UPDATE TO "PERCHLORATE TOXICOLOGICAL PROFILE AND HEALTH ASSESSMENT"

In support of:
Perchlorate Maximum Contaminant Level (310 CMR 22.06)
Perchlorate Cleanup Standards (310 CMR 40.0000)

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PREFACE

This document presents an update to MassDEP's 2004 *Draft Toxicological Profile and Health Assessment.* MassDEP postponed finalization of the 2004 document in order to consider the National Academy of Science's (NAS) report, *Health Implications of Perchlorate Ingestion,* which was released in January 2005, as well as additional information on perchlorate in breast milk that was not available to the NAS Committee. A separate MassDEP document, released concurrently with this document, titled *Addendum: Review of New Studies on Perchlorate*, addresses results from several even more recent scientific studies that were published during the internal review period for this updated document. This document reflects the scientific review and deliberation of MassDEP scientists, as well as distinguished members of the MADEP/DPH Advisory Committee on Health Effects. Two local members of the NAS committee met with MassDEP and its Advisory Committee to share information and perspectives on the health effects of perchlorate.

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PEER REVIEW: DEP/MA DPH ADVISORY COMMITTEE ON HEALTH EFFECTS

MassDEP employed its standing advisory committee on health effects to advise it during the preparation of this document. The Committee membership was augmented with a number of specialists with expertise in the subject areas covered in our assessment of the health effects of perchlorate and standard setting. Participants included:

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MassDEP is very appreciative of the participation of the members of the MassDEP/DPH Advisory Committee on Health Effects in the scientific peer review of MassDEP's draft updated toxicity assessment. Their generous commitment of time and tremendous expertise has been extremely helpful to our efforts on this important issue. The participation of independent public health scientists is a critical component of our state's efforts to protect public health and the environment in MA.

MassDEP also wishes to acknowledge two members of the National Academy of Sciences' Committee to Assess the Health Implications of Perchlorate Ingestion, Dr. Robert Utiger (Harvard University School of Medicine, Boston, MA) and Dr. Rosalind Brown (Children's Hospital, Boston, MA) for sharing information and perspectives on the toxicity of perchlorate with MassDEP and the Advisory Committee during the February 11, 2005 meeting. MassDEP is also grateful for the valuable input and information provided by Charles Emerson, MD, endocrinologist, University of Massachusetts Medical Center, Worcerster, MA.

EXECUTIVE SUMMARY

The following document provides an update of the Massachusetts Department of Environmental Protection's (MassDEP) 2004 *Draft Toxicological Profile and Health Assessment*, taking the National Academy of Sciences (NAS) report on perchlorate released in January 2005 into account, as well as other recent data.

Perchlorate inhibits iodide transport into the thyroid gland, which is hypothesized to be its primary mechanism of toxicity. The thyroid requires iodide to make thyroid hormones, which are essential for the normal growth and development of many systems in the body including the brain (MassDEP, 2004; U.S. Environmental Protection Agency, 2002a). Perchlorate is itself concentrated into breast milk in experimental animals and likely in humans as well; it may also be concentrated into thyroid cells, where it could impact other cellular functions. It may also interfere with iodide transport into breast milk, and interfere with a second iodide transporter found in the thyroid. Although not well understood, perchlorate also promotes the discharge of accumulated iodide from the thyroid gland at high doses. These effects of perchlorate, whether working in concert or independently, may, at sufficient doses, disrupt normal thyroid function and hormone dynamics, including altered synthesis of essential thyroid hormones (thyroxin (T4) and triiodothyronine (T3)).

Based on many lines of evidence premature infants and newborns are particularly at risk from perchlorate toxicity. Their sensitivity is attributable to the development of various organs, especially the brain, which occurs during fetal growth and early childhood. Appropriate levels of thyroid hormones are critical to these processes.

During the fetal and newborn periods the thyroid gland is also incompletely developed, increasing sensitivity to toxicants that disrupt the thyroid functions available. Pregnancy itself stresses maternal thyroid function increasing risks to the fetus. To further compound matters, breast milk is the sole source of the iodide a newborn needs to supply its capacity to synthesize thyroid hormones. Data suggests that maternal exposure to perchlorate may reduce iodide levels in breast milk, and thereby may increase neonatal sensitivity to perchlorate. This risk is further compounded by the fact that perchlorate is itself secreted, and may be concentrated, into the milk, presenting a double insult to the newborn's thyroid (Kirk et al. 2005).

Despite their likely sensitivity, there is essentially no good toxicity data on perchlorate exposures to pregnant women, the fetus and newborn. Thus, regulators must extrapolate from controlled studies on small numbers of healthy adults and/or data from animal (rodent) model systems. The current epidemiological data on larger groups of people exposed to perchlorate does not allow for safe exposure levels to be determined.

The MassDEP *Draft Toxicological Profile and Health Assessment* was undertaken as the result of a public request for guidance on drinking water perchlorate contamination, as no federal or state standards on this toxicant were available. In the MassDEP 2004 *Draft Toxicological Profile and Health Assessment*, MassDEP used a weight of the evidence approach in which data from animal and human studies were both assessed. These were found to yield overlapping toxicity values. Because the animal studies included exposures to the fetus and newborn, the

lifestages most at risk, Mass DEP relied on data from that research to derive an acceptable total human dose or RfD of 3×10^{-5} mg/kg-day.

While MassDEP was working on its 2004 Assessment the National Academy of Sciences (NAS) convened a scientific advisory committee to address perchlorate toxicity. The NAS completed its report in January 2005, and derived two possible RfD values using data from the 14-day perchlorate exposure study performed on small groups of healthy adults by Greer, et al. (2002). The majority of the NAS Committee members supported an RfD of 7×10^{-4} mg/kg-day, treating the lowest dose given to a group of 7 healthy adults in the Greer study as a no effect level and applying a total uncertainty factor of 10. One member of the NAS committee concluded that an additional uncertainty factor of 3, for a total uncertainty factor of 30, was needed to account for deficiencies in the database. Including this additional factor results in an RfD of 2.3×10^{-4} mg/kg-day.

The following document provides an update of MassDEP's *Draft Toxicological Profile and Health Assessment* taking the NAS report released in January 2005 into account, as well as even more recent data on perchlorate in breast milk and perchlorate in the U.S. food supply that was not available to the NAS Committee. In summary, MassDEP scientists in consultation with the MassDEP/DPH Advisory Committee on Health Effects concluded that a weight of the evidence approach should continue to be used to determine the most appropriate RfD value and corresponding drinking water limit. Thus, both the human and animal data were again considered. However, in part based on the NAS report, MassDEP is now placing more weight on the human data, which is used as the point of departure to derive MassDEP's current recommended RfD.

Upon consideration of the NAS report, MassDEP and the MassDEP/DPH Advisory Committee on Health Effects concurred with the NAS committee's view that the iodide uptake inhibition (IUI) data in the Greer et al. study constitute a reasonable basis for determining an RfD, provided that the inherent limitations and uncertainties in the data are appropriately accounted for. MassDEP believes the following factors must be addressed when using the Greer study to derive an RfD: protection of sensitive subgroups, since the Greer study involved adults; uncertainty in defining a true no adverse effect level, given the small number of subjects in the low dose group; and data gaps resulting from new information that became available after the NAS recommended an RfD.

Mass DEP is deriving an RfD for perchlorate by using the traditional approach and long-standing protocol also used for other chemicals. MassDEP supports the designation of iodide uptake inhibition (IUI) as a critical effect and point of departure (POD) for the development of an oral RfD for perchlorate. This position is consistent with U.S. EPA policy for setting RfDs (U.S. Environmental Protection Agency, 2002). The critical effect is defined as the first adverse effect, or its *known precursor*, that occurs to the most sensitive species, as the dose rate of the agent increases. We treat the lowest dose in the Greer study as a minimal Low Observed Adverse Effect Level (LOAEL), requiring application of an uncertainty factor of 3 to derive a No Observed Adverse Effect Level (NOAEL). MassDEP has concerns about the lowest dose in the Greer study being a no-effect level based on the facts that: due to the small number of subjects in the lowest dose group, there is low power to detect a statistically significant effect; averaging of

group responses obscures positive individual inhibition; a non-statistically significant IUI effect was observed at the lowest dose tested; and, good low dose corroborating data is lacking. Furthermore, we treat iodide uptake inhibition as an adverse effect, because to our knowledge, no human data exists that demonstrates the level of iodide uptake inhibition necessary to cause downstream effects, especially for the sensitive subgroups. At this time, the relationship between IUI and thyroid hormone synthesis, especially in the fetus and neonate, is unknown. Indeed the available data from studies on animals suggests that adverse effects may be associated with low levels of IUI. Thus, we treat IUI, which is an early event in the putative mechanistic pathway leading to perchlorate toxicity, as being causative of adverse effects and thus an effect to be avoided itself. Our use of this effect as a point of departure in deriving an RfD is consistent with US EPA policy for deriving RfDs.

MassDEP is also taking into account new information demonstrating perchlorate's widespread presence in breast milk in the US. Perchlorate was detected in 36 of 36 samples taken from a wide geographic area, at an average concentration of 10.5 µg/L and ranging up to 92 µg/L. These levels in breast milk substantially exceed recommended infant doses that can be derived based on the Mass DEP RfD *as well as* both those advanced by NAS.

The presence of perchlorate in breast milk and various foods, along with limited biomonitoring data collected by the U.S. Centers for Disease Control indicate that exposures to this compound are likely to be common. This data combined with the approximately 10-fold concentration of perchlorate from serum to milk that has been demonstrated to occur in animal bioassays raises significant concern about neonatal perchlorate exposure. Furthermore, based on a limited number of samples, the data suggest that perchlorate may also inhibit iodide transport into human breast milk. Because breast milk is the nursing infant's sole source of iodide and newborns are particularly sensitive to thyroid disruption due to their limited reserve capacity of thyroid hormones and developing thyroid function, reduced iodide levels in breast milk would increase neonatal sensitivity to perchlorate and other thyroid toxicants. Thus, perchlorate may present a double threat to nursing infants if their mothers are exposed, contributing to a reduced level of iodide available to the thyroid and inhibition of the thyroidal uptake of the iodide that is present. MassDEP has concluded that acceptable exposure levels for perchlorate should reflect the significant uncertainty and data gaps that exist relating to neonatal perchlorate exposures and toxicity attributable to nursing.

In part based on these data, MassDEP and the MassDEP/DPH Advisory Committee on Health Effects have concluded that an UF of 30, associated with the lower NAS RfD value, is the minimum that should be considered with this data. Taking the full range of uncertainties involved into account (e.g. including the short duration of the Greer study as well as several other uncertainties detailed in the following report) MassDEP has concluded that a total UF of 100 should be applied to account for variability in human sensitivity, uncertainty in the effect level and overall database deficiency[1]. Some members of the Mass DEP/DPH Advisory Committee on Health Effects indicated that an uncertainty factor of 300 could be supported and one suggested that 30 could be sufficient. Application of UF of 100 or 300 to the Greer data

[1] MA DEP's uncertainty factor choices are consistent with guidance from the US EPA (2002c), as well as from previous NAS committees who specifically evaluated the use of uncertainty factors in the development of drinking water guidance (National Research Council , 1980; National Research Council , 1986).

yields RfDs of 7 x 10^{-5} mg/kg-day and 2.3 x 10^{-5} mg/kg-day, respectively. A UF factor of 30 would yield an RfD of 2.3 x 10^{-4} mg/kg-day. MassDEP notes that, in recognition of the significant gaps in the toxicology database for perchlorate, the NAS report recommended that numerous additional studies be performed[2]. Data from these studies may ultimately help to reduce the current uncertainty on perchlorate exposure risks.

In summary, the human data can be interpreted, depending on how the fundamental uncertainties in the scientific data are addressed, to support RfDs of 2.3 x 10^{-4} mg/kg-day (based on the NAS lower value and also viewed as reasonable by one member of the MassDEP/DPH Advisory Committee on Health Effects); 7.0 x 10^{-5} mg/kg-day (MassDEP's recommended value, which all members of the MassDEP/DPH Advisory Committee on Health Effects supported) or 2.3 x 10^{-5} mg/kg-day (which some members of the MassDEP/DPH Advisory Committee on Health Effects indicated could be supported). RfDs derived from the animal data fall within a similar range (2.8 x 10^{-5} to 8.5 x 10^{-5} mg/kg-day, see main report). Based on the weight of the evidence MassDEP has concluded that an RfD value of 7.0 x 10^{-5} mg/kg-day, based on the Greer study data and a composite uncertainty factor of 100, provides a scientifically defensible basis for evaluating exposures. The MassDEP/DPH Advisory Committee on Health Effects unanimously concurred with this decision. The U.S. Environmental Protection Agency (US EPA) recently adopted the NAS higher RfD value (7.0 x 10^{-4} mg/kg-day), which is now included on the Integrated Risk Information System database. In part based on the new data on perchlorate in breast milk, MassDEP scientists and the MassDEP/DPH Advisory Committee on Health Effects unanimously concluded that this value did not fully account for the uncertainties surrounding science's understanding of perchlorate toxicity and exposures.

In the derivation of health based drinking water values, MassDEP considers the RfD, other sources of exposure, receptor body weight, and water ingestion rates. With respect to other sources of exposure, the available data indicates that perchlorate is frequently found in foods including many types of lettuce, store milk, fruits and other vegetables. To account for exposures to perchlorate from these foods, as well as data that indicates that perchlorate may be concentrated into breast milk, MassDEP is applying a 20% relative source contribution factor in its drinking water value derivation. This choice is consistent with how MassDEP has dealt with this issue in the derivation of drinking water guidelines and limits for other chemicals based on non-carcinogenic endpoints.

When the RfD values in the range noted above are translated into drinking water values, apportioning 20% of the acceptable dose to drinking water, the associated health based drinking water values are as follow:

Uncertainty Factor	RfD (mg/kg-day)	Drinking Water Limit (ppb)
10	7 x 10^{-4}	4.9
30	2.3 x 10^{-4}	1.6
100	7 x 10^{-5}	0.49
300	2.3 x 10^{-5}	0.16

[2] MA DEP and the Advisory Committee on Health Effects also noted that the NAS' extensive proposed research agenda serves to highlight fundamental uncertainties in the science on perchlorate toxicity and support a health-protective approach in deriving acceptable exposure limits to protect children's health.

The value of 0.49 µg/L is associated with MassDEP's recommended RfD[3]. The highest value, 4.9 µg/L, is derived from the NAS RfD, which is not supported by MassDEP or the MassDEP/DPH Advisory Committee on Health Effects. One member of the MassDEP/DPH Advisory Committee on Health Effects indicated that the NAS lower RfD alternative was reasonable. This RfD is associated with a drinking water value of 1.6 µg/L. Several members of the MADEP/DPH Advisory Committee on Health Effects suggested that a lower RfD could be derived, which would lead to a drinking water value of 0.16 µg/L, but viewed the MassDEP final RfD as appropriate.

In selecting final drinking water standards, Mass DEP considers the RfD, as well as additional factors. These additional factors include the frequency of contamination, analytical limitations, treatment options, costs, and for contaminants like perchlorate that may be introduced into drinking water as a result of treatments to address pathogens, comparative risks.[4]

In summary, MassDEP is recommending an RfD for perchlorate of 7.0 x 10^{-5} mg/kg-day, based on the Greer study data and a composite uncertainty factor of 100.

[3] Values for the bottle-fed infant, using infant exposure parameters, are 0.44 µg/L using the MA DEP RfD.
[4] MA DEP's previous drinking water advisory level of 1 ppb was based on the department's recommended RfD and a method reporting limit of 1 ppb, based on a modification of US EPA Method 314, which was then in effect. Improved analytical methods have now reduced detection limits to below 1 ppb.

1.0 INTRODUCTION

The Massachusetts Department of Environmental Protection's (MassDEP's) work on perchlorate began in 2002 when high levels of the chemical were detected in the groundwater on Cape Cod. Groundwater at the Massachusetts Military Reservation (MMR) contained perchlorate levels as high as 500 ppb, and 3 nearby public drinking water wells serving the town of Bourne were contaminated with levels in the low parts per billion range. Due to the fact that there were no federal or state standards for perchlorate, at the request of the Bourne Water District, MassDEP provided interim advice on drinking water, which stated that if levels of perchlorate exceed 1 ppb, that sensitive subpopulations should not consume the water. Sensitive subpopulations were defined as pregnant women, infants, children up to age 12 and individuals with hypothyroidism. MassDEP's Office of Research and Standards (ORS), a toxicology and risk assessment unit, based the interim guidance on the most current scientific studies that were presented in the U.S. Environmental Protection Agency's (US EPA) 2002 (2002a) draft health assessment for perchlorate.

In January 2003, MassDEP made a decision to set standards for perchlorate due to the need to clean up sources on Cape Cod and to protect the public health. Using established protocols, MassDEP ORS initiated an in-depth review of the scientific information on the toxicity of perchlorate towards establishing a reference dose (RfD), the starting point for setting standards to protect public health. The perchlorate standards that MassDEP intends to set are cleanup standards to support the state's hazardous waste site clean up program as well as a drinking water standard. As part of the regulation development effort, MassDEP has also collected test data on the occurrence of perchlorate in public water supplies in Massachusetts. The results indicate that approximately 1% of the public water systems contain perchlorate at levels greater than 1 ppb and as high as 1300 ppb.

As part of its RfD derivation process, MassDEP held three meetings of its MassDEP/DPH Advisory Committee on Health Effects, an external scientific peer review group, along with scientific representatives of the U.S. Department of Defense, on the toxicity of perchlorate. Information and comments received from these meetings were taken into account in the assessment and derivation of a draft RfD. In establishing the draft RfD, MassDEP utilized a weight of evidence approach where the available data on perchlorate toxicity was critically evaluated for consistency and biological plausibility in order to select an appropriate point of departure to derive an RfD for this compound that is appropriately health protective for all members of the population.

In its 2004 toxicity assessment, MassDEP noted that the concordance of effect levels observed for several endpoints, including iodide uptake inhibition, hormone effects and brain morphometry, as well as the consistency in observed effects with those that would be expected based on the proposed mode of action provides a strong basis for establishing an RfD. Mass DEP's assessment included consideration of both the human and animal data to ensure that the impacts of perchlorate on various life stages are taken into account. Whereas the human data (e.g. Greer et al., 2002) represents the effects of perchlorate on healthy adults (iodide uptake

inhibition) the available data is insufficient to directly address risks to sensitive groups. The animal data (e.g. Argus Research Laboratories, 2001) addresses the effect of perchlorate on the fetus and neonates who have limited thyroid hormone synthesis capacity. MassDEP's final draft document, "Perchlorate Toxicological Profile and Health Assessment" contained a recommended RfD of 3×10^{-5} mg/kg-day based on studies of the effects of perchlorate in animals (iodide uptake inhibition, alterations in hormone levels, and brain morphometry) and in humans (iodide uptake inhibition).

After MassDEP's effort to set perchlorate standards was well underway, US EPA's work on its draft health assessment for perchlorate was suspended and a federal level decision was made that the draft would be reviewed by a special committee under the National Academy of Sciences (NAS). The charge of the NAS Committee was to evaluate the scientific studies conducted on perchlorate and to assess the draft 2002 US EPA health assessment report.

In January 2005, NAS released its report, *Health Implications of Perchlorate Ingestion.* The report includes recommended RfD values, which was unexpected since this was not a task within the charge of the committee. The NAS Committee was not unanimous in its view of the RfD with the majority recommending an RfD value of 7×10^{-4} mg/kg-day. One dissenting member of the committee thought the RfD should be 3 times lower, or 2.3×10^{-4} mg/kg-day. The NAS recommended RfD values are approximately 7 and 20 times higher than MassDEP's 2004 recommended RfD.

To ensure that MA standards on perchlorate reflect current scientific data, MassDEP has reviewed the NAS report and recommendations as well as additional data on perchlorate including data on perchlorate in breast milk that was not available during the NAS review. Mass DEP's process for undertaking this review included a preliminary discussion with members of its external scientific advisory committee in late January 2005 and a meeting with members of the committee in mid-February 2005 to receive input and advice. Two NAS Committee members also attended the February meeting to share their perspectives on perchlorate with MassDEP and its Advisory Committee on Health Effects.

The following report updates MassDEP's draft perchlorate toxicological assessment and RfD derivation (MassDEP, 2004), based on MassDEP's consideration of the NAS's perspectives and views, new data on perchlorate in breast milk, recently published studies and feedback received from its external scientific review committee. In keeping with MassDEP's weight of the evidence approach to deriving an RfD for perchlorate, the report first addresses the use of the human data from the Greer study to derive an RfD, followed by further assessment of the animal data. A range of possible RfDs and potential drinking water values associated with these RfDs is derived based on other sources of exposure and dosimetry adjustments.

2.0 CALCULATION OF A REFERENCE DOSE FOR PERCHLORATE

2.1 HUMAN STUDY POINT OF DEPARTURE - GREER ET AL. (2002)

There are six primary human exposure studies involving perchlorate (Table 1). The study of Greer et al. (2002) is the most complete and has been chosen as the most suitable for development of a point of departure from the human studies. This was a 14-day exposure study involving a total of 37 healthy, euthyroid adult subjects. They were assigned to 4 perchlorate dose groups of 0.007, 0.02, 0.1, and 0.5 mg/kg-day perchlorate and given perchlorate daily in drinking water for 14 days. In 24 of the 37 subjects, 8- and 24-hour measurements of thyroidal ^{123}I uptake (RAIU) were made before exposure, on exposure days 2 and 14 and 15 days after exposure ceased. Both the day 2 and 8-hour measurements on post-exposure day 15 were omitted in another group of 13 subjects who were otherwise treated the same as the first group. Each group's pre-exposure baseline served as its own control. The results for the 24-hour RAIU measurements on day 14 of exposure have been chosen for further analysis and are presented in Figure 1. RAIU decreased relative to the baselines in the 3 highest doses. The low dose group had an 8 % decrease from the baseline mean, based upon only seven individuals. Greer et al.'s analysis determined that the small response seen in the low dose group was not statistically significantly different from the baseline for that group. Based upon this conclusion for the low dose, Greer et al. (2002) designated this dose of 0.007 mg/kg-day as a NOEL, a no observed effect level, even though they presented a more refined analysis which suggested that the true effect level was likely lower. This designation was subsequently seconded in an independent analysis of the Greer paper by the NAS peer review Committee (National Academy of Science, 2005).

MassDEP supports the designation of RAIU inhibition (iodide uptake inhibition or IUI) as a critical effect and point of departure (POD) for the development of an oral RfD for perchlorate. Our position is consistent with U.S. EPA policy for setting RfDs (U.S. Environmental Protection Agency, 2002). The critical effect is defined as the first adverse effect, or its *known precursor*, that occurs to the most sensitive species, as the dose rate of the agent increases. RfDs have been set based on precursor biochemical changes such as plasma or red cell cholinesterase inhibition for various organophosphates such chlorpyrifos and malathion and induction of liver enzymes such as for 1,4-dibromobenzene and other chemicals (U.S. Environmental Protection Agency, 2005).

While it has been argued that IUI is not an adverse effect (National Academy of Science, 2005) in adults, but rather a precursor to adverse effects, it most certainly would seem to have the potential to causally lead to adverse effects in some circumstances. In its report, NAS stated that iodide uptake would need to be inhibited to a level of 75% or more for extended periods in healthy adults in order to cause truly adverse effects. No experimental data or quantitative assessment was provided in support of this statement. To our knowledge no human data exists that demonstrates the level of iodide uptake inhibition necessary to cause downstream effects, especially for the sensitive subgroups. At this time the relationship between IUI and thyroid hormone synthesis, especially in the fetus and neonate, is unknown. No pharmacodynamic model is available to address this issue. In fact, physiologically-based pharmacokinetic (PBPK) modeling combined with experimental data suggests that, in rodents, iodide uptake inhibition of

less than 2% may be associated with adverse effects on thyroid hormone status and, possibly, neurodevelopmental endpoints. Thus, there are no objective data addressing the degree of IUI that would lead to adverse downstream effects. Furthermore it is likely that this level would be variable. Clearly IUI is an element of perchlorate's mechanism of toxicity and not merely indirectly associated with its adverse effects. It may thus predispose individuals to sensitivity to other thyroid toxicants or conditions that impact iodide status, could lead to transient, but physiologically significant, changes in downstream parameters and/or could lead to adaptive responses that themselves may have impacts not yet understood. MassDEP believes that it is prudent and appropriate to functionally treat IUI as an adverse effect in deriving a health protective RfD. Thus, for purposes of the remaining discussion in this report we will refer to this endpoint in the Greer study as a LOAEL. Because the effect observed in the Greer study is not a frank adverse effect it is treated as a minimal adverse effect in the assessment. This is consistent with established protocols for deriving RfDs. The MassDEP/DPH Committee on Health Effects concurred with this determination.

In addition to the issues described in the preceding paragraphs, we consider the Greer study as statistically weak for deriving an RfD and examine the strengths and weaknesses of two approaches for deriving a perchlorate RfD in the following discussion. The first method is the NOAEL/LOAEL[5] approach and the second the benchmark dose approach.

2.1.1 NOAEL/LOAEL Approach To RfD Derivation

2.1.1.1 *NOAEL/LOAEL Characterization*

MassDEP and its Advisory Committee on Health Effects note that the designation of the low dose of 0.007 mg/kg-day as a NOAEL has several weaknesses based upon: i. statistical considerations; ii. shortcomings of analysis of group-averaged data; iii. strength of supporting observations from other studies.

Statistical Considerations

Viewed from a statistical perspective, the Greer study had very low power to detect significant differences from the baseline levels of RAIU. Given the limited number of individuals in the low dose group (7) and the variability observed in measured RAIU about the mean baseline (18.1%) for the low dose group (standard deviation of 8.2), it is possible to determine the magnitude of change from the baseline mean that would be detectable within an acceptable level of error. Sample size calculation approaches presented in Green (1979) and Sokal and Rohlf (1995) can be used to determine the difference from the baseline mean that is detectable with a given error risk (alpha level). The minimum difference that could be discriminated from the baseline mean was ± 40% with a 1-in-20 chance of being wrong. This range is represented by the shaded box in Figure 1. Given the limitations of the Greer study, it was unable to reliably detect up to a 40% inhibition of iodine uptake. This interpretation is consistent with that of the U.S. Environmental Protection Agency (2002a) in its draft report on perchlorate. They concluded that a NOAEL could not be identified from this study and they designated the lowest dose as a

[5] NOAEL – no observed adverse effect level;; LOAEL – lowest observed adverse effect level.

LOAEL. Their peer review panel supported this conclusion (U.S. Environmental Protection Agency , 2002b).

Greer and coauthors themselves actually performed a more refined analysis to help identify what they called the true NOEL from this experiment using linear regression of RAIU against the logarithm of the doses. They extrapolated the derived equation to identify the dose associated with 0% inhibition of RAIU as in the range of 0.005 – 0.006 mg/kg-day. The authors note, however, that due to variability in the data, inhibition of RAIU of from 8.3-9.5% is possible at this dose. This interpretation presents what the authors call the true (or predicted) NOEL as less than the experimental NOEL. Given the slope of the dose-response (DR) curve, inhibition at 0.007 mg/kg-day could exist. The U.S. EPA in their response to comments on their draft perchlorate assessment offered the criticism of this approach that they saw no *a priori* reason to assume that the shape of the dose response curve was linear in the zone of extrapolation below the low dose group response values.

Group Averaging

The majority of the analyses of the Greer study results by others have focused on the dose-group averaged response values for purposes of deriving a NOAEL-based RfD. Greer presented individual subject response data in his Figure 2 (see Figure 2 in this report). A differential response to perchlorate exposure appears to exist which is a function of the baseline level of iodide uptake. Those 3 or 4 individuals (their figure is not entirely clear on this point) in the low dose group whose baseline RAIU was above about 15% exhibited a greater degree of inhibition of RAIU (approximately 18-26%) than those who had baseline uptakes below this level (7% inhibition to 40% increase). One possible interpretation of this result is that even in the healthy adult population, there is variation in iodide uptake efficiency with those having higher uptake efficiencies being more affected by perchlorate exposure-induced iodide uptake inhibition. Greer et al. do not present data on serum iodide concentrations, which would enable one to determine whether this differential response was a function of an individual's baseline serum iodide level. If such a relationship exists, then applying these results to the rest of the population would suggest that those who might be iodine deficient either through diet, life stage or as a result of their reproductive status might be more susceptible to perchlorate-induced harm. Included in this group of concern are pregnant women, their fetus and neonates.

In their statistically-based evaluation of the Greer data with the benchmark dose (BMD) approach the U.S. Environmental Protection Agency (2003) characterized the iodide uptake responses of two of the possibly three individuals whose uptake efficiency increased as a result of low dose perchlorate exposure as outliers. They noted that some dose groups were more highly variable in their responses to perchlorate exposure and that removing the responses of these two individuals improved the homogeneity of variances in the sample set across doses. An alternative view is that perhaps these "outliers" had low baseline iodide uptake efficiencies and were relatively insensitive to perchlorate exposure. The absolute changes in RAIU in these individuals with low baseline uptakes were not large (1-4%) so that perhaps what was being seen was statistical "noise" rather than aberrant responses. The effect of censoring them from the data set would be to drive the estimates of effect at the low dose towards higher response levels and

more closely support an interpretation that this dose was indeed a LOAEL, rather than a NOAEL.

Supporting Data

MassDEP thinks that the strength of the evidence cited to support Greer et al.'s (2002) results warrants critical appraisal. A consistent picture of the nature of perchlorate-induced inhibition in iodide uptake and responses of thyroid hormones from the five other human perchlorate exposure studies has been cited as support for the designation of the low dose of 0.007 mg/kg-day as a NOAEL and POD for the derivation of an oral RfD (National Academy of Science, 2005). One of the five studies is a repeat of one of the published studies with a longer, although unspecified duration and was reported through a personal communication (Brabant, 1994). Two others are short, non-peer reviewed publications (Lawrence et al., 2001; Braverman et al., 2004[6]). All three of these contained limited descriptions of the studies, data and statistical analyses. Interpretation of these results is difficult as the raw data for some have not been available and some data are not published. Information from such incomplete communications is not of the standard normally relied upon as a significant basis for setting regulatory standards.

The Lawrence et al. (2001) reference is a letter to the journal editor and the Braverman paper is the abstract for a poster presentation at a professional meeting. Only one of the studies (Braverman et al., 2004) contained an exposure at the same dose level as the Greer low dose. The level of detail reported in the abstract for that work and in the actual poster for the work was insufficient for determining whether or not the reported conclusions of no effect of perchlorate exposure on RAIU and thyroid hormone status were supported by the data. That study's low dose level is subject to the same criticisms listed for the Greer et al. (2002) study, in which small sample size limited its ability to discriminate experimentally-induced differences from the baseline RAIU. If the low dose results from the Greer et al. (2002) study and the Braverman et al. (2004) study were to be pooled, there would still only be a total of 12 (7+5) individuals from which inferences about perchlorate's low dose effects on iodide uptake and thyroid hormone status can be drawn. However, with no data on intersubject variance reported for Braverman's study and no data on RAIU inhibition at early time points post exposure, MassDEP does not view that study as sufficient supporting evidence for the designation of a NOAEL.

2.1.1.2 *Accounting for Uncertainty to Derive a Human Study*-Based *RfD*

Although a total uncertainty factor of 300 can be supported, MassDEP is recommending that a total uncertainty factor of 100 be applied to the lowest dose in the Greer et al. study (2002). Application of an uncertainty factor of 100 represents a change from MassDEP's 2004 toxicological assessment where a total uncertainty factor of 300 was applied. In determining how to apportion the total uncertainty factor of 100, MassDEP is assigning an uncertainty factor of **10 for variability in human sensitivity** (consistent with NAS) and a value of **3 for a LOAEL to NOAEL adjustment**. The remaining factor of **3 accounts for database deficiency**. An uncertainty factor of 3 for database deficiency is consistent with the view of the

[6] This study has now been peer-reviewed and published in J Clin Endocrin Metab (April 24, 2006); however, MassDEP's critique of the study (i.e., small sample size, low statistical power) still holds.

dissenting opinion of one member of the NAS Committee[7]. The bases for these designations are discussed below.

Interindividual Variability (Sensitive Individuals)

This uncertainty factor accounts for the variation in responses to exposure to a chemical in the population and for the possibility (given a lack of relevant data) that the database available is not representative of the dose/exposure-response relationship in the subgroups of the human population that are most sensitive to the health hazards of the chemical being assessed (U.S. Environmental Protection Agency, 2002c). The full human variability uncertainty factor of 10 is applied by the NAS to the Greer low dose and will be maintained by MassDEP.

This factor for human variability is intended to account for differences in inter-individual sensitivity across all life stages (neonates, children or adults). The human short-term study was conducted in a limited number of healthy volunteers who were iodine-sufficient. The study groups do not represent the sensitive subpopulations (pregnant woman, fetuses, children, hypothyroid individuals, and people with thyroid disease). Justification for the use of a 10 fold UF in the derivation of reference doses can be found in Krasovskij (1976) and Dourson and Stara (1983). More information to justify the use of this uncertainty factor for perchlorate can be found in MassDEP's toxicity report (MassDEP 2004).

The U.S. EPA in its guidance on deriving RfDs (U.S. Environmental Protection Agency, 2002c) provides the advice shown in Table 2 for evaluating evidence about susceptible subpopulations. Several of their criteria are germane to the knowledge base for perchlorate where greater weight should be reflected in the assignment of a higher UF when: the effect occurs at greater magnitude in one or more life stages, when different types of effects are seen in specific subpopulations, and when the effects differ in seriousness or degree of reversibility in specific subpopulations. For perchlorate, it is recognized that the pregnant mother, her fetus and the neonate are at particular risk from perchlorate-induced iodide deficiency. Since at these life stages, iodide deficiency may already exist in the absence of perchlorate exposure, added deficits as a result of perchlorate exposure could be beyond the compensatory capabilities of these individuals to withstand excursions in available serum iodide and concomitant changes in thyroid and pituitary hormones. The fact that we don't fully know the perchlorate dose-response relationship for this susceptible subgroup argues for conservativeness in the assignment of a UF.

The possible dependence of the perchlorate-associated IUI on the baseline level of iodide uptake seen in the individual responses from the Greer et al. (2002) study can also be viewed as either representing the variability across the human population, or alternately a unique subpopulation response.

[7] "One NAS committee member thought that the factor for database uncertainty should be greater than one and provided the following rationale: The RfD is derived from a study in which a group of only seven healthy adults was given 0.007 mg/kg of perchlorate daily for 14 days (Greer et al. 2002). Although two other studies had similar results, the total number of subjects is still small. In addition to the small number of subjects, no chronic exposure studies have been published. An uncertainty factor of 3 could account for the uncertainty surrounding the small number of subjects and the absence of a long-term study."

LOAEL to NOAEL Extrapolation

An uncertainty factor is normally employed in this category when the starting point for the derivation of an RfD is a LOAEL or LOEL in order to extrapolate the results based on effects observed at a low dose to the dose where no effect would be observed. MassDEP's view that the lowest dose in the Greer study (0.007 mg/kg-day) may not be a true no observed effect level has not changed**. MassDEP prefers to view this lowest dose as a minimal Lowest Observed Adverse Effect Level (LOAEL) and to maintain an uncertainty factor of 3**, for reasons developed in Section 2.1.1.1 (low study power to detect an effect, positive individual inhibition responses at the NOAEL obscured by group-averaging, and a lack of good low dose corroborating data) and as further described in MassDEP's 2004 draft toxicological assessment (MassDEP, 2004).

Derivations of RfDs take into account the nature of the critical effect, such as whether it is a biochemical change versus a frank adverse effect and whether the effect is on the causal pathway to more serious effects by way of an uncertainty factor. For example, when deriving an RfD that is based on a precursor effect, the uncertainty factor to extrapolate from a lowest observed adverse effect level to the no observed adverse effect level may be lower (e.g., three-fold) than if you were starting with frank effects such as brain damage (e.g., ten-fold).

When the responses of seven healthy individuals to perchlorate exposure are examined, it is apparent that even at low perchlorate doses, there are some individuals whose uptake of iodine can be substantially affected by perchlorate exposures. This observation calls into question the designation of 0.007 mg/kg-day as a NOAEL. An alternative analysis of the perchlorate dose response data from the Greer et al. (2002) study by the U.S. EPA (benchmark dose analysis) identified a BMDL dose of 0.002 mg/kg-d which suggests that biologically significant inhibition of iodine uptake can't be ruled out at a dose of 0.007 mg/kg-day (U.S. Environmental Protection Agency, 2003).

Database Insufficiency

This factor may be employed to adjust a NOAEL or LOAEL downwards to reflect the level of uncertainty in the database for the chemical. **MassDEP is recommending an uncertainty factor of 3 for database deficiency** due to concerns over:

(1) lack of chronic data. The duration of exposure in the Greer study was only 14 days, during which time iodide uptake inhibition of the thyroid was measured. Long-term effects due to iodide uptake inhibition, thyroid accumulation of perchlorate, thyroid hormone perturbation, or direct chronic effects of perchlorate in various other organs cannot be determined from this study. No oral chronic studies at this low dose level exist;
(2) emerging data indicating potentially widespread contamination of breast milk by perchlorate and related uncertainties regarding the degree to which perchlorate may interfere with iodide transport into breast milk, which could increase the sensitivity of the

neonate to perchlorate thyroid disruption, and data that indicates perchlorate may be concentrated into breast milk (see more detailed discussion in Sections 3.1 and 3.2)[8].

In addition, there is limited information on the interactive effects of perchlorate with other goitrogenic chemicals in the environment, such as nitrate.

Two other reviewers of the human perchlorate exposure literature (California Environmental Protection Agency, 2004; National Academy of Sciences, 2005) have concluded that, if the initial step of perchlorate-induced inhibition of thyroid uptake of iodide is limited, then the potential succeeding events that could lead to thyroid hormone changes and subsequent neurodevelopmental effects in offspring of mothers exposed to perchlorate or neonates directly exposed to perchlorate, will be prevented. This conclusion seems to reflect an assumption that pregnant women and the neonate, who in some cases may already be iodine deficient or have lower iodine reserves to supply hormone production needs during periods of perchlorate-induced iodine deficiency, will respond in the same way as healthy adults to perchlorate exposure. MassDEP has seen no data or argument that supports this assumption. There is a significant gap in our knowledge of how much IUI these susceptible subgroups can withstand and by extension, how they will respond to low-level perchlorate exposures.

2.1.2 Benchmark Dose Approach

Analysis of dose-response data with the benchmark dose approach has been coming into greater use in regulatory toxicology since its earlier introduction by authors such as Crump (1984) and Barnes and Dourson (1988). Setzer and Kimmel (Setzer et al., 2003) provide a more recent perspective on its use and note that it has the advantages over the NOAEL/LOAEL approach of using the entire set of data over all doses tested and as being more transparent and quantitative. It models the dose response curve in the range of observable data and then uses a model to predict the dose associated with a chosen level of response – the benchmark dose (BMD) (e.g., 5 or 10%). Confidence limits can be calculated and the lower confidence limit on the dose used as the BMD is called the BMDL. This BMDL value is used as the point of departure for calculation of an RfD. Unlike the NOAEL, especially as designated from the Greer et al. (2002) study, the BMDL accounts for the uncertainty in the estimate of the dose response that is due to the characteristics of the experimental design such as sample size.

[8] The detection of perchlorate in breast milk raises several issues. First of all, the degree to which it may interfere with iodide transport into the milk, which may *increase the sensitivity* of the neonate to the direct action of thyroid toxicants, including perchlorate, is a concern that has not been adequately addressed to date. The data suggesting that perchlorate may furthermore be concentrated into breast milk also raises a related but separate concern regarding *overall exposure levels to the neonate* (see Section 3.1 and 3.2). Neonate exposures to perchlorate in breast milk could ultimately be accounted for through the use of a quantitative adjustment to the maternal RfD to ensure that maternal to neonatal exposures do not exceed acceptable levels. However, sufficient data to do this is not currently available. Alternatively, an additional UF could be applied in the derivation of the maternal RfD and/or adjustments made to the proportion of the total maternal perchlorate exposure allowed from drinking water. As is discussed above and in section 3.1 and 3.2, MA DEP has concluded that the uncertainty regarding perchlorate's effect on iodide transport into milk provides partial support for the use of a database deficiency uncertainty factor. Uncertainty in perchlorate's concentration into breast milk supports the use of a conservative relative source apportionment factor to limit potential neonatal exposures from breast milk.

In cases where there are either problems or difficulties with the NOAEL/LOAEL approach for RfD derivation such as with perchlorate, the BMD advantages can be attractive. The challenge with using a BMD analysis on the Greer data is getting agreement on a POD for RAIU inhibition.

The U.S. EPA (U.S. Environmental Protection Agency, 2003) in its response to comments on its 2002 draft evaluation of perchlorate's effects and California (California Environmental Protection Agency , 2004) performed benchmark dose analyses on the Greer et al. (2002) data. US EPA censored two of the data points for the low dose group for statistical reasons and identified a BMDL 0.002 mg/kg-day for a 5% response rate. Irrespective of whether one calls a 5% response a no effect level or low effect level, the implication of this analysis is that the 0.007 mg/kg-day of the Greer et al. study is not a no effect level.

California's BMD analysis employed the U.S. EPA's BenchMark Dose analysis software, Ver. 1.3.1 and found the Hill model provided the best fit to the data, similar to EPA's conclusion. A BMDL of 0.0037 mg/kg-day was identified as a POD for the development of a RfD. This dose, like that identified by EPA with censoring, was below the NOAEL of 0.007 mg/kg-day identified by Greer et al. (2002) and NAS (2005) for this data set. It should be noted that the basis for the choice of a 5% response level in the modeling was to have a minimal biologically significant change as a point of departure.

The conclusion that MassDEP derives from these BMD analyses of the Greer et al. (2002) data is that a dose of 0.007 mg/kg-day cannot be confidently declared a no effect level for perchlorate exposure. This fact should be recognized either by designating the 0.007 mg/kg-day dose as a minimal LOAEL and assigning a partial amount (3) of the total UF (10) which could be assigned for extrapolating low effect data to a no effect level, or by employing a BMDL as the POD for assignment of uncertainty factors.

2.1.3 MassDEP Recommended Derivation of a Reference Dose Based Upon Human Data

The low dose of 0.007 mg/kg-day in the Greer et al. (2002) study is chosen as the point of departure for derivation of an RfD based upon human data. For a variety of reasons including poor statistical power of the study, the strong positive responses seen at this dose in some of the small study group and the weakness of potentially corroborating data for calling this a NOAEL, MassDEP views this dose as a minimal LOAEL.

The following uncertainty factors can be applied to adjust this dose downwards to an RfD such that it should be protective of all members of the human population:

10 - for sensitive individuals
3 - for minimal LOAEL to NOAEL extrapolation
3 - for database insufficiency.
100 TOTAL UF

$$\text{RfD} = \frac{0.007 \text{ mg/kg-day}}{100} = \mathbf{7 \times 10^{-5} \text{ mg/kg-day.}}$$

An alternative and supportable interpretation would be to apply a full factor of 10 for database insufficiency as has been recommended by some of our peer review committee members to produce a total UF of 300 and associated RfD of **2.3 x 10^{-5} mg/kg-day**:

10 - for sensitive individuals
3 - for minimal LOAEL to NOAEL extrapolation
10 - for database insufficiency
300 TOTAL UF

$$RfD = \frac{0.007 \text{ mg/kg-day}}{300} = \mathbf{2.3 \times 10^{-5} \text{ mg/kg-day}}$$

The lower of the two NAS RfDs, which relied on a total UF of 30 and which was supported by one member of the NAS Committee and one member of our Committee was **2.3x10^{-4} mg/kg-day.**

10 – for sensitive individuals
3 – for database uncertainty
30 TOTAL UF

$$RfD = \frac{0.007 \text{ mg/kg-day}}{30} = \mathbf{2.3x10^{-4} \text{ mg/kg-day.}}$$

2.2 USE OF ANIMAL STUDIES FOR SUPPORTING AN RfD BASED ON HUMAN DATA

2.2.1 Background

Information on the toxicity of perchlorate on the most sensitive subgroups of the human population is inadequate. Data on iodine deficient populations as well the mechanism of action believed to be responsible for perchlorate toxicity indicate that the most sensitive subgroups of the population for iodine deficiency are:

- pregnant women especially when marginally iodine-deficient or iodine-deficient,
- fetuses and infants of these women,
- premature infants,
- children, and
- hypothyroid individuals.

Due to a lack of adequate data on the toxicity of perchlorate in the sensitive subgroups, various rodent studies were conducted in the late 1990s and early 2000 to fill data gaps (U.S. Environmental Protection Agency , 1998a, 2002a). These studies are extensively discussed in the 2004 draft MassDEP perchlorate health assessment document (MassDEP 2004).

2.2.2 Justification for Use of Animal Data in the Weight of Evidence Assessment and RfD Designation Process

MassDEP is considering the rodent studies as supporting evidence in the perchlorate risk assessment process because: (1) the rat may be a good model to represent the most sensitive subgroups, especially the iodine deficient pregnant woman and the congenitally hypothyroid fetus which may depend only on maternal thyroid hormones during *in utero* development; (2) sensitive subgroups are being directly tested (the pregnant rat and its fetus and the neonate); and, (3) the observed results in the animals are consistent with the proposed mode of action of perchlorate, including the upstream effect of iodide uptake inhibition followed by downstream effects such as thyroid and pituitary hormone perturbations and thyroid and brain structural alterations. Appendix A contains further information on the justification for using the animal studies as supporting evidence in the derivation of an RfD for perchlorate.

2.2.3 Studies Conducted in Rats at Different Life Stages

The studies of perchlorate toxicity in animals demonstrated the following effects consistent with its proposed mode of action of perchlorate:

- inhibition of iodide uptake (Yu et al. 2000);
- decrease of serum T4 and T3 levels and increase of TSH levels (Caldwell et al. 1995; Springborn Laboratories, 1998; Argus Research Laboratories, 1998a,b, 2001);
- thyroid hypertrophy (increased cell size), hyperplasia (increased cell number) across life stages, and tumors[9] in F1 generation rats (rats exposed *in utero* and throughout lactation)[10] (Caldwell et al. 1995; Argus Research Laboratories, 1998a,b);
- alteration in brain morphometry (form and structure) and behavior in rat pups that were exposed *in utero* and after birth (Argus Research Laboratories,1998b, 2001; Bekkedal et al. 2000).

2.2.4 Critical Study (Argus Research Laboratories, 2001)

[9] It is suggested that thyroid cancer in humans resulting from perchlorate exposure is unlikely, since rats are sensitive to the development of thyroid tumors because their thyroid function is easily disrupted. Based on this argument, humans are much less susceptible than rats to disruption of thyroid function and, therefore, are not likely to develop thyroid tumors as a result of perchlorate exposure (NAS, 1995). The comparative sensitivity of humans and rats is discussed Appendix A. The database on long-term effects of perchlorate either on the sensitive population or the general population is insufficient to make such conclusions. A number of studies (Pendergast et al., 1960, Wahner et al., 1966; Williams et al., 1977; Williams, 1985; Levi et al., 1991; Pettersson et al., 1996; Vigneri et al., 1998) conducted in various parts of the world found that iodine deficiency may increase the incidence of thyroid malignancy and alter the type of cancer produced. Since perchlorate can contribute to functional iodine deficiency, its ability to cause thyroid cancer cannot be ruled out. Moreover, perchlorate was found to be a potent promoter of thyroid tumors in animals (Hiasa et al. 1987) and its effect in people with already initiated tumors is unknown. Based on its mode of action, perchlorate has the potential to contribute to thyroid tumors at doses sufficient enough to decrease thyroid hormone levels and change thyroid structure.

[10] Perchlorate was found to be nongenotoxic in various *in vitro* and *in vivo* studies, suggesting that the mechanism of tumor formation might be perturbation of the thyroid and pituitary hormone homeostasis (ManTech Environmental Technology , 1998; Zeiger, 1999).

Of the above listed studies, the critical study selected for perchlorate reference dose determination by MassDEP in 2004 and now in 2005 is the Argus (2001) neurodevelopmental study, which investigated sensitive endpoints at different life stages. In this study, perchlorate produced changes in pituitary and thyroid hormone levels and thyroid morphometry at different life stages. Brain mophometry changes were also reported, but these have been questioned due to experimental design and execution issues. The study is extensively discussed in the MassDEP (2004) document and is summarized below.

2.2.4.1 *Thyroid and Pituitary Hormone Alterations and Changes in Thyroid Structure*

Female Sprague-Dawley rats were treated with ammonium perchlorate at 0, 0.01, 0.1, 1.0, or 30 mg/kg-day in drinking water two weeks prior to cohabitation and continuing through the day of sacrifice. F1-generation pups were not directly dosed but might have been exposed *in utero* during gestation and via maternal milk and maternal water during the postpartum period. Significant changes were observed in thyroid and pituitary hormone levels at the lowest dose at different life stages (Table 3). Thyroid histopathology was also altered in both dams and pups, although at higher doses.

2.2.4.2. *Brain Morphometry Changes*

Because thyroid hormones are important for normal neurodevelopment, the Argus (Argus Research Laboratories , 2001) study also investigated treatment-related changes in brain morphometry. Significant changes, especially in the corpus callosum and striatum, were observed at the lowest dose. Similar changes in various brain regions, especially in the corpus callosum and striatum, were also observed in the Argus (Argus Research Laboratories, 1998a) study. These are summarized in Appendix B. However, various reviewers of the 2002 US EPA perchlorate toxicity document have challenged the brain morphometry data, especially the plane of cut of the corpus callosum, leading to considerable uncertainty in the quantitative interpretation of this data. . The NAS also reviewed several studies to establish biological plausibility of the effects of perchlorate on the corpus callosum, and concluded that although not widely recognized as a classic marker of neonatal hypothyroidism, increased thickness of the corpus callosum appears to be a biologically plausible effect. Based on the NAS report and further input from the MassDEP/DPH Advisory Committee on Health Effects, MassDEP has concluded that the brain morphometry data should not be quantitatively used in the derivation of an RfD. That data does however qualitatively support concern over perchlorate neurodevelopmental toxicity.

2.2.5 MassDEP Selected Animal Endpoint For Supporting RfD for Perchlorate

Changes in thyroid and pituitary serum hormone levels have been detected in several perchlorate treated species (Table 4) at different life stages, indicating that the hormone changes (Table 3) observed in the critical study (Argus Research Laboratories , 2001) are not isolated incidents. The NAS report also concurred that hormone changes were observed in the animal studies.

One issue with the hormone data is that the effects observed may be of too low a magnitude (Table 3, Table 5) to cause any further downstream effects (NAS, 2005). However, small and transient changes in thyroid hormone levels in pregnant women have been associated with

neuropsychological impairments (Haddow et al. 1999, Klein et al. 2001), and brain structural changes in rats (Auso et al. 2004). These studies are discussed in detail in Appendix C.

MassDEP views changes in thyroid hormone status as an adverse effect and has used these as the POD for deriving animal-based RfD values.

2.2.6 Summary and Conclusions

Perchlorate treatment in rats produces changes in thyroid hormone levels, thyroid morphometry and thyroid tumors consistent with its proposed mode of action. In the 2004 MassDEP draft perchlorate health assessment, MassDEP selected the Argus (2001) study as the primary basis for its draft recommended RfD for perchlorate. MassDEP utilized this study since it provided information on the direct effects of perchlorate on sensitive subgroups. MassDEP is continuing to use information from this study as part of its weight of the evidence approach but as supporting evidence for the human data rather than a primary point of departure for an RfD. MassDEP is making this revision in part based upon the National Academy of Sciences report (NAS, 2005).

Due to the controversy surrounding the brain morphometry data, MassDEP has concluded that the significant changes in thyroid and pituitary hormone levels are the preferable endpoint to use. The lowest observed adverse effect level (LOAEL) for this endpoint is 0.01 mg/kg-day, and this value is used as a point of departure to derive animal based RfDs for perchlorate (in support of the human data) as discussed below.

2.2.7 Derivation of RfD Values Based on the Animal Data

MassDEP has derived possible animal-data based RfD values using total uncertainty factors of 100 and 300, applied to the LOAEL dose in the Argus Laboratory study (2001). MassDEP and the MassDEP/DPH Advisory Committee on Health Effects concluded that an UF of at least 100 was needed to account for the uncertainties inherent in the use of the animal data with some participants supporting a UF of 300. Thus, both UFs have been used in the following calculations. Use of an uncertainty factor of 100 represents a change from MassDEP's 2004 toxicological assessment where a total uncertainty factor of 300 was applied.

For the total UF of 300 the apportionment is the same as presented in DEP's 2004 assessment. In summary, uncertainty factors included values of:

- 10, applied to adjust the LOAEL. This is the standard approach used when a NOAEL is not available. The size of the LOAEL-to NOAEL uncertainty factor may be altered, depending on the magnitude and the response at the LOAEL (U.S. Environmental Protection Agency, 2002c). In this case, a factor of 10 was deemed appropriate given that effects on multiple endpoints were observed including impacts on thyroid hormone levels.
- 10, to account for variations in susceptibility within the human population (intra-human variability) and the possibility (given a lack of relevant data) that the database available is not representative of the dose/exposure-response relationship in the subgroups of the

human population that are most sensitive to the health hazards of the chemical being assessed (U.S. Environmental Protection Agency, 2002c).
- 3, to account for interspecies extrapolation.

In the case of the total uncertainty factor of 100, MassDEP apportioned this as follows:

- an uncertainty factor of 10 for the LOAEL to NOAEL adjustment
- a composite value of 10 to account for variability in human sensitivity and interspecies extrapolation. This reduction in the UF for these factors is appropriate if the NAS contention that the rodent model is particularly sensitive to perchlorate-induced alterations in thyroid hormone status is correct.

As discussed in preceding sections the NAS position on this matter is, however, open to question, in which case a total UF of 300 would be justified.

Applying a total UF of either 300 or 100 to the study LOAEL results in RfD values of 2.8×10^{-5} mg/kg-day and 8.5×10^{-5} mg/kg-day, respectively. The derivation of these values is presented below:

For total UF of 300:

10 - for sensitive individuals
10 - for LOAEL to NOAEL extrapolation
3 - for interspecies extrapolation
300 TOTAL UF

$$\text{RfD} = \frac{0.0085 \text{ mg/kg-day}}{300} = \mathbf{2.8 \times 10^{-5} \text{ mg/kg-day}}$$

For Total UF of 100:

10 - LOAEL to NOAEL extrapolation
10 - composite UF for sensitive individuals and interspecies extrapolation
100 TOTAL UF

$$\text{RfD} = \frac{0.0085 \text{ mg/kg-day}}{100} = \mathbf{8.5 \times 10^{-5} \text{ mg/kg-day}}$$

3.0 ASSOCIATED DRINKING WATER VALUES

Drinking water values (or health based limits for contaminants in drinking water) are derived by converting a dose of the chemical in question to a concentration in water using exposure parameters and apportioning a fraction of the total reference dose (the RfD or total dose of the chemical in question allowed from all sources per day per kilogram body weight) to the drinking

water pathway. Typically, drinking water values are calculated using a standardized approach based on a 70 kg adult consuming 2 liters of water per day. In some cases adjustments may be made to account for differences in water intake rates and body weights of infants, children and pregnant women as compared to other adults. Although no current drinking water standard reflects the breast milk exposure pathway, MassDEP notes that further adjustment for exposures to nursing infants may also be warranted as they may experience quantitatively different exposures to chemicals, such as perchlorate, that are expressed in breast milk.

3.1 RELATIVE SOURCE APPORTIONMENT FACTOR

US EPA guidance recommends the use of a source apportionment factor in the range of 20% – 80% in the derivation of drinking water values for chemicals where exposures are likely to occur through additional pathways (U.S. Environmental Protection Agency, 1998a). A source apportionment factor is needed in the case of perchlorate because other exposures are likely.

Specifically, perchlorate has been found in common food items. In a preliminary study by the US Food and Drug Administration, 116 out of 128 samples of four types of lettuce from California, Arizona, New Jersey, and Florida contained perchlorate ranging in concentration from 1-129 ppb. Of 104 samples of cow's milk from across the country, 101 contained perchlorate with concentrations ranging from 3.1-11.3 ppb (U.S. Food and Drug Administration, 2004). In a separate study perchlorate was detected in all seven brands of dairy milk randomly purchased from grocery stores in Lubbock, Texas (Kirk et al. 2003). Most recently, perchlorate was detected in 46 out of 47 dairy milk samples from 11 states, with an average concentration of 2 ppb (Kirk et al. 2005). Another study focused on the development of analytical methods tested for perchlorate in a variety of food products, e.g., fresh fruits, vegetables, milk, alcoholic beverages, baby foods and other products harvested or processed in many parts of the world. The analysis showed the presence of perchlorate in most food products. Higher perchlorate levels were found in products from California (e.g., green grapes having 19 ppb), Mexico (e.g., 62.8 ppb in red tomato and 39.9 ppb in asparagus), and some South American countries, with cantaloupe from Guatemala having approximately 463 ppb of perchlorate. Foods produced in Canada and China showed the lowest level of perchlorate. Food products from Europe also showed relatively low perchlorate levels. The study also found that perchlorate can survive in food even after processing at a high temperature (El Aribi and Sakuma 2005). Although the data is limited, other food items are also likely to contain perchlorate. Recently, a Texas Tech investigator found perchlorate in samples of commercially grown wheat and alfalfa. In this study perchlorate concentrations ranged into the ppm range, considerably higher than those observed in the studies previously mentioned on lettuce and milk (Jackson et al. 2005). Although the database is limited for quantifying food and beverage perchlorate exposures, it does appear that perchlorate may be very pervasive in the U.S. food supply (U.S. and imported products). Concerns about exposures to perchlorate due to food ingestion are also supported by a recent study conducted by the U.S. Centers for Disease Control (CDC) on perchlorate in urine (Valentin-Blasini et al. 2005). CDC analyzed urine samples collected anonymously from healthy adult donors from Atlanta, Georgia, an area with no known perchlorate drinking water contamination. The limited sampling event of only 61 samples produced results showing that perchlorate was detectable in all of the urine samples ranging from 1 to 35 µg of perchlorate/g of creatinine.

The available human data also indicate that neonatal exposures to perchlorate in breast milk are likely to be widespread. Perchlorate has been detected in the breast milk of subjects from the Chilean cities of Chantaral at 19 µg/L (ppb) (where the water concentration was 5-7 µg/L (ppb)) and in Taltal at 104 µg/L (ppb) (where the water concentration was 100-120 µg/L (ppb)). Data also demonstrates that perchlorate is also common in breast milk in the US. In the study noted above, breast milk samples from all 36 individuals tested contained perchlorate. The maximum concentration was 92 µg/L with an average concentration of 10.5 µg/L (Kirk et al, 2005). The individuals tested were from 18 states indicating that perchlorate contamination of breast milk is likely to be widespread. These data indicate that breast-fed neonates may experience significant perchlorate exposures from breast milk. Neonatal consumption of breast milk with the average concentration of perchlorate reported in the Kirk et al study would result in exposures that exceed both the RfD values recommended by the NAS, as well as that recommended by Mass DEP.

Thus, the available data clearly demonstrates that perchlorate is prevalent in common foods and suggests that exposures to perchlorate from such consumables may be substantial. In addition, other dietary and environmental thyroid toxicants acting through a similar mechanism (e.g. nitrate) exist and will contribute to overall levels of thyroid disruption. Mechanistically, exposures to all such agents should be considered. Unfortunately the data is insufficient at this time to derive population-based exposure distributions from consumption of other perchlorate containing food items or to evaluate aggregate exposures to other thyroid toxicants.

In situations where other exposures are likely but the data is insufficient to quantitatively evaluate the relative contributions of other sources, federal and state drinking water programs often use a 20% source apportionment factor in drinking water limit derivations. In such situations, MassDEP has consistently used 20% in all of its drinking water guidelines based on non-cancer endpoints. Given the available data, MassDEP has concluded that a 20% factor is appropriate in the case of perchlorate.

3.2 ADJUSTMENTS FOR NEONATAL EXPOSURE

MassDEP scientists and members of the MassDEP/DPH Advisory Committee on Health Effects have noted that perchlorate risks to neonates are of particular concern due to their limited reserve capacity of thyroid hormones, incompletely developed thyroid functions, and higher liquid consumption rate per unit of body weight (of water in formula and/or breast milk). Although a concern, MassDEP did not focus on the issue of neonatal dosimetry in its earlier 2004 Draft Assessment because the proposed drinking water limit guideline of 1 ppb was determined by analytical limitations. Adjustment for neonatal dosimetry would have resulted in values below this limit, in which case the final guideline would still have defaulted to the analytical based reporting limit of 1 µg/L (ppb) in drinking water. In light of the higher RfDs proposed by the NAS and the data on perchlorate in breast milk, MassDEP has revisited this issue.

The US EPA in their 2002 draft assessment concluded that dosimetric adjustment for neonates was not needed in the case of perchlorate because the available PBPK models indicated that infant internal doses would likely be less than those of adults at a given exposure. Thus, for

water at a given perchlorate concentration, although infants would be expected to consume a greater daily dose of perchlorate on a per kilogram body weight basis, other pharmacokinetic factors (such as protein binding levels; kidney clearance rates; volumes of distribution etc.) would be expected to reduce the resulting delivered dose and IUI in the infant compared to the adult. MassDEP, however, notes that the available PBPK data supporting this position are by no means definitive because the models have not to date been fully developed or verified with respect to the human fetus and neonate.

The pharmacokinetics of toxicants vary considerably across life stages and across species. Of particular relevance to this discussion is the fact that very significant changes in pharmacokinetic functions occur during fetal and neonatal development and these vary considerably between rodent and human neonates (Ginsberg et al. 2002). Although the NAS did not address the issue of neonatal dosimetry adjustment in their report (NAS, 2005), they also noted limitations of the PBPK modeling data, stating:

> "Although no formal sensitivity analysis was performed on the human PBPK model, it is likely that, in addition to the skin compartment, urinary clearance of both [*sic*. perchlorate and iodide] and the plasma-protein binding of perchlorate may be important for additional future research. Furthermore, the PBPK model was developed for adult males and females (primarily healthy subjects although one subject with Graves disease was simulated) but not for pregnant or lactating females, human fetuses, neonates or children." (MassDEP note: the model under-predicted iodide uptake inhibition in the Graves disease patient modeled) and,
>
> "Given the important species differences in developmental biology and the current inability to validate extrapolation to human fetuses and neonates, such an approach (PBPK modeling) should be used with caution for these potentially sensitive populations."

The uncertainties in the PBPK models raise significant doubts regarding the use of the rodent neonate model outputs to conclude that human neonate internal doses will be less than in adults for a given intake. This is clearly an area where further research is needed. Given the available data MassDEP has concluded that it is appropriate to consider the default assumption of equal dosimetry in infants and adults (i.e., that the neonate will receive the same internal dose per unit of ingested dose as the adult) which necessitates adjustment of human infant exposures to account for their higher consumption of fluids per body weight compared to adults (and thus higher total ingested dose).

MassDEP notes that US EPA has previously included neonatal dosimetry adjustment in the derivation of a drinking water standard (for nitrate). To provide consistency, MassDEP has concluded that the body weight and liquid intake parameters used in the derivation of the US EPA nitrate limit are appropriate for dosimetric adjustment for perchlorate as well. Thus, an infant BW of 4 kg and consumption rate of 0.64 L/day were used to assess neonatal exposures.

These values are in the range of those reported in the 2002 interim US EPA Child Specific Exposure Factor Handbook (U.S. Environmental Protection Agency, 2002d)[11].

Formula-fed Infants. In the case of formula fed infants, a 100% apportionment to the drinking water used to make formula was selected. This is clearly appropriate, as exposures due to other foods are not a concern.

As noted in Table 6, possible drinking water values derived using these parameters are only marginally lower than those calculated using adult body weight and water consumption rates with a 20% source apportionment value.

Breast fed-Infants. The situation in the case of a breast fed infant is more complex as one must derive an acceptable drinking water value for the lactating mother that appropriately accounts for the potential concentration of perchlorate into breast milk. As noted earlier, perchlorate is expressed into breast milk and may also inhibit iodide transfer into the milk[12]. Furthermore, animal data indicate that perchlorate may be *concentrated* into breast milk[13]. Breast feeding infant exposures to perchlorate will therefore reflect the total maternal perchlorate exposure and the degree to which it is concentrated into breast milk and may well, on both an absolute and an adjusted body weight basis, exceed the exposures experienced by adults consuming contaminated water. Therefore, to account for the potential concentration of perchlorate into breast milk and ensure that breast milk concentrations do not exceed levels acceptable for the neonate, total maternal exposures may need to be well below RfDs for adults.

With respect to neonatal exposures, an acceptable breast milk perchlorate concentration can be derived for any selected RfD value. For example, based on a 4 kg infant consuming 0.64 L of breast milk and using the lower of the two NAS RfDs (2.3×10^{-4} mg/kg-day, which is the highest RfD that MassDEP and its MassDEP/DPH Advisory Committee on Health Effects believe can be supported), the breast milk concentration of perchlorate would need to remain below 1.4 µg/L (ppb) to keep infant exposures below the RfD. Based on MassDEP's recommended RfD of 7×10^{-5} mg/kg-day, the breast milk value would be 0.42 µg/L (ppb). Based on the average breast milk concentration from the US samples of 10.5 µg/L, a 4 kg newborn consuming 0.64 L of milk per day would receive a dose of 1.68×10^{-3} mg/kg-day, exceeding the MassDEP RfD and both of the NAS RfD values (2.3×10^{-4} mg/kg-day and 7×10^{-4} mg/kg-day).

[11] The 2002 interim US EPA Child Specific Exposure Factor Handbook (U.S. Environmental Protection Agency, 2002d) recommends a breast milk consumption rate of 0.74 L/day for 1-6 month old infants. The mean value for 1-month old infants was reported to equal 0.70 L/day. This reference presents smoothed 50th percentile body weights for one-month old male and female infants as 4.29 and 3.98 kg, respectively.

[12] Based on a *very limited* number of samples from this study population, for breast milk samples with a perchlorate content greater than 10 µg/L, the iodide content of the breast milk (the nursing infants sole source of iodide) was reported to be inversely proportional to the perchlorate concentration (r^2 of >0.9; $n = 6$) (Kirk et al. 2005). Thus, nursing infants may be subjected to a "double insult" if their mothers are exposed to sufficient perchlorate; a reduced level of iodide available to the thyroid and inhibition of thyroidal iodide uptake by perchlorate.

[13] The perchlorate concentration in the milk of lactating rats exposed to a perchlorate dose of 0.01 mg/kg-day was close to 10-fold higher than the concentration in the maternal serum (Clewell et al. 2003, Fig 2 panels A and E). In this case the PBPK model under predicted the observed breast milk concentrations.

Because of limited data on serum and breast milk perchlorate concentrations in people and the uncertainties with the PBPK models as they relate to fetal and neonatal life stages, as noted above[14], MassDEP has concluded that it is not possible at this time to derive meaningful quantitative estimates of infant perchlorate exposures from breast milk that would result from maternal drinking water exposures. However, the experimental data that indicates perchlorate is concentrated into milk from the serum suggests that, in order to keep the breast milk concentration of perchlorate below 1.4 µg/L, the perchlorate concentration in the drinking water of the nursing mother may need to be below this concentration[15].

Although, as noted throughout this discussion, there are significant data gaps that preclude robust quantitative assessment of this exposure pathway, the qualitative data emphasize its potential significance relative to exposures and risks to those considered to be among the most sensitive to perchlorate toxicity. This argues for a conservative, health protective approach. As previously discussed, uncertainty regarding perchlorate's inhibition of iodide transport into milk, which could increase neonatal sensitivity to perchlorate thyroid effects, is one of several uncertainties that support MassDEP's use of a database deficiency uncertainty factor. The levels of perchlorate detected in breast milk also support the use of a conservative relative source apportionment factor for maternal drinking water exposures to perchlorate.

3.3 RfDS AND ASSOCIATED DRINKING WATER LIMITS.

In light of the issues discussed above, MassDEP scientists in consultation with the Advisory Committee on Heath Effects concluded that a weight of the evidence approach should continue to be used to determine the most appropriate RfD value and corresponding drinking water value. Thus, both the human and animal data were considered by MassDEP in its assessment. In part based on the NAS report, MassDEP is now placing more weight on the human data.

[14] At the 2/05 meeting of the MA DEP-DPH Advisory Committee on Health Effects, one participating NAS Committee member noted that premature infants constitute an additional group at risk. To our knowledge, PBPK models addressing perchlorate exposures to premature infants have not been developed.

[15] Note: Although the following calculations are very uncertain because of limited data, they support a health-protective approach to the selection of the uncertainty factors used to derive a final RfD and exposure apportionments used to derive any drinking water limit for perchlorate for lactating women. Serum perchlorate concentrations in individuals (9 men) exposed to approximately 0.14 mg/kg-day of perchlorate in drinking water averaged 0.61 mg/L (Lawrence et al. 2000). Based on this data and using a linear extrapolation, exposures to perchlorate at the NAS lower RfD of 2.3×10^{-4} mg/kg-day would be expected to result in a serum concentration of approximately 0.001 mg/L (note: the PBPK models suggest that proportionally higher serum concentrations may result at lower doses, so this value may be an underestimate of the serum concentration). Assuming that the 10-fold serum to breast milk concentration factor observed in rodents applies to humans as well, this would imply a breast milk perchlorate concentration of 10 µg/L. This value is 7-fold higher than the acceptable breast milk target concentration of 1.4 µg/L, which can be derived based on the lower of the two NAS RfDs, and 23 times higher than that which can be derived based on MA DEP's RfD. As the adult drinking water limit associated with the NAS lower value is 1.6 µg/L, the final drinking water limit for lactating women would, in this scenario, be well below 1 µg/L. The final drinking water limit for lactating mothers would have to be below 1 µg/L even on the basis of the higher NAS RfD, which is associated with an adult drinking water limit of 4.9 µg/L based on standard exposure parameters that do not account for breast milk. These preliminary calculations are by no means definitive but do suggest a need for further research to better delineate the relationship between maternal perchlorate exposures, levels in breast milk and exposures to the nursing infant.

MassDEP and its MassDEP/DPH Advisory Committee on Health Effects, noting that the statistical power of the Greer study was such that a 40% inhibition of iodide uptake would have had to occur to be statistically detectable with a reasonable level of confidence, have concluded that an UF of 30 is the minimum that should be used with this data. One Committee member supported this value over others. Based on the full range of uncertainties involved, including data which indicates that perchlorate may be concentrated into breast milk, the remaining members of the Advisory Committee on Health Effects recommended that a total UF of at least 100 should be applied. Some members indicated that a higher UF of 300 could be supported. Application of UFs of 100 or 300 to the Greer data yields RfDs of 7×10^{-5} mg/kg-day and 2.3×10^{-5} mg/kg-day, respectively. As discussed in preceding sections, the animal data can support RfDs of 2.8×10^{-5} and 8.5×10^{-5} mg/kg-day. These values are in the same range as those that can be derived from the human data.

These RfD's along with the associated drinking water values are summarized in Table 6. The higher of the two NAS RfDs is footnoted in the Table for comparative purposes although MassDEP and the Advisory Committee on Health Effects unanimously concluded this value does not adequately account for the uncertainties in the data, including the breast milk exposure pathway[16].

Based on the weight of the evidence as discussed in this report, MassDEP has concluded that the RfD value of 7×10^{-5} mg/kg-day, based on the Greer study data and a composite uncertainty factor of 100, provides a scientifically defensible basis for evaluating exposures. This value is associated with a drinking water limit of 0.49 µg/L (adults) and 0.44 µg/L (formula-fed infants). Based on an RfD of 2.3×10^{-4} mg/kg-day, the highest value supported by the DEP/DPH Advisory Committee on Health Effects (one member) and the lowest value supported by the NAS Committee (one member) a drinking water value of 1.6 µg/L (adults) or 1.4 µg/L (formula-fed infants) would be supported.[17]

As noted earlier, upon completion of the above document and during the internal review period, a few new perchlorate studies and assessments were published and/or made available. For completeness, MassDEP reviewed these studies in light of their importance towards setting a perchlorate RfD and also received input from the MassDEP/DPH Advisory Committee on Health Effects. The results of this detailed review are presented in a separate Addendum along with conclusions that the newer studies do not present information sufficient to warrant altering MassDEP's proposed RfD value.

[16] To reiterate, breast milk exposure is an emerging issue that was not considered by NAS. Data released in February 2005, and thus not available to the NAS Committee, indicate that perchlorate is prevalent in breast milk at concentrations that frequently exceed values that would be appropriate for the neonate.

[17] MassDEP notes that if an adjustment is conducted using standard default values for the child body weight (10 kg) and child drinking water consumption (1 L/day), resulting drinking water limits are 0.046 ppb and 0.14 ppb based on total uncertainty factors of 300 and 100, respectively.

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Table 1. Comparative Summary of Human Perchlorate Exposure Studies.

*not peer reviewed; Key: O – no effect observed; ↓ - decrease in value of variable; NR – not reported; ** calculated by MassDEP assuming 70 kg body weight

Study	N	Daily Dose mg	Mg/kg/d	Duration (wks) 0 2 4 6 8 // 12 24 28	Effects RAIU	Total Serum T4	Free Serum T4	Total Serum T3	Free Serum T3	TSH	other
Brabant et al., 1992	5♂	0.2	0.003** iodide	----------|	-	O		O			O (Thy. vol)
		910	13** perchl.	|----------|	-	↓			O	↓	↓ (Thy I^- content) ↓ Serum thyroglobulin
Brabant pers. comm. 1994 (cited in U.S. EPA, 2002)*	NR	NR	12	----------.>4 wks	-	-	-	-	-	O	↑ Thyroid volume
Lawrence et al., 2000	9♂	10	0.1**	-------|……| follow-up	↓ + post recovery		O	O		O	O urine Iodine
Lawrence et al., 2001*	8♂	3	0.04**	-------|……| follow-up	↓ NS + post exp. ↑	O	-	O	-	O	-
Greer et al., 2002	7	NR	0.007	--------|……..| +15d follow-up	O	O	O	O		O	-
	10	NR	0.02	--------|……..| +15d follow-up	↓	O	O	O		O	
	10	NR	0.1	--------|……..| +15d follow-up	↓ + recov.	O	O	O		O	
	10	NR	0.5	--------|……..| +15d follow-up	↓+ recov	O	O	O		↓+ Recov.	
Braverman et al., 2004*	4	0	0	----------|……| post exp.	O	O		O		O	
	5	0.5	0.007**	---------- ----------|……| post exp.	O	O		O		O	
	4	3	0.04**	---------- ------|……| post exp.	O	O		O		O	

DOSES:	0	0.007	0.02	0.04	0.1	0.5	13
Σn		12	10	12	19	10	5

Table 2. Factors for Evaluating Evidence Regarding Identification and Characterization of Susceptible Subpopulations. Source: U.S. Environmental Protection Agency , 2002c

Factor	Increased weight	Decreased weight
Timing (life stage) - response relationship	Effects occur at greater magnitude at one or more life stage(s)	No difference in effects at different life stage(s)
Type of effect	Different types of effects in specific subpopulations	Same effect(s) across all potential subpopulations
Dose-response relationship	Effect occurs at lower exposures in one or more subpopulation(s)	No evidence for differential dose-response across different subpopulations
Latency of effect	Latency to observed effect different in specific subpopulations	No difference between subpopulations in latency to effect
Seriousness/ reversibility of effects	Effects different in seriousness or degree of reversibility in specific subpopulations and/or differences in later consequence of an initially reversible effect	No differences between subpopulations in seriousness and/or reversibility of effects, or in later consequences of an initially reversible effect

[a] Subpopulations may be defined by gender, individuals at different life stages (fetus, child, adult, elderly), differences in genetic polymorphisms, and/or pre-existing diseases or conditions that may result in differential sensitivity to adverse effects from exposure to a specific toxic agent.

Table 3. Perchlorate Dose Levels Associated with Significant Changes in Thyroid and Pituitary Hormone Levels at Different Life Stages

Generation	T3 LOAELmg/kg-day	T4 LOAEL mg/kg-day	TSH LOAEL mg/kg-day	Brain morphometry (corpus callosum, striatum, cerebellum) LOAEL mg/kg-day	References
GD21 (Dams)		0.01 0.004 (BMDL)	0.01		Argus Research Laboratories, Inc., 2001
GD21 (PUPS)	0.01				Argus Research Laboratories, Inc., 2001
PND4, PND9	0.01				Argus Research Laboratories, Inc., 2001
PND21		0.01 (LOAEL) 2.86×10^{-7} (BMDL)	0.01 (female)	0.01	Argus Research Laboratories, Inc., 2001

Note: Dose values in the table are for potassium perchlorate, which translates into 0.0085 mg/kg-day of perchlorate

Table 4. Qualitative Consistency of Effects of Perchlorate on Thyroid and Pituitary Serum Hormones* (Table reproduced from U.S. Environmental Protection Agency, 2003)

Study	Time Point	T4	T3	TSH
Rat 14 Day (Caldwell et al., 1995)	14-Day	⇓	⇓	⇑
Rat Subchronic (Springborn, 1998)	14-Day	⇓	⇓	⇑
	90-Day	⇓	⇓	⇑
Rat Neurodevelopmental (Argus, 1998a)	PND5	⇓	⇓	⇑
Rat Argus"Effect Study" (Argus, 2001)	Dams - GD21	⇓	⇓	⇑
	Dams - PND10	⇓	⇓	⇑
	Dams - PND22	⇓	⇓	⇑
	F1 - GD22	⇓	⇓	⇑
	F1 - PND5	⇓	⇓	⇑
	F1 - PND10	⇓	⇓	⇑
	F1 - PND22	⇓	⇓	⇑
Rat 2-Generation Study	P0 Males	⇓	--	⇑
	P0 Female	--	--	--
	P1 PND21	⇓	--	⇓
Mouse Subchronic	14-Day	⇓	⇓	⇑
	90-Day	⇓	⇓	NA
Rabbit Developmental (Argus, 1998b)	Gestation Day 28	⇓	⇓	⇑

*see Tables 5-2and 5-4 of the 2002 ERD provided herein in Appendix 4B as Tables 4B-1 and 4B-2 for details.
NOAEL and LOAEL estimates were determined by Agency ANOVA for the individual studies.
NA = not available

Table 5. Effects of Perchlorate on Rat Pituitary and Thyroid Hormone Levels Relative to Controls (Argus, 2001)

PND 21 dams	0.01 mg/kg/day CLO_4^{-dose})	0.1 (mg/kg/day CLO_4^- dose)	1 (mg/kg/day CLO_4^- dose)	30 (mg/kg/day CLO_4^- dose)
% Decrease in T4 levels	11	45	48	54
% increase in TSH levels	35	50	64	146

Table 6. Range of Possible RfDs and Associated Drinking Water Values Derived from Human and Animal Studies with MassDEP and NAS Assigned Uncertainty Factors

STUDY		Total Uncertainty Factor Applied			
		UF 300[1]	UF 100[2]	NAS UF 30[3]	Notes
Greer study	**RfD mg/kg-day**	2.3×10^{-5}	7.0×10^{-5}	2.3×10^{-4}	-
	a) Health Based Adult Drinking Water Value (µg/L or ppb)	0.161	**0.490**	1.610	For 70 kg adult; 2 L/day water consumption; 20% source apportionment factor
	b) Formula-fed Infant Health Based Drinking Water Value	0.137	**0.438**	1.373	For 4 kg infant; 0.64 L/day formula consumption; 100% source apportionment factor
Argus study	**RfD mg/kg-day**	2.8×10^{-5}	8.5×10^{-5}	NA	-
	a) Health Based Adult Drinking Water Value (µg/L or ppb)	0.196	0.595	NA	For 70 kg adult; 2 L/day water consumption; 20% source apportionment factor
	b) Formula-fed Infant Health Based Drinking Water Value	0.167	0.507	NA	For 4 kg infant; 0.64 L/day formula consumption; 100% source apportionment factor

Table notes:

1 RfD value that would result from use of an UF of 300, an option that some members of the MADEP/DPH Advisory Committee on Health Effects indicated could be supported by the data.

2 Final MassDEP proposed RfD value, unamimously supported by MADEP/DPH Advisory Committee on Health Effects.

3 RfD value that one member of the DEP/DPH Advisory Committee on Health Effects and one member of the NAS Committee supported.

4 If the RfD, proposed by the NAS Committee (based on the Greer study) and adopted by USEPA were used, the associated drinking water limits for adults and infants would be approximately 4.8 µg/L and 4.2 µg/L, respectively.

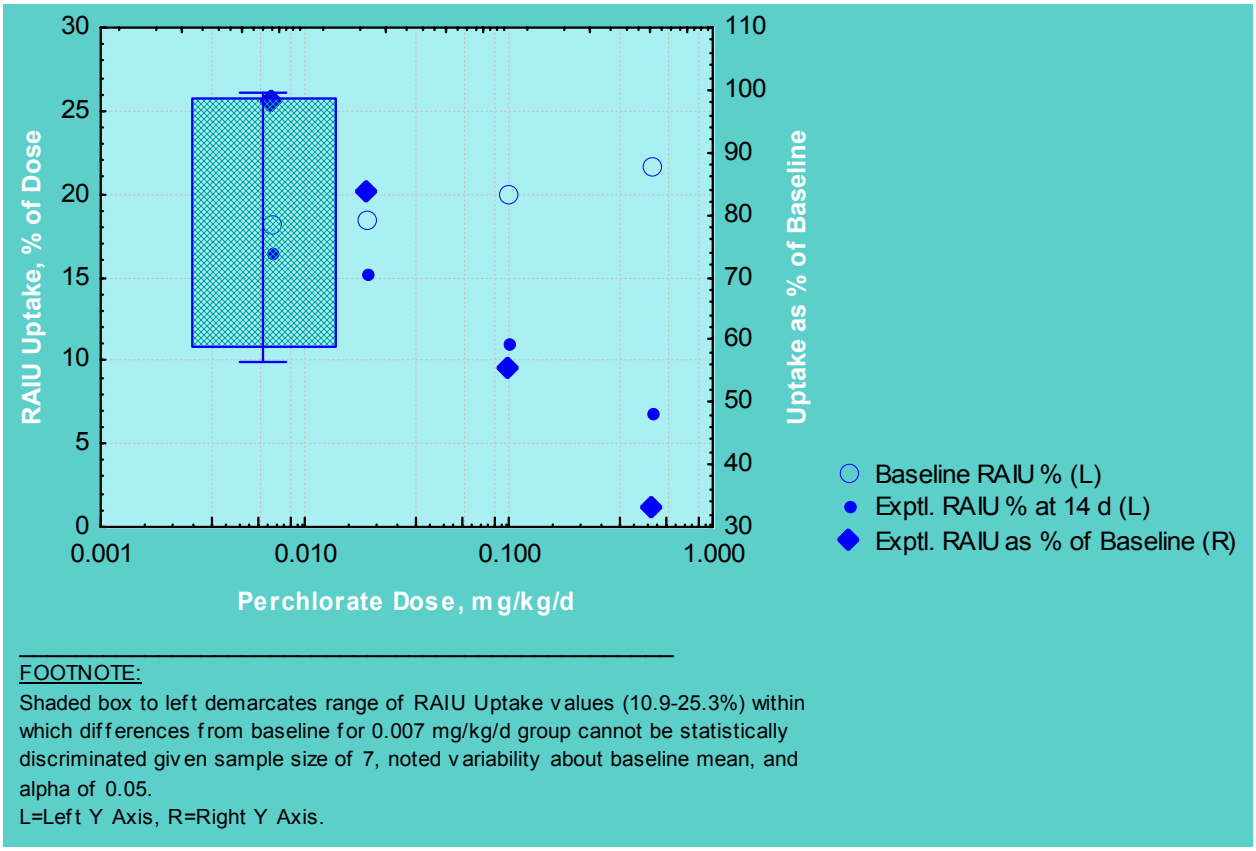

Figure 1. Iodide Uptake Data from Greer et al 2002. Means with Low Dose Group Baseline Std. Dev.

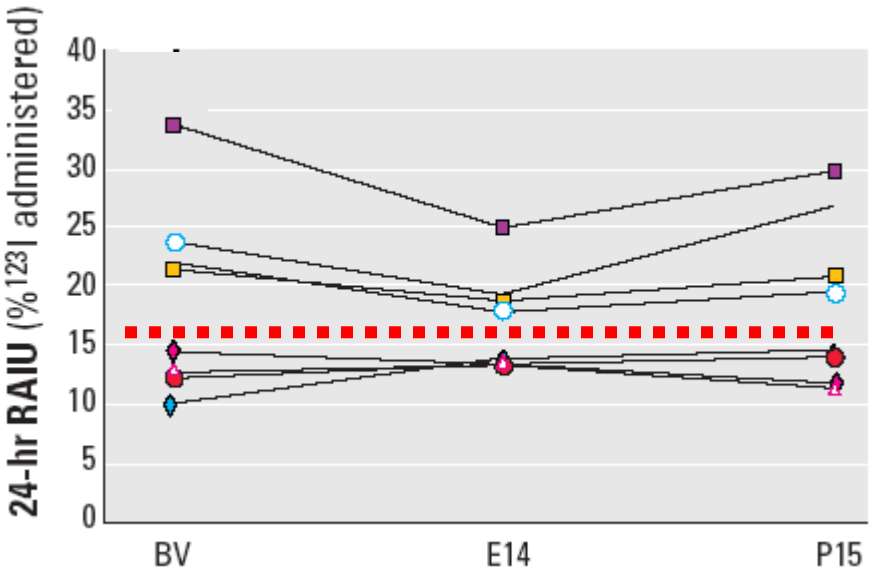

Figure 2. Individual Baseline (BV), 14 day Exposure (E14) and 15 day Post-Exposure (P15) 24-hour RAIU Responses for 0.007 mg/kg/d Dose Group. Dotted Line Added to Emphasize Differences Between Individuals Based on Baseline RAIU Level (Adapted From: Greer et al. 2002).

APPENDICES

Appendix A

The Rat as an Animal Model for Perchlorate Toxicity

The NAS Committee stated that the rat is more sensitive than humans to perchlorate disruption of thyroid function and thus concluded that the rat was not a good model to study perchlorate toxicity. The NAS noted that rats exhibit:

(a) higher thyroid hormone turnover rate and lower thyroid storage capacity than observed in humans;
(b) lower thyroid hormone levels in normal pregnant rats compared to normal pregnant women; and,
(c) delayed thyroid gland development in rat fetuses compared to human fetuses.

These issues are briefly discussed below:

Thyroid Hormone Turnover Rate and Thyroid Storage Capacity

Greer et al. (2002) pointed out that: (a) the rat thyroid is much more responsive to perturbation of iodine metabolism, leading to decreased hormone formation; (b) if thyroid hormone synthesis is prevented, the rat thyroid contains only enough hormone to last a few days, while the iodide sufficient healthy human thyroid has enough thyroid to last several months; and (c) the rat thyroid is rapidly upregulated in response to multiple treatments with perchlorate, a phenomenon not observed in humans treated for 14 days with perchlorate. These differences were also discussed by the National Academy of Sciences (2005). In the comparisons made between rats and humans above, it is not clear if the rat is expected to have thyroid hormone storage capacity and thyroid upregulation time frames exactly similarly to humans, or whether there are known human-equivalent time frames for these parameters that the rat failed to meet.

These conclusions on rat versus human sensitivity to perchlorate-induced thyroid function disruption seem to contrast with the general physiological literature on inter-species scaling. It is well known in physiology that physical dimensions, and physiological and biochemical functions in different species are functions of metabolic rate which in turn relates to various exponents of body weight (Kleiber, 1947; Adolph, 1949; Krasovskij, 1976[18]). This biological regularity provides a basis for the use of small animals as models for human toxicological and pharmacological studies and for interspecies extrapolation, provided appropriate adjustment is made.

[18] Intake of water, urine output, urea clearance, creatinine clearance, Diodrast clearance, hippurate clearance, oxygen consumption-basal, heartbeat duration, breath duration, ventilation, tidal volume, gut beat duration, total nitrogen output, endogenous nitrogen output, creatinine nitrogen output, sulfur output, oxygen consumption in liver slices, hemoglobin weight, myoglobin weight, cytochrome weight, nephron number, renal corpuscle diameter, and weight of heart, kidneys, lungs, liver, thyroid, adrenals, pituitary, stomach, intestine, and blood (Adolph , 1949); duration of pregnancy, number simultaneously born offspring, latent period of tumor formation, nerve and muscle cell dimension, maturation time of bone marrow, cellular elements, duration of erythrocyte life (Krasovskij, 1976).

Much of the fundamental work on this topic came from studies of metabolism in different sized organisms (Kleiber,1947). The work of others expanded to other physiological and morphometric variables relationships with size. Adolph (1949) reported over 30 mammalian biological parameters[17] to be mathematical functions of body weight. Adolph's work was verified by Krasovskij (1976). This author also established linear relationships among 100 mammalian biological parameters and body weight and described this relationship as "biological regularity". Krasovskij expressed this "biological regularity" by an allometric exponential equation of the form $x = ay^b$ or by a linear-line equation $\log x = \log a + b \log y$. When the biological parameter is standardized by dividing by body weight, the relationship becomes described by a negative exponential curve, or by a straight line with negative slope if both the biological variable and weight are log transformed. This weight standardization is the basis for the well- recognized rule that smaller organisms have higher metabolic rates per unit of body mass than larger organisms, whereas their absolute metabolic rates are of course less than those of much larger sized organisms. The generalized results from two classic interspecies sensitivity studies (Pinkel, 1958; Freidreich et al.,1966) found that smaller animals were less sensitive (i.e, require greater effective doses to produce the same effect than in humans) to chemical toxicity than larger ones when compared on a mg/kg body weight basis: an important point vis-à-vis perchlorate. Concomitantly, smaller animals like rats have greater weight-specific physiological parameter rates than humans.

These generalizations relate directly to thyroid functioning. Basal metabolic rate (BMR) was historically used as a clinical tool for determining thyroid status in humans. The production and secretion of thyroid hormone is related to body mass with the same exponent of 0.75 that holds for BMR (Hulbert et al., 2004). From this relationship, one can infer that the rates of these thyroid hormone processes are greater in rats (and likely other small animals) than in larger animals when expressed on a body weight basis. There does not, however, seem to be any significant relationship of either total or free thyroid hormone concentrations in the plasma of animals to weight (Hulbert et al., 2004). This finding for thyroid hormone production rate is entirely consistent with the classic metabolic-size and dose-size paradigm, yet it is a high turnover rate of thyroid hormones in rats that was cited by National Academy of Science (2005) as one reason for characterizing rats as being potentially more sensitive to perchlorate-induced disruption of thyroid function than humans. The existence of these two apparently contrasting views of thyroid function sensitivity in rats compared to humans suggests this issue deserves closer scrutiny and that it may be premature to dismiss rats as models for human perchlorate toxicity because of their greater responsiveness or sensitivity than humans. The situation that exists is what is expected based upon classic interspecies scaling relationships.

Thyroid and Pituitary Hormone Levels in Pregnant Women and Pregnant Rats

In humans, normal pregnancy is accompanied by a rise in serum levels of T4 and T3. However due to an increase in T4-binding globulin, the free T4 and T3 levels decrease during pregnancy (Glinoer et al., 1990). In contrast to findings in humans, serum T4 and T3 levels in the rat during 17 to 22 days of gestation are decreased, leading to reduced concentration of T4 and T3 in tissues, except for T3 in the brain (Calvo et al., 1990). If this late gestation phenomenon in the rat also occurs during other stages of pregnancy, the pregnant rat can serve as a good model to study the effects of perchlorate in marginally iodine sufficient and subclinically hypothyroid women

who would be expected to exhibit comparatively lower levels of thyroid hormones. Hollowell et al. (2002) estimated that 4.6 percent of the United States population has hypothyroidism (0.3 percent clinical and 4.3 percent subclinical). Kung et al. (2000) studied marginally iodine sufficient pregnant women and found that pregnancy in these women decreased T4 and T3 levels (Table 1), as was observed in pregnant rats by Calvo et al. (1990) and Versloot et al. (1994).

The usefulness of the rat as a model for pregnant women cannot be ruled out. Pregnancy itself puts pressure on the thyroid gland. Among other changes in thyroid function, pregnancy increases the demand for iodine due to pregnancy-associated iodide clearance by the kidney (because of increased glomerular filtration rate), and transfer of maternal iodide to the fetus (Glineor, 1990). Any decrease in iodide uptake inhibition caused by perchlorate could create a negative iodide balance at any stage in pregnancy, causing a decrease in thyroid hormone synthesis.

Delayed Rat Fetal Thyroid Gland Development

Much of the knowledge regarding the maturation of thyroid function in the fetus and neonate is derived from studies in rats. The rat is a good model to study human thyroid gland development and differs primarily in the timing of events. The thyroid gland of the rat fetus starts to function between days 17 and 18 of gestation (Morreale de Escobar et al., 1985). Because of this late development of the thyroid gland, the rat fetus depends entirely on the mother's thyroid hormone during the greater part of *in utero* development. The rat fetus may thus provide an animal model to study the congenitally hypothyroid human fetus. Congenital hypothyroidism is one of the commonest causes of mental retardation in humans and its causes and prevalence are presented in Table 2. Since the rat fetus has some contribution of its own thyroid hormone during the last stages of its *in utero* development, this fact should be taken into consideration when extrapolating data from the animals to humans. The human hypothyroid fetus may be more sensitive to perchlorate because of a lack of any other thyroid hormone sources except its mother's, throughout gestation.

In conclusion, data on perchlorate toxicity derived from studies on the rat should not be completely discounted. In fact, the rat may be a reasonable model to study the toxicity of perchlorate during sensitive human lifestages such as the pregnant woman and the congenitally hypothyroid fetus. MassDEP is therefore continuing to use the animal bioassay data in its analysis but as supporting information. In acknowledgement of the uncertainty about the sensitivity of the rat model in comparison to healthy adults, MassDEP has considered alternative uncertainty factor adjustments for the animal data.

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Table 1. Changes of Thyroid Function Tests, Thyroidal Volume and Urinary Iodine Level of Marginally Iodine sufficient Women During and After Pregnancy (Kung et al, 2000)

Parameter	First trimester	Second trimester	Third trimester	Postpartum 6 weeks	Postpartum 3 months
Total T4 (nmol/L)	154	126*	125*	89**	92**
Free T3 (pmol/L)	3.9	3.4*	3.3*	4.0	4.0
Free T4 (pmol/L)	13.4	11.9*	11.7*	14.5	14.4
TSH (mIU/L)	0.49	0.96*	0.95**	1.15**	1.14**
Urine iodine μgl /L	10.6	11.5*	12.4*	10.5	10
Thyroid volume	9.5	10.3*	11.2*	11.0*	10.6*

Results are median, * $p < 0.05$, ** $p < 0.01$, vs first trimester

Table 2. Thyroid Disorders And Their Approximate Prevalences In The Human Neonatal Period (Post et al. 1996)
Table adopted from US EPA (2002).

Thyroid Dysgenesis	1:4000
Agenesis	
Hypogenesis	
Ectopia	
Thyroid Dyshormonogenesis	1:30,000
TSH unresponsiveness	
Iodide trapping defect	
Organification defect	
Defect in thyroglobulin	
Iodotyrosine deiodinase deficiency	
Hypothalamic-Pituitary Hypothyroidism	1:100,000
Hypothalamic-pituitary anomaly	
Panhypopituitarism	
Isolated TSH deficiency	
Thyroid hormone resistance	
Transient Hypothyroidism	1:40,000
Drug induced	
Maternal antibody induced	
Idiopathic	

Appendix B

Brain Morphometric Data from Animal Studies

The NAS (2005) document extensively discussed the Argus Research Laboratories (1998, 2001) brain morphometric analyses, and pointed out that there was no dose-effect relationship and no consistent effect across age and sex except for the corpus callosum which increased in size at the highest dose tested (10 mg/kg/day) at different life stages. The NAS also noted that the Argus (2001) study showed significant increase in the corpus callosum across life stages at all doses except the highest dose tested (30 mg/kg/day). The NAS was concerned about the lack of a dose-effect relationship after comparing the Argus (1998) and (2001) studies. In the Argus (1998) study the thickness of the corpus callosum was increased only at 10 mg/kg/day and not at 3 mg/kg/day. In contrast, the corpus callosum size was increased at doses ranging from 0.01 to 1 mg/kg/day, but not at 30 mg/kg/day in the Argus (2001) study.

However, the NAS did not consider the dosing protocols (duration of exposure and dose spacing) used in the two studies. In the Argus (1998) study, female rats were exposed to perchlorate beginning on gestation day 0, while in the Argus (2001) study, female rates were exposed 2 weeks before cohabitation with male rats. In the Argus (2001) study, female rats were made hypothyroid before pregnancy while the female rats in the Argus (1998) study, were euthyroid at pregnancy. Previous studies by Springborn Laboratories (1998) have shown that rats treated with perchlorate for two weeks had significant changes in thyroid and pituitary hormone levels at 0.01 mg/kg/day, which is the dose that resulted in changes in brain morphometry in the Argus (2001) study. These observations suggest that the two studies should only be qualitatively compared. Lack of dose-response concordance between the two studies should not be a valid reason to discredit the results of the studies.

The NAS (2005) document also discussed other issues surrounding the brain morphometry data including:

(a) method of brain tissue fixation and time since fixation before sectioning and slicing of tissues;
(b) variation in plane of section;
(c) differences in plane of sectioning across animals;
(d) coronal versus sagittal sectioning of the corpus callosum; and
(e) lack of non-blinded morphometric measurements.

The NAS report indicated that the Committee did not think issues (a) and (b) above were of concern in the overall evaluation of the brain morphometry data. Regarding issue (c), the NAS stated that the US EPA resectioned and reanalyzed brain tissues from PND 22 pups, which helped to dispel some of the concerns that have been raised about systematic differences in the plane of section among treatment groups. However, the NAS was concerned that the reanalysis of the brain tissues has contributed to the inconsistencies in the data set because the thickness of the striatum were decreased in the 2001 brain section and increased in the 2003 brain section from the same pups. Like the NAS, MassDEP also observed these inconsistencies while

reviewing the US EPA (2003) document in 2004. The data in the 2002 and 2003 EPA documents on the corpus callosum were consistent while the data on the striatum were inconsistent. MassDEP previously contacted US EPA scientists involved in the perchlorate toxicity assessment for clarification of the divergent results of the striatal data set. Dr. Geller (personal communication) explained that the 2002 analysis was based on the combined male and female data, while the 2003 analysis was based only on male data. As seen in Table 1, perchlorate dosing was associated with a decrease in striatal size in females and an increase in males. It is not clear why the male and female striatal data were combined for analysis when there is clear evidence of treatment and sex interaction.

Of note is the observation that the effects of perchlorate on the striatum are reproducible at multiple doses and multiple life-stages, as it is with the corpus callosum. Wahlsten (2002) also reproduced the results on the striatum. Moreover, the coronal sectioning of this region of the brain is said to be not as controversial as it is with the corpus callosum. Issues (d) and (e) are of concern, but these issues may not be sufficient grounds to discount the brain morphometry data.

While MassDEP is still concerned with some methodological problems of the brain morphometry data, the consistent observation of perchlorate-related effects in the corpus callosum and the striatum observed in two separate studies at different life stages, and the concordant morphometric measurements performed by two separate pathology laboratories cannot be ignored. The data suggest that perchlorate may change brain morphometry in rats exposed *in utero* and postnatally. Moreover, the NAS (2005) also stated that the corpus callosum thickness was increased at multiple doses and at several stages of development (PND10-12, PND22, PND 82-85) in two studies and is suggestive of a relationship between perchlorate exposure and altered neurodevelopment. The NAS also reviewed several studies to establish biological plausibility of the effects of perchlorate on the corpus callosum, and concluded that although not widely recognized as a classic marker of neonatal hypothyroidism, increased thickness of the corpus callosum appears to be a biologically plausible effect. Thus MassDEP views the brain morphometry data as qualitatively informative but is no longer relying on this data to quantitatively assess perchlorate risks.

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Table 1. Summary of Morphometric Findings in Rat Pups Exposed to Perchlorate

Brain regions	Argus 1998		Argus 2001		EPA 2003
	PND10 -12 LOAEL	PND82 – 85 LOAEL	PND 10 LOAEL	PND22 LOAEL	PND 22 LOAEL
Frontal Cortex	No change	↑ 10 (m)	↑ 1.0	0.1 ↑	Not measured
Parietal cortex	No change	No change	↑ 1.0	No change	Not measured
Striatum	↓ *3.0 (f)	↑ 10 (m)	↑ 1. 0 (m) ↓0.1(f)	↓ 0.01 (m +f)	↑ 0.01 (m)
Corpus callosum	↑ 10	↑ 10 (m)	↑ 0.1 (m)	↑ 0.01	↑ 0.01
Hippocampus gyrus	↓ 3.0	No change	No change	No change	Not measured
Dental gyrus	Not measured	Not measured	No change	No change	Not measured
CA1 portion	Not measured	Not measured	↓1.0 (f) ↑(m)	No change	Not measured
CA3 portion	Not measured	Not measured	No change	↓ 0.1	Not measured
External germinal layer of cerebellum	Not measured	Not measured	↓ 1.0	No change	Not measured
Anterior/posterior cerebellum	↑ 3.0	No change	No change	↑ 0.01	Not measured

↑ = Increase in size; ↓ = decrease in size; m = male; f = female
* Numbers in the table are doses in mg/kg/day at which effect was noted

Appendix C

Modest Thyroid Hormone Decrement in Pregnant Women and Impaired Neurodevelopment in the Offspring

Haddow et al.(1999) measured thyrotropin (TSH) levels in stored serum samples collected from 25,216 pregnant women (during the second trimester) in Maine between January 1987 and March 1990. They then located 47 women with serum thyrotropin concentrations at or above the 99.7th percentile of the values for all the pregnant women, and 15 women with values between the 98th and 99.6th percentile, inclusive, in combination with low serum T4 and free T4 levels. The 7-9 year old children of these women were further investigated, none with hypothyroidism at birth The authors then conducted 15 tests on the children of these women as well as controls, relating to intelligence, attention, language, reading ability, school attainments, and visual-motor performance. The staff giving the tests did not know whether the children's mothers were women with hypothyroidism or control women. To establish a suitable control group, the authors selected 124 children born to euthyroid mothers, matched for various items such as the women's age at delivery, gestational age at blood sampling, and duration of serum storage. A scoring system was used to check for possible differences in socioeconomic status of the families (number of years of education and occupation of both parents). They found that the children of the 62 women with high serum thyrotropin concentrations performed slightly less well on all 15 tests and scored, on average, 4 IQ points lower compared to controls. Of the 62 women with thyroid deficiency, 48 were not treated for hypothyroidism during pregnancy. The average IQ score of their children was 7 points lower than the controls. Haddow *et al*. (1999) concluded that that even mild and probably asymptomatic hypothyroidism in pregnant women could adversely affect the neuropsychological performance of their offspring. From the Haddow et al. study it can be seen that T4 decreases in the pregnant mother as low as 30% may be associated with neuropsychological deficits in offspring, which is in the range of the T4 decreases observed in the critical Argus Research Laboratories (2001) study (Table 1).

In a follow-up study, Klein et al. (2001) studied serum TSH concentrations of pregnant mothers at 17 weeks of gestation and performed the standard neuropsychological testing in the offspring at mean age of 8. These authors found that the severity of neuropsychological deficits in the offspring was associated with maternal hypothyroidism. The hormonal status of subjects in this study might not be different from those in the Haddow et al. (1999) study, as both studies appear to have used subjects from the same sample pool. The results of the Klein et al. study support a causal association of low thyroid hormone level in the pregnant woman and poor neuropsychological development in the progeny.

Modest and Transient Thyroid Hormone Decrement in Pregnant Rat and Altered Brain Structure in Offspring

Auso et al. (2004) designed an experiment where mild transient hypothyroidism was induced in pregnant rats by treating these rats with a known goitrogen (2-mercapto-1-methyl-imidazole) for

only 3 days, from gestation day 12 to gestation day 15. Maternal thyroid hormones transiently decreased by about 30% in this study, without clinical signs of hypothyroidism. The pups born to treated dams were tested for audiogenic seizure susceptibility 39 day after birth and killed after postnatal day 40 to examine brain structure. The structure and distribution of the cortex and hippocampus were altered in 83% of the pups. Pups born to the treated dams (52%) also responded to an acoustic stimulus, followed in some by seizures. The authors concluded that even mild hypothyroxinemia should be prevented before and during pregnancy. Such study designs could in the future help identify the threshold for thyroid hormone decreases associated with brain structure alterations and other thyroid hormone deficiency-related effects. Until then, the 11% (Table 1) decrease in T4 and the 35% increase in TSH observed in the Argus (2001) study should remain of concern. The dose of perchlorate (0.01 mg/kg/day) associated with these effects provides a reasonable point of departure for deriving an animal data based RfD for perchlorate.

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Table 1. Effects of Perchlorate on Rat Pituitary and Thyroid Hormone Levels Relative to Controls (Argus, 2001)

PND 21 dams	0.01 mg/kg/day CLO_4^-)	0.1 (mg/kg/day CLO_4^- dose)	1 (mg/kg/day CLO_4^- dose)	30 (mg/kg/day CLO_4^-) dose
% Decrease in T4 levels	11	45	48	54
% increase in TSH levels	35	50	64	146