Quantitative Evaluation of Perchlorate Risk Assessment

Comments Submitted by

Toxicology Excellence for
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Executive Summary: Key Findings Presented in Comments

- Decreased serum T4 should be designated as the critical effect. Pregnant animals or women are a sensitive subpopulation.

- EPA's weight-of-evidence analysis in support of a point-of-departure is flawed because it ignores data that does not support its position and fails to evaluate the adverse nature of each of the endpoints discussed. Adverse effects actually identified by the body of perchlorate data include:
  - decreased habituation of motor activity; LOAEL of 3 mg/kg-day
  - immune enhancing activity; LOAEL of 30 mg/kg-day
  - thyroid hyperplasia; LOAEL of 1-3 mg/kg-day
  - decreased serum T4; statistically significant changes at 0.01 mg/kg-day

- The quality of the human database is sufficient for deriving a human-based RfD. Dramatic dynamic differences between rats and humans in their response following iodine uptake inhibition suggest that using animal data as the basis of the RfD will introduce an unnecessary degree of uncertainty and excess conservatism in the assessment.

- The most appropriate point-of-departure for a perchlorate RfD is a benchmark dose analysis on the data from Greer (2002). A BMDL of 0.02 mg/kg-day based on 20% inhibition of iodine uptake inhibition was identified as an appropriate point-of-departure since, no effect was observed on serum T4 levels in Greer (2002). In the rat data are used, the most appropriate point-of-departure is a BMDL of 0.007 mg/kg-day based on 20% decrease of serum T4 in pregnant rats.

- EPA's choice of an uncertainty factor of 3 for human variability is supported by data following the IPCS approach of Compound-specific Adjustment Factors. Data from the Greer (2002) study could support human variability up to 10-fold.

- If the animal data is used for the point-of-departure, then the data supports an uncertainty factor for interspecies variability of 0.1.

- Uncertainty factors for duration, extrapolation from a LOAEL, and incomplete database are not needed.

- Based on a total UF of 10, a RfD from the Greer (2002) study of 0.002 mg/kg-day is appropriate.

- If animal data is used for the point-of-departure, a total UF of 0.3 resulting in a RfD of 0.02 mg/kg-day is appropriate.

Mode of Action – Critical Effect

Observation

On page 7-3, EPA presents an overall mode-of-action for perchlorate that includes inhibition of iodine uptake in the thyroid as the key event. Subsequent events include decreases in serum T4 (and T3), which leads to altered neurodevelopment if observed in either dams or fetuses/neonates, and increases in serum TSH, which may lead to thyroid hyperplasia and tumors. On page 7-9 (Lines 23-30), EPA list a series of observations from the body of rat studies, including alterations of hormones, increased thyroid weight, alterations of thyroid histopathology (including tumors), and neurodevelopmental effects as providing supporting evidence for the proposed mode-of-action, and confirming that the perturbation of thyroid hormone economy as an adverse effect. EPA then indicates that a weight-of-evidence approach will be used to determine a point of departure.

Findings

We agree with EPA's statement of overall mode of action. We also agree that the available data provide evidence that perchlorate is operating by this mode of action in both animals and humans. We disagree, however, with EPA's designation of a host of effects as critical. As clearly shown in background EPA documents (e.g., Barnes and Dourson, 1988), the critical effect(s) is the first adverse effect(s) or its known precursor(s) as dose increases. In the case of perchlorate, the first adverse effects are hyperplasia in the thyroid and neurological changes in offspring. The clear precursor to these adverse and adaptive effects is hormone changes, specifically decrease in serum T4 and increase in serum TSH.

We encourage EPA to clearly define those effects which are adverse, rather than adaptive, and be consistent with its proposed mode-of-action. We encourage EPA to clearly show the critical effect – the adverse effect, or its known precursor, that occurs at the lowest dose, and to clearly state that by preventing the critical effect, one prevents all adverse sequelae. Following the proposed mode-of-action, inhibition of iodine uptake in thyroid, the key event, can be considered as a biological marker of perchlorate exposure, but is not a critical effect because there is some, as yet unquantified, link to the reserve of T4 thyroid hormone. Specifically, we do not yet know what levels of iodine uptake inhibition would decrease T4 levels. The next step in the proposed mode-of-action involves the decrease of serum T4, leading to neurodevelopmental effects, and the increase of serum TSH, leading to thyroid hyperplasia and ultimately tumors. In defining which of these represents the critical effect, it is important to consider which event is most relevant to human public health.

In its policy on assessing thyroid follicular tumors, U.S. EPA (1998) notes, "that the consequences of long-term antithyroid action [in humans] are harder to interpret and controversy exists whether the enlarged human thyroid gland undergoes conversion to cancer. Thyroid enlargements and nodules have been implicated as possible antecedents to thyroid cancer in humans, but direct evidence of conversion of these lesions to cancer is lacking." In contrast, a series of recent reviews suggest that children born to mothers who had decreased levels of serum

T4 during pregnancy were more susceptible to deficits in neuropsychological development (Haddow et al., 1999; Morreale de Escobar et al., 2000). (Separate comments have been submitted on the strength of conclusions that can be drawn from these studies.) Therefore, of the two pathways to altered structure and function proposed for perchlorate's mode-of-action, the assessment of decreased serum T4 followed by potential neurodevelopmental effects is more relevant to an assessment of human health and should be considered as the critical effect.

Figures 1-7 (Appendix 1), below, plot T4 data from all studies as a function of percent change relative to the control animals in each study. These values are plotted against administered dose. From these plots, it is clear that dams on GD 20 are the most sensitive group of animals to decreases of serum T4. This effect becomes even more obvious when data from the effects study are plotted against serum perchlorate AUC as estimated by the pregnant/lactating rat pbpk model (Figure 8).

Figures 9 and 10 are adapted from EPA's document and show the human equivalent exposures that were calculated using the pbpk models from data in various life stages of the rat. Figure 9 was calculated using serum perchlorate AUC as the dose metric and Figure 10 was calculated using iodine uptake inhibition as the dose metric. Essentially, both figures demonstrate that at the lower end of the dose range, all stages of rat are equivalent at predicting HEE. At doses greater than 3 mg/kg-day, the pregnant and lactating rat appear to generate more conservative estimates of HEE, based on serum AUC as the dose metric, than other life stages of rat. The neonate rat appears to give the least conservative estimate of HEE using both serum AUC and Iodine uptake inhibition as dose metrics.

The critical effect has been analyzed using three different approaches: relevance of effect to human health based on mode-of-action, evaluation of the empirical data, and evaluation of the life stage of rat that results in the most conservative HEE. Conclusion, decreases in serum T4 in pregnant population should be considered to be the critical effect most relevant to human health, both based on an analysis of mode of action, and an evaluation of the empirical data that this is the effect that occurs at the lowest doses. By developing a RfD based on the critical effect of decreased serum T4, all subsequent potential adverse effects will be prevented.

Observation - Designation of LOAEL from Animal Studies

Using a weight-of-evidence approach, EPA identified several studies that suggest 0.01 mg/kg-day as the exposure dose that is a level of concern for the adverse effects of perchlorate (page 7-16, line 25). EPA evaluates a number of endpoints that contribute to their overall weight-of-evidence argument including brain morphometry changes; motor activity changes, thyroid hormone changes, thyroid histopathology changes, and immunotoxicity.

Findings

Under the banner of the "weight-of-evidence" approach, EPA relies on any change in any parameter to justify calling the lowest doses tested a LOAEL without conducting an evaluation of whether the particular change in question is, in fact, adverse. In addition, EPA singles out effects that support its position while ignoring other data do not support its position. Here, we will evaluate each of the endpoints to assess what is adverse, what is the dose level that causes adverse changes, and what does the entire body of the data show.

Brain Morphometry. More detailed comments on the adversity of this endpoint are provided in a separate document by Wahlsten (2002). Five experts in neurodevelopment were asked to review the brain morphometry results of the "effects" study, and their comments were submitted to EPA (TERA, 2001). In general, these reviewers concluded that issues related to the design of the neurodevelopmental component of the study prevented drawing any conclusions about the effects of perchlorate on neurodevelopment. Reviewers pointed to several areas where the effects observed were inconsistent with the proposed mode-of-action for perchlorate, questioning whether any effects observed were in fact related to perchlorate exposure. Reviewers noted that there is no foundation in the literature suggesting that the size of most of the brain regions measured in the effects is affected by hypothyroidism.

EPA cites the guidelines on assessment of neurotoxicity in order to avoid a detailed analysis of the biological significance and adverse nature of the brain morphometry results from the effects study (page 5-65, line 15). Although the guidelines may specify that alterations in brain structure should be considered adverse, a closer reading would indicate that only alterations that are clearly treatment related should be considered adverse. Five neurological experts submitted comments, supported by evidence in the literature, which suggested that the observations in the effects study were sporadic and unrelated to perchlorate treatment. The EPA report did not address these review comments, nor did it provide support from the literature to suggest that five neurodevelopmental experts were wrong. Although the EPA report states that alterations in brain structure are consistent with the perchlorate mode-of-action (page 5-65, line 17), wishing cannot make it so. In fact, the observations from the effects study demonstrate a lack of consistency with perchlorate's mode-of-action. Specifically, sporadic changes were observed in brain regions that are completely unrelated to the role of thyroid hormones on brain development as defined by 50 years of literature on effects of hypothyroidism. The effects study was not designed to adequately evaluate neurological changes due to altered thyroid action. Yet, it could have detected changes in External Granular Layer thickness and alterations in degree of myelination, which are well known effects of hypothyroidism. However, these effects were not observed.

Motor Activity. EPA cites increases in motor activity observed in both the Argus neurodevelopmental study (Argus, 1998a) and the Bekkedal motor activity study (Bekkedal, 2000) as supporting a LOAEL of 0.01 mg/kg-day. Based on reanalysis using a Bayesian hierarchical model EPA identifies a NOAEL of 1.0 mg/kg-day for decreased habituation. More detailed comments on this analysis are provided in Bekkedal (2002); however, both the author and EPA agree that the NOAEL is 1.0 mg/kg-day. This conclusion is supported by the mode-of-action analysis, which suggests that neurodevelopmental effects will occur subsequently to

changes in serum T4 – at higher doses than changes in T4. However, these behavioral effects, observed only at doses of 3 mg/kg-day and higher, do not support designating a dose of 0.01 mg/kg-day as a LOAEL.

Immunotoxicity. EPA identifies effects in a series of immunotoxicity assays on perchlorate as supporting the weight-of-evidence conclusion that a dose of 0.01 mg/kg-day is a LOAEL. Specifically, following 14 and 90 days of drinking water exposure, perchlorate enhanced the effect of a contact sensitizer in the local lymph node assay at doses of 0.06, 0.2, and 50 mg/kg-day but not at doses of 0.02 or 2 mg/kg-day. Based on the results of this study, EPA suggests that perchlorate has the potential to be a contact sensitizer. On page 7-19 (line 27), EPA designates the 0.02 mg/kg-day dose level from this study as a NOAEL and the 0.06 mg/kg-day dose as a LOAEL. In contrast, on page 5-108 to 109, EPA indicates that the effects in the local lymph node assay cannot be designated as a LOAEL because it is not clear that this is a physiologically relevant adverse effect. First, the effects of perchlorate in the LLNA assay do not provide information on whether perchlorate is a contact sensitizer. Second, we would agree that this effect is not biologically significant and the effect should not be considered adverse (see review comments from Immunotoxicologists, Appendix 7):

> As stated above both immune suppression and immune enhancement can be adverse effects. Immune suppression can increase the host's susceptibility to microbial pathogens and cancer. Likewise, immune enhancement can, under certain instances, lead to hypersensitivity and autoimmunity. In light of the absence of immune inhibition observed throughout the battery of immune function assays, many of which are critically dependent on phagocytosis of antigen by macrophages, I do not believe that the inhibition observed in the *in vitro* phagocytosis assay is an adverse effect. Likewise, based on the absence of an increase by perchlorate treatment in serum anti-sRBC IgM or IgG, I do not believe the increase in the sRBC PFC assay alone constitutes an adverse effect. However, as a follow-up to the increase in plasma cell formation by perchlorate treatment observed in the anti-sRBC PFC response, additional measurements of serum IgE would be appropriate to further assess the potential for an adverse effect. Lastly, the enhancement of the LLNA response, as performed, in my opinion does not constitute an adverse effect and should be performed again utilizing an appropriate experimental design.

On the other, a variety of effects in the immunotoxicity assays suggest that, at doses 0f 30 mg/kg-day or higher, perchlorate may possess adjuvant-like activity, which could be considered adverse effects:

> With the exception of the *in vitro* macrophage phagocytosis of *L. monocytogenes*, the anti-sRBC IgM PFC assay, enhancement of the DTH response to SLA (30 mg/kg-day only) and LLNA, no consistent alterations attributable to perchlorate treatment were observed in any of the immune function assays as compared to the appropriate control groups. Based on the fact that none of the immune function responses, with the exception of 50 mg/kg-day in the 90 day LLNA study were inhibited and many of the responses are critically dependent on phagocytosis of

antigen by macrophages, it is unlikely that the impairment of phagocytic activity observed in the *in vitro* phagocytic assay is biologically significant. The results from the anti-sRBC IgM PFC assay, LLNA and DTH response to SLA suggest that perchlorate under certain exposure regimens – most likely extended exposure (i.e., 90 days) – possesses adjuvant-like or immune enhancing activity. Although immune enhancement as immune suppression can be an adverse effect, in light of the fact that neither enhancement nor immune suppression was observed in virtually any of the other immune function assays, the biological significance of the immune enhancement in the sRBC PFC assay, LLNA and DTH response to SLA is questionable. Moreover, there was neither a significant increase in serum anti-sRBC IgM nor IgG detected due to perchlorate treatment nor an increase in spleen cellularity, which further bring into question whether the enhanced anti-sRBC PFC response is biologically significant. Enhancement of the DTH response to SLA was only observed at the 30 mg/kg-day dose. Lastly, it is notable that the experimental design employed for the LLNA cannot ascertain whether perchlorate is a dermal contact sensitizer. Based on the available data sets, there is no experimental evidence to suggest that perchlorate is a dermal contact sensitizer.

However, note that there is no evidence to support the conclusion that these effects are related to the perchlorate mode of action. In addition, even though a dose of 30 mg/kg-day could be considered a LOAEL for immunotoxicity, this does not provide support of a LOAEL of 0.01 mg/kg-day.

Thyroid Histopathology. EPA stated that the 0.01 mg/kg-day LOAEL was also supported by the observations of thyroid histopathology in the database. However, EPA completely disregarded the conclusions of its 1998 Peer Review panel in basing its LOAEL designation on observations of colloid depletion and hypertrophy. The 1998 Peer Review Panel (RTI, 1999) concluded, "thyroid cell hypertrophy (increase in cell size) was not a good biomarker for adverse effect of perchlorate, but rather suggested the use of hyperplasia (increase in cell number)." "The variable used by EPA in the determination of the RfD (thyroid hypertrophy) was not considered by the Panel to represent an adverse effect, nor was it demonstrated to be correlated with an adverse effect such as hyperplasia." Thus, the panel recommended that the Pathology Working Group be convened in order to clearly establish dose-response curves for hyperplasia, the adverse effect.

Using the observation of statistically significant increase in incidence of thyroid hyperplasia as the designation of an adverse effect on thyroid hyperplasia, evaluation of both the PWG report and the Effects study demonstrate that thyroid hyperplasia was only observed in the following datasets (see Appendix 2):

- 90 day study (Siglin, 1998) – male rats
- Two-generation study (Argus, 1999) – P1 females, F1 male and female pups
- Rabbit developmental study dams (York, 1998)
- Effects study dams (York, 2000) – PND 10 and PND 22.

The lowest LOAEL for thyroid hyperplasia identified for any dataset is 1 mg/kg-day in PND 22 dams from the effects study; although this LOAEL was not confirmed in any other study. The next lowest LOAEL was 3 mg/kg-day for male F2 pups from the two-generation study.

In contrast, no statistically significant increase in the incidence of hyperplasia in the following datasets at the highest doses tested (up to 30 mg/kg-day):

- 14 day studies – males and females in both Caldwell (1996) and Siglin (1998) reports
- Neurobehavioral developmental study (Argus, 1998) – dams and PND 5 pups
- Two-generation study (Argus, 1999) – P1 males, F1 male and female adults, F2 pups
- 90 day exposure Immunotoxicity study (Siglin, 1998) – female mice
- Effects study (York, 2000) – Dams at GD 21, pups at PND 5, 10, 22.

Thus, while there are clearly data that demonstrate thyroid hyperplasia and identify a LOAEL for this endpoint, there are also data at identical doses and times, which demonstrate no effects at all on thyroid hyperplasia. The weight-of-evidence does not clearly point to a LOAEL for thyroid hyperplasia and does not clearly support a LOAEL of 0.01 mg/kg-day for thyroid histopathology. A conservative LOAEL for thyroid hyperplasia would be 1 mg/kg-day, but this level was only identified in one data set, and not confirmed in any other studies.

EPA conducted extensive benchmark dose analysis of the thyroid histopathology and cites BMDL estimates for colloid depletion and hypertrophy in the range of 0.01 mg/kg-day from several studies as support for designation of 0.01 mg/kg-day as the LOAEL/ point of departure. However, evaluation of the data reported by the PWG and in the Effects study demonstrates that **no statistically significant increased incidence of either colloid depletion or thyroid hypertrophy was observed at a dose of 0.01 mg/kg-day in any study**. As with thyroid hyperplasia, statistically significant increased incidences of colloid depletion or hypertrophy were not observed in all of the studies. Although, both endpoints were observed more consistently in females than males.

Significantly increased incidence of colloid depletion was observed in the following data sets:

- 14 day study, Siglin (1998) – female rats
- Neurobehavioral developmental (Argus, 1998) – rat dams and PND 5 pup
- Two-generation study (Argus, 1999) – P1 males and females, F1 male and female adults, F2 male and female pups
- 90 day study (Siglin, 1998) – males and females
- Effects study (York, 2000) – dams and pups at all times
- Developmental toxicity (York, 1998) – rabbit dams

The lowest dose in any study at which statistically significant increased incidence of colloid depletion was observed was 1 mg/kg-day in PND 10 dams, GD 21 pups, and PND 5 pups from the Effects study. Colloid depletion was not significantly increased in the remaining data sets from the Effects study until the 30 mg/kg-day dose. A significant increase in colloid depletion was observed at 3 mg/kg-day in dams from the neurobehavioral study and F1 adult male rats and F2 male and female pups from the two-generation study. In the remaining datasets where colloid depletion was observed, it did not occur until doses ≥10 mg/kg-day.

In contrast, no statistically significant increase in the incidence of colloid depletion in the following datasets at the highest doses tested (up to 30 mg/kg-day):

- 14 day study, Caldwell (1996) – male and female rats
- 14 day study, Siglin (1998) – male rats
- 90 day study, Keil (1999) – female mice
- 90 day study recovery group, Siglin (1998) – male and female rats
- Developmental study (York, 1998) – rabbit dams

Significant increased incidence of thyroid hypertrophy was observed only in the following datasets:

- 14 day study, Caldwell (1996) – female rats
- 14-day study, Siglin (1998) – female rats
- Two-generation study (Argus, 1999) – P1 female rats, F1 male and female adult rats, F1 and F2 male and female pups
- 90 day study, Siglin (1998) – male and female rats
- Neurobehavioral study (Argus, 1998) – PND 5 pups
- Effects Study (York, 2000) – dams at GD 21, PND 10, PND 22

The lowest dose at which statistically significantly increased incidence of hypertrophy was observed was 3 mg/kg-day in the P1 females and F1 and F2 pups from the two-generation study. In the remaining datasets where hypertrophy was observed, it did not occur until doses ≥10 mg/kg-day.

In contrast, no statistically significant increase in the incidence of hypertrophy were observed in the following datasets at the highest doses tested (up to 30 mg/kg-day):

- 14 day study, Caldwell (1996) – male rats
- 14 day study, Siglin (1998) – male rats
- Two-generation study (Argus, 1999) – P1 male rats
- 90 day study, Keil (1999) – female mice
- 90 day study recovery group, Siglin (1998) – male and female rats
- Effects study (York, 2000) – male and female pups at GD 21, PND 5, PND 10, PND 22

In summary, EPA bases its conclusion that the thyroid histopathology supports a LOAEL of 0.01 mg/kg-day on the fact that BMDL estimates for colloid depletion and thyroid hypertrophy from a relatively few datasets in the database are close to a value of 0.01 mg/kg-day. However, the 1998 Peer review panel concluded that hyperplasia, but not hypertrophy, should be considered as an adverse effect in rodent thyroids; EPA has not addressed these comments, nor indicated why they have disregarded the recommendations of their earlier panel. Although thyroid histopathology is clearly expected to be an effect of perchlorate based on its mode of action, the full weight-of-the evidence from considering the effects in all of the studies do not clearly demonstrate thyroid pathology as a consistent effect in all datasets observed. When all of the datasets are considered, no statistically significantly increase in any of the parameters of thyroid histopathology occurs in any data at doses lower than 1.0 mg/kg-day. And a true weight-of-

evidence analysis of the adverse effect, hyperplasia, demonstrates that for all but a single data set, doses ≥ 3 mg/kg-day are necessary before thyroid hyperplasia will develop.

Thyroid Hormones. The EPA assessment also points to alterations of thyroid hormone levels as supporting the designation of 0.01 mg/kg-day as a LOAEL. Specifically, EPA notes that T4 and TSH changes were observed in dams and pups in the effects study and in the 90-day study by Siglin. While it is true that statistically significant changes in hormones were observed at 0.01 mg/kg-day in some datasets, hormone changes were not consistently observed at these doses at all datasets (see T4 and TSH Figures in Appendix 1). As many datasets were observed that had no significant alterations of hormones at a dose of 0.01 mg/kg-day as there were datasets finding significantly different hormone levels at this dose. Thus, the weight-of-evidence does not clearly support EPA's conclusion. However, it is clear from these data that pregnant dams are the sensitive population for this effect, demonstrating T4 and TSH changes at lower doses and at a steeper dose response than other populations evaluated. This observation confirms a conclusion that T4 changes in pregnant dams should be designated as the critical effect in the sensitive population.

Although decreases in T4 as the critical effect are consistent with the mode of action, the degree of T4 decrease that can result in adverse effects should be analyzed. Schwartz (2002) indicates that although the T4 is the predominant hormone secreted from the thyroid, T3 is the more active hormone at the tissue and nuclear level. T3 in both human and rat is produced locally in the brain by monodeiodination of T4. In brain, the enzyme type II-5' deiodinase (5'D-II) is primarily responsible for this process. The 5'D-II activity is regulated by the intrabrain T4 levels so that a fall in T4 leads to an increase in enzyme activity and compensates for the diminished serum T4 seen in conditions such as hypothyroidism. In the normal adult rat brain, as much as 80% of the receptor-bound T3 in the cerebrum and 70% in cerebellum may be generated by local production of T3. Therefore, it appears that there can be a significant decrease in serum T4 levels before local production of T3 in the brain is compromised. Calvo et al. (1990, as cited in Schwartz, 2002) demonstrated that in fetuses of dams treated with methimazole, infusion of T4 to the dam results in fetal brain T3 that is normalized when the plasma T4 is still only 40% of normal (i.e., a 60% decrease of T4). These data would suggest that a decrease in serum T4 would not be adverse until there is a 60% decrease from normal. Evaluation of the entire database (Figures 1-7 in Appendix 1) demonstrate that in the entire body of data, T4 levels did not decrease more than about 50%, even at the highest dose levels in any study. Again, the pregnant dams appear to be the most sensitive, with a T4 decrease approaching 50% of control starting to occur at doses of 0.1 mg/kg-day.

Choice of Data

A primary point for discussion regarding EPA's risk assessment for perchlorate is the choice to base the risk assessment on the animal data. In our opinion, EPA erred in discounting the quality of the epidemiological, clinical, and occupational studies conducted on perchlorate (*TERA*, 2002). The quality of the body of human data is equal to or better than that used in many other RfDs based on human data. And for perchlorate, dramatic differences in the response of rats and humans to inhibition of iodine uptake suggests that using animal data as the basis of the risk assessment will introduce an unnecessary degree of uncertainty that can be reduced by relying on the human data.

Comparison of Animal and Human Responses

EPA's mode-of-action analysis correctly states that all potential adverse effects flow from the alterations of thyroid hormones by perchlorate exposure and that the key event in this cascade is the inhibition of iodine uptake by the thyroid. However, EPA incorrectly concludes that the only important species comparison to consider is the action of perchlorate at the NIS, resulting in iodine uptake inhibition (page 3-23, lines 1-4). EPA notes the equal sensitivity of both rats and humans to inhibition of iodine uptake at the NIS, and the PBPK modeling of these effects as support for use of the animal data in developing the RfD (page 7-27, lines 11-18). However, this approach effectively ignores the demonstrated dynamic differences between rats and humans. Not only is the time course for iodine uptake inhibition very different between rats and humans, but once iodine uptake has been inhibited, rats and humans differ greatly in the response of thyroid hormones. Note that the use of PBPK modeling does not address this issue of dynamic differences, since it only models the kinetics at the NIS.

Although rats and humans appear to be equally sensitive at the NIS, the time course of inhibition is quite different between rats and humans. For both rats (Yu et al., 2000) and humans (Greer, 2002), dose response curves for iodine uptake inhibition were plotted by duration (Figure 18 in Appendix 1 and Figure 7 in Appendix 3). For rats, iodine uptake inhibition data were available for days 1, 5, and 14 of drinking water exposure. The figure 18 (Appendix 1), shows that rats up-regulate iodine uptake very quickly and that inhibition actually decreases with time. In fact, following perchlorate exposures for durations longer than 14 days, iodine uptake inhibition could not be measured, because iodine uptake by the thyroid had returned to normal levels (Yu, personal communication). For humans, iodine uptake inhibition data were available following 2 and 14 days of perchlorate exposure (Greer, 2002). The figure 7 (Appendix 3) shows, that in contrast to rats, humans do not up-regulate iodine uptake within the times measured – dose response curves for iodine uptake are identical for the two points evaluated.

The difference in response between rats and humans becomes apparent when the iodine uptake curves and the hormone response levels are plotted against serum perchlorate levels rather than external dose. Figures 8-10 in Appendix 3 represent human iodine uptake, T4 and TSH dose response curves, as percent of control values, plotted against measured serum perchlorate levels. From these Figures, it is apparent that in humans, no effect on either T4 or TSH was observed at serum perchlorate concentrations ranging over two orders of magnitude – from 0.01 to 1 µg/mL.

These same serum perchlorate levels result in iodine uptake inhibition ranging from about 15% to 70%.

In contrast, the thyroid hormone response in rats shows a very different pattern. Figures 19-24 in Appendix 1 represent T4 and TSH dose response curves, as percent of control, plotted against serum perchlorate levels. Actual iodine uptake inhibition could not be measured in these animals because at the time the hormone data was collected, up-regulation of iodine uptake had occurred. Therefore, PBPK-derived % iodine uptake inhibition in male rats after i.v. dose (Merrill CL-2001-0010, 2001) was used for the comparison with human iodine uptake inhibition. In pregnant dams on GD21 (Yu et al., 2000; York, 2000), serum perchlorate levels of about 0.04 µg/mL, equivalent to an iodine uptake inhibition of about 3%, resulted in significantly decreased T4 levels that were about 50-70% of control values. In pregnant dams on GD 21 and dams on PND 5 (Yu et al., 2000), serum perchlorate levels of 1 µg/mL, equivalent to iodine uptake inhibition of about 32%, results in decreased serum T4 levels that were about 60-80% of control values.

Therefore, when equivalent serum perchlorate levels are reached in humans and rats, very different degrees of iodine uptake inhibition and hormone response are observed. Serum perchlorate levels in the range of 0.01 to 1 µg/mL in rats result in iodine uptake inhibition ranging from about 3% to about 32%; the same range of serum perchlorate levels in humans results in iodine uptake inhibition ranging from 15% to 70%. Pregnant rats, one of the apparent sensitive subpopulations, respond to a 3% iodine uptake inhibition with statistically significant hormone changes. In contrast, humans have no hormone response even at iodine uptake inhibition approaching 70%. The differences in the time course of iodine uptake inhibition, the rapid up-regulation of iodine uptake by rats, and the dramatic differences in hormone response between rat and human at corresponding serum perchlorate levels all suggest that, given the availability of adequate human data, human data represent the best approach to perchlorate risk assessment.

Confounding of Animal Studies

Soy has been known to be a goitrogen since clinical reports were published in the medical literature in the early 1960s. In 1981, Filisetti and Lajolo demonstrated that feeding rats water soluble fraction from raw soy flour caused an enlargement of thyroid weight, a decrease of ^{131}I uptake and altered thyroid hormones. In 1997 Divi, Chang and Doerge working with the NTP determined that an isoflavone present in soy, genistein, inhibits microsomal thyroid peroxidase (TPO) at levels similar to isoflavone levels measured in plasma from humans consuming soy products. In 2000, Chang and Doerge reported that male and female rats consuming a standard soy-based rodent diet (NIH 31) had TPO activity approximately 50% lower than rats consuming a soy-free diet and this loss was commensurate with measured serum levels of isoflavones. That same year, Ikeda (2000) at the National Institute of Health Sciences in Tokyo reported a dramatic synergism between soy intake and iodine deficiency regarding induction of thyroid hyperplasia in rats.

Most standard rodent chows are soy-based diets. For example, the "Certified Rodent Diet" from PMI nutrition, which was fed in the Effects study lists the ingredients as:

> Ground corn, dehulled soybean meal, ground wheat, fish meal, wheat middlings, brewers dried yeast, cane molasses, wheat germ, dried beet pulp, dehydrated alfalfa meal, ground oats, dried whey, soybean oil, ground soybean hulls….

The studies on soy-based diets suggest that when combined with abnormal conditions like iodine deficiency (or perchlorate exposure), the normal homeostatic mechanisms in the rat are overwhelmed. This effect seems to be supported by the perchlorate rat studies, which seem to indicate an incredible sensitivity to perchlorate compared to humans. At doses that result in only about 1% I uptake inhibition in rats, T4 and TSH are significantly changed in many rat studies; at doses that result in about 70% inhibition, no effect at all in human studies. Therefore, the data on soy diets suggests that perchlorate alone may not be affecting the thyroid as severely as we think but that it is acting synergistically with the soy diet. Therefore, it seems possible that the soy-based diets are confounding the animal studies, contributing to the overall unreliability of the rat as a model. Due to the soy diet it is impossible to adequately define the NOAEL/LOAEL boundary of perchlorate alone.

Point of Departure Analysis

The available data on the effects of perchlorate in both rats and humans demonstrates that rats respond to perchlorate exposure in a very different manner than humans. Although there is no question that the rat can serve as an adequate model for the human response to perchlorate in the absence of human data, using human data as the basis of developing a RfD for perchlorate will reduce the uncertainty inherent in extrapolating from the rat data. Therefore, this section presents a benchmark dose analysis of iodine uptake in humans that can serve as the point of departure for a RfD based on human data. For comparison purposes, a benchmark dose analysis of decreased T4 in pregnant rats is also presented.

Benchmark Dose (BMD) Analysis of Iodine Uptake Inhibition in Humans

In order to assess the health effects of perchlorate in healthy humans, Greer et al. (2002), administered perchlorate in drinking water at doses of 0.007, 0.02, 0.1, and 0.5 mg/kg-day to 37 male and female volunteers for 14 days. Iodine uptake was measured in test subjects prior to exposure, and on exposure days 2 and 14. Serum levels of T3, T4, and TSH were measured periodically through out the study. More details of the study design and results are presented separately (Greer, 2002). This study is a well-conducted study that underwent a rigorous quality assurance audit and conforms to the "Common Rule", the Federal Agency Guidelines on the ethical conduct of human studies (*TERA*, 2002).

Even at the highest dose tested, the Greer study observed no statistically significant effects in serum T4, T3, or TSH (Appendix 3). Although, when serum T4 and TSH are plotted against serum area under the curve (AUC) values predicted by the human pbpk model (Merrill, 2001), there was a non-significant trend toward decreasing TSH and increasing T4 levels with dose– an observation that is consistent across all human studies (See Figure – Plot of Human TSH and T4 in Appendix 3). In keeping with the mode-of-action analysis, and the designation of decreased serum T4 as the critical effect leading to the potential for neurodevelopmental effects, this study defines a NOAEL of 0.5 mg/kg-day for the healthy adult human population. Because, essentially no effect was observed on serum hormone levels, and the hormones of interest tend toward changing in the opposite direction than expected, the hormone data are not amenable to BMD analysis. However, the study adequately characterizes the dose-response curve for iodine uptake in humans, the key event for subsequent toxicity as defined by EPA's mode-of-action analysis. Thus, a BMD analysis of the I uptake data can be used to estimate a conservative "point of departure" for derivation of a RfD based on human data.

For the data of Greer et al. (2002), three models were used to develop BMDs and their 95% lower limits (BMDLs). Currently, insufficient data exist to adequately define the level of iodine uptake inhibition in humans that can be tolerated for a lifetime without altering serum T4 and TSH levels. Greer et al (2002) demonstrated that for 14-day exposure, inhibition of iodine uptake up to about 70%, has no effect on serum T4 or TSH. Occupational studies (Gibbs et al, 1998; Lamm et al., 1999) demonstrated that workers exposed to perchlorate for several years demonstrated no altered T4 or TSH serum levels. When the serum hormone levels from these studies are plotted against serum perchlorate AUC predicted by the human pbpk model (Figures

4 and 5 in Appendix 3), it can be seen that chronic exposure in workers had no effect on serum T4 or TSH at serum AUC values that resulted in approximately 50% I uptake inhibition. Thus, it might be reasonable to conclude that an appropriate benchmark response would be the perchlorate dose that resulted in a 50% inhibition of iodine uptake. Nonetheless, benchmark response levels of 10%, 15%, and 20% inhibition of iodine uptake were modeled in order to be public health protective and take into account the uncertainties involved in extrapolating data from healthy adults to potential sensitive populations such as iodine deficient people, pregnant women, and neonates.

The Hill and Power models successfully modeled the data, whereas the polynomial model failed (Printouts from model runs in Appendix 4). The Power model gave goodness-of-fit value of 0.57, indicating good fit. The Hill model was unable to provide a goodness-of-fit analysis because of too few degrees of freedom; however the Hill model gave a good visual fit. Modeling results are presented in Table 1. At 10% inhibition, there is a slight difference in BMDL values between the two models; at inhibition of 15% or 20%, the BMDLs from both models are almost identical. Since the Hill model is good for modeling the receptor binding response, this model might be preferred over the Power model in estimating the BMD for Iodine inhibition response. However, either model is acceptable.

The perchlorate dose that is modeled to cause a 10% inhibition of iodine uptake is rounded to 0.01 mg/kg-day; the BMDL estimate ranges from 0.004 to 0.008 mg/kg-day. These results are consistent with the conclusions of Greer et al. (2002), which indicated that the no effect level for iodine inhibition was 0.007 mg/kg-day; based on subject variability, Greer et al. (2002) estimated a 95% probability that iodine uptake inhibition will be ≤10% at the no effect level. The BMDL estimate for 20% inhibition of iodine uptake is 0.02 mg/kg-day.

Table 1. Benchmark Doses and Their Lower Limits for Iodine Inhibition In Adult Males and Females.

Data from Greer et al. (2002) (all values in mg/kg-day)				
	Endpoint	Hill model	Power model	Average
10% inhibition	BMD	0.014	0.012	
	BMDL	0.0037	0.0078	0.0054
15% inhibition	BMD	0.020	0.017	
	BMDL	0.013	0.012	0.012
20% inhibition	BMD	0.027	0.023	
	BMDL	0.019	0.017	0.018

One criticism of the Greer study by EPA is that the number of subjects was too small to allow sufficient power**.** Therefore, a second BMD analysis was conducted combining the iodine uptake inhibition data from both Greer et al. (2002) and Lawrence et al. (2000, 2001) studies. Although Greer indicated that significant differences in the shape of distributions of the two data sets, plots of iodine uptake against serum AUC from both studies suggested a reasonable similarity between the two data sets. As with the Greer data alone, both the Hill model and the Power model, at benchmark responses of 10%, 15% and 20% inhibition were modeled. The

Hill model provides a relatively good fit of the combined data; the goodness-of-fit value is marginal at 0.05, but the visual fit of the model is good, especially at the lower doses. The Power model only models a straight line and did not provide an adequate fit of the combined data. Therefore, only Hill model results are presented in Table 2.

Table 2. Benchmark Doses and Their Lower Limits for Iodine Inhibition In Adult Males

Data from all the human data (mg/kg-day)		
	Endpoint	Hill model
10% inhibition	BMD	0.024
	BMDL	0.010
15% inhibition	BMD	0.036
	BMDL	0.016
20% inhibition	BMD	0.048
	BMDL	0.024

The perchlorate doses that result in a 10% and 20% inhibition of perchlorate are 0.02 and 0.05 mg/kg-day respectively; the BMDLs are 0.01 and 0.02 mg/kg-day. Although, modeling the combined data resulted in slightly higher BMD estimates at 10% and 20% response levels, the BMDL at 20% response is essentially the same using the combined data and the Greer study alone. This strengthens a conclusion that the BMDL estimate at 20% inhibition of iodine uptake, 0.02 mg/kg-day, is a reasonable point-of-departure for a RfD based on human studies.

BMD Analysis of T4 Changes in Rats

Using rat data as the basis of a RfD for perchlorate introduces a higher degree of uncertainty into the assessment than using human data. Considering that adequate human data is available, use of the rat data is for developing the RfD is not recommended. However, this analysis is included to demonstrate an appropriate approach to using rat data. As discussed in the previous section, a careful, point-by-point analysis of the rat database does not adequately support the designation of a LOAEL at a dose level of 0.01 mg/kg-day. Rather the overall weight-of-evidence suggests that adverse effects probably occurs between the doses of 1 and 3 mg/kg-day. However, decreases in serum T4 do appear to occur at lower doses in pregnant rats. Consistent with the mode-of-action analysis, this endpoint is considered to be the critical effect in a sensitive subpopulation. Using this critical effect in a sensitive subpopulation reduces uncertainty associated with intraspecies variability. Using a benchmark dose analysis reduces the uncertainty associated with extrapolating from a LOAEL to a NOAEL.

Benchmark dose analysis was conducted using data on serum T4 levels from pregnant dams in the Effects study (Argus, 2001) and from pregnant dams in a parallel study conducted at the Wright Patterson Air Force Base (Yu et al., 2000a). Data suggests that rats can tolerate a 60% inhibition of serum T4 and still maintain normal T3 levels in the brain (cited in Schwartz, 2002). Although this could be considered to be a reasonable level for estimating a benchmark response, responses of 10%, 15%, and 20% inhibition were actually modeled. BMD analyses for the Effects study were done with Hill model; the data from the highest dose level were removed to

optimize model fit at the low dose part of the dose-response curve. The Power model was failed to provide an adequate fit to the data from the Effects study. In contrast, BMD analysis for the combined data were done with Power model; responses from the two high dose levels were removed to optimize fit at the low dose part of the dose-response curve. The Hill model was not used with the combined data because it could not provide a good model fit either for the complete data set or with the two high dose responses removed. (Model printouts and curves are presented in Appendix 4)

Table 3. BMD Analysis of Decreased Serum T4 in Pregnant Rats (GD 21) in the Effects Study

		Oral dose (mg/kg-day)	Serum AUC (ug/L)	Serum Peak Concentration (ug/L)
10%	BMD	0.0094	46.4	55.2
	BMDL	0.0070	39.7	47.5
15%	BMD	0.013	55.7	65.8
	BMDL	0.010	48.9	58.0
20%	BMD	0.018	64.7	76.2
	BMDL	0.011	N/a	N/a

Table 4. BMD Analysis of Decreased Serum T4 in Pregnant Rats (GD 21) in the Effects Study and Yu et al. (2000), Combined

		Oral dose (mg/kg-day)	Serum AUC (ug/L)	Serum Peak Concentration (ug/L)
10%	BMD	0.0020	20.9	24.8
	BMDL	0.0006	11.0	13.1
15%	BMD	0.0062	37.6	44.3
	BMDL	0.0025	23.7	28.0
20%	BMD	0.014	57.1	66.9
	BMDL	0.0071	40.6	47.6

Table 3 presents the modeling results from just the Effects study data; Table 4 presents the modeling results for combining data from the two studies. Combining the two datasets provides a lower estimate for the BMD and BMDL at the 10% response level than using data from the Effects study alone. However, the BMDs and BMDLs for the 20% response level are essentially the same for the Effects study alone or for the two studies combined. Thus, a reasonable point-of-departure is the BMDL estimated for a 20% decrease in serum T4 in pregnant rats, 0.007 mg/kg-day.

Choice Of Uncertainty Factors

Uncertainty factors are used in risk assessment to account for areas in which our lack of knowledge reduces our certainty that the dose level selected as the point of departure will be protective of human health. EPA's choices of factors are summarized in Table 5; these choices were based on the designation of 0.01 mg/kg-day as a LOAEL in rats. This section will discuss each choice uncertainty factor and present analysis based on different recommended points-of-departure for human and animal studies as well analysis of additional data not considered by EPA.

Table 5. EPA's Choices of Uncertainty Factors for the Perchlorate RfD

Area of Uncertainty	**US EPA (rat study)**
Intraspecies (kinetics = 3, dynamics = 1)	3
Interspecies (kinetics = 1, dynamics = 1)	1
LOAEL to NOAEL (shallow dose response slope)	10
Subchronic to Chronic (tumors and imprinting)	3
Missing Data Base (immunotoxicity)	3
Modifying Factor	1
Total Uncertainty	300

Observations - Intra-species Uncertainty (Human Variability)

EPA considers an uncertainty factor for intra-species uncertainty of 3-fold to be appropriate based on the observed variability in toxicokinetics and in the unknown toxicokinetics in the sensitive populations of pregnant women and their fetuses. EPA presumably considers that the choice of sensitive experimental model for thyroid dysfunction, the pregnant and neonatal rat, is

sufficiently representative of the sensitive human populations that an uncertainty factor for toxicodynamics of 1-fold is appropriate.[1]

Findings

EPA uses an uncertainty factor of 3-fold to account for intra-species extrapolation (human variation). EPA and others recognize that this factor is composed of toxicokinetic and toxicodynamic sub-factors of approximately 3-fold (1/2 the log based value of 10) each. Do data exist to suggest that either of these sub-factors should be changed, as suggested by EPA for one of them? Are data available to determine compound specific adjustment factors (CSAFs) based on the work of IPCS (Meek et al., 2001)? Two approaches to these related questions are possible.

One approach is to look at specific differences in either kinetic or dynamic parameters of perchlorate that most closely tie into the critical effect and its sensitive population(s). For perchlorate, the critical effect is T4 hormone decrease in pregnant women and the resulting neurological disturbances in their fetuses.

Perchlorate kinetics have been studied somewhat in humans. The relevant parameter to more closely investigate is the variation in perchlorate AUC or peak exposure when individuals are given the same perchlorate dose. Since AUC is the dosimetric adjustment between rats and humans chosen by EPA for its calculation of HEE, we focus on this.

Preferably this measurement of AUC should be done at doses near the LOAEL chosen as the point of departure, or lower, since this is the area where the variation in AUC is most relevant to the RfD (Meek et al., 2001; IPCS, 2001). However, human studies have only measured half-life of perchlorate in humans (e.g., Greer et al., 2002), and such measurements have been made it too few individuals to give a sense of the expected variability in the sensitive population. Thus, development of an uncertainty factor different than EPA's default value of 3-fold for toxicokinetics based on these data is not possible. This is EPA's position with which we agree.

Perchlorate dynamic differences among humans have been even less well studied. The relevant parameter to more closely investigate is the variation among individuals in effective internal doses or serum AUC that cause a certain T4 level change. Alternatively, variations among individuals in the effective internal doses that cause a certain level of inhibition of iodine uptake by the thyroid, recognized as the precursor to changes in T4, could be measured. Unfortunately,

[1] EPA's distinction of the intraspecies uncertainty factor into components of toxicokinetics and toxicodynamics is based on its pioneering work on dosimetric adjustments in the development of Reference Concentrations (RfCs) in the late1980s, as publish by Jarabek and colleagues (Jarabek et al., 1989; Jarabek, 1994, 1995; US EPA, 1994). Subsequently and independently the International Programme on Chemical Safety (IPCS, 1994) also determined the need for toxicokinetic and toxicodynamic components analysis work based on the publications of Renwick (1991, 1993). Guidelines have been developed by IPCS on the use of data and judgments in the determination of compound specific adjustment factors (CSAFs). These guidelines have the imprimatur of many national organizations, including the EPA, FDA and ATSDR. A brief version of these guidelines has been published by Meek et al. (2001), and is available on the IPCS website. Appendix 1 gives more information about these guidelines.

while the available human data are very useful for determination of NOAEL and BMDL values, and offer much insight into perchlorate kinetics, these same data do not offer any picture of dynamic differences among individuals in terms of inhibition of iodine uptake or changes in T4 level.

However, a second approach to answering the question of whether either of these sub-factors should be changed, is possible. One can measure the variation in the susceptibility to the critical effect, decreases in T4 hormone, within human populations exposed to different levels of perchlorate. This variation is recognized as due to both variation in kinetics and dynamics differences among individuals. We have a wealth of data for this latter approach to the question.

Table 6 shows the variation in the critical effect within different human populations as a function of perchlorate dose. Mean values are as low as 6.6 ug/dl and as high as 17.12 ug/dl. Coefficients of Variation are as low as 6.0 ug/dl and as high as 31.5 ug/dl. Relatively speaking, the means and standard deviations are reasonably consistent among studies.

Unfortunately, the information in Table 6 cannot be used to develop Compound Specific Adjustment Factors (CSAFs) directly. This is because no significant change in T4 levels is apparent with dose, thus, no effect dose can be identified, and no uncertainty factor for within human variation to administered perchlorate dose is directly possible from these data. Figure 1 in Appendix 1 shows this lack of response more clearly for human T4 levels in response to perchlorate dose.

Table 6. Variation of the Critical Effect of T4 Changes With Administered Perchlorate Dose

Study	Population	n	Dose[2]	T4 Level, Mean, S.D.	Coefficient of Variation
Crump et al., 2000	All Schoolchildren	53	Non detect	9.1, 1.3	14.3
	All Schoolchildren	49	5.5 ppb water	10.4, 1.4	13.5
	All Schoolchildren	60	112 ppb water	9.3, 1.2	12.9
	Lifetime residence Schoolchildren	36	Non detect	9.0, 1.4	15.6
	Lifetime residence Schoolchildren	41	5.5 ppb water	10.3, 1.3	12.6
	Lifetime residence Schoolchildren	50	112 ppb water	9.3,1.1	11.8
Li et al., 1999	Newborns-Reno	5,882	Non detect	17.12, n/a[3]	
	Newborns-Las Vegas	17,308	9-15 ppb water	17.11, n/a	
Brabant, 2000	Males Day 7	7	1.0	7.0, 1.3	18.6
	Males Day 14	7	1.0	6.7, 0.9	13.4
	Males Day 7	7	12.0	7.5, 1.0	13.3
	Males Day 14	7	12.0	7.4, 0.9	12.2
Brabant,1992			12		
Lawrence et al., 2001	Males-low dose RAIU	8	3mg/day	8.8, 1.7	25.8
	Males-high dose	9	3mg/day	6.6, 1.7	25.8
Lawrence et al., 2000	Males Day 7	9	10 mg/day	6.7, 0.4	6.0
	Males Day 14	9	10 mg/day	6.6, 0.5	7.6
Gibbs et al., 1998	Employee Control Group	83	Non detect	7.5, 1.8	24.0
	Employee Exposed Group	18	36	7.0, 1.4	20.0
	Employee 1996 Control Group	120	Non detect	8.1, 1.4	17.3
	Employee 1996 Low-dose Group	26	3,500 ug/kg	8.3, 1.4	16.9
	Employee 1996 High-dose Group	22	38,000 ug/kg	8.1, 2.1	26.0
	Employee 1997-1998 Control Group	72	Non detect	7.5, 1.6	21.3

[2] Units given by investigators

[3] SD is not available.

	Employee 1997-1998 Low-dose Group	18	3,500 ug/kg	7.9, 1.5	19.0
	Employee 1997-1998 High-dose Group	31	38,000 ug/kg	7.2, 1.6	22.2
Greer et al. 2002	Males/Females	8	0.5	7.4, 1.9	26.0
	Males/Females	8	0.1	7.1, 1.2	16.9
	Male/Females	8	0.02	7.2, 2.2	31.0
	Males/Females	7	0.007	7.3, 2.3	31.5
Lamm et al., 1999	Employees	21	0.0173	6.73, 1.48	22.0
	Employees	13	0.0568	7.13, 1.58	22.2
	Employees	8	0.2548	7.34, 1.12	15.3
	Employees	15	5.4139	7.03, 1.30	18.5

However, one can also study the variability of inhibition of iodine uptake as a function of different perchlorate doses (Greer et al., 2002; Lawrence et al., 2000, 2001). An analysis of these data can also be used to develop a CSAF. Based on the work of Greer et al. (2002), value of a potential CSAF is approximately 5-fold. This is determined from the difference between the dose of 0.15 mg/kg-day for an estimated 50% of the population with a 50% decreased iodine uptake and the dose of 0.03 mg/kg-day for an estimated 5% of the population with this 50% decrease (Table 7, unpublished observations of Zhao, 2002).

This potential CSAF is developed from data on healthy male and female adult humans. It reflects both inter-individual variability in kinetics and dynamics, and thus supports a reduction in the 10-fold uncertainty factor for intraspecies variability of a magnitude that is similar to EPA's selection of a 3-fold. However, variability in these individuals in the inhibition of iodine uptake with perchlorate dose may or may not reflect the expected variability of sensitive subgroups.

Table 7. Development of Potential CSAFs based on variation in the inhibition of iodine uptake as a function of perchlorate dose. Data are from Greer et al. (2002). Twenty or 50 percent inhibition of iodine uptake is considered to be adverse for this estimation

Dose (mg/kg-day)	n	14 day Iodine Uptake as a % of Baseline Mean, SD	Estimated % of Individuals with <80% of baseline uptake	Estimated % of Individuals with <50% of baseline uptake
0.007	7	98.2, 22.0	20	1
0.02	10	83.6, 13.0	39	0
0.1	10	55.3, 11.7	98	33
0.5	10	32.9, 12.0	100	92
CSAF Estimation		Estimated Dose at 50% response	0.03	0.15
		Estimated Dose at 5% response	n/a	0.03
		CSAF	n/a	~5

n/a = could not be determined

The judgment of appropriate intraspecies uncertainty factor also depends in part on the choice of study as the basis of the RfD. For example, if the study is on a sensitive experimental animal subgroup, and if variability in toxicokinetics is observed and the toxicokinetics in the sensitive populations of pregnant women and their fetuses is unknown, then an overall uncertainty factor of 3 may be reasonable. This would represent a 3-fold for toxicokinetic variability due to observed variability and unknown kinetics in the sensitive subgroup, and a 1-fold for toxicodynamic variability due to the choice of the pregnant rat's LOAEL as the basis of the RfD. This appears to represent EPA's judgment.

If, however, the study is on a likely sensitive human subgroup such as children found in Crump et al. (2000), then an uncertainty factor of 3 seems reasonable for both toxicokinetic and toxicodynamic variability together. This is because both are already addressed in the choice of the NOAEL and this group represents a more sensitive subgroup when compared with adults. This factor should not be reduced to 1-fold for perchlorate exposure, however, because yet a more sensitive subgroup may exist, that is pregnant women and their fetuses.

Specific information that would allow the further development of CASFs would be the variation in perchlorate AUC when individuals are given the same perchlorate dose. This measurement of AUC should be done at doses near the LOAEL chosen as the point of departure, or lower. Another relevant parameter to more closely investigate is the variation among individuals in T4 levels with the change of perchlorate AUC. Alternatively, variations in the inhibition of iodine uptake by the thyroid with the change of perchlorate AUC among individuals, recognized as the precursor to changes in T4, could be measured.

Observation - Inter-species Uncertainty (Experimental Animal to Human Variability)

DOD (Table 7-1 of EPA, 2002) calculates human equivalent exposures (HEEs) to various experimental doses in the male rat for a 70 kg human base on perchlorate serum area under curve (AUC). At an external dose to the male rats of 0.01 mg/kg-day (the dose chosen by EPA as the point of departure to develop an RfD), the 70 kg human HEE is 0.021 mg/kg-day. DOD also calculates HEEs to various experimental doses in the male rat for a pregnant female rate based on perchlorate serum AUC. At an external dose to the male rats of 0.01 mg/kg-day, the HEE calculated from the pregnant female rat is 0.01 mg/kg-day. This latter calculation is important since pregnant females and their fetuses are considered as the sensitive populations.

Based on these evaluations and general confidence that the extrapolation base on perchlorate distribution and iodide inhibition is accurately characterized by the PBPK modeling, EPA considers a 1-fold default uncertainty factor for inter-species extrapolation to be appropriate. We presume that this statement reflects the toxicokinetic differences between rats and humans. EPA's choice of serum AUC between rats and humans as the human equivalent exposure, rather than iodine inhibition was discussed but not further explained other than either choice was similar to the other (the difference in these choices is approximately 2-fold).

EPA did not discuss the selection of an uncertainty factor of 1-fold for toxicodynamic differences between these two species. However, if the overall uncertainty factor for rats to humans is 1-fold, then presumably this is what EPA intended. On an administered dose basis, rats are shown to be much more sensitive than humans to T4 decrease, the critical effect of perchlorate.

Findings

We feel that EPA's choice of rat data as the basis of its RfD for perchlorate (U.S. EPA, 2002a) is a second-best approach. Rather, we feel that the recent studies in humans by Greer et al. (2002), supported by Crump et al. (2000), should be used instead. In this we agree with suggestions by Cal EPA (Ting et al., 2001). Our belief is also consistent with the general practice of EPA and others in the choice of human data as the basis of noncancer risk assessments when appropriate data are available.

However, if we were forced to base a perchlorate RfD on rat data, we would use EPA's mode of action understanding, as shown in its Figure 7-1, to select the critical effect, that is the first effect or its known precursor as dose increases. The critical effect is determined to be T4 decrease in the pregnant rat. Rather than choosing the LOAEL of 0.01 mg/kg-day for this critical effect, we feel that a superior point of departure would be a 20% BMDL of 0.007 mg/kg-day. This choice is superior both because the effect is critical (the first adverse effect as per EPA's Figure 7-1) and the pregnant rat is clearly the most sensitive subgroup as discussed in previous sections.

One way to determine the inter-species uncertainty factor is to directly compare the toxic effect doses in rats and humans. The available data indicates that the LOAEL for T4 changes, the critical effect, in rats was 0.01 mg/kg-day, while the LOAEL for T4 changes in humans would be greater than 10 mg/kg-day (see Figure 11 in Appendix 3). Thus, humans are less sensitive to perchlorate-induced T4 changes than rats, and the sensitivity difference between these two species would be at least 1000 fold based on this comparison. Because even the highest test dose in human studies could not induce a significant T4 change, more accurate comparison is not possible at this time.

A conservative way to estimate inter-species uncertainty factor is to use IPCS's approach. Our choices of uncertainty factor for rat to human extrapolation would follow that by EPA, but only in part. For example, we agree with EPA's use of a toxicokinetic factor of 1-fold (EPA, 2002a, Table 7-3), which reflects the lack of differences in Human Equivalent Exposure (HEE) associated with perchlorate serum AUC between the male rat and the pregnant rat at the LOAEL dose of 0.01 mg/kg-day. In contrast with EPA, however, we would use a toxicodynamic uncertainty factor different than 1-fold, to reflect the average difference in plasma T4 half-lives between the rat of 12 to 24 hours (midpoint is 18 hours) and human of 5 to 9 days (midpoint is 168 hours), and differences in functional requirements of this hormone between these two species.

This average difference in plasma T4 half-lives is due primarily to the presence of thyroxine-binding globulin (TBG) found in the human but not the rat (Capen, 2001), where the affinity of TBG is approximately 1000 times higher that for prealbumin, one of the carrier proteins for T4 in the rat. Because of this difference in part, a rat without a functional thyroid requires about 10 times more T4 for full substitution than a similarly deficient adult human. The specific value of the CSAF for toxicodynamic differences between the rat and human that we propose is 0.1-fold based on the functional requirements in thyroid deficient rats and humans. This value is further supported by the differences in T4 half lives (i.e., 18 hours / 168 hours ~ 0.1) in rats and humans.

An alternative toxicodynamic uncertainty factor might be proposed on the basis of thyroid colloid T4 storage, which is dramatically different between rats and humans with rats being more sensitive. However, thyroid colloid is very difficult to quantitate accurately based upon the 3-dimentional structure of follicles, thickness of histological section, location in thyroid lobe, staining method, among other factors. Certainly the follicles in the human thyroid have more stored colloid than rats but this again is related to the relatively slow turnover of T4 in humans compared to rats. The rat thyroid has to work much harder and secrete more hormone in order to maintain normal blood levels due to the lack of the high affinity thyroxine required to supplement a hypothyroid rat compared to humans. This is a graphic documentation of the interspecies differences (Capen, 2002).

The combined interspecies uncertainty factor for toxicokinetics and toxicodynamics is 0.1 fold (i.e., 1 x 0.1 = 0.1). This combined factor suggests that rats are approximately 10-fold more sensitive than humans, due to toxicodynamics. This difference is supported in part by the apparent dramatic difference in response between rats and humans to T4 levels as a function of administered perchlorate dose of at least 1000-fold show previously in this text (See Figure 11, Appendix 3).

Observation – LOAEL to NOAEL

EPA considers an additional uncertainty factor to be necessary because the basis of the RfD is a LOAEL for brain morphometry, thyroid histopathology, and hormone changes. EPA considers that a full 10-fold factor is reasonable because of the shallow slope of the dose response curve(s) at these lower levels.

Findings

We agree with EPA that an additional uncertainty factor would be necessary if the point of departure as the basis of the RfD is a LOAEL for brain morphometry, thyroid histopathology, and hormone changes. However, we feel that a superior point of departure is available. Specifically, the 20% BMDL of 0.007 mg/kg-day in T4 decrease in pregnant rats is superior because T4 decrease in pregnant rats is the critical effect in a sensitive population. Protecting against this effect in this population is entirely consistent with EPA's mode of action model for perchlorate (Figure 7-1 of U.S. EPA, 2002b), and consistent with established EPA methods for determination of RfDs and RfCs (Barnes and Dourson, 1988; Dourson, 1994; Jarabek, 1994 and 1995). Selection of a 20% BMDL of 0.007 mg/kg-day in T4 decrease in pregnant rats as the point of departure also obviates a number of potential controversies in the choice of multiple critical effects, the principal one being that multiple critical effects are not consistent with EPA's mode of action model for perchlorate. If a BMDL is used as a basis of an RfD, then an uncertainty factor for LOAEL to NOAEL is not needed as per EPA guidelines and practice.

Observation – Subchronic to Chronic

EPA also considers an additional uncertainty factor of 3-fold to be necessary because of the biological importance of the statistical significance increase in tumors in the F1 generation pups at 19 weeks and 30 mg/kg-day, and the possibility of *in utero* imprinting with endocrine disruption. Furthermore, decreases in the NOAEL/LOAEL estimates for hormone perturbations and histopathology were observed between the 14-day and 90-day time points in several of the animal studies. These decreases indicate that shorter term studies may not be as effective as longer term studies in elucidating the critical effect, decreases in T4 hormone, with perchlorate exposure.

Findings

We fundamentally disagree with EPA's judgment that such a factor is needed. Although tumors are ultimately caused by perturbations in hormone status, initiated by a decrease in T4, they are far distant in dose from that which affects T4 changes in pregnant rats, the critical effect for perchlorate exposure. In fact, the dose difference between these two endpoints is 3000-fold. It does not make biological sense to depend on the evocation of tumors from *in utero* imprinting after such high dose as one reason to lower the projected NOAEL or BMDL for the critical effect by 3-fold more. Nor is the use of an uncertainty factor for tumors consistent with EPA's stated mode of action model (Figure 7-1 of U.S. EPA, 2002a), nor EPA's determination that the tumors caused by perchlorate do not have a genetic basis. Quite simply put, if a NOAEL for T4 decrease is achieved in the sensitive population, then the production of tumors in offspring is not possible. Dividing this NOAEL by an additional uncertainty factor does not further decrease an already zero possibility.

Moreover, the changes in NOAEL/LOAEL estimates for hormone perturbations and histopathology observed in some animal studies between shorter and longer time points, were not observed in other studies going from longer to shorter time points. While variations in hormone responses among studies are not necessarily unexpected, of more importance, is the clear indication that the pregnant rat is the most sensitive animal when compared with other rats (*v. supra*). These figures show clearly that decreases in T4 are more effectively evoked by perchlorate in pregnant rats than in any other rat group or exposure duration, including other shorter term or longer-term rat studies. Thus, the need for an uncertainty factor for duration of effect does not exist. Perchlorate exposure has occurred during the complete period of sensitivity for the critical effect and its sensitive subgroup. The 20% BMDL that we suggest as the starting point for the determination of the RfD captures both.

Finally, the need for a duration UF can be examined by evaluating the effect that increasing the duration of exposure has on perchlorate's inhibition iodine uptake, the first identified step in the sequence of perchlorate's effects. For both rats (Yu et al., 2000b) and humans (Greer, 2002), dose response curves for iodine uptake inhibition were plotted by duration (Figure 18 in Appendix 1 and Figure 7 in Appendix 3). For rats, iodine uptake inhibition data were available for days 1, 5, and 14 of drinking water exposure. The figure 18, shows that rats up-regulate iodine uptake very quickly and that inhibition actually decreases with time. In fact, following

perchlorate exposures for durations longer than 14 days, iodine uptake inhibition could not be measured, because iodine uptake by the thyroid had returned to normal levels (Yu, personal communication). For humans, iodine uptake inhibition data were available following 2 and 14 days of perchlorate exposure (Greer, 2002). The figure 7 shows, that in contrast to rats, humans do not up-regulate iodine uptake within the times measured – dose response curves for iodine uptake are identical for the two points evaluated. However, these data do show that iodine uptake inhibition does not increase with increasing duration in either rats or humans. Therefore, an uncertainty factor for duration is not required since the key event in the sequence of effects does not increase in severity with duration of exposure in either rats or humans.

Observation – Database

EPA also considers an additional uncertainty factor of 3-fold to be necessary because the potential for perchlorate to cause immunotoxicity remains a concern. Specifically, even though an immunotoxicity NOAEL of 0.02 mg/kg-day can be judged, with a LOAEL at 0.06 mg/kg-day, deficiencies in this study raise concern for this characterization of NOAEL. EPA recognizes this as a database deficiency.

Findings

We fundamentally disagree with EPA's judgment that such a factor is needed. The overall database for perchlorate is complete. Although immunotoxicity studies are not generally considered to be necessary for a complete database, a complete battery of immunotoxicity assays were conducted to address concerns over the potential for immune effects caused by perchlorate. Kaminsky (Appendix 7) indicates that collectively, the studies performed by the two laboratories represent a broad-based evaluation of the effects of perchlorate on innate, humoral and cell-mediated immune functions in mice. The assays utilized by the two laboratories are widely accepted and routinely employed by immunotoxicologists for evaluating the immunotoxicity of xenobiotics. Although technical problems and issues were raised primarily with several of the experiments performed in the Keil laboratory, for the most part these issues were corrected through the replication of those experiments and/or by statistical reanalysis of the data sets that were in question. In general, both Keil and Kaminsky (Appendix 7) indicate that sufficient data has been generated to conclude that perchlorate is not immunotoxic.

Based on some anecdotal evidence in early clinical studies with perchlorate in Graves disease patients, there is some concern that perchlorate is a contact sensitizer. The local lymph node assay does not adequately address this issue; although the results obtained in that assay are not considered to be biologically significant. Kaminsky (Appendix 7) indicates that to resolve the concern of contact sensitization, "I recommend repeating the LLNA, using an experimental design that would genuinely evaluate whether perchlorate is a skin sensitizer. Collectively and in the absence of additional data, there is no evidence to suggest that perchlorate is a dermal contact sensitizer, especially at doses of 0.01mg/kg-day. In light of the absence of any observable effects on the immune system in any of the other immune function assays at doses of 0.01 mg/kg-day, addition of a 3-fold uncertainty factor due to an "incomplete characterization" of immunotoxicity in this reviewer's opinion is excessive."

Conclusions

We believe that the critical effect of perchlorate is a decrease in serum T4 hormone level in pregnant women, leading to more severe thyroid effects in themselves and neurological toxicity in their fetuses. We also agree with EPA that the inhibition of iodine uptake by perchlorate is an even more sensitive endpoint on which a RfD might be based, similar to what is proposed by Cal EPA (Ting et al., 2001).

We conducted a somewhat limited analysis of individual toxicokinetic and toxicodynamic parameters as shown above. This analysis was conducted within the recent guidelines of the IPCS (Appendix 5), and the more extensive history of such judgments with the EPA. One of the important requirements of the IPCS guidelines and EPA judgments is a firm understanding of the toxic form of perchlorate. Another important requirement for either group is for kinetic and dynamic data that are known to directly relate to the evocation of the critical effect, that is T4 serum decrease in pregnancy.

Fortunately, data on perchlorate are sufficient for the determination of both the toxic moiety and appropriate kinetic modeling relating to T4 serum decrease. EPA's analysis with both of these is well done. Thus, EPA's use of data, in part, instead of default uncertainty factors of 10-fold for intraspecies (within human) and interspecies (rat to human) toxicokinetic extrapolation seems reasonable to us. Our development of a CSAF based on the human response in the inhibition of iodine uptake with perchlorate exposure of ~5 for a healthy group of humans supports a factor other than 10-fold for intraspecies variation (within human) in toxicodynamics. We do not suggest the use of this latter CSAF rather than EPA's default of 3-fold, however, because the CSAF value is based on a small, but healthy group on humans and not sensitive individuals. We expect that a larger group of healthy humans would tend to decrease the value of this CSAF, but that information from sensitive individuals may tend to increase this CSAF. Still, this CSAF suggests that the use of a 10-fold factor for intraspecies variability might be excessive, especially if the inhibition of iodine uptake was chosen as the critical effect.

We would also extend EPA's analysis on toxicokinetics similarities between rats and humans to include the known toxicodynamic differences between these two species, if the RfD is ultimately based on rat data. This is because of the demonstrated huge differences in response between rats and humans in the critical effect, T4 hormone decrease, with perchlorate exposure. Additional specific data that might allow the further determination of Compound Specific Adjustment Factors (CASFs) for toxicodynamics in humans would include variations in susceptibility to T4 level changes among pregnant women given perchlorate, and better understanding of neurological status in children as it relates to *in utero* perchlorate exposure.

We specifically disagree with EPA's choice of uncertainty factor for tumors at high dose and decreasing hormone levels with increasing duration of exposure in some studies. Although tumors are ultimately caused by perturbations in hormone status, if a NOAEL or BMDL for T4 decrease is achieved in the pregnant animal, then the production of tumors in offspring is not possible according to EPA's proposed mode of action and our understanding of the underlying biology of this endpoint. Moreover, decreases in T4 are more effectively evoked by perchlorate in pregnant rats than in any other rat group or exposure duration. The BMDL that we suggest as

the starting point for the determination of the RfD from the rat studies captures both the sensitive time point and subgroup.

In addition, we specifically disagree with EPA's choice of uncertainty factor for deficiencies in the immunotoxicity database. If immunotoxicity is caused by changes in hormone status, then protecting against T4 decrease, the critical effect, will prevent the immunotoxicity. If immunotoxicity is not caused by perturbations in hormone status, it is still not the critical effect as defined by U.S. EPA (2002b). Thus, EPA's use of an uncertainty factor for potential immunotoxicity as the critical effect is not reasonable. If EPA uses immunotoxicity as the critical effect, it is not health protective.

Choice of uncertainty factors for various areas of uncertainty depend in part on the point of departure chosen as the basis of the RfD.

Developing an RfD

Table 8 summarizes our suggested uncertainty factors with different choice of critical effect and study. The application of these factors represents a good acceptance of the data available yet it allows for the concerns inherent in the analysis to be addressed.

As shown by extensive animal studies, the critical effect of perchlorate is T4 serum decrease. Pregnant rats are demonstrated to be the most sensitive subgroup, likely followed by the young rat. Several human studies exist that monitored for this critical effect. These studies do not include pregnant women, but they do include children. In addition, our review of comparative data between the experimental animal and human clearly indicate that humans are not more sensitive than the experimental animal species tested to T4 serum decrease by perchlorate; in fact based on toxicodynamics parameters they are much less sensitive. This supports the use of the human data for development of a RfD. We agree with EPA's policy when developing RfDs that the use of human data is first and foremost in the determination of critical effect and choice of uncertainty factors.

The most relevant data for judging the RfD for perchlorate exposures comes from human epidemiology and clinical studies, supplemented with available and extensive information on experimental animals. Specifically, we believe that a NOAEL of 0.006 mg/kg-day for T4 changes in children from the Crump et al. (2000) study, or a 20% BMDL of 0.02 mg/kg-day for inhibition of iodine uptake in adults from the Greer et al. (2002) study provide the most appropriate and relevant basis for the perchlorate RfD. The use of the Crump et al. (2000) study in children has the advantage of a population closer in response to the sensitive subgroup, pregnant women. The choice of the Greer et al. (2002) in adults has the advantage of selection of a known precursor to the critical effect, inhibition of iodine uptake, with a well-established dose response curve.

We adhere to the recent guidelines of the International Programme on Chemical Safety (IPCS) on the determination of compound specific adjustment factors (CSAFs), and on the more extensive history on uncertainty factor judgments within EPA when given available data. In either case, this adherence allows for the use of specific data or judgment on intraspecies and interspecies differences in toxicokinetics and toxicodynamics. We feel that the data are sufficient to estimate an overall uncertainty factor of 3-fold with the choice of T4 serum decrease and the use of Crump et al. (2000). This factor reflects the expected differences in toxicokinetics and toxicodynamics between children, a potential sensitive subgroup for perchlorate exposure, and pregnant women and their fetuses. We also suggest the use of a 3 to 10-fold uncertainty factor with the choice of inhibition of iodine uptake and the use of Greer et al. (2002). This factor reflects the expected differences in toxicokinetics and toxicodynamics between the adults tested in the Greer et al. (2002) study and pregnant women and their fetuses and potentially children.

Table 8. Choices of Uncertainty Factors for Different Possible Perchlorate RfDs by Toxicology Excellence for Risk Assessment (*TERA*)

Item / Group	EPA 2002 proposed	*TERA* 2002 possible	*TERA* 2002 possible	*TERA* 2002 possible
Critical Effect	Weight-of-evidence: thyroid perturbation	20% T4 decrease in pregnant	T4 decrease in children	20% adult iodine inhibition
Study	York, 2000	York, 2000; Yu et al. (2000)	Crump et al., 2000	Greer et al., 2002
BMDL[a], NOAEL, or LOAEL (mg/kg-day)	rat LOAEL 0.01	rat BMDL 0.007	human NOAEL 0.006[b]	human BMDL 0.02
Area of Uncertainty:				
within human (H)	3K[c] x 1D	3K x 1D	3	10 K&D
animal to human (A)	1K x 1D	1K x 0.1D	1	1
subchronic to chronic (S)	3	1	1	1
LOAEL to NOAEL (L)	10	1	1	1
data base (D)	3	1	1	1
modifying factor (MF)	1	1	1	1
Total Factor	300	0.3	3	10
RfD (mg/kg-day)	0.00003[d]	0.02	0.002	0.002
Confidence in RfD	Medium	Medium to High	High	High

[a] BMDL = benchmark dose, lower 95% confidence limit; NOAEL = no observed adverse effect level; LOAEL = lowest observed adverse effect level

[b] Based on Taltal exposure of 0.112 mg/L, consumption of 1.5 liter per day for a 28 kg child. This dose would be 0.0037 mg/kg-day and the RfD would be 0.001 mg/kg-day based on 60 kg adult female.

[c] K = kinetic component of this uncertainty factor, D = dynamic component

[d] Includes an adjustment since AP was given to rats as a salt.

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