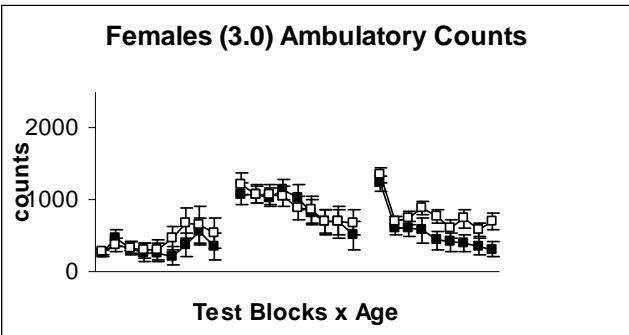
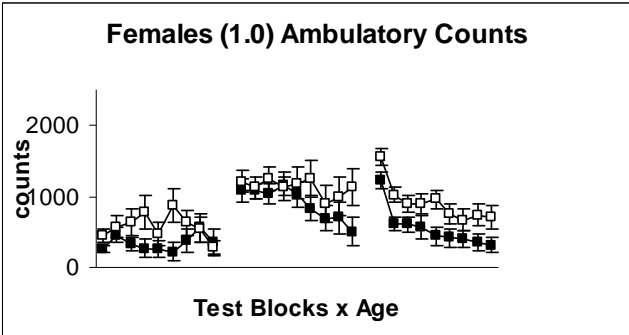
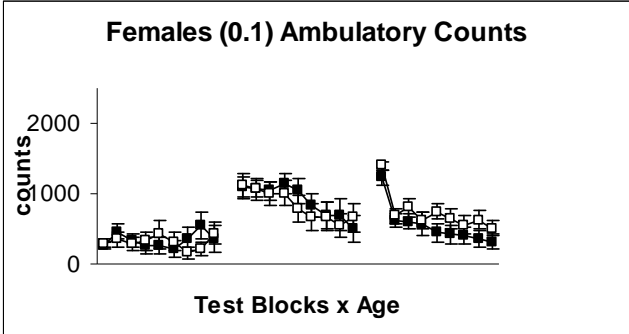
	<b>NHRC/TD</b>	<b>ARGUS</b>
Ages tested	14, 18, 22 days	14, 18, 22, 59 days
Test duration/ analysis divisions	90 minutes/every 10 minutes	90 minutes/every 5 minutes
Test system	17" x 17" (approx 43 x 43 cm) open field: sensors spaced 2.4 cm along lower and upper perimeters: Separate and different from home cages	40.6 x 25.4 x 17.8 cm wire-bottomed cage (solid flooring for pre-weaning ages) sensors on front of cage: DO NOT KNOW IF USED HOME CAGES OR CAGES SIGNIFICANTLY DIFFERENT FROM HOME CAGES
Target Doses	0, 0.1, 1.0, 3.0, 10.0 mg/kg-day in drinking water	0, 0.1, 1.0, 3.0, 10.0 mg/kg-day in drinking water
Measures	Time & freq of ambulatory mvmts, time & freq of stereotypic mvmts, freq of horizontal mvmts, distance traveled, rears, freq of vertical plane mvmts, time resting	# of movements and time spent moving
Lighting Conditions	Dim, red lighting	UNKNOWN
Treatment Duration	2 weeks prior to gestation – post-natal day 10	Gestation day 0 – post-natal day 10

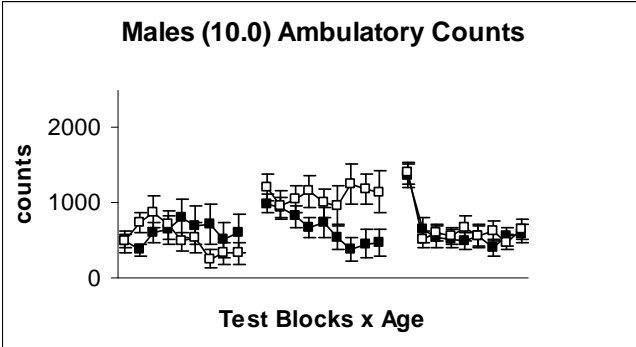
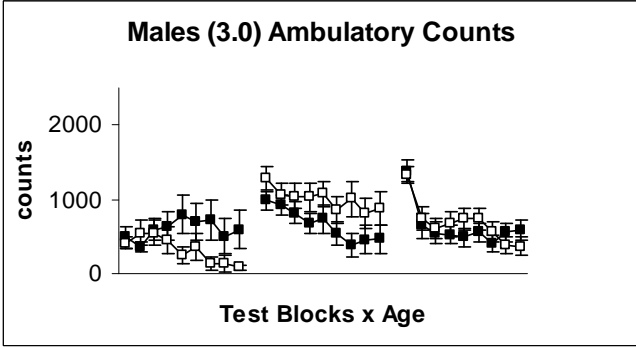
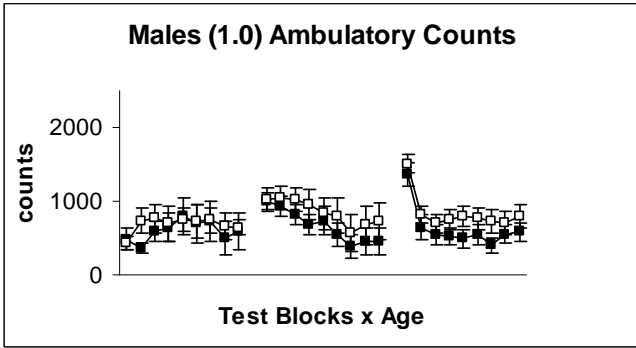
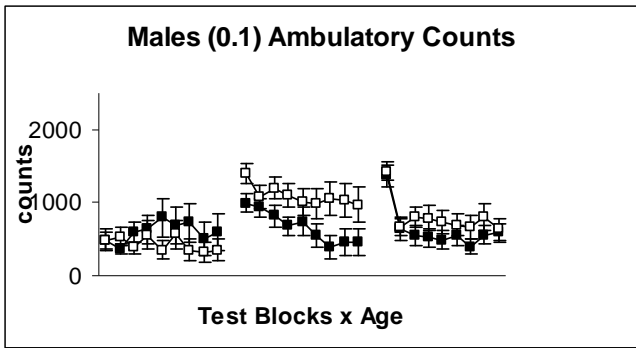
**Table 2. Evidence for Reduced Habituation in Treated Groups as Measured by Number of Ambulatory Movements.**

<b>Sex</b>	<b>Age</b>	<b>Dose</b>	<b>NHRC/TD</b>	<b>Argus</b>
Female	PND14	0.1 mg/kg-day	no	no
Female	PND14	1.0 mg/kg-day	no	no
Female	PND14	3.0 mg/kg-day	no	no
Female	PND14	10.0 mg/kg-day	no	no
Female	PND18	0.1 mg/kg-day	no	no
Female	PND18	1.0 mg/kg-day	no	no
Female	PND18	3.0 mg/kg-day	no	no
Female	PND18	10.0 mg/kg-day	no	no
Female	PND22	0.1 mg/kg-day	no	no
Female	PND22	1.0 mg/kg-day	yes	no
Female	PND22	3.0 mg/kg-day	yes	no
Female	PND22	10.0 mg/kg-day	yes	no
Male	PND14	0.1 mg/kg-day	no	no
Male	PND14	1.0 mg/kg-day	no	no
Male	PND14	3.0 mg/kg-day	no	yes
Male	PND14	10.0 mg/kg-day	no	yes
Male	PND18	0.1 mg/kg-day	yes	no
Male	PND18	1.0 mg/kg-day	no	no
Male	PND18	3.0 mg/kg-day	yes	no
Male	PND18	10.0 mg/kg-day	yes	no
Male	PND22	0.1 mg/kg-day	no	no
Male	PND22	1.0 mg/kg-day	no	no
Male	PND22	3.0 mg/kg-day	no	no
Male	PND22	10.0 mg/kg-day	no	no

**Table 3. Evidence for Increased Habituation in Treated Groups as Measured by Number of Ambulatory Movements.**

<b>Sex</b>	<b>Age</b>	<b>Dose</b>	<b>NHRC/TD</b>	<b>Argus</b>
Female	PND14	0.1 mg/kg-day	no	no
Female	PND14	1.0 mg/kg-day	no	no
Female	PND14	3.0 mg/kg-day	no	no
Female	PND14	10.0 mg/kg-day	no	no
Female	PND18	0.1 mg/kg-day	no	yes
Female	PND18	1.0 mg/kg-day	no	yes
Female	PND18	3.0 mg/kg-day	no	yes
Female	PND18	10.0 mg/kg-day	no	no
Female	PND22	0.1 mg/kg-day	no	no
Female	PND22	1.0 mg/kg-day	no	no
Female	PND22	3.0 mg/kg-day	no	no
Female	PND22	10.0 mg/kg-day	no	no
Male	PND14	0.1 mg/kg-day	no	no
Male	PND14	1.0 mg/kg-day	no	no
Male	PND14	3.0 mg/kg-day	no	no
Male	PND14	10.0 mg/kg-day	no	no
Male	PND18	0.1 mg/kg-day	no	no
Male	PND18	1.0 mg/kg-day	no	no
Male	PND18	3.0 mg/kg-day	no	no
Male	PND18	10.0 mg/kg-day	no	no
Male	PND22	0.1 mg/kg-day	no	no
Male	PND22	1.0 mg/kg-day	no	no
Male	PND22	3.0 mg/kg-day	no	no
Male	PND22	10.0 mg/kg-day	no	no





**5.3.3 The 2001 "Effects Study":** The document implies that up regulation of the thyroid after exposure to perchlorate is a compensatory response that made it more difficult to compare previous data. This is misleading as hypothyroidism, which involves an iodine deficiency, begins with up regulation of the thyroid. The thyroid was up-regulated by the increase in TSH. The hormone changes that the EPA uses as precursor effects are part of the compensatory response in the rat after exposure to perchlorate. The second paragraph should be removed or rewritten as it not consistent with the rest of the document as it is currently written.

## **5.5 Two-Generation Reproductive Toxicity Study**

**5.5.2 Evaluation of Thyroid Histology:** With respect to the tumors seen in this study, a number of questions were not addressed by the document. Why didn't the document address the fact that the first pathologist didn't see any tumors? Would every pathologist agree with Dr. Wolf on these adenomas? Why did the tumors only appear in the F1 generation and not at the end of the study (F2) if *in utero* imprinting is a possible explanation for the tumors in the F1?

**5.6 Immunotoxicity Studies:** The sponsor for the work conducted by Dr. Keil was not AFRL/HEST. A U.S. Army program, not a contract study, funded this effort as a research grant. The use of the term contractor throughout this section is misleading. The study was a research effort at an academic institution and was not a series of toxicity tests at a contractor laboratory. The investigator agreed to cooperate with the Air Force and EPA to provide interim data for the first risk assessment. Interim reports were voluntarily submitted to EPA to provide data as soon as possible instead of waiting for the final combined report and statistical analysis. A statistical analysis using all of the data will often be different than interim analyses because of the larger final n and a better understanding of how to conduct the analysis.

The BRT has issues not addressed by the document. The LLNA assay had conflicting results for both perchlorate and CP. Also there was no explanation of the dose range for the BRT studies. An error in determining doses for the studies resulted in a high dose of only 2 mg/kg-day (instead of 10), a dose too low to be the highest dose for an animal study with perchlorate. The 50 mg/kg-day dose was then conducted as a second study to get a true high dose value that was sufficiently high enough to cause effects in thyroid as well as immune endpoints. Variability and conflicting results could be the result of the dose range being conducted as two studies, one after the other instead of at the same time.

**5.6.5 Results for Evaluations of Hematological Parameters:** The document did not mention the results of the 90-day perchlorate study, which examined hematological parameters in male and female rats. The rat study also did not see any perchlorate effects on hematological parameters.

**5.6.6 Results Summary:** The document tries to compare the human case studies of the 1960s with the present mice studies of Keil and BRT. However, the clinical use of perchlorate involved large pharmacologic doses of perchlorate at 1000 mg/day or greater (more than 20 times greater dose). The comparison becomes an extrapolation from high dose to low dose

instead of a true comparison. The possibility of a contaminant in the pharmacological dose cannot be ruled out for the patients who suffered hematological and/or skin rashes as the cases were clustered in time after no such reports in the literature previously. Perchlorate was given to patients with thyroid disease, not healthy subjects. The form of the perchlorate was either potassium or sodium perchlorate rather than ammonium. This difference in cation was also seen in earlier cancer studies in rodents such as Weetman *et al.* (1984) mentioned earlier in Chapter 5. The form of the cation could have an effect under certain conditions. In aquatic studies, there are different effects seen between cations (Mattie, D.R. 2001. Consultative Letter, AFRL-HE-WP-CL-2001-0003, The Effects of Perchlorate on Amphibian Development and Growth Draft Report by J.N. Dumont, Oklahoma State University).

## 6. Construction of PBPK Models to Address Perchlorate's Mode-of-Action

### 6.2 Adult Rat and Human Model Structures

**Draft Document Table 6-1 - (Page 6-13):** The percent body fat for female humans should be 32.7, not 2.7. The volumes of the thyroid follicle and colloid for the human are switched; the follicle volume should be 15.0% and the colloid volume should be 57.3%. This error was propagated from the source document.

**Draft Document Table 6-1 continued - (Page 6-14):** The blood flows for the slowly and richly perfused tissues in the human are missing. Therefore, the next four human parameter values are shifted up two rows. Slowly and richly perfused blood flows should be 24.0 and 76.0%, respectively. Skin volume is also missing; it should be 5.8%. This caused the last three human parameter values in the table to be shifted up three rows. The corrected values are provided in Table 4, below.

**Table 4. Corrected Human Physiological Parameters**

Cardiac Output QCc (L/hour-kg)	16.5	Brown <i>et al.</i> , 1997; Hanwell & Linzell, 1973
Slowly Perfused QSc (%QC)	24.0	Brown <i>et al.</i> , 1997
Richly Perfused QRe (%QC)	76.0	Brown <i>et al.</i> , 1997
Fat QFc (%QC)	5.2	Brown <i>et al.</i> , 1997
Kidney QKc (%QC)	17.5	Brown <i>et al.</i> , 1997
Liver QLc (%QC)	22.0	Brown <i>et al.</i> , 1997
Stomach QGc (%QC)	1.0	Leggett & Williams, 1995; Malik <i>et al.</i> , 1976
Skin QSkc (%QC)	5.8	Brown <i>et al.</i> , 1997
Thyroid QTc (%QC)	1.6	Brown <i>et al.</i> , 1997
Adjusted Slowly Perfused QS (%QC)	13.0	Calculated, using 24% QC as flow to all slowly perfused tissues (Brown <i>et al.</i> , 1997)
Adjusted Richly Perfused QR (%QC)	33.0	Calculated, using 76% QC as flow to all richly perfused tissues (Brown <i>et al.</i> , 1997)

**Figure 6-33 (Page 6-72):** The top simulation should read “Control” and the bottom simulation should read “Perchlorate”.

**Figures 6-42 and 6-43 (Pages 6-98 and 6-100):** The figure legends should indicate an iv dose of  $1.0 \times 10^6$   $\mu\text{g}/\text{kg}$  perchlorate. It appears that when the document was compiled, all fonts were standardized, changing  $\mu$  to m or  $\mu\text{g}$  to mg.

**Figure 6-46 (Page 6-115):** The x-axis caption should read ng/kg-day, not mg/kg/day.

## 7. Dose-Response Assessments for Human Health

**7.1.2.1 Choice of Dose Metric - Page 7-15 (Lines 12-14):** The sentence should be revised to “...regarding the thyroid perchlorate values in the human neonates and fetuses because these values were not validated against experimental data. Thyroids of humans, fetal rats and neonatal rats were never actually analyzed...”.

**7.1.4 Application of Uncertainty Factors - Page 7-22 (Lines 3-31):** It is important to provide uncertainty factors that ensure that adverse effects are not seen. Information is lacking as to why the EPA used multiple UFs for potential adverse effects that are believed to occur at the same concentration. The need to include a 10-fold UF for the protection of brain morphology should usurp the need to include additional UFs. This UF exceeds the 3-fold UF for the protection of tumors and immunotoxicity. It also is logical to include only one UF in light of EPA’s description that adverse effects in brain morphology, neurotoxicity and thyroid hormonal effects occur at roughly the same level and are precursors to tumor effects and immunotoxicity. So, if effects that occur at the lowest concentration are prevented, i.e., changes in brain morphology, then you will protect against tumorigenic and immunotoxic effects. (The use of UFs as presented suggests that the endpoints one wishes to protect against cannot be pooled together, such that 5 endpoints would result in a total UF of at least  $3^5$  (or 243-fold) for an intraspecies UF.) A more logical composite UF should exclude two uncertainty factors (3-fold factor for duration in F1 tumors observed and 3-fold for immunotoxicity database insufficiency), which results in a 10-fold reduction in the composite UF, based on the EPA’s rounding of the four original UFs. The resultant composite UF would be 30 (intraspecies variability and brain morphology, which is inclusive of all effects) compared to the 300 composite UF calculated and modified by EPA NCEA.

**Medical Review, Preliminary Draft Comments**  
**Navy Environmental Health Center (NEHC-EP)**  
**8 February 2002**

- 1.** NEHC-EP was asked to review the U.S. EPA Perchlorate draft and provide comments back to AFRL/HEST, WPAFB. We are grateful for the opportunity to comment on this U.S. EPA Perchlorate External Review Draft. We have been able to complete only a cursory review of the EPA document due to the size (about 500 pages) and the short response time (driven by the 19 February 2002 U.S. EPA deadline for submission of comments to the U.S. EPA Docket for review by the Perchlorate External Peer Review Panel prior to the U. S. EPA proposed 5-6 March 2002 External Peer Review).
- 2.** Unfortunately, when we first tried to download the document from the U.S. EPA/NCEA Internet Site, we experienced great difficulty. In addition, we have been unable to review the original scientific papers referenced due to the U.S. EPA imposed time constraints. We feel that the U.S. EPA is doing the public a disservice by not allowing for questions to be formulated for the Expert Panel's consideration. We do not consider this tactic to be fair to the Panel members themselves, who are tasked to formulate an opinion without the benefit of all considerations of others, or to the general public, who may address issues that have been overlooked.
- 3.** We believe that we may not be alone (that is, among other perchlorate stakeholders) in requiring additional time to address (and/or obtain more information on) the many complicated issues that have impacted the selection of a "draft" perchlorate RfD (e.g., results, significance, and uncertainties associated with the perchlorate PBPK modeling data and other modeling simulations of relevance to this risk characterization). Unless the U.S. EPA postpones the External Peer Review of 5-6 March 2002, the document complexity and length makes the task of thoroughly reviewing it and trying to obtain and digest the significance of the data presented and/or discussed in the studies very difficult.
- 4.** Based on the limited quantity of sound scientific research data presented by the U.S. EPA in their document, we strongly feel that the draft reference dose (RfD) for perchlorate proposed by the U.S. EPA greatly overestimates the potential human health risk (overly conservative RfD, which would equate to a risk value in drinking water of 1 microgram/liter ( $\mu\text{g/L}$ ) or 1 part per billion (ppb) using standard default assumptions).
- 5.** Due to the relatively recent release of the perchlorate ecological toxicity data, the results of the ecological data may have been insufficiently reviewed for "soundness." Also, the relevance of these issues to human health risk assessment is unclear at this time. These results must be thoroughly reviewed and replicated by other researchers in these fields of endeavor.
- 6.** The overall goal of the U.S. EPA's human health risk assessment (HHRA) framework is to consider how to practice and communicate the "best science" in predicting credible human health risks. Customarily, data sets are limited. Even when the general scheme of toxicological events is known, species-specific mechanisms applicable to humans may not be fully understood.

**7.** The “best science” dictates having reliable estimates of exposure to accurately and comprehensively characterize the human health risks. Thus, premature release of a “draft” RfD for perchlorate at this time, when such a magnitude of uncertainty is associated with the risk numbers, may unjustifiably increase public concerns and fears concerning their health. Clearly, more research is needed to greatly reduce the uncertainty associated with the human health dose-response estimate proposed in the document.

**8.** Explain in the “Acknowledgements” Section of the document that although individuals and/or organizations are recognized for their specific efforts on behalf of the risk characterization of perchlorate, this does not imply that the DOD, etc. also supports the U.S. EPA’s quantification of potential health risk and the proposed “draft” RfD value selected.

**9.** The “Executive Summary” should clearly identify the “key” studies used to derive the “draft” perchlorate reference dose (RfD). The first page of the executive summary should state that the epidemiological, occupational, and clinical data presented in Chapter 4 were not used to derive the draft perchlorate RfD.

**10.** Most stakeholders and/or decision makers most likely do not have the time or desire to read through the 12-page “Executive Summary” Section. The main points should be presented in the first few paragraphs (overall concise summary). This cannot be emphasized strongly enough. List up front the key data used to derive the risk estimates. (Both Page E-6 and Table 3-8 are very helpful). Include a “Summary of Key Conclusions and Recommendations” Section, similar to an abstract (e.g., a few paragraphs summarizing the “Executive Summary” Section) up front. This section of the “Executive Summary” also should summarize the significance of the ecological toxicity data to the derivation of the draft perchlorate RfD and its impact/importance to the HHRA.

**11.** The following issues are of key importance when evaluating the magnitude of the toxicity of perchlorate, the relevance of the toxicity data to humans, and the dose-response for low environmental perchlorate concentrations: human exposure scenario information (e.g., frequency, duration, pattern, concentration in the environment necessary to achieve a “critical” internal dose of perchlorate); perchlorate scientific external peer review and evaluation of the quality of the recent individual animal and ecological toxicity studies (e.g., 2000, 2001); perchlorate scientific external peer review of the PBPK and other modeling simulations used and the uncertainties associated with these data; and perchlorate scientific external peer review of the magnitude of the uncertainty associated with the “draft” RfD proposed based on the dose-response data used; and the perchlorate scientific external peer review and evaluation of the potential reversibility/irreversibility of developmental, carcinogenic, and other applicable physiological and toxicokinetics effects.

**12.** Demonstrate/discuss how measurements of perchlorate exposure are applicable to potential environmental exposure scenarios and indicate the consistency with which the weight of evidence is related to health outcomes considered to pose an appreciable risk. Differentiate more clearly between which evidence strongly supports this association and which evidence may just “point to” an association of actual disease outcome.

**13.** More thoroughly discuss the ability to correctly quantify, within a specified estimated range, the HHRs from environmental exposure through applicable human health exposure scenarios (e.g., ingestion of drinking water, incidental ingestion of soils and/or sediments, incidental ingestion of surface water, etc.).

**14.** The potential impact to HHRA (that is, food-chain consumption issues) of the toxicity results derived from the ecological risk assessment are unclear and unproven at this time. We believe that the document should stress this more. We also feel that a great deal of uncertainty would be associated with any HHR numbers that are derived from these ecological toxicity data based on the data gaps and other potential differences in these health risk assessment methodologies (e.g., fish tissue sampling procedures recommended, differences in receptor exposure scenarios (exposure frequency and duration), dose-response variabilities, species sensitivities, etc.).

**15.** Address the magnitude of the uncertainties in the perchlorate HHRA/risk characterization (e.g., dose/response for endpoints considered to present an appreciable risk of an adverse effect over a lifetime). To assure appropriate consideration by decision makers, comment on the likelihood of an appreciable human health risk (HHR) occurring as a result of the dose-response functions measured in the key supporting literature (that is, animal, epidemiological, clinical, and mode of action/mechanism/physiologically-based pharmacokinetic (PBPK) modeling research).

**16.** Discuss the potential impact (e.g., for bias) pertaining to including versus excluding the results of select perchlorate clinical studies based on ethical issues (non-scientific data quality issues).

**17.** There are wide variations in the reported epidemiological results (about five studies presented), likely due in part to differences in data captured and other uncontrolled “confounders.” Some of the potential “confounders” discussed in the document have not been proven to impact the thyroid. It is a leap of faith to try to associate some of the “developmental” and “adult” “main symptoms and effects of hypothyroidism” listed in Table 3-4 with exposure to very low concentrations of perchlorate in the environment. We consider it inappropriate to try to regulate for some of the potential “confounders” addressed in Chapter 4 that may prove to have either a synergistic or an antagonistic effect in the presence of low concentrations of perchlorate in the environment (and/or some of the many other U.S. EPA regulated chemicals that have health impacts). The health effects of some of the various potential “confounders” by themselves (that is, in the absence of perchlorate in the environment) may impact or cause developmental effects (e.g., diet variabilities, ambient temperature variabilities; socio-economic status, health, age, effect of birthing process on newborn (degree of complications, etc.), other potential environmental exposures and lifestyle habits (e.g., alcohol/medication consumption), etc.). It would be a real challenge to differentiate between the effect of these potential “confounders” by themselves and the effect of exposure to low concentrations of perchlorate in the environment plus these “confounders.”

**18.** Explain the statement that “New studies since 1999 have confirmed that the inhibition of iodide uptake by perchlorate at the [sodium-iodide symporter] NIS is essentially the same sensitivity across species” (page E-5). Only certain species (those reported) may have exhibited

the same sensitivities. The text also should explain that different species, to include humans, have different mechanisms to compensate for/respond to changes/level fluctuations. The first complete paragraph on Page 3-18 does bring up the subject of species variability and the pharmacokinetic and pharmacodynamic differences between humans and laboratory animals. We believe that this concept should be explained in the executive summary section and in greater detail in Chapter 3 of the document (and refer the reader to Chapter 6, which does address this concept in much greater detail). Concerned stakeholders without scientific and/or familiarity with modeling simulations, etc. may inadvertently (or intentionally) skip one of the most important chapters in the document.

**19.** A great deal of uncertainty and variability in quantifying HHRs are associated with the use of data obtained from modeling simulations (e.g., the potential effects of differences in parameters used with different modeling systems, the current lack of models equipped with the capability to account for up-regulation of the thyroid, etc.). We suggest a more thorough treatment of the “uncertainty” associated with deriving a quantitative dose-response estimate for perchlorate thyroid effect from PBPK modeling. This complex issue needs to be explained as clearly as possible to enable concerned stakeholders to understand how important an issue this is. Both the “Executive Summary” Section and Chapter 6 should summarize these uncertainties.

**20.** None of the individual studies demonstrate sufficient weight of evidence to quantify the association between a significant health outcome (that is, not a transient, short term physiological response with questionable health consequence) and perchlorate environmental exposure. A few epidemiological papers suggest a slight association. Most of the epidemiological and occupational studies on humans included for review have been faulted for one reason or another. More questions have been legitimately raised concerning the potential role of “confounders” (such as, ambient temperatures, birth weights, dietary effects) on the human thyroid and its ability to compensate for these effects. Limitations exist in data derived from human studies. Limitations also exist in data derived from laboratory animal studies, not only in the experimental data themselves, but in their extrapolation and application to human health risk issues. These limitations greatly impede the establishment of an accurate quantitative dose-response estimate for the effects of perchlorate on thyroid hormones.

**Review of the Toxicological Review and Risk Characterization  
Of Perchlorate Environmental Contamination  
The U. S. Army Center for Health Promotion and Preventative Medicine  
11 Feb 02**

**General Comments**

The 2002 risk characterization for perchlorate was the product of a long-term collaboration between the DOD and the EPA. Many of the studies cited in this document were done very recently for the specific purpose of obtaining data needed to support this assessment. In general we have found that this review was thoroughly done and well written. It is important to note that the treatment of the data for risk characterization purposes was quite sophisticated and relied on some methods new to many reviewers. The information on the biochemistry and endocrinology of perchlorate toxicity presented in this document was impressive and the manner in which this information was used in the risk assessment appeared to be sound. However, the author's use of uncertainty factors for cancer risk and immunotoxicity do not seem consistent with the biochemical mechanism of the mode of action of this compound.

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**Specific Comments and Issues**

**1. Fate, Transport and Persistence in the Groundwater:** The physicochemical characteristics of the perchlorate anion presented in Chapter 2 of this review were accurate and consistent with information from other sources. The presentation of these data early in the document assists the reader in understanding later assessments of exposure and toxicity. In general, the characterization of perchlorate as a rapidly dissolved salt that exists in water as a free (largely uncomplexed) anion and leaches effectively to groundwater is accurate. The author's contention that this molecule is unreactive and remains unchanged for decades is well supported by the referenced documentation and the chemical description (tetrahedral orientation, low charge density, etc.).

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**2. Toxicokinetics/Toxicodynamics and Mechanism of Toxicity.** The author's contention that the sole mechanism of toxicity of perchlorate is the competitive inhibition of iodine uptake by the NIS (sodium/iodide symporter) was well supported in the document. This contention is fundamental to the remaining parts of this risk assessment because it implies that all of the toxic effects attributed to this compound stem from insufficient uptake of iodine by the thyroid gland with a resultant decrease in the production of the thyroid hormones T<sub>3</sub> and T<sub>4</sub>. According to the author, decreased levels of thyroid hormone produce both direct and indirect toxic effects. The lack of thyroid hormone affects development directly because of the effect of T<sub>3</sub>/T<sub>4</sub> on protein synthesis. The indirect effects also result from decreases in levels of T<sub>3</sub>/T<sub>4</sub> because neoplastic growth of the thyroid (evidenced in adenoma and carcinoma) is known to be caused by an excessive decrease in the concentrations of circulating thyroid hormones. Thus, the neoplastic effects are secondary to deficiencies in the generation of T<sub>3</sub>/T<sub>4</sub> and the serious toxic endpoints of adenoma of the pituitary hormone TSH. Increased levels of TSH are themselves generated in response to decreased thyroid hormone levels and cancer observed at higher toxicant concentrations need not be addressed separately from the formulation of a reference dose for the

non-neoplastic endpoints that occur at lower doses. Consequently, if perchlorate is present at concentrations insufficient to cause developmental effects (the most sensitive effects), one can also assume that this concentration is insufficient to generate neoplastic effects.

**3. Mechanism Across Species:** The author's contention that the mechanism for perchlorate's toxicity is the same across species is supported by evidence presented. This is important with perchlorate because of the large amount of animal and human data that are available for generation of a reference dose. Having a single toxic mechanism that is conserved across species supports the EPA's argument that the newer and well-designed studies with rodents and lagomorphs should take precedence (for the purposes of this risk characterization) over less rigorous epidemiology studies with humans. However, this argument may not be entirely valid because human populations are probably less sensitive due to the presence of thyroid binding globulin and the increased levels of thyroid hormone stored in the colloid.

**4. Brain Morphometric Measurements:** The most sensitive endpoint (lowest LOAEL) found in this study was the abnormal growth of the corpus callosum in the 21-day-old F1 animals. The author's initial intention to use a parallel vectors approach in the morphometric analysis did not work because this region does not seem to behave as the other regions of the brain. The corpus callosum became enlarged with exposures to perchlorate while in the remainder of the brain, the trend was the opposite effect. We agree with the author's contention that the multivariate analysis (addition of growth vectors) would probably have been the most reliable measure for assessing these effects and with the contention that this method could not be used for this study because of the inconsistency in the pattern of brain growth between the various regions. We also concur that the results from the univariate analyses of brain morphometry appear to have identified a significant occurrence. However, we do have some concerns about these studies. It should be noted that the corpus callosum is not of uniform thickness from front to back and it is critically important for the purposes of morphometry to tightly control the sectioning of this region. Error in identification of anatomical landmarks or in sectioning through these landmarks could easily bias the resulting morphometry data. In addition to the technical difficulties associated with sampling, we are somewhat concerned by times of sacrifice for these studies. The 9 and 21 day ages of the animals used in this study include periods of rapid brain development. Use of the PND21 age may very well be relevant because it is a sensitive period of development, but it is important to note that studying young animals during stages of rapid brain development creates uncertainty because this is also a time of high developmental variation between individuals in the test population. These studies would have been improved by examination of additional animals at PND40 to see if these differences persist after early development is completed.

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**5. Cancer Effects:** According to the document, the sole carcinogenic effect of perchlorate results from the neoplastic effects of increased levels of TSH. In the vernacular of modern cancer theory, TSH is acting as a tumor "promoter". It is known that tumor promoters require sustained action (often for much of the animal's life) in order to generate a neoplastic event. If promoters are withdrawn before a cancer occurs, the risk of developing cancer usually returns to normal. Thus, if TSH were to act as a tumor promoter, we would expect induction of carcinogenesis only after long-term chronic elevation. However, it is unclear from the text if sustained ingestion of low levels of perchlorate actually causes chronically elevated levels of

TSH above the normal range. It is certain that the circulatory levels of T<sub>3</sub> and T<sub>4</sub> do not remain chronically elevated. In animals that are exposed to perchlorate at low levels, the uptake of iodide is inhibited and the synthesis of T<sub>3</sub> and T<sub>4</sub> is reduced. However, this deficiency is rapidly detected and TSH is secreted, bringing the level of thyroid hormones back to normal. The entire cycle of decrease and return to normal takes less than 10 days even when the intake of perchlorate is sustained. If increased TSH is secreted in response to diminished T<sub>3</sub> and T<sub>4</sub>, why would it remain elevated after their levels return to normal? Are we certain that it does? If it does not remain elevated it is unlikely that a tumor "promoter" such as TSH would induce cancer. Conversely, if the levels of TSH are known to be chronically elevated above the normal range by sustained ingestion of perchlorate one must assume that the cancer risk is real.

## 6. Risk Assessment:

a) The LOAEL for the effect of perchlorate in rats is 0.01 mg/kg-day. The toxic endpoints seen at this level were anomalies in regional development of the brain, evidenced by morphometric determination (LOAEL), decreases in T<sub>3</sub> (LOAEL) and elevations in TSH (NOAEL). This evidence is indicative of potentially serious developmental effects associated with perchlorate at low levels in drinking water.

b) The composite uncertainty value used to formulate an RfD from the 0.01 LOAEL was 300. There was a factor of 3 for intraspecies variability (sensitive human subpopulation). This value is supported by the fact that transient and long-term hypothyroid states are not uncommon in women and further decreases in thyroid hormone caused by ingestion of perchlorate would place *in utero* children of these women at greater risk. There was a factor of 10 applied for something the authors call "extrapolation of the LOAEL for adverse effects". This is probably a different way of describing the traditional LOAEL to NOAEL uncertainty factor. A factor of 10 is commonly applied in LOAEL to NOAEL conversions and is probably appropriate for use here. The 3-fold factor applied because of tumors in the F1 generation may or may not be valid.

The potential for perchlorate to induce tumors was addressed in Section e, above. We are concerned that a cancer endpoint is assumed even though only two of the test animals (6.7% incidence) were seen to develop this condition. Moreover, it is important that this condition occurred only in the highest dose group (30 mg/kg-day), which is 300 times the lowest dose. It is important to remember that the author's initial contention that all effects, including cancer effects, stem from decreases in absorption of iodide. It has been shown that perchlorate acts only as a threshold carcinogen and that elevation of TSH is the only mode of action. Therefore, is there any evidence that cancer effects could occur at levels lower than the NOAEL for TSH?

c) The final 3-fold uncertainty factor for "inaccurate characterization of immunotoxicity data" may be difficult to support. As with the cancer endpoint, these effects occur only at doses in excess of the 0.01 mg/kg-day level upon which the RfD is based. As with the cancer assessment, we do not understand the reasoning in adding additional uncertainty factors for effects that occur only at higher levels than the 0.01 mg/kg-day level of the most sensitive endpoint.

**Review of the Ecological Risk Assessment**  
**The U. S. Army Center for Health Promotion and Preventative Medicine**  
**11 Feb 02**

**General Comments**

The document is well written and very thorough providing excellent background and adequate explanations. Clearly, the focus of the document is on the protection of human health and the derivation of a Reference Dose. The methodology and logic for this seem sound although EPA did not provide adequate time for review for a document of such magnitude. This review focused on Chapter 8, Screening Ecological Risk Assessment for Perchlorate. Detailed comments are provided below. Generally, this section followed the others in terms of readability and thoroughness. It is more than apparent that what is needed most is more quality research on the effects of perchlorate on wildlife and aquatic species. The lack of inclusion of amphibians as an important assessment endpoint was surprising given that these animals are in the public eye, likely to be exposed to perchlorate and are seemingly susceptible to the deleterious effects of perchlorate. Moreover, recent data are robust and could be used to derive a reasonable benchmark. Also, there are concerns regarding the screening benchmark for herbivorous mammals. The benchmark is based on levels of perchlorate that affect thyroid function but do not result in what some consider ecologically relevant effects (survival, growth or reproduction). Hence the benchmark value would likely suffer criticism among those in the ecological risk assessment arena. It would be prudent to reevaluate the screening benchmark with respect to levels of perchlorate known to alter development and reproduction.

**Specific Comments**

**8. Screening Ecological Risk Assessment for Perchlorate**

**8.2 Problem Formulation**

**8.2.1 Assessment Endpoints:** It seems surprising that amphibians are not mentioned in this section. It is well recognized that (1) amphibian biomass can be extremely high in certain ecosystems, (2) amphibian populations are on the decline and (3) developmental effects of contaminants can be significant. Given these facts and the likely deleterious effects of perchlorate on amphibian development (see Goleman, W.L. *et al.* 2002. *Environmental Toxicology and Chemistry* 21(2): 424-430), amphibian populations would be a warranted assessment endpoint. Another reason warranting the inclusion of amphibians as assessment endpoints is the likely exposure of certain species (some salamanders for example) to perchlorate contaminated soil and surface waters. These species may prove valuable in assessing ecological effects of perchlorate as they could easily receive the same exposure from water or soil. It seems likely that, given recent data, a Toxicity Reference Value for amphibians could be derived.

It is important to ensure that the final report on perchlorate includes available information on amphibian populations as viable assessment endpoints. Mention the studies on the effects of

perchlorate on amphibian development and that amphibians could prove useful in assessing the effects of perchlorate due to life histories that would lead to exposures via surface waters and soil.

Similar to the previous comment, birds are not mentioned as a viable assessment endpoint. These are typically high profile organisms that the public often has interest in conserving. Also, data indicate that perchlorate can reach high levels in plant seeds (Smith, P.N. *et al.* 2001. *Ecotoxicology* 10: 305-313). Seed-specializing birds may then be exposed to high levels of perchlorate. Although there are no data on the effects of perchlorate on birds, a small discussion is warranted to indicate to the reader that the potential ecological impacts of perchlorate have been thoroughly thought out; however, current conclusions are limited by data availability.

The final report should include a discussion of birds as potential assessment endpoints. Although you may not choose to include this group, a more detailed discussion will show a more thorough consideration the potential ecological effects of perchlorate.

### 8.3 Analysis

**Section 8.3.2 Characterization of Effects:** This section may benefit from a change in title. Primarily, the important information in this section is related to the derivation and presentation of benchmarks. It may be useful to include “Benchmark” in the title of this section as the reader could more easily find the important benchmark values. For example, “Screening Ecological Benchmarks” or “Screening Benchmarks for Characterization of Effects” are suggestions.

**Section 8.3.2.2 Terrestrial Organisms:** Why, when deriving benchmarks for ecological receptors, is the proportion of perchlorate in the test compound (sodium perchlorate, for example) not factored into the equation? This was done for the RfD., but not for ecological receptors. It is fairly common practice to consider the relative proportion of compound of interest to test compound when deriving benchmark values such as toxicity reference values. Include the proportion perchlorate/test compound in the derivation of ecological screening benchmarks or offer a brief explanation for why this was not done.

**Section 8.3.2.2 Terrestrial Organisms - Soil Invertebrates:** The derivation of the soil screening benchmark and the equivalent aqueous phase benchmark is not clear. Although generally the method is sound, the origin of the exact numbers requires a more transparent presentation. Although it is not too difficult to trace the steps taken, a more thorough presentation would help the reader remain focused on the important points of the document instead of wondering where certain numbers were obtained. Include more detail on the derivation of the soil screening benchmark and the equivalent aqueous phase benchmark (i.e. what are the numbers contributing to the acute-chronic ratio). Further elucidate the relationship between the soil screening benchmark and the aqueous phase benchmark; why and how are these related?

**8.3.2.2 Terrestrial Organisms - Herbivores:** The dietary screening benchmark for herbivores is based on the same results that the RfD is based on: perturbations in thyroid and pituitary hormones, thyroid histopathology and changes in brain morphometry in dams and pups. The document states that the population-level consequence of these effects is unknown, although these effects are likely to result in reductions in populations. Some in the ecological risk assessment arena would argue that if the endpoint is not ecologically relevant than it is not appropriate for deriving a benchmark. On page 7-4, it is stated that “developmental and reproductive NOAEL and LOAEL values were higher than those associated with thyroid toxicity per se.” Hence, it seems that for ecological benchmarks, the higher NOAELS and LOAELS associated with endpoints known to be ecologically relevant (i.e., reproduction) would be the better choice. From an ecological standpoint, is a slight alteration in thyroid function really going to impact the population? The data presented, particularly from the multi-generation test, suggest it would not. Results showed that alterations in development and reproduction occurred at levels higher than alterations in thyroid function, so it also suggests that the alterations in thyroid function at the lower levels of perchlorate were not severe enough to impact survival or reproduction.

Consider deriving the screening benchmark for herbivores using toxicity values associated with endpoints known to be ecologically relevant (development, reproduction). If this is not done, then a robust explanation should be provided as one could argue the derivation of the benchmark contradicts the data presented. Since it is likely that this value may drive terrestrial screening level eco-risk assessments, care should be taken in generating a robust, defensible benchmark.

**Ecological Risk Assessment Comments**  
**Air Force Institute for Environment, Safety and Occupational Health Risk Analysis Health**  
**Risk Assessment Branch (AFIERA/RSRE)**

**General Comments**

The weight of evidence approach should incorporate all information. This perchlorate characterization assumes that the same methodologies used to develop the human health RfD are applicable to ecological risk. Because the assessment endpoints for the human health risk assessment are focused at the individual and because ecological assessment endpoints relate to population survivability, growth, and reproduction, it is not appropriate to substitute one for the other. Two separate risk assessments are required.

The risk assessment approach used by EPA NCEA in this evaluation differs from the EPA's 1998 Guidelines for Ecological Risk Assessment (US Environmental Protection Agency. Guidelines for Ecological Risk Assessment. EPA/630/R-95/002F, April 1998) and 2000 Soil Screening Level (SSL) draft guidance

<http://www.epa.gov/oerrpage/superfund/programs/risk/ecorisk/guidance.pdf>

Chapter 5 of the EPA Guidelines for Ecological Risk Assessment describes the need to provide clear information to risk managers. The meadow vole evaluation should not be used to determine effect concentrations, because they are qualitatively linked to the assessment endpoints (i.e., population sustainability). Text Box 4-17 of the guidelines for ERA provides a list of "criteria strongly affirming causality". The first three items, "strength of association, predictive performance, and demonstration of a stressor-response relationship", cannot be determined based on the meadow vole risk assessment findings. These criteria are not met in this EPA NCEA evaluation.

Qualitative evaluation of toxicity information should be used when causality cannot be adequately extrapolated to the ecological endpoints of interest, defined as population level effects throughout the report. This approach should be used to evaluate *Pimephales promelas* and meadow voles, and is discussed more below.

Use of a weight of evidence approach is discussed in several sections of the report. The report describes how progression of effects at one target appears to be related to other outcomes. To visualize this information, a table or figure should be constructed which follows the EPA's thought process. This must include all evaluation measurements (i.e., those that are significant and those where there does not appear to be a difference).

Observations of "redness and swelling" in test organisms in the chronic assay using *Pimephales promelas* do not indicate an adverse toxicological effect. The endpoints of the assay demonstrated that there was no impairment to growth, survival, or reproduction. Likewise, changes in thyroid function in laboratory rodents do not necessarily indicate a probability of adverse effects at the ecosystem/population level. Interpretations such as these result in overly

conservative estimates of levels of perchlorate that are likely to result in adverse effects in ecological receptors.

It should be noted that the most contaminated sites studied by Parsons (2001) are also the sites that had the most robust populations of ecological receptors. Rodent trap success in the vicinity of the Las Vegas Wash with the highest levels of perchlorate in terrestrial plants was the highest of any site studied. In the surface water seeps in the same area, water bodies with the highest concentrations of dissolved perchlorate also had the highest observed numbers of the mosquitofish (*Gambusia affinis*). Since these site investigations were single efforts covering one week, we recommend follow up studies that investigate seasonal effects and also develop definitive information on the relationship between media concentrations and concentrations found in ecological receptors. This information is not found in the documents reviewed for preparation of the risk characterization and is mentioned here only to provide some site specific observations that are indicative of healthy habitats at the most contaminated site studied.

Throughout the document there are inconsistencies in the units used to report information from the toxicological literature and in the calculations. For example, mg/g and mg/kg are used inconsistently and interchangeably. Nor is there consistent use of scientific notation. For clarity, we recommend that an appropriate expression of the units of measurement be applied consistently throughout the document.

**Terminology:** Several terms should be defined within the report. These minimally should include bioconcentration and screening benchmark. Bioconcentration appears to be used to represent a greater concentration within a receptor; however, it can merely reflect the observation of the contaminant in a receptor and be at lower concentration than the exposure medium. Benchmark has been used to identify knowledge of percent of a species affected at a particular concentration (US Army Center for Health Promotion and Preventive Medicine. Standard Practice for Wildlife Toxicity Reference Values. Technical Guide 254. Environmental Health Risk Assessment Program, Health Effects Research Program. Aberdeen Proving Ground, MD. October 2000. <http://chppm-www.apgea.army.mil/> listed under publications). The EPA defines the benchmark as the “concentration (that) is intended to represent the location on the dose-response curve that is the threshold between absence and presence of the effects of concern for a relevant ecological endpoint” (Page A-6, Appendix 3-1, Plant and Soil Invertebrate Standard Operating Procedure # 3: Literature Evaluation and Data Extraction. In US Environmental Protection Agency. Ecological Soil Screening Level Guidance. Draft. Office of Emergency and Remedial Response, Washington, DC. June 27, 2000. <http://www.epa.gov/oerrpage/superfund/programs/risk/ecorisk/ecossl.htm>.)

## **Specific Comments**

### **7. Dose-Response Assessments for Human Health**

**7.1.1 Key Events and Weight of the Evidence:** “Developmental and reproductive NOAEL and LOAEL values were higher than those associated with thyroid toxicity per se.” This

suggests that the effects evaluated by ecological risk assessors who look at potential impact to the individual and try to interpret effects on populations and communities, should consider that the “screening benchmark” described in the ecological section should not be based on thyroid effects. However, potential adverse effects that are associated with reproduction and survivability should be used to develop the “screening toxicity reference values”.

## **8. Screening Ecological Risk Assessment for Perchlorate**

**Pages 8-2 (Line 13), 8-6 (Line 24-26), 10-14 (Lines 1-6), 10-15 (Lines 2-11):** Page 8-2 (Line 13) “...assessment was categorized as screening-level.” Page 10-14 provides a description suggesting the screening benchmark, derived for the meadow vole, may be inappropriate. It also indicates the need to provide more information to the risk assessor who is attempting to determine cleanup levels. Page 8-6 (Line 24) states that the endpoint of interest is population productivity. This lack of understanding of impact to potential species and need to address population effects for herbivorous wildlife should be described in Section 10.2.5.2 (Research Needs: Effects), Page 10-15.

**Page 8-2 (Line 1):** Replace “USAF Armstrong Laboratory” with “USAF Institute for Environment, Safety and Occupational Health Risk Analysis.”

**Page 8-8 (Line 16):** Change “form” to “from”; change “report” to “reports”.

**Pages 8-8 (Lines 16-18), 8-11 (Lines 16-18), 8-12 (Lines 1-11):** The variable nature of perchlorate in natural systems needs to be considered. Care should be taken to describe the lack of understanding of transient/variable exposures when comparing water concentrations (especially at seeps and streams) due to the rapid movement of perchlorate in these systems. Perchlorate-contaminated waters were being released at the Longhorn Army Ammunition Plant at known rates; however, the variability in the concentrations monitored at the same points downgradient from the release, ranged from 24 to 711 µg/L over a five-week period (Wireman JR. Advancement of Ecological Risk Assessments within the Department of Defense. Dissertation. Texas Tech University. Lubbock, Texas. December, 2001.). Factors, which likely influenced the concentrations, were related to water flow of the stream and periodicity of discharge (four days per week).

**Pages 8-8 (Lines 16-19), 8-12 (Lines 5-11):** The Condike (2001) study did not analyze whole fish, but mostly the head and surrounding tissues. Consequently, these data are not directly comparable to whole fish tissue analyses and may not adequately reflect bioconcentration.

**Page 8-10 (Line 8):** The “INF Pond” is an engineered, clay-lined, surface water impoundment.

**Page 8-11 (Lines 16-18):** Clarify what media were high and their association to the once contaminated INF pond at the Longhorn Army Ammunition Plant.

**Page 8-13 (Line 14):** Caps are not required for “Eastern Cottonwood”.

**Pages 8-15 (Line 9):** We agree that the 100-fold plant uptake factor is conservative. A linear regression of the aquatic plant data and surface water samples was performed on the Parsons (2001) data by Wireman (2001). Incorporation the 16 and 280 µg/L water concentrations used in this report on Page 8-14 (Lines 28-30) into the model resulted in aquatic plants with 18.4 and 7.6 times the surface water dry-weight perchlorate concentration. Linear regressions were also performed on dry-weight surface soil and terrestrial plants from Parsons (2001). Using the range of concentrations (1 to 1470 mg/kg) reported on Page 8-12 (Line 22) would result in an increase of 25-fold at 1 mg/kg and 17-fold at 1470 mg/kg.

**Page 8-15 (Line 23):** A citation for the data presented in this paragraph should be provided.

**Page 8-15 (Lines 28-30):** Assumptions used to estimate perchlorate exposures should be restated.

**Page 8-17 (Line 5):** Methodology used to develop Tier II water quality should include the latest USEPA guidance (Title 40--Protection Of Environment; Chapter I—Environmental Protection Agency; Part 132—Water Quality Guidance for the Great Lakes System)

**Page 8-17 (Line 23):** Standard endpoints for potential ecological effects (growth, survival and reproduction) showed no significant effects. The subtle effects described here (redness and swelling) are not significant with regard to population endpoints. 40CFR132.2 indicates that an adverse effect "...does not include nonharmful effects such as tissue discoloration alone...". We recommend providing a justification for defining these observations as adverse.

**Page 8-22 (Line 22):** The factor for interspecies variance (242) is used here as it was in the previous document. Please provide a reference for use of aquatic uncertainty factor for evaluation of soil invertebrate toxicity.

**Page 8-22 (Line 29):** The LOAEL of 0.01 mg/kg-day used in the human health risk assessment is not appropriate for evaluating population effects of perchlorate exposure to wild rodents. To further apply an uncertainty factor of 10 to this number is being overly conservative. The sublethal effects seen in laboratory animals are not likely to cause adverse impacts in wild rodent populations.

## **9. Evaluation of Evidence for Indirect Exposures**

**Page 9-12 (Line 22):** The plural of leaf is leaves. Please correct.

**Page 9-14 (Line 6):** The final s in "accumulations" should be deleted.

**Page 10-17 (Line 2):** The sentence fragment is confusing. Please correct.

## **10. Major Risk Characterization Conclusions**

### **10.2 Ecotoxicology**

#### **10.2.3 Terrestrial Life**

**10.2.3.3 Herbivores: Page 10-10 (Line 17)** - This sentence implies that there is some toxicity to plants at environmentally relevant concentrations of perchlorate. As discussed earlier in this section, there is little known toxicity to terrestrial plants. Therefore, there is little threat to either the loss of habitat or the loss of food for terrestrial herbivores. Please rewrite this section to clarify any potential effects.

#### **10.2.4 Uncertainties**

**10.2.4.1 Uncertainties Surrounding Aquatic Risks: Page 10-12 (Line 8)** - The sublethal effects described here (redness and swelling) cannot be described as “adverse.” The results of the test indicated that all test animals matured. Please justify the use of this endpoint in determining the LOAEL for aquatic vertebrates.

## APPENDIX A Point of Departure and HEEs

The point of departure used by the EPA in the calculation of the perchlorate reference dose (RfD) for the human is 0.01 mg/kg/day. This daily dose, which is referred to as the human equivalent exposure (HEE) is intended to represent the human drinking water exposure that would be expected to produce serum perchlorate concentrations (i.e., AUC) equivalent to those achieved in the rat dams from the dose of 0.01 mg/kg/day, at which hormonal effects were observed in the developmental effects study (Argus, 2001). This HEE was calculated in four steps. First the adult male rat PBPK model was run to determine the perchlorate AUC in the serum of an adult male rat at a drinking water dose of 0.01 mg/kg/day. Second, the human PBPK model was run to determine the non-pregnant human adult (70 kg) drinking water exposure that would result in the same AUC for perchlorate as that calculated in step 1. The resulting "male rat based" HEE of 0.021 mg/kg/day is shown in Table 7-1 (row 1, column 3). Third, the pregnant rat PBPK model was run to determine the perchlorate AUC in the serum of the pregnant dam at a drinking water dose of 0.01 mg/kg/day, and this value was divided into the male rat AUC calculated previously to obtain the male rat:pregnant rat AUC ratio of 0.63 (Table 7-2). Fourth, this ratio was then multiplied by the adult male rat based HEE to obtain the "pregnant rat based" HEE of 0.01 mg/kg/day in Table 7-3. Unfortunately, the male rat:pregnant rat ratio should have been divided into the adult rat based HEE to obtain a pregnant rat based HEE of 0.04 mg/kg/day, so the value used by the EPA as the human point of departure was calculated erroneously.

A similar set of calculations yielded an alternative HEE of 0.02 mg/kg/day based on the perchlorate AUC in the serum of the neonatal rats associated with the LOAEL for brain morphometry effects of 0.01 mg/kg/day reported in the same study. In this case, the male rat:neonate rat AUC ratio is close to unity, so the miscalculation mentioned above does not effect the result. Thus the neonatal effects become the most conservative basis for the human point of departure, which would be 0.02 mg/kg/day.

However, there is no clear justification for applying either the male rat:pregnant rat ratio or the male rat:neonate rat ratio in the calculation of the HEE. The use of this ratio in the rat should be matched by a similar calculation in the human in order to obtain the cross species adjustment. In the case of perchlorate, however, there are no human models for pregnancy and lactation available, so the non-pregnant adult model calculations must be used in every case. Thus the "HEE" based on the pregnant rat, if calculated as described above, is actually the human drinking water exposure that would result in a perchlorate AUC in a non-pregnant human adult that is the same as in the pregnant rat. To apply it to a human in the same life stage (i.e., pregnant) tacitly assumes that there are no pharmacokinetic differences between pregnant and non-pregnant humans.

In the absence of information on perchlorate kinetics in the human during pregnancy and lactation, it would seem more appropriate to assume that the differences in dosimetry observed across life stages in the rat would also be expected to be similar in the human. That is, if differences between the pregnant rat and the adult male rat lead to an AUC for perchlorate in the

pregnant rat that is higher than that in the male rat, it is likely that a similar difference would exist between pregnant humans and adult male humans.

Given the above discussion, it appears that the adjustments for the different life stages are highly uncertain due to the lack of information on the relationships in the human. In the absence of human models for pregnancy and lactation it would therefore be more appropriate to simply calculate the HEE based on the calculation with the human PBPK model for a 70 kg adult of a drinking water exposure equivalent to an adult male rat dosed at 0.01 mg/kg-day. This HEE, which is provided in Table 7-3 as 0.02 mg/kg-day, which represents a rat-to-human dosimetry adjustment that can be performed with high confidence, should serve as the human point of departure for the risk assessment.

**APPENDIX B**  
**Human Equivalent Exposures**

An analysis of model parameter sensitivity on AUC serum and thyroid concentrations was performed with the male rat model (Merrill *et al.*, 2001a) using a 240 hour time point and drinking water concentrations of 0.1 and 1.0 mg/kg-day ClO<sub>4</sub><sup>-</sup>. Tables 7-1 through 7-4 of this appendix correspond to and replace the tables of the same number in the EPA document. HEEs based on thyroid AUCs are removed from Table 7-1, due to the uncertainty of this parameter in humans, as described earlier. Table 7-2 is unchanged from the EPA document and provides a referral point for the corrections made in Table 7-3. Table 7-3 shows HEEs across life stages based on the correct use of the serum AUC ratios in Table 7-2. Table 7-4 represents HEEs based on a continuous 12-hour drinking water exposure and an 8-hour inhibition time point on day 12 for both the species. This was done to using a non-upregulated thyroid in the male rat to provide the same exposure comparison in the human.

**Table 7-1 (Revised): PBPK Calculated Human Equivalent Exposures (HEE) to Various Experimental Doses in the Male Rat for 15 and 70 kg Human Based on Perchlorate Area Under the Curve (AUC) in Serum or Thyroid as the Dose Metric**

<b>Adult Male Rat DW Dose (mg/kg-day)</b>	<b>Human 15 kg HEE (mg/kg-day) based on serum AUC</b>	<b>Human 70 kg HEE (mg/kg-day) based on serum AUC</b>
0.01	0.030	0.021
0.10	0.145	0.1
1.00	0.745	0.505
3.00	2.050	1.35
5.00	3.350	2.25
10.00	6.750	4.45
30.00	20.300	13.2
100.00	65.000	43.8

**Table 7-2 (Revised): Ratio of PBPK Derived Perchlorate Area Under the Curve (AUC) Serum Concentrations in Drinking Water for Various Exposure Concentrations in Drinking Water for Various Experimental Life Stages**

<b>Rat DW Dose (mg/kg-d)</b>	<b>Male Rat: Pregnant Rat</b>	<b>Male Rat: Lactating Rat</b>	<b>Male Rat: Lactating Rat</b>	<b>Male Rat: Fetal Rat</b>	<b>Male Rat: Neonate Rat</b>	<b>Pregnant Rat: Fetal Rat</b>	<b>Lactating Rat: Neonate Rat</b>
0.01	0.63	0.58	1.44	1.44	1.16	2.28	1.99
0.1	0.73	0.54	1.06	1.06	0.85	1.46	1.56
1	0.90	0.84	1.44	1.44	1.01	1.61	1.20
3	0.94	0.95	1.67	1.67	1.71	1.77	1.80
5	0.95	0.98	1.74	1.74	2.14	1.82	2.18
10	0.96	1.00	1.79	1.79	2.70	1.87	2.69
30	0.97	1.02	1.84	1.84	3.33	1.90	3.26
100	0.97	1.03	1.86	1.86	3.65	1.92	3.55

**Table 7-3 (Revised): PBPK-Model Calculated Human Equivalent Exposures (HEE) to Various Experimental Life Stages in the Rat Using Serum Perchlorate Area Under the Curve (AUC) as the Dose Metric**

<b>Dose (mg/kg-day)</b>	<b>Human Equivalent Exposure (mg/kg-day)</b>				
	<b>Adult Male Rat</b>	<b>Pregnant Rat</b>	<b>Fetal Rat</b>	<b>Lactating Rat</b>	<b>Neonate Rat</b>
0.01	0.021	0.033	0.015	0.036	0.018
0.1	0.1	0.138	0.094	0.184	0.118
1	0.505	0.564	0.350	0.599	0.500
3	1.35	1.437	0.809	1.416	0.788
5	2.25	2.364	1.296	2.292	1.053
10	4.45	4.641	2.481	4.430	1.649
30	13.2	13.663	7.179	12.925	3.959
100	43.8	45.196	23.599	42.603	12.003

**Table 7-4 (Revised): PBPK Calculated Human Equivalent Exposures (HEE) to Various Experimental Doses in the Adult Male Rat for 15 and 70 kg Human Based on % Iodide Uptake Inhibition in the Thyroid from DW ClO<sub>4</sub><sup>-</sup> Exposure in a Non-upregulated Male Rat**

<b>Adult Male Rat DW Dose (mg/kg-day)</b>	<b>Human 15 kg HEE (mg/kg-day) based on % Iodide Inhibition</b>	<b>Human 70 kg HEE (mg/kg-day) based on % Iodide Inhibition</b>
0.01	0.008	0.01
0.10	0.085	0.06
1.00	0.800	0.5
3.00	2.200	1.5
5.00	3.500	2.3
10.00	8.000	4.8
30.00	25.000	14
100.00	75.000	40