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U. S. Environmental Protection Agency
Office of Ground Water and Drinking Water
Standards and Risk Management Division
1200 Pennsylvania Avenue NW.
Washington, DC 20460

November 23, 2016

RE: Request for Public Comments to be sent to EPA on Peer Review Materials to Inform the Safe Drinking Water Act Decision Making on Perchlorate, Docket ID Number: EPA-HQ-OW-2016-0438

Dear Sir or Madam:

The Chlorine Institute ("CI" or the "Institute") is a 190 member, not-for-profit trade association of chlor-alkali producers worldwide, as well as packagers, distributors, users, and suppliers. The Institute's North American Producer members account for more than 93 percent of the total chlorine production capacity of the U.S., Canada, and Mexico. The Institute's mission chemicals, namely chlorine, sodium hydroxide and potassium hydroxide, and hydrogen chloride, are used throughout the U.S. economy and are paramount to the protection of public health.

While The Chlorine Institute appreciates EPA's efforts in developing a standardized model for the purpose of determining an appropriate perchlorate drinking water level, the Institute believes there are flaws in the BBDR model that should be considered. CI provides the following comments to further explain the flaws in the model.

Lack of data or limited data is noted for the life-stage human values.

- The proposed BBDR model used physiological parameters to set the life-stage-specific human values. However, throughout the document on several occasions, the lack of data or limited data is noted for the life-stage human values. As noted by EPA, "Physiological parameters are life-stage specific, and were taken from appropriate sources (identified in the data tables) where possible. However, many of the iodide/thyroid hormone and perchlorate parameters where not available for specific life-stages, and so were extrapolated from other life-stages or in-vitro data." (US EPA, 2016)[1]. One parameter with limited or no data is hypothyroxinemia during pregnancy, maternal lactation or infancy as noted by EPA. In addition, there is no clinical definition of hypothyroxinemia and, as a result, various ranges of free T4 (fT4) are found in the literature. As noted by EPA, " within the context of pregnancy, some authors use the

2.5th percentile of the fT4 population distribution while others use the 10th percentile and the reference ranges vary between populations and studies (Moleti et al., 2011)[2]. Elevated TSH levels, the marker of hypothyroidism, can occur in women with fT4 levels in the same range as other women with normal TSH levels (e.g., see 9 Figure 1 in Moleti et al. (2008), so there is also not a specific fT4 level below which this transition occurs" (US EPA, 2016). Therefore, assumptions have been made to set the parameters for the model that may weaken the predictive outcomes of the model.

The BBDR model does not account for both aggregate and cumulative exposures to chemicals with the same mode of action to perchlorate.

- The proposed BBDR model does not account for chemicals with similar modes of action that inhibit the sodium iodide symporter (NIS) which transports iodine into the thyroid. Perchlorate is known to inhibit the NIS. However, so are nitrate and thiocyanate which are ubiquitous in a healthy diet and drinking water. Background levels of these chemicals can be much higher than perchlorate and are unlikely to be distinguishable. In regards to their potency, in vitro studies comparing the relative abilities of perchlorate, nitrate, and thiocyanate to inhibit cellular iodine uptake show that, after adjusting for biological half-life, perchlorate is half as potent as thiocyanate and 240 times as potent as nitrate (De Groef et al. 2006)[3]. In addition, ATSDR (2008)[4] noted that, "Nitrate and thiocyanate are widely distributed in nature and, because both anions also inhibit RAIU [radioactive iodide uptake], as demonstrated by Tonacchera et al. (2004)[5], should also be included in the discussion of the effects of inhibition of the NIS by anions." The BBDR model does not account for both aggregate and cumulative exposures to chemicals with the same mode of action to perchlorate.

Appropriate data are not available to validate the model.

- The infant, 7 to 90 days of age, breast-fed and formula fed, as well as the lactating mother were selected for the BBDR model development to address the most sensitive period of thyroid function maturation that may have consequences if exposure to perchlorate occurs. There is, however, a lack of epidemiologic data that current environmental exposure to perchlorate results in adverse effects to infant thyroid function (Tarone et al. 2010[6]; Charnley 2008[7]). Without the appropriate data, the model cannot be validated. If the appropriate data are not available to validate the model, then data must be collected from biomonitoring studies or generated in animal prior to use of the model to most accurately predict outcomes from exposures to perchlorate.

Homeostatic control mechanism does not account for in the proposed BBDR model.

- Clearly, a reduction in thyroid hormones over a substantial amount of time during fetal development is potentially deleterious to the developing fetus. However, the thyroid

has stores of iodide received from the mother such that short-term fluctuations in iodide uptake in the first post-natal days will cause no effect. Physiologically, when uptake of iodine is decreased, the body adapts by upregulating the number of the NISs to pump more iodide into the thyroid. This is a homeostatic control mechanism and is not accounted for in the proposed BBDR model.

Additional resources to be reviewed and considered in proposed BBDR model.

- Lumen et al. (November 2016)[8] have published a population based pregnancy model that estimates iodine nutrition and thyroid status in late gestation pregnant women in the United States. The BBDR model accounts for a wider range of iodine deficiencies and homeostatic mechanisms. To characterize total (T4) and free (fT4) thyroxine levels for a given iodine status at the population-level, the distribution of iodine intake for late-gestation pregnant women in the U.S was reconstructed using various reverse dosimetry methods and available biomonitoring data. The reconstructed distributions of iodine intake allowed for the estimation of nutrient inadequacy for late-gestation pregnant women in the U.S. via the probability approach. The prevalence of iodine inadequacy for third-trimester pregnant women in the U.S. was estimated to be between 21% and 44%. This paper should be reviewed and considered for incorporation into the proposed BBDR model (Lumen, 2016).

- In May 2011, The Chlorine Institute commissioned an evaluation (attached) conducted by ENVIRON International Corporation on the health advisories and standards for perchlorate levels in drinking water. This evaluation was submitted to EPA in response to its Final Regulatory Determination, which was issued in February 2011 for the agency to proceed with derivation and proposal of a National Primary Drinking Water Regulation. In that evaluation, ENVIRON provides what the Institute believes to be an acceptable perchlorate drinking water level based on appropriate data. The ENVIRON evaluation is another resource that should be reviewed and considered by EPA to further develop the BBDR model.

Peer review process

- The Federal Register notice of September 30, 2016 states that "Versar, Inc., will provide a report to EPA summarizing the peer reviewer's [sic] evaluation of the scientific and technical merit of the draft model and draft report and their responses to the charge questions." Due to the complexity of the model and the SAB Perchlorate Panel's call for transparency, like the SAB process, the panel should be responsible for drafting the report, not the contractor Versar. Additionally, it is unclear whether Versar has the necessary expertise to summarize the complex science and science policy questions

involved with the BBDR model and therefore may not accurately reflect the issues, conclusions, and recommendations of the panel.

Conclusion

In conclusion, the Institute believes the BBDR model does not account for both aggregate and cumulative exposures to chemicals with the same mode of action to perchlorates and that there is a lack of data to validate the model. CI recommends that EPA consider the comments and resources noted above, as well as review the attached ENVIRON study, to further improve the BBDR model in the agency's effort to propose an appropriate perchlorate drinking water level.

Thank you for the opportunity to comment on this proposed model and your careful attention.

Best Regards,

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4 Agency for Toxic Substances and Disease Registry (ATSDR) Toxicological profile for perchlorates. U.S. Department of Health and Human Services, Public Health Service. September 2008.

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Evaluation of Health Advisories and Standards for Perchlorate Levels in Drinking Water

Prepared for:
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Prepared by:
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Date:
May 13, 2011

Contents

ACRONYMS

ADHD	Attention Deficit Hyperactivity Disorder
BMD	Benchmark Dose
BMDL	Lower statistical limit on a benchmark dose
Cal EPA	California Environmental Protection Agency
CATS	Controlled Antenatal Thyroid Screening Study
DWS	Drinking Water Standard
HA	Health Advisory
kg	Kilogram
Mass DEP	Massachusetts Department of Environmental Protection
MCL	Maximum Contaminant Limit
mg/kg/day	Milligrams per kilogram per day
µg/g	Micrograms per gram
µg/L	Micrograms per liter
NHANES	National Health & Nutritional Examination Survey
NIS	Sodium-Iodide Symporter
NOAEL	No Observed Adverse Effect Level
NOEL	No Observed Effect Level
NPDWR	National Primary Drinking Water Regulation
NRC	National Research Council
PCH	Primary Congenital Hypothyroidism
PHG	Public Health Goal
RAIU	Radioactive Iodine Uptake
RfD	Reference Dose
RSC	Relative Source Contribution
TBG	Thyroxine Binding Globulin
T_3	Triiodothyronine
T_4	Thyroxine
Tg	Thyroglobulin
TSH	Thyroid Stimulating Hormone
TRH	Thyrotropin Releasing Hormone
UCMR 1	First Unregulated Contaminant Monitoring Regulation
UF	Uncertainty Factor
USEPA	United States Environmental Protection Agency
USFDA	United States Food and Drug Administration

Executive Summary

Perchlorate's effect on the thyroid to inhibit iodide uptake has been known for decades and has been the reason for the use of high doses of perchlorate, therapeutically, to treat hyperthyroidism, in particular in the treatment of Graves disease, a medical condition resulting from hyperthyroidism. Other non-medical uses of perchlorate, such as in rocket fuel and other applications as an oxygen source in high burn applications, may have resulted in releases of perchlorate to the environment, particularly to ground water.

Studies in laboratory rats have raised a concern that exposure to perchlorate may result in adverse effects in people with normally functioning thyroids. While the function of the thyroid and the homeostatic mechanisms for maintaining thyroid function at biologically relevant levels are similar across species, rats are more sensitive to perchlorate-induced hormone changes than humans. Fundamental differences between rats and humans are the reduced ability in rats to store thyroid hormones in the thyroid gland and the inability to have a substantial "backup" supply maintained by special proteins in the blood. Thus, the dose-responses for adverse effects reported in rats may not be directly applicable to use in human risk assessment of exposure to perchlorate.

Human epidemiological, occupational, and clinical studies, taken together, indicate that perchlorate exposures at environmental and occupational levels do not produce changes in thyroid function that would be considered adverse and affect normal thyroid functioning. While very short-term exposure to perchlorate may produce a transient change in thyroid hormone levels, the resilient, adaptive mechanisms that maintain homeostasis (a dynamic and controlled balance) in humans restore the balance of thyroid hormone levels in the thyroid and circulating blood. In people, even with long term exposure to perchlorate, the thyroid system is able to control these fluctuations and conserve thyroid hormone levels in the blood to maintain normal function. Thus, inhibition of thyroid iodide uptake is not an adverse effect unless extreme conditions occur, such as chronic inhibition from extremely high (i.e., therapeutic levels) exposures. In the case where adequate iodine is in the diet, exposure to perchlorate at environmentally relevant levels should not be of concern.

Despite these experimental and physiological data, the U.S. Environmental Protection Agency (USEPA), California Environmental Protection Agency (Cal EPA), and Massachusetts Department of Environmental Protection (Mass DEP) have derived perchlorate drinking water advisories or standards based on human clinical data for inhibition of iodide uptake into the thyroid from a 2-week drinking water exposure. This biological endpoint was recommended by a National Research Council (NRC) committee in 2005, which noted that the effect was not adverse, but was conservatively useful for developing a safe drinking water level. However, data published at the same time or after the NRC recommendation clearly indicated that environmental perchlorate exposure levels can be handled without injury by adaptive biological mechanisms and confirmed the NRCs conclusion that such levels do not have an adverse impact on thyroid function, even in iodide-deficient pregnant women and infants.

This present report evaluated the data considered by the NRC committee and regulatory agencies and more recent data published since their evaluations. We are proposing an

acceptable drinking water level of 620 µg perchlorate/L, based on a dose-response analysis of the No Observed Adverse Effect Level (NOAEL) from human occupational studies. While our proposed value is higher than those proposed by USEPA, Cal EPA, and Mass DEP, our recommended drinking water level is scientifically defensible and was derived with conservative choices for defining the effect threshold that would be protective of sensitive subpopulations.

1 Introduction

The ability of perchlorate to affect human thyroid function has been known for decades. In the 1950s and 60s, high oral doses of perchlorate (2000 mg/day in adults) were used medicinally to treat hyperthyroidism ("overactive thyroid"). Although high-dose treatments were discontinued in the late 1960s, lower doses (600 mg/day) continued to be used without serious side effects to treat anti-thyroid effects of the drug, amiodarone (Leung et al., 2010). Perchlorate does not affect organ systems in humans other than the thyroid.

The use of perchlorate in non-medical applications, such as an oxygen source in high burn rate combustion applications, such as solid rocket fuels, explosives, road flares, pyrotechnics, and air bag inflation systems, may have resulted in the release of perchlorate in the environment, where it has been detected in ground water of 26 states (Trumbo, 2009; Braverman, 2007).

Regulatory agencies in the United States, based largely on animal data and data in a very short-term study in human volunteers, have assumed that the presence of perchlorate in drinking water could have an impact on normal thyroid function with resultant adverse impacts on health. While the potential amount of perchlorate intake from drinking water is likely to be orders of magnitude lower than those historically used in medical therapy, California Environmental Protection Agency's (Cal EPA) Office of Environmental Health Hazard Assessment (OEHHA) derived a Public Health Goal (PHG) for perchlorate in drinking water in 2004, which became the Maximum Contaminant Level (MCL) in 2005. Likewise, Massachusetts Department of Environmental Protection (Mass DEP) derived a perchlorate drinking water standard in 2006. In 2008, the U.S. Environmental Protection Agency (USEPA) issued a Preliminary Regulatory Determination (USEPA, 2008a), indicating that a national primary drinking water regulation for perchlorate would "not present a meaningful opportunity for health risk reduction for persons served by public water systems." USEPA did calculate a Health Advisory (HA) Level for drinking water (USEPA, 2008b). IN February of 2011, USEPA issued a Final Regulatory Determination to proceed with derivation and proposal of a National Primary Drinking Water Regulation (NPDWR) (USEPA, 2011a) within 24 months.

Although the USEPA, Cal EPA and Mass DEP values differ, they are all based on the same clinical study in which volunteers ingested perchlorate in drinking water for two weeks, (Greer et al., 2002). The differences between the values derived by these groups result from different methodological approaches used to determine the allowable total daily dose of perchlorate, the proportion of that dose allowed in drinking water, the drinking water ingestion rate, and treatment of data uncertainties (Sections 2.1-2.3). The regulatory values are based on opinions of the National Research Council (NRC) presented in a 2005 assessment of the perchlorate data in humans and animals (NRC, 2005).

Newer and more relevant data are now available. For this report, ENVIRON examined and performed a critical review of the latest data. A summary of the biology and function of the thyroid hormone system relevant to perchlorate, relevant animal and human data used by the USEPA, Cal EPA, and Mass DEP to set acceptable values for perchlorate in drinking water, and data published since the NRC (2005) assessment are presented in the Appendix. A critical evaluation of the existing USEPA, Cal EPA, and Mass DEP perchlorate drinking water

standards is presented in Section 2. Based on this review, ENVIRON has proposed a scientifically defensible acceptable drinking water level for perchlorate, presented in Section 3.

2 Evaluation of the Drinking Water Levels for Perchlorate by Regulatory Agencies

As detailed in the Appendix, the effect of perchlorate on the thyroid is to inhibit the uptake if iodide from the blood into the thyroid follicle cells, where iodine is used to produce the hormone thyroxine (T_4), which is then converted enzymatically to triiodothyronine (T_3). The USEPA, Cal EPA, and Mass perchlorate drinking water values are all based on the data from Greer et al. (2002), in which 37 adults were given 0.007, 0.02, 0.1, or 0.5 mg/kg/day perchlorate (groups of 7-10 subjects) in drinking water for 14 days. After 2 and 14 days of exposure, the uptake of radioactive iodide (RAIU, a representative fraction of iodide in the whole body) into the thyroid was measured. In addition, blood levels of perchlorate and thyroid hormones T_3, T_4, and thyroid stimulating hormone (TSH) were measured before, during, and after cessation of exposure. While significant inhibition of RAIU was observed at ≥0.02 mg/kg/day, there were not associated with changes in thyroid hormone levels. A lack of change in thyroid hormones is not surprising, as healthy adult thyroids contain sufficient stores of T_3 and T_4 to maintain normal hormone levels in the body for up to six months.

Studies published since the NRC (2005) assessment indicate that the human thyroid system compensates for inhibited iodide uptake from chronic environmental and occupational perchlorate exposure, maintaining adequate thyroid function in clinical volunteers (Braverman et al., 2006), perchlorate workers (Lamm et al., 1999; Braverman et al., 2005), and in iodine-deficient women and pregnant women and their infants (Crump et al., 2000; Tellez et al., 2005; Buffler et al., 2006; Van Landingham and Gibbs, 2008; Pearce et al., 2008, 2010, 2011). Compensation for inhibition of iodide uptake is accomplished by stimulation of the thyroid to increase the capacity of iodide transport into the thyroid follicle cells. This is one of the functions of adaptive, resilient biological feedback mechanisms that are inherent in all humans to maintain homeostasis and normal thyroid health, as discussed in the Appendix. Thus, a 2-week exposure (Greer et al., 2002) is not characteristic or predictive of long term functioning at such low levels of perchlorate exposure. Nevertheless, the NRC derived a Reference Dose (RfD) of 0.0007 mg/kg/day (0.7 µg/kg/day) based on a perchlorate No Observed Effect Level (NOEL) producing no significant short-term inhibition (less than 2%) of RAIU. The NOEL of 0.7 µg/kg/day was divided by an uncertainty factor (UF) of 10 to account for sensitive subpopulations. In the same assessment, NRC stated that

> *"**it is not likely that the decreases in thyroid iodide uptake reported in short-term studies would be sustained; rather, iodide uptake would be expected to return to normal. To cause declines in thyroid hormone production that would have adverse health effects, iodide uptake would most likely have to be reduced by at least 75% for months or longer."***

Derivation of a scientifically-valid, acceptable perchlorate drinking water level should be based on a dose estimate that is close to, but below, the actual threshold for onset of the most sensitive adverse effect (subclinical hypothyroidism). For perchlorate, this calls for chronic exposure data in humans that identifies an exposure level in which the thyroid has achieved non-adverse compensation for iodide uptake inhibition, while maintaining adequate thyroid hormone production. Nevertheless, USEPA, Cal EPA, and Mass DEP adopted inhibition of thyroidal iodide uptake reported by Greer et al. (2002) as the critical endpoint for derivation of their respective drinking water values. Short-term inhibition of iodide uptake is an inappropriate endpoint for deriving a safe drinking water level for perchlorate because (1) it is not an adverse effect, (2) it does not result in changes in thyroid hormone levels or function, and (3) the thyroid system compensates for the inhibition to maintain adequate thyroid hormone levels. The USEPA, Cal EPA, and Mass DEP perchlorate drinking water levels, based on a short-term NOEL, are no more health protective than higher levels that are based on a long-term No Observed Adverse Effect Level (NOAEL). As discussed in Section 3 and the Appendix, the available human data for perchlorate indicate higher exposure levels than the Greer et al. (2002) NOEL that result in initial inhibition of iodide uptake, but produced no adverse effect on thyroid hormone levels or thyroid tissue health (i.e., a NOAEL).

2.1 U.S. EPA Interim Drinking Water Health Advisory for Perchlorate

The USEPA (2008) Interim Health Advisory (HA) for perchlorate in drinking water is 15 µg/L. The HA is based on the NRC (2005) and USEPA (2005) RfD of 0.7 µg/kg/day (i.e., Greer et al. [2002] NOEL divided by 10). The fraction of the total dose (such as an RfD) of a regulated chemical allowable in drinking water, accounting for other non-drinking water exposures, is called the Relative Source Contribution (RSC). For example, if a hypothetical daily estimated perchlorate exposure from sources other than drinking water were 25% of the RfD, the perchlorate exposure allowed from drinking water would be 75% of the RfD, resulting in an RSC of 0.75. In the U.S., the main source of non-drinking water perchlorate exposure is food, such as vegetables and dairy products, with daily intake estimates ranging from 0.08-0.4 µg/kg/day (Trumbo et al., 2010). Data for perchlorate intake in food are available from the National Health and Nutritional Examination Survey (NHANES), the First Unregulated Contaminant Monitoring Regulation (UCMR 1), and the U.S. Food and Drug Administration (USFDA) Total Diet Study. USEPA analyzed the NHANES and UCMR 1 databases to derive an estimate of 0.263 µg/kg/day for dietary perchlorate ingestion in pregnant women. This value is 38% of the USEPA RfD, allowing for 62% of the RfD to be allowed in drinking water (RSC = 0.62). Finally, USEPA used a body weight (BW) and daily drinking water ingestion rate (IR) of 70 kg and 2L/day, respectively, both of which are default values used in deriving drinking water health advisories. Using these values, the interim drinking water HA level was calculated as:

$$HA = \frac{\frac{NOEL}{UF} \times RSC \times BW}{IR}$$

$$HA = \frac{\frac{7\mu g/kg/day}{10} \times 0.62 \times 70kg}{2L/day} = 15\ \mu g/L$$

USEPA selected the inappropriate endpoint (inhibition RAIU) on which to base the RfD and the HA, for reasons discussed in Section 2.0. The selection of an RSC of 0.62 is based on NHANES and UCMR 1 data for pregnant women living in areas unlikely to have perchlorate in drinking water. With an estimate of perchlorate in food of 0.263 µg/kg/day, selection of an RfD of 0.7 µg/kg/day allows for only 62% of total perchlorate intake to come from drinking water. However, there is uncertainty as to whether an RSC is at all necessary, as the perchlorate levels in diet of volunteers were not monitored and may have resulted in total daily perchlorate intake higher than the known doses administered in this study. Thus, the Greer et al. (2002) NOEL on which the USEPA RfD was based may have been underestimated.

2.2 Evaluation of the California EPA Drinking Water MCL for Perchlorate

In 2004, Cal EPA promulgated a primary MCL for perchlorate in drinking water, based on the derivation of a Public Health Goal (PHG) of 6 µg/L, as described by OEHHA (OEHHA, 2004) and Ting et al. (2006). The MCL was based on Cal EPA's benchmark dose (BMD) modeling of the data from the study of Greer et al. (2002), in which a 5% decrease in RAIU was considered biologically significant. From the BMD modeling, a statistical lower limit (i.e., confidence bound) of the benchmark dose (BMDL), 3.7 µg/kg/day, was estimated. The BMDL incorporates information on the shape of the dose-response curve (i.e., potency), as well as variation in the experimental measurements. The BMDL served as a surrogate No-Observed-Effect-Level (NOEL) (USEPA, 2000), and was divided by an UF of 10 to account for uncertainties in sensitivity of iodide-deficient hypothyroid pregnant women and their fetuses and infants. Data for perchlorate in some foods were analyzed by Cal EPA (Kirk et al., 2003, as cited by USEPA, 2008) to qualitatively determine that more than half of environmental perchlorate exposure is likely to come from drinking water rather than food. Thus, an RSC of 0.60 was selected (OEHHA, 2004). Finally, Cal EPA used a body weight-to-daily drinking water ingestion rate ratio of 25.2 kg/day/L (61kg body weight; 2.42L/day) to represent drinking water consumption for the 95th percentile of pregnant women (OEHHA, 2000). Using these values, the Cal EPA primary MCL was calculated as:

$$MCL = \frac{\frac{3.7\mu g/kg/day}{10} \times 0.60 \times 61\ kg}{2.42L/day} = 6\ \mu g/L$$

Cal EPA selected the inappropriate endpoint (inhibition of RAIU) on which to base MCL. Further, Cal EPA's selection of an RSC of 0.60 is based on the assumption that perchlorate in drinking water accounts for most of the perchlorate exposure. Data for perchlorate in the diet of various U.S. subpopulations, including adults, children, and pregnant and lactating women, are now available from the NHANES and UCMR databases, as well as the U.S. Food and Drug Administration (USFDA) Total Diet Study. Selection of an RSC should be based on a reasonable quantitative estimate of relative food contribution to the total allowable perchlorate dose, rather than an arbitrarily-chosen fraction.

2.3 Evaluation of the Massachusetts DEP Drinking Water MCL for Perchlorate

In 2006, Massachusetts DEP promulgated a Drinking Water Standard (DWS, Massachusetts' nomenclature for an MCL) for perchlorate of 2 µg/L, as described by Zewdie et al. (2008). Mass DEP defined an RfD for perchlorate by dividing the Greer et al. (2002) NOEL of 7 µg/kg/day for inhibition of RAIU by a composite UF of 100. In addition to lowering the NOEL by a factor of 10 to account for uncertainties in sensitivity of iodide-deficient of hypothyroid pregnant women and their fetuses and infants (as done by USEPA and Cal EPA), another factor of 10 was applied for "database insufficiencies", extrapolation of results from healthy adults, and basing the drinking water standard on what Mass DEP defined as an adverse effect level rather than a NOEL. An RSC of 0.20 was applied to the Mass DEP RfD, allowing for 20% of the total daily perchlorate dose to come from drinking water. Finally, Mass DEP used default adult body weight and daily drinking water ingestion rate values of 70 kg and 2L/day, respectively. Using these values, the Mass DEP DWS was calculated as:

$$DWS = \frac{\frac{7\mu g/kg/day}{100} \times 0.20 \times 70kg}{2L/day} = 0.49\ \mu g/L$$

Although the calculated DWS is <0.5 µg/L, Mass DEP set the DWS at 2 µg/L. Mass DEP stated that standard use of sodium hypochlorite for public drinking water disinfection may result in finished drinking water with perchlorate levels in excess of 1 µg/L. In order to avoid creation of compliance issues creating a disincentive for necessary water supply disinfection, the DWS was set at 2 µg/L.

Mass DEP selected the inappropriate endpoint (short-term inhibition of RAIU) on which to base their RfD and DWS. In addition to applying an UF of 10 for uncertainties in sensitivity of subpopulations, Mass DEP applied an additional UF of 10 for data gaps. Mass DEP determined inhibition of RAIU in 4 of 7 volunteers in the low-dose group of the Greer et al. (2002) study to be an adverse effect rather than a NOEL, in spite of the fact that the group as a whole did not exhibit significant RAIU inhibition or any reduction in thyroid function. Categorizing short-term inhibition of RAIU as an adverse effect is in opposition to the findings of the NRC (2005) panel, as well as the occupational and epidemiological studies discussed above and in the Appendix that indicate the compensatory ability of the thyroid hormone system to maintain normal thyroid function in adults, pregnant women, and their infants. This decision resulted in an inappropriately conservative RfD on which to base the DWS.

The 2004 selection of an RSC of 20% is based on Mass DEP's determination at the time that the available data did not support the derivation of a less conservative value. In retrospect, Zewdie et al. (2008) discussed the NHANES, UCMR, and USFDA Total Diet Study data. These authors determined that, given the low Mass DEP RfD of 0.07 µg/kg/day relative to food perchlorate intake estimates of 0.2-0.4 µg/kg/day, an RSC of 20% or less was justified. However, Mass DEPs choice to use an inappropriate endpoint and an unnecessarily high UF also resulted in an unnecessarily conservative RSC.

2.4 Limitations of Existing Drinking Water Advisories and Standards

The perchlorate drinking water health advisory and standards derived by USEPA, Cal EPA, and Mass DEP are overly conservative and not based on appropriate interpretation of the available human clinical, epidemiological, and occupational data. All are flawed because they are based on a short-term, non-adverse effect (inhibition of RAIU) from a 2-week exposure in healthy adult volunteers that does not inform on the compensatory mechanisms of the thyroid hormone system to maintain homeostasis during chronic exposure, which are indicated in the human data from chronic exposures. In addition, Mass DEP incorrectly categorized short-term inhibition of RAIU as an adverse effect and applied an additional, unnecessary UF of 10. A consequence of selecting the Greer et al. (2002) NOEL of 7 µg/kg/day as the point-of-departure for deriving a drinking water standard, and applying UFs of 10 or 100, is that the resulting total allowable perchlorate dose (0.07-0.7 µg/kg/day) is at or less than the levels consumed in food (0.08-0.4 µg/kg/day). This forces down the fraction of total perchlorate allowed in drinking water by application of smaller and unnecessarily conservative RSCs. These issues resulted in allowable drinking water levels of 2-15 µg/L that may be problematic for compliance by some public water systems, while providing little, if any, real meaningful reduction in public health risk.

3 Acceptable Drinking Water Level for Perchlorate

ENVIRON derived an acceptable drinking water level for perchlorate based on dose-response data from chronic human exposures. The present assessment is based on data from chronic human exposures reported by Braverman et al. (2004) and Lamm et al., 1999. These data are preferable for several reasons. The study subjects, perchlorate production workers, were exposed for a minimum of 1.7 years and an average of 6 years.

Exposure of the perchlorate workers were primarily via inhalation. However, estimates of the total daily perchlorate dose were calculated using perchlorate levels measured in blood and urine by the study authors (Lamm et al., 1999; Braverman et al., 2004). Regardless of whether perchlorate is inhaled or ingested, equivalent levels in the blood will have the same effect on the thyroid system. Thus, inhalation exposures can be extrapolated to oral exposures using data for perchlorate blood and urine levels of perchlorate as well as knowledge of how quickly perchlorate is cleared from the body (as done by Crump and Gibbs [2005], discussed below).

The perchlorate workers and controls were observed before, during, and after three consecutive 12-hour work shift exposures for inhibition of RAIU (Braverman et al., 2004) and thyroid function (T_3, T_4, TSH, FTI [free T_4 index], and thyroglobulin levels) (Lamm et al., 1999; Braverman et al., 2004). Blood and urinary perchlorate levels for individual workers were available for analysis. Braverman et al. (2004) also measured levels of the inhibitors of iodide uptake that are ubiquitous in the diet, thiocyanate and nitrate, in order to isolate the effects of perchlorate exposure. Because thiocyanate and nitrate levels were not different between control and exposed workers, any differences seen RAIU or thyroid function could be attributed to perchlorate exposure.

Although RAIU inhibition was reported in the exposed workers, upregulation of iodide uptake capacity was also exhibited. Prior to the first observed work shift, the average thyroid RAIU for twenty-nine exposed workers was 21.5%, compared to 14.4% for twelve controls (Braverman et al., 2004). Even though the median exposures of 0.33 mg/kg/shift occurred in 3-day on/3-day off shifts, the workers experienced a compensated ability to get the required amount of iodide into the thyroid, even in the presence of perchlorate-induced inhibition. The study authors suggested that higher pre-shift values of RAIU in exposed workers are indicative of upregulation of iodide uptake. During the exposure, the inhibition of iodide uptake by perchlorate reduced the rate of RAIU from 21.5% down to 13.5%, which was essentially equal to the unexposed controls (14.4%). These findings, coupled with the lack of change in thyroid hormone levels or thyroid gland size, showed that chronic occupational perchlorate exposure was dealt with effectively by the hypothalamus-pituitary-thyroid axis, providing protection from subclinical hypothyroidism.

Crump and Gibbs (2005) analyzed the occupational data of Braverman et al. (2004), Lamm et al. (1999), and the data of Greer et al. (2002). They used simple, single-compartment mathematical models of the body to extrapolate the inhaled daily doses from the blood and urine perchlorate levels. The accuracy of the models to predict daily perchlorate doses were confirmed against the multi-day perchlorate blood level data from subjects in the Greer et al. (2002) study. They then performed BMD modeling of the worker dose data to estimate a perchlorate dose that would have no effect on thyroid function. Rather than modeling data for inhibition of iodide uptake (as was done by Cal EPA), Crump and Gibbs (2005) modeled changes in blood levels of thyroxine (T_4) or thyroid stimulating hormone (TSH), two thyroid hormones that are the primary indicators of thyroid function. Because no clinically abnormal T_4 or TSH levels were measured in the workers, Crump and Gibbs (2005) selected blood levels that were different from 95% of the study population as their surrogate threshold of response for the purposes of modeling the data. The resulting BMDLs were 24 mg/day (based on free T_4 in the blood) and 57 mg/day (based on TSH in the blood). Assuming an average worker body weight of 96 kg (201.3 lbs for exposed workers in Braverman et al., [2004]), the more conservative of the two no-response levels, 24 mg/day, would result in a body weight-adjusted BMDL of 0.25 mg/kg/day, or 250 µg/kg/day, that could be used as a point of departure for deriving an RfD and acceptable drinking water level. An RfD of 25 µg/kg/day was calculated by dividing the BMDL of 250 µg/kg/day by an uncertainty factor of 10 to be conservatively protective of potentially sensitive subpopulations, if any. The ENVIRON RfD is higher than the RfD USEPA (2005, 2008) an Cal EPA (2004) because of the selection of subclinical hypothyroidism from chronic perchlorate exposure as the critical endpoint, which is a more appropriate endpoint than inhibition iodide uptake from short-term exposure.

The derive an appropriate drinking water level for perchlorate, representative body weights and ingestion rates from the most sensitive population should be used, if data are available. Although the human data do not indicate that infants exposed *in utero* of iodide-deficient women are affected by environmental perchlorate exposures (see Appendix), this subpopulation was selected to base an acceptable drinking water level. Thus, ENVIRON calculated an acceptable perchlorate drinking water level using estimates of perchlorate in food, body weight, and water ingestion rate data for pregnant women.

Using data from the NHANES and UCMR data sets, USEPA estimated perchlorate intake from food in 90% of pregnant women in the U.S. would likely be 0.263 µg/kg/day or less (USEPA, 2008a). This is 1% of the ENVIRON RfD of 25 µg/kg/day. Thus, with 99% of the total perchlorate dose allowable in drinking water, an RSC of 0.99 was applied to the RfD to calculate an acceptable drinking water level.

The representative body weight and daily drinking water ingestion rate used in the drinking water calculation were taken from the USEPAs *Estimated Per Capita Water Ingestion and Body Weight in the United States – An Update* (USEPA, 2004). For pregnant women, 50% percentile for body weight was 63 kg, while the 95th percentile for daily drinking water ingestion rate was 2.5 L/day, respectively (USEPA, 2004). These are conservative choices, assuming higher drinking water (and perchlorate) ingestion than the majority of pregnant women.

Using the values discussed above, an acceptable drinking water level was calculated as:

$$acceptable\ drinking\ water\ level\ (\mu g/L) = \frac{\frac{BMDL}{UF} \times RSC \times BW}{IR}$$

$$acceptable\ drinking\ water\ level\ (\mu g/L) = \frac{\frac{250\ \mu g/kg/day}{10} \times 0.99 \times 63kg}{2.5\ L/day}$$

$$acceptable\ drinking\ water\ level\ (\mu g/L) = 620\ =\ 620\ \text{parts per billion}$$

The proposed acceptable drinking water level of 620 µg/L is 2-3 orders of magnitude higher than the federal and state drinking water advisory and standards for perchlorate. The reason for the marked difference in the proposed drinking water level and those of Cal EPA, Mass DEP, and USEPA is the choice of study and critical endpoint. The published data for humans, including iodine-deficient pregnant women and their infants, support the choice of subclinical hypothyroidism (an adverse effect) as the critical effect, rather than RAUI, a non-adverse effect which has been shown to occur in the absence of any adverse effects on human growth, development, or metabolism (Braverman et al., 2004; Tellez et al. 2005; Buffler et al. 2006; Cao et al. 2010; Pearce et al. 2010, 2011). The proposed value was calculated based on conservative parameter value choices, including a BMDL modeled using a conservative threshold for thyroid hormone response, addition of an UF of 10 (assuming existence of perchlorate-sensitive subpopulations), and an assumed high drinking water ingestion rate (95th percentile for pregnant women).

4 Summary and Conclusions

The weight-of-evidence from the human clinical, occupational, and epidemiological data does not support the hypothesis that perchlorate in drinking water has resulted in adverse effects, including subclinical hypothyroidism, even in subpopulations that are presumed by others to be more sensitive to changes in thyroid iodide uptake. The critical endpoint on which the proposed drinking water level is based is the potential for subclinical hypothyroidism, as would be indicated by decreased blood T_4 concentration and/or increased blood TSH concentration. Changes or lack thereof in thyroid hormone levels is a more appropriate biomarker for potential effects on the thyroid. Inhibition of iodide uptake is not the appropriate endpoint for toxicity assessment in humans because of the ability of the thyroid hormone system to adapt to transient changes because of normally functioning compensatory, feedback, and homeostatic mechanisms. Thus, inhibition of iodide uptake is not necessarily an indicator of impending downstream toxicity at exposure levels in which thyroid hormone levels are maintained without overstressing the compensatory mechanisms. The human data discussed previously and in the Appendix provide strong evidence that normal thyroid hormone function can be maintained during chronic exposures to perchlorate that may also cause inhibition of iodide uptake in adults (Lamm et al., 1999; Braverman et al., 2004; Tellez et al. 2005; Pearce et al. 2010, 2011;) and newborn infants (Buffler et al. 2006; Cao et al. 2010) but without indications of adverse changes in thyroid hormone levels or thyroid function.

The proposed acceptable perchlorate drinking water level of 620 µg/L differs from the USEPA, Cal EPA, and Mass DEP values of 2-15 µg/L because of the selection of subclinical hypothyroidism as the critical endpoint, rather than inhibition of thyroidal iodide uptake, and because of the relatively small contribution that dietary perchlorate likely makes to the allowable daily dose. The proposed value is preferable to the existing regulatory values because it takes into account non-adverse compensatory mechanisms of thyroid biology, is based on observations of chronic exposure, and protects against an adverse, versus non-adverse, effect.

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Appendix A

A.1 Thyroid Biology and Function

Thyroid hormones leave the thyroid, circulate throughout the bloodstream, and enter and interact with organ systems throughout the body. Thyroid hormones are thyroxine (T_4) and triiodothyronine (T_3). Thyroxine is produced in the thyroid and then, with assistance of specific enzymes produced in the liver, convert to T_3. The thyroid uses specific proteins (called sodium-iodide symporters, or NIS) found on the surface of thyroid cells (thyroid follicles) to actively take iodine from the blood. The NIS molecules are the targets of perchlorate, with inhibition of iodide uptake demonstrated in short-term volunteer data (Greer et al., 2002) or long-term therapeutic use (discussed below). Other proteins within the thyroid follicles, along with a hormone from the pituitary gland (thyrotropin, or TSH), integrates the iodine into the formation of T_3 and T_4 molecules. In humans, the thyroid can store significant quantities of T_3 and T_4 in the thyroid cells.

In the cells of the body, T_3 is the active thyroid hormone that interacts with specific molecules in the cell, including portions of DNA, to turn on and off processes that initiate, increase, or decrease growth, development, and energy metabolism. T_4 has little, if any, biological function itself; it is at the ready when needed to be transformed within the cells into T_3 by the removal of one iodine atom. The liberated iodine atom re-enters the circulation where it can be taken back up into the thyroid and re-cycled into new T_3 or T_4, or it may be eliminated in the urine or, to a lesser extent, the saliva and breast milk. Iodine has no other known function in the human body.

In humans and other mammals, most of the thyroid hormone in the blood is in the form of T_4. Further, over 99% of T_4 and T_3 in human blood is bound to specific proteins to act as a ready source of thyroid hormone. As free, unbound T_3 and T_4 are taken from the circulation into body tissues, it is replaced by release of bound hormone. Thus, the body maintains a constant, low level of free T_3 and T_4 in the blood. This capability of healthy humans to maintain a ready supply of thyroid hormone bound in the blood as well as stored in the thyroid itself is not present in laboratory rodents. In rats, the thyroid is constantly stimulated to maintain adequate T_3 and T_4 levels in the circulation. Thus, rats are more susceptible than humans to effects arising from subtle changes in thyroid function and thyroid hormone levels. This difference between species is important when comparing the effects of perchlorate, or any other thyroid-acting agent, in humans and laboratory animals, as is discussed below in greater detail.

Mechanisms are at work in the human body to precisely maintain adequate levels of thyroid hormone in the blood as needs change. The pituitary gland located in the brain can stimulate the thyroid to increase production of thyroid hormone should blood levels of T_4 decrease. Falling T_4 levels in the blood signal the hypothalamus, a specialized region of the brain, to secrete a hormone, TRH, which signals the pituitary to begin secretion of TSH, which stimulates

a number of functions in the thyroid, including increasing the capacity for iodine uptake (through increasing the number of NIS molecules on the thyroid cell surfaces), increasing the production of T_3 and T_4, and increasing production of special proteins that bind T_4 in the blood. Adequate blood T_4 levels signal the hypothalamus and pituitary gland to stop production of TRH and TSH, respectively, which halts the stimulation of the thyroid. This tightly controlled set of feedback and feed-forward mechanisms is known collectively as the hypothalamus-pituitary-thyroid axis.

If conditions occur such that T_4 levels in the blood are too low to maintain proper regulation of growth, development, or metabolism, a medical condition known as hypothyroidism results. Hypothyroidism has numerous causes, including autoimmune thyroid disease, congenital abnormalities of thyroid or pituitary function, treatment with iodine-containing drugs or radiographic contrast agents, or iodine deficiency in the diet. Hypothyroidism can be further graded as subclinical, mild, or overt indicated by slight, moderate, or high levels of TSH in blood and, for mild or overt cases, low blood T_4 levels (Ross, 2005).

A.2 Toxicity Data for Perchlorate

According to the animal and human data discussed below, perchlorate at high doses inhibits the uptake of iodine by NIS proteins on the surface of thyroid follicle cells, thereby, inhibiting the transport of iodine into the thyroid cell. However, the magnitude and duration of reduced iodide uptake into the thyroid must be high enough to overcome compensatory mechanisms that act to maintain homeostatic control to sustain normal thyroid function. One of the ways that the hypothalamus-pituitary-thyroid axis compensates for reduced thyroid uptake is to stimulate an increase in the number of NIS proteins, resulting in more entry points for iodine and reducing the inhibitory effect of perchlorate. Studies in humans have shown that, while perchlorate exposure can result in inhibition of iodide uptake in the short term (Greer et al., 2002), there are transient and adaptive homeostatic mechanisms to offset in humans increases in iodine uptake (Braverman et al., 2004, 2006). This capability can be overwhelmed (as in the case of therapeutic uses of high doses of perchlorate), but at environmental levels of perchlorate exposure, decreased T_4 in the blood does not necessarily follow inhibition of iodide uptake into the thyroid.

The scientific literature for perchlorate toxicology includes many studies in laboratory animals and humans, which are summarized below. Human studies are preferred to determine the dose-response of any chemical. Data from animal studies in the appropriate species should be reviewed as well, bearing in mind the physiological differences between species. As is discussed below, perchlorate interacts similarly with the NIS molecules in human and animal thyroid cells, but the resulting changes in available iodide and resulting levels of thyroid hormone in the circulation are very different between laboratory rodents and humans.

A.2.1 Summary of Human Data

A.2.1.1 Clinical Uses for Perchlorate in the Treatment of Hyperthyroidism

In the 1950s and 1960s in patients with hyperthyroidism, potassium perchlorate was administered to inhibit thyroid iodide uptake, thus reducing the production of T_4 and T_3. Administration of potassium perchlorate was typically at doses ranging from 400 to 2000 mg/day for several weeks or months disease (Leung et al., 2010). Few side effects were noted in these

patients, with the frequency of side effects reported increasing with increasing dose. However, because of the observation of some side effects and the development of better anti-thyroid drugs, the use of perchlorate to treat hyperthyroidism ceased by the late 1960s.

Although not used as frequently, perchlorate is still used today diagnostically to detect defects in the synthesis of thyroid hormones (NRC 2005). It is also used specifically to treat hyperthyroidism resulting from the administration of the anti-arrhythmic drug, amiodarone. While perchlorate continues to be used for these specific applications, the Food and Drug Administration does not recognize perchlorate as a pharmaceutical for the treatment of endocrine or metabolic disorders (NRC 2005).

A.2.1.2 Occupational Studies

Several occupational studies have been conducted to evaluate thyroid function in workers following long-term exposure to ammonium perchlorate (Gibbs et al. 1998; Lamm et al. 1999; Braverman et al. 2005). Regardless of the characterization of exposure in these cohorts (estimated daily doses approximately 0.3 mg/kg/day), no significant association between estimated exposure to perchlorate and alterations in any of thyroid hormone or TSH measures was reported. In the Gibbs et al. (1998) study, only duration of shift was related to changes in TSH concentrations, with this observation possibly being associated with an increase in serum TSH concentrations expected for workers' changes in the day/night cycle.

The only significant change in an endpoint related to thyroid function reported in any of the occupational studies, was a significant decrease in thyroid radioactive iodine uptake (RAIU) reported in workers following three 12-hour shifts compared to pre-exposure measurements (Braverman et al., 2004). However, serum TSH, serum thyroglobulin (Tg) (a protein essential for thyroid hormone formation) and thyroid volume were not affected. In addition, the RAIU measured in workers during exposure, while lower than preshift values, was comparable to those of community controls. These findings indicated a compensatory response to inhibition of iodide uptake occurring early on in every 3-day work shift, but not result in adaptive changes seen in adults that would result in the development of hypothyroidism or goiter.

A.2.1.3 Ecological Epidemiology Studies

Ecological epidemiology studies have been conducted examining possible associations between environmental exposure to perchlorate in drinking water (4-120 µg/L) and various effects, including thyroid function, thyroid disease, and cancer in children (including low-birth-weight or preterm newborns and offspring of mothers who were hypothyroid or had iodide deficiency during gestation) and adults (Rockette and Arena, 1983; Li et al., 2000a, 2000b, 2001; Morgan and Cassidy, 2002; Lamm and Doemland, 1999; Brechner et al., 2000; Crump et al., 2000; Schwartz et al., 2001; Chang et al., 2003; Kelsh et al., 2003; Lamm, 2003; Buffler et al., 2004, as cited by NRC 2005). NRC (2005) noted that while ecologic study design may be limited in their ability to establish cause-and-effect of toxicity, the association, or lack thereof, of exposure and toxic endpoint can be informative when combined with other data on the biology of the thyroid gland and experimental studies of the effects of acute exposure to perchlorate. The NRC Committee concluded that the available epidemiologic data did not indicate a causal association between perchlorate exposure and congenital hypothyroidism, perturbations of

thyroid hormone and TSH production in normal newborns, or thyroid diseases or hypothyroidism in normal adults. NRC (2005) further determined that there was inadequate evidence to motivate the conduct of a study of possible associations between perchlorate exposure and neurodevelopmental outcomes in children (e.g., autism, ADHD) or thyroid cancer in adults.

Ecological epidemiological studies published since the NRC (2005) assessment have focused on evaluating the potential association between perchlorate exposure and indicators of thyroid function in pregnant women (Pearce et al. 2010, 2011; Tellez et al. 2005) and newborn infants (Buffler et al. 2006; Cao et al. 2010). In the Pearce et al. (2010) study, subjects were a subset of pregnant women involved in the Controlled Antenatal Thyroid Screening Study (CATS). This prospective study was designed to determine whether L-T_4 for hypothyroid or hypothyroxemic pregnant women during pregnancy improves child development. In 2002, a total of 22,000 women with single pregnancies at less than 16 weeks were enrolled in this program. For the subset 1002 women considered by Pearce et al. (2010), urinary iodine, thiocyanate, and perchlorate and serum TSH, free T_4 and thyroperoxidase antibodies (an indicator of thyroid function) were measured. Urine perchlorate was detectable in all women studied, with a median concentration of 2-5 µg/L, depending upon location (Turin, Italy versus Cardiff, Wales). No associations between urine perchlorate concentrations and serum TSH or free T_4 were observed. In addition, perchlorate in urine was not a predictor of free T_4 or TSH in blood. The authors concluded that low-level perchlorate exposure is ubiquitous, but it is not associated with alterations in thyroid function among iodine-deficient women in the first trimester of pregnancy. They also noted that their sample is the largest to date in women with low urinary iodine levels, all of whom were pregnant, and that it is in contrast to a smaller study conducted in the United States by Blount et al. (2006). Pearce et al. (2011) reported similar results in a study conducted in smaller groups of pregnant women from Los Angeles, California (N=134) and Cordoba, Argentina (N=107). In nonpregnant women with similar urine iodine (less than 100 µg/L) and perchlorate concentrations as those reported by Pearce et al. (2010), Blount et al. (2006) reported increases in serum TSH and lower serum T_4 concentrations. However, a re-analysis of the same data by Pearce et al. (2007) in which urinary perchlorate levels were more appropriately corrected for creatinine excretion (reducing the effect of variable urine volume among subjects), did not find an association urinary perchlorate and T_4 and TSH levels in blood.

Tellez et al. (2005) conducted a longitudinal epidemiological study among pregnant women in three areas of Chile with drinking water concentrations of perchlorate that varied from 0.5 to 114 µg/L. It was the same areas evaluated by Crump et al. (2000) to evaluate potential effects in school children from exposure to perchlorate. No increases in thyroglobulin (Tg) or TSH or decreases in free T_4 were observed among women in early pregnancy (approximately 16 weeks) or late pregnancy (approximately 32 weeks). In addition, no significant changes in the thyroid hormone indicators were seen in newborns related to perchlorate drinking water concentration. These studies have been criticized for focusing on a population having higher iodine ingestion than in the U.S. Further analysis, however, by Van Landingham and Gibbs (2008) of only iodide-deficient women within these data found no association between urinary perchlorate and T_4 or TSH levels.

In ecologic studies focused on the evaluation of indicators of thyroid function in infants (Cao et al. 2010; Buffler et al. 2006), Cao et al. (2010) reported a weak but significant association

between higher urinary perchlorate (expressed as log perchlorate in µg/g creatinine) and higher TSH concentrations in infants with lower urinary iodine (<100 µg/L) in children enrolled in the Study of Estrogen Activity and Development (Cao et al. 2009). This study design was partly cross-sectional (offering a "snapshot" of data in time) and partly longitudinal (following the subjects over time), since some children were evaluated at multiple visits. Blood and urinary TSH and T_4 were measured in each child at each visit.

The Buffler et al. (2006) study included 342,257 newborns screened by the California Newborn Screening program in 1998, whose mothers resided in communities in which ground water was tested for perchlorate in 1997 or 1998. The endpoints of interest for evaluation in the study were the presence of clinically-diagnosed primary congenital hypothyroidism (PCH) and serum TSH concentrations in the newborns. In evaluating any correlation TSH and perchlorate ground water concentrations, the authors considered the time in which perchlorate was measured in the infant after birth as a critical factor in evaluating the results, because a normal postnatal surge of TSH is present within the first 24 hours after birth that could impact the interpretation of the results. Therefore, only infants whose TSH was measured at a time point greater than or equal to 24 hours of age was considered. Calculations were controlled for confounding factors such as sex, ethnicity, birth weight, and multiple birth status. The study authors reported no association between estimated average perchlorate concentrations of greater than 5 µg/L in drinking water supplies and the prevalence of clinically diagnosed PCH or high TSH concentrations.

A.2.1.4 Clinical Volunteer Studies

Several clinical studies have been conducted to evaluate the potential effects of potassium perchlorate in healthy subjects, with exposure duration ranging from 2 weeks to 6 months (Brabant et al. 1992; Lawrence et al. 2000, 2001; Greer et al. 2002; Braverman et al. 2006). Doses in these studies ranged from 0.007 to 0.5 mg/kg/day in drinking water. Endpoints considered in these studies included measurements of serum T_4, T_3, and TSH concentrations, as well as evaluation of changes in RAIU.

No statistically significant changes in thyroid hormone levels (T_4, T_3, TSH levels in blood) that would indicate an impact on thyroid function leading to hypothyroidism were reported in any of these clinical studies. Some authors noted slight decreases in TSH (Brabant et al. 1992; Greer et al. 2002), which is inconsistent with hypothyroidism, in which a significant increase in TSH would be expected. Significant transient decreases in 24-hour RAIU were noted in clinical studies in which perchlorate was administered for 2 weeks, with a return to baseline levels following cessation of exposure (Lawrence et al. 2000, 2001; Greer et al. 2002). However, most importantly, in the study with the longest duration of exposure (6 months) (Braverman et al. 2006), no statistically significant effect on 24-hour RAIU was observed. While this study is limited by small sample size (N=13, 9 treated and 4 placebo controls), it does provide information on levels of perchlorate ingestion (up to approximately 0.04 mg/kg/day) to which healthy individuals can be exposed for a longer-term duration with no impact on indices of thyroid function.

USEPA (2005) based the current Reference Dose (RfD) on changes in RAIU uptake reported in the 2week clinical study by Greer et al. (2002), which provided evidence that there were no

other significant changes in T_4, T_3 and TSH concentrations. USEPA (2005) also noted that chronic exposure will have no greater effect than that resulting from short term exposure; lack of the precursor event of inhibition of iodide uptake will result in no changes in thyroid function in the short or long term. In fact, prolonged exposure will actually have less effect because of the capacity of the hypothalamus-pituitary-thyroid axis to compensate for iodide deficiency by increasing iodide uptake, as seen in the Braverman et al. (2005) study of perchlorate workers. USEPA (2005) further noted that if some inhibition of iodide uptake by the thyroid did occur at the minimal dose at the point of departure, data from humans indicated that longer exposures were not likely to result in a greater or more severe response.

A.2.2 Summary of Laboratory Animal Data

The following sections include a brief review of the animal studies pertaining to perchlorate health effects. The studies evaluated for these sections were those included in the NRC (2005), *Health Implications of Perchlorate Ingestion* report and those studies, published post-2005 located in a literature review. The information summarized in this section includes results of rodent studies that look for changes in thyroid hormone levels, reproductive and developmental effects, brain changes, and cancer.

A.2.2.1 Effects on Thyroid Hormone Levels

An evaluation of the effects of perchlorate on thyroid hormones and thyroid histopathology was conducted in pregnant rats and their offspring (Argus, 2001; York et al., 2001a). Argus (2001) gave pregnant rats perchlorate in drinking water at doses ranging from 0.01 to 30 mg/kg/day throughout pregnancy (gestation) and lactation. Argus (2001) reported dose-related increases in serum TSH in the mothers (dams), fetuses, and pups at ≥0.01 mg/kg/day, decreases in T_4 at ≥0.01 mg/kg/day, and decreases in T_3 at 30 mg/kg/day. The NRC (2005) summary of the Argus (2001) study did not indicate whether statistical analyses used individual pups or litters as the experimental unit for comparison of thyroid hormone levels. Similar significant decreases in T_4 were also reported in pregnant rabbits at doses ≥30/mg/kg/day (York et al. 2001b).

Studies in non-pregnant rats or pregnant prairie voles (James-Walke et al., 2006) and deer mice (Smith et al., 2006) did not report changes in thyroid hormone levels. Drinking water concentrations that resulted in an estimated intake of perchlorate of 0.05 or 0.4 mg/kg/day for 21 days did not produce significant changes in blood T_4 hormone levels in female rats (James-Walke et al. 2006). Similar results were reported in prairie voles and deer mice given perchlorate at concentrations resulting in 0.7-1.1 mg/kg/day in either food or drinking water (Smith et al. 2006).

Two studies in rats have investigated the effects of iodide deficiency on the thyroidal effects of perchlorate exposure(Paulus et al., 2007; Wu et al., 2010). Rats fed iodine-deficient diets absorbed greater amounts of iodide into the thyroid at about twice the rate of rats fed normal diets (Paulus et al. 2007). Following administration of perchlorate at concentrations of 1.1-28 µg/L in drinking water (0.1-3 mg/kg/day), the normal-diet rats showed significant inhibition of thyroidal iodide uptake at all doses, while the iodide-deficient rats had significant inhibition of iodide uptake only at 28 µg/L. These findings indicated that iodide-deficiency in rats stimulates

a compensatory increase in iodide uptake capacity that offsets perchlorate-induced inhibition up to a certain level.

Wu et al. (2010 reported similar reductions in T_4 and increases in TSH in treated and in non-treated iodine-deficient rats. They also reported differences in genetic expression of two enzymes and proteins involved with T_3 and T_4 production in the thyroid at different doses, but not in the iodine-deficient animals. These study authors speculated that, in addition to iodide uptake inhibition, perchlorate may affect thyroid hormone balance by another mechanism. However, they offered no explanation as to why the differences in genetic expression markers were not expressed at the same levels that affected T_4 and TSH, or why iodine deficiency resulted in similar changes to T_4 and TSH if there were, in fact, another mechanism responsible to effects associated with perchlorate exposure in laboratory rats.

McLanahan et al. (2009) used a mathematical computer model to investigate the influence of iodine uptake inhibition on thyroid hormone levels. Their results corroborated the conclusions of Wu et al. (2010), suggesting that the effects of perchlorate in the thyroid may to occur through more thanone mode of action, by blocking iodide transport as well as interacting in some way with thyroid hormone production and release.

A.2.2.2 Effects on Brain Structure

Argus (1998) reported an increase in thickness of specific brain tissues of rat pups born to dams exposed 0.1-10 mg/kg/day perchlorate in drinking water throughout pregnancy. Argus (2001) extended their 1998 analysis by performing brain measurements on additional groups of rats using a higher dose (30 mg/kg/day) and additional sampling times during pregnancy. Again, they reported significant increases in thickness of brain tissues, but could not demonstrate an increase in effect as doses increased.

Concerns have been raised about the Argus (1998, 2001) studies from outside reviewers (Harry, 2001; Wahlsten, 2002; Elberger, 2003; Gellar, 2003). In general, the concerns were: 1) The systematic differences in how brain samples were measured between treatment groups; 2) the lack of a dose-response relationship, and 3) concerns about the biologic plausibility of the changes that were reported. Based on their review of Argus (1998, 2001), NRC (2005) concluded that the data from Argus (1998, 2001) were inadequate to identify a causal relationship between maternal perchlorate exposure and pup neurodevelopmental abnormalities.

A.2.2.3 Effects on Neurobehavior

Previous research has suggested that changes in thyroid hormone levels occurring during fetal development could interfere with normal brain development, including changes in the brain areas that may be involved in motor coordination (Chan and Kilby, 2000). The Argus (1998) study discussed above included a battery of behavioral tests performed on the offspring of rats exposed to perchlorate in drinking water during pregnancy. Significant changes were not observed during several standard tests following doses of 0.1 to 3 mg/kg/day, the highest dose tested. However, Argus (1998), but not Bekkedal et al. (2000), observed significant changes in locomotor activity in litters of rat pups born to dams exposed to 10 mg/kg/day.

The effects of perchlorate on developmental neurotoxicity reported by Argus (1998) were not observed in a longer exposure study by York et al. (2005a, 2005b). Offspring from female rats exposed to up to 30 mg/kg/day perchlorate from 2 weeks prior to mating through day 10 of lactation exhibited no changes in a variety of motor activity variables, suggesting that perchlorate exposure in the womb and through lactation did not significantly affect the development of simple muscle movements in pups (York et al., 2005b).

Exposure to perchlorate during gestation did not impair behavioral test endpoints in the male offspring of females exposed to drinking water concentrations up to 1000 ppm (Gilbert and Sui, 2008). Significant reductions in electrophysiological assessments (suggesting adverse effects in the hippocampal region of the developing brain) were reported for the offspring at all dose levels. However, a consistent dose-response relationship was not observed, and the lack of behavioral changes (sensitive to deficiencies in the brain hippocampus) did not comport with measured electrophysiological changes (Mavis and DeSesso, 2009).

To evaluate the thyroid hormone action in the brain, Sharlin et al. (2010) measured gene expression, indicators of thyroid hormone action in the brain, in the offspring of pregnant rats administered methimazole and potassium perchlorate to induce hyperthyroidism during gestation. There was a significant decrease in blood T_4 levels and body weight in young pups. However, the findings did not support the notion of the developing rat brain being capable of compensating for low T_4 levels.

A.2.2.4 Effects on Reproductive and/or Developmental Function

Studies in several species examined the effect of perchlorate exposure in the diet or drinking water on reproduction. No changes in reproductive endpoints were see in rats (York et al. (2005a, 2005b), deer mice or prairie voles (Smith et al., 2006), or rabbits (York et al., 2001b).

A.2.2.5 Thyroid Gland Tumors

Cancer and noncancer effects on the thyroid gland have been reported in both mice and rats following long term exposures to high doses of perchlorate (Kessler and Kruskemper, 1966; Pajer and Kalisnik, 1991; Fernandez-Rodriguez, 1991). Thyroid cellular changes and nodules were reported in female rats administered 1% perchlorate in drinking water for up to 12 months (Fernandez-Rodriguez, 1991). In rats, daily drinking water doses of 1,339 mg/kg/day for a lifetime produced benign thyroid tumors in 4 of 11 treated rats (Kessler and Kruskemper, 1966), while thyroid carcinomas were observed in 5 of 6 mice exposed to 2,147 mg/kg/day in drinking water for 46 weeks (Pajer and Kalisnik, 1991). In both studies of mice and rats, no tumors were observed in the control animals.

Thyroid benign tumors were reported in male rats in a two-generation reproductive study (Argus 1999). In this study, male rats in the first generation were given perchlorate in drinking water from 70 days before mating until age of 24-25 weeks. The male offspring were exposed to the same duration of dosing, with additional exposure through pregnancy and lactation. The microscopic tissue changes, which were re-evaluated by USEPA (Wolf, 2000; USEPA, 2002), reported that two first-generation males in the 30 mg/kg/day group and one second-generation male control rat had benign thyroid tumors. Both the USEPA (2002) and the NRC (2005) concluded that while the thyroid tumors seen in the treated male rats were not statistically

significant, the appearance of tumors as early as week 19 indicated that they were likely treatment-related.

A.3 Differences in Animal and Human Perchlorate Toxicokinetics

The function of the thyroid in rats and humans is qualitatively similar; however, some differences between the two species in chemical binding of perchlorate to thyroid proteins in the blood and rates of clearance from the body present important quantitative differences (NRC 2005). The principal protein that binds T_4 in human, thyroxine-binding globulin (TBG) is not present in rats. Most of the T_4 in rats is bound to two other bold proteins: albumin and transthyretin, which have a T_4 binding affinity ("tightness of binding") of about 100 times less than TBG in humans. Therefore, T_4 is cleared from the body more quickly in rats than in humans, requiring a higher production rate of T_4 per unit body weight. In the liver, rats and humans join T_4 to a specific carbohydrate molecule to form a compound class (glucuronide conjugate) that may then be eliminated in the bile. This mechanism is used by the body to promote elimination of many chemicals. However, about 50% of T_4 in the rat gets eliminated in the bile, compared to only 10-15% in humans.

The lower binding capacity for T_4 blood as well as the much smaller thyroidal storage capacity of T_3 and T_4 in rats, compared to human, requires that rats produced thyroid hormone on a continual basis, with almost constant stimulation by TSH. In studies of perchlorate-induced iodide uptake inhibition, rats exhibited compensation for reduced iodide availability within 5 days (York et al., 2002) of exposure, while human volunteers did not compensate by 14 days of drinking water exposure, even though iodide uptake was inhibited by as much as 67% (Greer at al., 2002). Longer durations of perchlorate exposure in human volunteers (6 months) (Braverman et al., 2006) and perchlorate workers (≥1.7 years) (Braverman et al., 2004) did result in evidence for compensatory mechanisms by increasing the thyroid's capacity to take up iodide from the blood.

Changes in blood concentrations of T_3, T_4, and TSH following perchlorate exposure are different in rats and humans. Following 14 days of perchlorate exposure in rats, Siglin et al. (2000) reported significant decreases in T_3 and T_4 levels, and significant increases in TSH. However, no significant changes in TSH, T_3, or T_4 have been reported in adult humans exposed to up to 0.5 mg/kg/day perchlorate for 14 days (Lawrence et al. 2000; Greer et al. 2002), six months (Braverman et al., 2004), or ≥1.7 years (Lamm et al., 1999; Braverman et al., 2004).

There are also differences between rats and humans in pituitary-thyroid function during pregnancy and in the fetal development of the thyroid. In humans, blood T_3 and T_4 levels increase during pregnancy and remain elevated through most of pregnancy (Glinoer 1997). In rats, blood T_4 levels significantly decrease during gestation days 17-22 (4 days leading up to birth), compared to non-pregnant rats (Fukuda et al. 1980; Calvo et al. 1990; Versloot et al. 1994). These differences are mostly related to the lack of TBG in rats. At birth, thyroid function in the rat is relatively immature, and equivalent to that of a third-trimester human fetus. In humans, thyroid function in the womb supported by maternal thyroid hormones, while T_4 and T_3 levels in newborn rats don't increase until 5 to 15 days after birth due to an increase in a blood thyroid hormone binding protein (Obregon et al. 1991).

Computer models capable of simulating perchlorate toxicokinetics in rats and humans, termed physiologically based pharmacokinetic (PBPK) models, have been developed (Clewell et al., 2007) and evaluated by USEPA (2009). These models allow for prediction of perchlorate doses to various tissues, including transfer of perchlorate and iodide to the developing fetus and breastfeeding infant, under a variety of exposure scenarios. However, the models were not designed to predict compensation of the hypothalamus-pituitary-thyroid axis for inhibited RAIU or the effect that these mechanism have on thyroid hormone levels. Because of this, the models are limited in their application to simulate and compare chronic doses and effects (or lack) reported in occupational or epidemiological studies.

A.4 Relevance of Animal Data to Human Risk

With regard to non-cancer and cancer effects of perchlorate, the rodent data should not be used in the quantitative estimation of human risk. Comparison of differences between the species of the physiology of the of the thyroid gland, capacity of thyroid hormone binding in blood, rate of elimination from the system, and onset of hormone production and availability of maternal hormones in the developing offspring indicate that rats are clearly more sensitive than humans to perturbations of iodide uptake into the thyroid. Because the rat thyroid is under near-constant stimulation by the pituitary gland to maintain blood T_4 levels, not having the reserve of thyroid hormone in the thyroid and the blood found in humans, additional chemical-induced stresses on the hypothalamus-pituitary-thyroid axis are likelihood to result in adverse effects not seen in humans or at much lower doses and shorter exposure durations (USEPA, 1998).

For thyroid carcinogenicity, an International Agency for Research on Cancer (IARC) working group stated that chemicals found to induce thyroid tumors in rodents by interfering with thyroid hormone balance can, with some exceptions (i.e., sulfonamide drugs), also interfere with human thyroid balance if given long enough at sufficiently high doses. However, these agents can be assumed not to cause cancer in humans at exposure levels that do not lead to changes in human thyroid hormone balance (IARC, 2001). This was echoed in a USEPA science policy document (1989) and by NRC (2005), stating that humans may not be as sensitive quantitatively to thyroid cancer development of thyroid-pituitary disruption as are rodents.