

**RESPONSE TO THE ENVIRONMENTAL PROTECTION AGENCY OFFICE OF  
INSPECTOR GENERAL SCIENTIFIC ANALYSIS OF PERCHLORATE**

**FINAL**

Prepared for:

*PERCHLORATE STUDY GROUP*

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*INTERTOX, INC.*  
600 Stewart St.  
Suite 1101  
Seattle, WA 98101

206.443.2115 phone  
206.443.2117 facsimile

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## 1.0 INTRODUCTION

Intertox appreciates the opportunity to comment on the United States Environmental Protection Agency (U.S. EPA) Office of Inspector General (OIG) Scientific Analysis of Perchlorate (External Review Draft) released December 30, 2008. We commend the OIG for the seriousness of its approach to the important issues relating to perchlorate remediation and public health protection.

The OIG analysis uses a cumulative risk assessment to estimate the overall risk from all stressors to the sodium-iodide symporter (NIS), the molecular pump that actively transports iodide into the thyroid. Those stressors include ingestion of high levels of perchlorate, ingestion of thiocyanate and nitrate, as well as iodine deficiency.

The OIG undertook this analysis in response to the May 1, 2007 Federal Register notice in which the U.S. EPA noted more information was needed on relative source contribution (RSC) to determine whether to regulate perchlorate under the Safe Drinking Water Act (SDWA).

In its review of the perchlorate reference dose (RfD), OIG questions the basis for the 2005 U.S. EPA RfD which was the National Academy of Sciences (NAS) National Research Council's (NRC) assessment of perchlorate, titled *Health Implications of Perchlorate Ingestion* (2005). The NAS review of the health effects of perchlorate was requested by the U.S. EPA in response to significant criticism of the scientific basis for the U.S. EPA's 2002 draft risk assessment for perchlorate.

The NRC recommended an RfD of 0.0007 mg/kg-d based on iodide uptake inhibition (IUI). In what it characterized as an unconventional move intended to provide a level of safety exceeding customary practice, the NRC chose to base this RfD on a no observed effect level (NOEL), rather than the no observed adverse effect level (NOAEL) or lowest observed adverse effect level (LOAEL), which EPA has traditionally used as the point of departure. The NOEL is the dose at which no effect occurs, adverse or otherwise, and results in a more conservative RfD than the traditional approach of the NOAEL or LOAEL.

The OIG report expresses several possible concerns with the current RfD. The OIG was unsure that the RfD would be protective of human health at all life stages; they express the view that the first adverse effect to occur should be hypothyroxinemia in pregnant women. Most critically, they conclude that the single chemical risk assessment does not, in fact, provide a scientifically accurate assessment of possible human health risk, and suggests a broader examination of all goitrogens. In short, the OIG analysis is a cumulative risk assessment for total NIS stressors using hypothyroxinemia in pregnant women as the first adverse effect.

The OIG has requested "scientific comments on the use and application of a cumulative risk assessment approach to characterizing the public health risk from a low [total iodide uptake] during pregnancy and lactation" (OIG, 2008). We believe the OIG is correct in their assessment of other NIS stressors and in their scientifically based conclusions that the current perchlorate RfD is conservative. We also agree with the OIG in that decreasing the concentration in drinking water from 24.5 ppb to 6 ppb would not offer any meaningful opportunity to lower the public's risk. There are certain aspects of the OIG argument that could benefit from additional information, such as proper/improper interpretation of iodine data based on spot urine samples and the failure to incorporate NIS up-regulation, which we later discuss in this document. As discussed below, we

believe the OIG is scientifically accurate in its assessment and in using an alternative approach. The OIG “confirmed that U.S. EPA’s perchlorate RfD is conservative and protective of human health, but limiting perchlorate exposure does not effectively address this public health issue [adverse thyroid effects due to total goitrogen load].”

## 2.0 THE NRC APPROACH

It is well understood that there are several levels of conservatism inherent in the existing perchlorate RfD, namely the use of a NOEL as a point of departure, the application of uncertainty factors to the NOEL, and the use of a reversible biochemical event (IUI) that is several steps prior to an actual adverse effect as the basis for the NOEL. Furthermore, perchlorate is not bioaccumulative. Rather, it has a relatively short half-life and is eliminated from the body quickly (*i.e.*, the half-life is 6 to 8 hours).

The determination of an RfD was based on the use of a single chemical risk assessment, which was considered the best science available at the time the NRC committee finalized its report in 2005. Between 1997 and 2002, at least 13 toxicological studies of perchlorate were conducted. The most sensitive target organ was determined to be the thyroid gland (U.S. EPA, 2002). There were also several clinical human exposure, epidemiologic, and ecological studies, some in occupational settings and some in populations exposed to perchlorate via the community drinking-water supply.

The U.S. EPA released a draft risk assessments for perchlorate in 1998 and 2002 based on the results of animal studies. In the 2002 draft risk assessment, U.S. EPA interpreted these studies as showing adverse effects in the pups of rats exposed during pregnancy to a perchlorate dose as low as 0.01 mg/kg-day, resulting in a proposed RfD of 0.00003 mg/kg-d (U.S. EPA, 2002). However, serious concerns about the validity of using these animal studies for a human health risk assessment were raised and, as a result, U.S. EPA and other federal agencies requested a review of the underlying science by the NRC (NRC, 2005).

The NRC committee on the perchlorate panel reached a consensus and recommended an RfD of 0.0007 mg/kg-d in the report *Health Implications of Perchlorate Ingestion* (2005). The NRC report recommended an RfD of 0.0007 mg/kg-d based on a NOEL, or the dose at which no effects occur, adverse or otherwise (NRC, 2005). The panel also took care to differentiate between a NOAEL and a NOEL, finding that there was confusion between the two and stating that the NOAEL is based upon an adverse effect, whereas the NOEL is based upon a nonadverse effect (NRC, 2005).

One of the critical studies that served as the basis for this RfD was the study by Greer *et al.* (2002), which demonstrated there was no inhibition of iodide uptake by the thyroid at a dose of 7 µg/kg-d. Importantly, the NRC did not examine Greer *et al.* in isolation. According to the NRC report, the findings in Greer *et al.* are supported by other clinical, occupational, and environmental epidemiologic studies and studies of long-term perchlorate administered to patients with hyperthyroidism.

Furthermore, the NRC emphasizes, “Inhibition of iodide uptake by the thyroid clearly is not an adverse effect; however if it does not occur, there is no progression to adverse health effects.” The committee views its recommendation to use IUI by the thyroid as the basis of the perchlorate risk assessment to be the most health-protective and scientifically valid approach (NRC, 2005).

In using a NOEL, the NRC committee deemed its approach as conservative and health protective as this dose is already lower than any dose in which adverse effects occur. Furthermore, a safety factor of 10 is applied to the NOEL to account for the most sensitive individuals in a population, in this case, hypothyroid or iodine-deficient pregnant women and their developing fetuses. The NRC panel stated that “the NOEL value from Greer *et al.* (2002) is a health-protective and conservative point of departure is supported by...extensive human and animal data that demonstrate that there will be no progression to adverse effects if no inhibition of iodide uptake occurs” (NRC, 2005).

Some have argued that Greer *et al.* was of short duration and have suggested a subchronic to chronic extrapolation. On that issue, the NRC concluded that since the point of departure is based upon IUI—a short-term event—any chronic effects “would have no greater effect” than any short term effects that may occur (NRC, 2005). Thus, the use of IUI as a point of departure is a more cautious and health protective approach than using changes in thyroid hormones (a precursor to possible adverse effects) or to some adverse effect such as hypothyroidism.

The U.S. EPA has based its current RfD for perchlorate and Integrated Risk Information System (IRIS) summary on the NRC report (U.S. EPA, 2005).

### 3.0 THE OIG APPROACH

The OIG approach is scientifically-based and appropriately incorporates most of the major thyroid related considerations needed in a risk assessment. It focuses on IUI, which is the mechanism of action for perchlorate and numerous other chemicals. As a method to cross check the human clinical data, it sufficiently supports the body of human *in vivo* literature using an *in vitro* approach. Other chemicals inhibit IUI; some of those chemicals, including perchlorate, are naturally found in food and also inhibit iodide uptake as was assessed and reported by Belzer *et al.*, (2004).

The OIG undertook this analysis in response to the May 1, 2007 Federal Register notice in which U.S. EPA noted that more information was needed on RSC to determine whether to regulate perchlorate under the SDWA. The OIG felt that a cumulative risk assessment was an appropriate measure of total stress to the NIS. While uncommon, the cumulative risk assessment has been recognized by the U.S. EPA as a method to assess total risk from multiple chemicals with the same mechanism of action (U.S. EPA, 2007).

As noted in the OIG report, perchlorate, thiocyanate, nitrate and low iodine intake fit the criteria of a cumulative risk assessment. OIG reports that thiocyanate, nitrate, and iodine are found naturally in food and water sources, assuming that people are not exposed to any one of these chemicals alone, but in combination. Thus, the focus on one chemical does not in fact provide a scientifically accurate assessment of possible human health risk. A cumulative risk assessment is defined as “an analysis, characterization, and possible quantification of the combined risks to health or the environment from multiple agents or stressors” (U.S. EPA, 2003).

The OIG uses the results from Tonacchera *et al.* (2004) to model and weigh each “stressor” [perchlorate, thiocyanate, nitrate, and iodine] by the strength of each chemical’s ability to inhibit iodide uptake. When each of these factors is applied in the model, a total effect can be estimated. The U.S. EPA and the NRC have both supported use of physiologically-based pharmacokinetic (PBPK) models for risk assessment purposes. Once the OIG had its complete model with all four

NIS stressors accounted for, they were able to estimate the overall effect of IUI on thyroid hormones. Consistent with the NRC, the OIG believes the first adverse effect is a change in thyroid hormones. The OIG was conservative in the interpretation of the iodine data, NIS up-regulation, and protein binding. The OIG determined that perchlorate alone had very little impact on the overall effect on the thyroid when the other three stressors were present.

The two major conclusions in OIG’s report:

1. “EPA’s perchlorate RfD is conservative and protects human health”
2. “...limiting perchlorate exposure does not effectively address this public health issue... lowering the perchlorate drinking water limit from 24.5 ppb to 6 ppb does not provide a meaningful opportunity to lower the public’s risk.”

The OIG assessment supports the weight of evidence from peer-reviewed science. The current body of literature has demonstrated that other chemicals do affect IUI and that pharmacological-dosing studies demonstrate levels at which side effects and adverse effects may occur. These pharmacological doses are 1000-fold or more greater than the RfD.

However, concerns regarding exposure to low levels of perchlorate continue to be an issue, which stresses the importance that in this alternative approach to risk assessment, the OIG came to the same conclusion as the NRC (2005), the U.S. EPA (2005), and the Agency for Toxic Substances and Disease Registry (ATSDR, 2008): the current RfD of 0.0007 mg/kg-d is health protective and also conservative by a factor of 6.6 times. Regardless of the method used, the current RfD is not expected to cause any adverse health effects, even in the most sensitive population (pregnant women and their fetuses).

There are several scientific points to highlight in the OIG report. First, Tonacchera *et al.* (2004) provided the relative potencies of the various inhibitors of iodide uptake at the NIS using Chinese Hamster Ovary (CHO) cells which were transfected with human NIS.<sup>1</sup> This is a common and accepted cell culture methodology. The OIG reports that Total Iodine Uptake (TIU) is the key biochemical event that, if perturbed sufficiently by the NIS stressors, should be used as the basis for the risk assessment of this public health issue. There is a large body of literature that supports this scientific approach.<sup>2</sup>

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<sup>1</sup> The potencies scientifically obtained in Tonacchera *et al.* (2004) were essentially the same as those derived by Belzer *et al.* (2004) based on a review of the literature at the time.

<sup>2</sup> For over half a century, the relative NIS inhibition potencies of prevalent nutritional and environmental NIS inhibitors (*i.e.*, perchlorate, thiocyanate, and nitrate) have been studied and quantified repeatedly with excellent agreement in the results. These key studies are referenced in the OIG report as well as in Tonacchera *et al.* (2004) and can be found there. Of these studies, Tonacchera *et al.* (2004) is the most robust and provides the most suitable starting point for a cumulative risk assessment.

The sole exception, to our knowledge, is a recent paper which, using an atypical modeling approach, agrees with IUI, but suggests a different level of potency for these stressors.<sup>3</sup>

Second, the OIG provides an effective and scientifically-supported demonstration of the overall effect on inhibition of iodide uptake that would occur by reducing the drinking water equivalent level (DWEL) based on the RfD from 24.5 ppb<sup>4</sup> to 6 ppb or lower. The OIG estimates that “potentially regulating perchlorate at a DWEL of 6.0 ppb instead of 24.5 ppb prevents about a 1% change in TIU in pregnant women; a 1% TIU change is only a small fraction of the  $\pm$  55% normal variation, observed in the % TIU at baseline in Greer *et al.* population. Therefore, decreasing the perchlorate drinking water concentration from 24.5 ppb to 6.0 ppb will not have a significant effect on the % TIU observed in people.” The OIG also found that perchlorate was the least significant of the four NIS stressors (thiocyanates, nitrates, perchlorate, and iodine deficiency) on TIU. The OIG further states that perchlorate “does not provide a meaningful opportunity to lower the public’s risk” (OIG, 2008). The OIG draft report concluded that “lowering the public’s nitrate exposure provides a more meaningful opportunity to lower the public’s NIS inhibition load (*i.e.*, a more meaningful opportunity to lower public risk) than to lower the public’s perchlorate exposure below the perchlorate RfD” (OIG, 2008).

Third, the OIG agrees with the NRC in stating that IUI is not an adverse effect. The OIG reiterates the NRC statement that identifies “NIS inhibition as a nonadverse effect that precedes all adverse effects from perchlorate exposure” and further contends that “hypothyroxinemia is the first adverse effect from a low uptake of iodide by the thyroid” (OIG, 2008).

#### 4.0 COMMENTS FOR OIG CONSIDERATION

We have several comments to offer the OIG, relating to their analysis.

##### 4.1 Conducting a Cumulative Risk Assessment

From a scientific standpoint, if there is a clearly defined pathway that leads to an adverse effect and several chemicals have a mechanism of action that affects that pathway, then it becomes less important what those chemicals are and more important that the pathway has been affected. A benefit of the cumulative risk assessment approach is that it allows for the assessment of possible neurological effects such as decreased intelligence quotients (IQ) and decreased motor performance for chemicals that all have the same mechanism of action. The OIG cumulative risk assessment considers the most environmentally-relevant chemicals that cause NIS inhibition. The OIG report

<sup>3</sup> A recent study by Dasgupta *et al.* (2008) found “transport selectivities are an order of magnitude lower than those indicated by in vitro studies, suggesting that the impact of both these anions on inhibiting iodide transport in milk may have been overestimated in the extant literature.” The study also states that “the very low fSCN<sub>m</sub> [fraction of thiocyanate in breast milk] despite high SCNT<sub>i</sub> in [total thiocyanate in the infant] suggests that the effective selectivity factor for thiocyanate over iodide transport must be considerably less than that suggested in ref 5 [Tonacchera *et al.*, 2004].” However, the conclusions of Dasgupta *et al.* (2008) are based on the amounts of each anion found in breast milk which may reflect a difference in transport of each rather than their ability to block iodide uptake by the NIS. The authors compare their results to that of Tonacchera *et al.* (2004) who found that perchlorate was 30 times more potent than iodide at blocking uptake of radioactive iodide. The two studies are not measuring the same thing and the quantitative results are not comparable. The Dasgupta *et al.* (2008) study was not designed to determine how each anion affects the transport of others, but rather, to evaluate the relative concentrations of each in breast milk. A further analysis of the conclusions drawn from Dasgupta *et al.* (2008) is available (Gibbs, 2009).

<sup>4</sup> 24.5 ppb is the drinking water equivalent level of the RfD of 0.0007 mg/kg-d assuming a 70 kg adult drinks 2 L of water per day.

stands on sound toxicological principles from this standpoint (*e.g.*, dose-response assessment of IUI).

At the same time, there are a number of considerations that this cumulative risk assessment (specifically and in general) should consider.

First, the OIG analysis is based on an *in vitro* study which, in isolation, should not be used as the basis of an RfD when there are human data to derive such values. Since there are quality human clinical and epidemiological studies available for perchlorate, those are the most appropriate to base a human health risk assessment upon for regulatory purposes. The OIG assessment, based on the model used in Tonacchera *et al.* (2004), is an excellent and valuable tool for verifying the results of several other studies and risk assessments. And, using a cumulative risk assessment allows the use of important epidemiological studies on iodine deficiency which would otherwise be unusable in a single chemical risk assessment.

Second, the OIG draft report defines the “total goitrogen load” as the combined NIS inhibition from perchlorate, thiocyanate, nitrate, and iodine deficiency acting on the thyroid. This definition is somewhat misleading because other substances inhibit IUI (*e.g.*, pertechnetate, perrhenate, boron tetrafluoride, iodide, bromide, chloride, chlorate, selenocyanate, periodate, and bromate among others (OIG, 2008)). IUI is only one mechanism of action that may lead to adverse effects in the thyroid. Other goitrogens affect other biochemical pathways, such as iodination of thyroglobulin, deiodination of thyroxine, glucuronidation of thyroid moieties, *etc.*<sup>5</sup> Another example is the antithyroid interaction of soy (which does not effect IUI) combined with iodine deficiency has been further elucidated by Ikeda *et al.* (2000, 2001).<sup>6</sup> Thus, being able to define all the parameters that would affect thyroidal function is important in a cumulative risk assessment. At a minimum, we suggest using a more accurate term, such as “total NIS inhibition load” or something similar as well as defining and supporting what is being evaluated in a cumulative risk assessment.

Third, there are several general considerations to be considered when proposing a cumulative risk assessment. While OIG presents numerous U.S. EPA guidance documents to support the use of a cumulative risk assessment, it has not been widely used. The traditional risk assessment evaluates one chemical in a systematic manner with accepted methodologies, while also accounting for uncertainty. The result is the development of a dose that is not considered to cause any adverse effect in the most sensitive population even when exposed daily over a lifetime. Traditional risk assessments have a history with demonstrated validity.

In a cumulative risk assessment, some questions arise that require some thoughtful consideration.

- Would evaluating multiple chemicals mean having to account for multiple sources of uncertainty?
- What chemicals should be included or excluded from a cumulative risk assessment? For

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<sup>5</sup> The OIG report lists lindane, malathion, and mancozeb as chemicals that inhibit iodide oxidation by thyroid peroxidase (TPO). The draft report does not mention soy protein in the context of other goitrogens anywhere in their report, although soy protein is a well documented TPO inhibitor and goitrogen. Two recent reviews of the TPO inactivation / goitrogenic action of soy isoflavones are Doerge and Sheehan (2002) and Doerge and Chang (2002).

<sup>6</sup> Ikeda *et al.* (2000, 2001) showed that rats fed an iodine-deficient diet containing soy bean developed a severe hypothyroid state characterized by decreased T4, increased TSH, increased thyroid weight, increased cell proliferation and marked histopathological changes. Another report from Son *et al.* (2001) tested the ability of isolated soy isoflavones to act synergistically with iodine deficiency to produce a hypothyroid state. These results suggested that only whole soy, but not soy isoflavones alone, is sufficient to produce a hypothyroid condition in rats under conditions of iodine deficiency.

example, other chemicals, such as thiocyanate, bromide, chloride, chlorate, and bromate (OIG, 2008) are known to inhibit IUI.

- How does one rank the importance of a particular mechanism of action? Is IUI more important in thyroid physiology than reduction of detoxification mechanisms?
- How does one evaluate multiple chemicals that have different qualitative (animal *versus* human data) and quantitative data in scientific literature?
- How does this cumulative risk assessment account for high doses of iodine which may adversely affect the thyroid? Although it is counterintuitive, iodine at high doses can also cause IUI.<sup>7</sup>
- How does one evaluate other possible confounders in a cumulative risk assessment such as smoking and diet?

The OIG does address and discuss some of these issues. It is also important to stress that this is not a chemical-specific risk assessment; it is an IUI risk assessment. Nonetheless, the science of a risk assessment is continually improving and, based on U.S. EPA guidance (U.S. EPA, 2007), a cumulative risk assessment does have scientific validity.

## 4.2 Use of Animal Studies

The draft OIG report implies that the NRC committee simply had a preference for human studies over rodent studies, and that the Argus rodent studies on which the 2002 U.S. EPA draft risk assessment is based continue to be relevant to perchlorate risk assessment. The OIG states “due to the scientific controversy surrounding the concentration at which perchlorate should be regulated, the National Academy of Science *Committee to Assess the Health Implications of Perchlorate Ingestion* was charged to assess the current state of the science regarding potential adverse effects of disruption of thyroid function by perchlorate in humans and laboratory animals at various stages of life.”

First, it should be recalled that U.S. EPA’s own guidelines state that human studies, when available and conducted properly, are preferred to evaluate chemical agents for the deriving the RfD. “If adequate human studies (confirmed for validity and applicability) exist, these studies are given first priority in the dose-response assessment, and animal toxicity studies are used as supportive evidence” (U.S. EPA, 1989).

Second, the NRC’s 2005 report clearly stated that “the committee considered several of the animal studies on which U.S. EPA based its point of departure to be flawed in their design and execution. Conclusions based on those studies, particularly the neurodevelopmental studies, were not supported by the results of the studies ...”

Lastly, the NRC further stated “although studies in rats provide useful qualitative information on potential adverse effects of perchlorate exposure, they are limited in their utility for quantitatively

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<sup>7</sup> “The main factor regulating the accumulation of iodide in the thyroid (*i.e.*, NIS activity), other than TSH, has long been considered to be iodide itself. Stated simply, high doses of iodide cause diminished thyroid function. Acute inhibition of organic iodide binding depends on the intrathyroidal rather than the plasma concentration of iodide. The Wolff-Chaikoff effect and the ensuing escape constitute a highly specialized intrinsic autoregulatory system that protects the thyroid from the deleterious effects of I overload but at the same time ensures adequate iodide uptake for hormone biosynthesis. The level of iodide capable of inhibiting iodide organification and concomitantly stopping thyroid hormone synthesis depends on the previous iodide supply status of the animal” (Dohán *et al.*, 2003).

assessing human health risk associated with perchlorate exposure.”

We recommend the OIG consider revising their comments to accurately reflect these facts.

### 4.3 Hypothyroxinemia

The OIG method is in agreement with the NRC analysis (2005) that the most sensitive subpopulations are pregnant women and their fetuses, particularly if they are iodine deficient; although they differ from the NRC conclusions and methods by assuming that the first adverse effect is not maternal hypothyroidism, but hypothyroxinemia.

Hypothyroidism is defined as low free T4 and increased TSH and hypothyroxinemia is characterized by low T4 levels with normal levels of TSH. The OIG reports that although hypothyroxinemia in the mother is a “less stressful thyroid condition,” they opine that effect may be frank hypothyroidism in the fetus as the mother would have “difficulty meeting her own T4 needs and [would be] unable to meet the fetal demand for T4 for proper brain development” (OIG, 2008).

The draft OIG report clearly states that hypothyroidism is an adverse effect from an extremely low uptake of iodide by the thyroid; however, in asserting that hypothyroxinemia and not hypothyroidism is the first adverse effect from perchlorate exposure or low iodide by the thyroid, they appear to be in conflict with the NAS committee conclusions. However, as both assessments evaluate IUI and hypothyroidism in the fetus, we believe the better view is that both the NAS committee and the OIG draft report are correct and that the differences are contextual or a matter of semantics.

There are more recent studies such as Vermiglio *et al.* (2004) and Kooistra *et al.* (2006) that may provide more insight on the issues of hypothyroxinemia even though there are questions of how well iodine sufficiency was evaluated. (Moreover, these studies do not evaluate any IUI or any of the other NIS stressors that the OIG has defined). However, it also appears that it would be useful to consult with clinical thyroid endocrinologists to further elucidate the subtleties of these clinical studies.

Regardless of whether hypothyroidism or hypothyroxinemia are defined as the first adverse effect, both the NRC and the OIG use IUI as a critical biochemical step and if a chemical or chemical does not cause an increase in IUI, then there can be no progression to an adverse effect.

### 4.4 Iodine Deficiency

Iodine sufficiency is an important human health topic. The manner in which iodine sufficiency is measured in people and the determination of what is sufficient are two key subjects that often are not discussed adequately. OIG correctly points out that none of the stressors (nitrate, thiocyanate, or perchlorate) directly cause iodine deficiency. The cause of iodine deficiency is a lack of iodine in the diet which is easily remedied by eating a balanced diet or taking supplemental vitamins with iodine. A cumulative risk assessment for chemicals that inhibit iodide uptake is a useful tool for scientific assessment to determine what concentrations are sufficient to inhibit iodide uptake and what duration is needed to deplete iodide stores that might lead to changes in thyroid hormones.

The manner in which iodine sufficiency is measured is by quantifying the amount of iodine excreted in urine. Iodine levels in urine are highly variable during the day and even day to day. Creatinine

adjustments can be made to account for some of the variability.<sup>8</sup> The standard method for measuring iodine in urine is the collection of urine over a 24-hour period. The advantage of total urine collected over the 24-hour period is that it accounts for iodine excretion and its variability during the day; however, it still cannot account for day-to-day variations which may be significant. The disadvantage and a common reason that it is not conducted is that it requires substantially more effort on the part of the study subjects and the investigators, which translates into more time and resources to conduct the study. Twenty-four-hour urine collections are generally limited to clinical studies, while epidemiological studies rely on the more common, but inferior method of “spot urine collection.” As the term indicates, urine is collected once. Creatinine adjustment and controlling the time of day the sample is collected help to minimize some of the variability, but there remains no method to give an accurate depiction of the status of iodine sufficiency in a single subject.

How is iodine deficiency defined? The World Health Organization (WHO) criteria for assessing iodine status within any given population is that a population’s median urine iodine value based on spot urine iodine measurements is optimal between 100 and 200 µg/liter, with no more than 20% of individual values less than 50 µg/liter (WHO/UNICEF/ICCIDD, 1994). According to WHO criteria, a population is mildly iodine deficient when its median urine iodine based on spot urine samples is between 50 and 99 µg/liter; moderate deficiency is a population median between 20 and 49 µg/liter; and less than 20 µg/liter is severe deficiency (WHO/UNICEF/ICCIDD, 1994).

Given the great variability of iodine content of food in the United States (Pearce *et al.*, 2004; Pennington *et al.*, 1991), it would be unlikely that a given individual would continue to ingest foods with low iodine over an extended period of time. This is confirmed in a one-year study of 15 euthyroid men in Denmark (an area of mild to moderate iodine deficiency) that shows long-term iodine status cannot be determined from a single 24-hour urine measurement (Andersen *et al.*, 2001).

With that basis, we suggest OIG review their assessment. First, the OIG considers a single spot urine iodine test to be useful in assessing an individual’s iodine status. As evidence, the OIG report states that an “approach to avoiding this potential for excess iodide exposure during pregnancy is to simply measure the [urine iodine concentration] UIC during prenatal care. If the UIC is below 150 µg/L, the use of an iodide-containing prenatal vitamin is warranted. If the UIC is above 150 µg/L, the use of an iodide supplementation may not be necessary, and eliminates the potential risk of inducing excess iodide intake.” We agree that this would be an ideal solution if a clinical test was available that accurately assessed an individual’s iodine status.

Second, the OIG should reevaluate the significance of individuals within a population with cross-sectional spot urine iodine levels below a certain cut-off, specifically levels less than 100 µg/L or less than 50 µg/L. The OIG report states that the “NHANES III survey documents that 8.1% of males and 15.1% of females are moderately iodide deficient (*i.e.*, urinary iodide concentrations < 50 µg/L) (Hollowell, 1998). The NHANES III survey identifies that 14.9 % ± 1.2 of the U.S. women of childbearing age are moderately iodide deficient and that 6.9 % ± 1.9 of pregnant U.S. women are moderately iodide deficient (Hollowell, 1998).” Thirdly, the OIG also states the NRC noted that “... distribution of iodide values measured in a spot urine sample is broader than values measured repeatedly in individual subjects (Anderson *et al.*, 2001), this leads to overestimation of the number of subjects with both low and high values (emphasis added; NRC, 2005).” In short, the NRC opinion

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<sup>8</sup> Creatinine is usually produced at a fairly constant rate by the body and actively secreted as fairly constant rate in urine whereas iodine is not.

demonstrates that the percentage of U.S. women of childbearing age and pregnant women who are moderately iodide deficient due to the limitations of a single urine spot test is overestimated. Several published papers by noted researchers have stated agreement with the NRC in recent years as discussed below. Other examples exist.<sup>9</sup>

The comparison of spot and 24-hour urine measurements from three studies (Greer *et al.*, 2002; Tellez *et al.*, 2005; Costeira *et al.*, 2009), found there was no correlation in urine iodine measurement in individuals from one spot urine sample to the next. Overall, it appears (and one would expect) that 24-hour urine excretion measurements are more reproducible than spot urine measurements. Most significantly, urinary iodide levels in subjects from Greer *et al.* (2002) were not different based on perchlorate dose.

#### 4.5 Protein binding by other anions

Proteins present in the serum are able to bind free anions to render the anions biologically unavailable. Even if an individual receives a certain dose or has a measured concentration in the body, if proteins (*e.g.*, albumin) in the serum bind, for example, 50% of the free anion, the effective dose is 50% of the total dose. If it is known that a certain proportion of perchlorate, thiocyanate, and nitrate are all bound to serum proteins, this proportion must be carefully evaluated (*e.g.*, the concentration of the stressor to the thyroid adjusted) from the total dose in the cumulative risk assessment.

The OIG is correct in their assumption that free perchlorate, thiocyanate, and nitrate are the critical entities that compete for iodide transport at the NIS. This critical concept was not included in Tonacchera *et al.* (2004) or in any of the other supporting studies. The OIG report is likely correct in their application of an estimate that thiocyanate is approximately 50% protein bound, but it is less clear that the absence of protein binding factors for nitrate and perchlorate are justifiable. Although there are known significant differences between rodents and humans with respect to protein binding, enough is known regarding protein binding of various anions to estimate parameters for the Tonacchera model.

The 2005 NRC report states that “plasma-protein binding is often a source of substantial species differences in chemical disposition, as was evident for perchlorate. On the basis of the serum data of Greer *et al.* (2000), humans had a lower capacity for binding perchlorate than did rats (for example, the capacity for plasma-protein binding was 500 ng/hr-kg in humans *versus* 3,400 ng/hr-kg in rats),

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<sup>9</sup> Laurberg *et al.* (2007) illustrated this misinterpretation by citing “a recent study of iodine deficiency in Spanish schoolchildren (Serra-Prat *et al.*, 2008). A cross-sectional study of 987 four-year-old children gave a mean UIC of 214 µg/L (median 189 µg/L), which is not low. Nevertheless, it was concluded that 7.8% of the children had iodine deficiency, because 7.8% of urinary samples had an iodine concentration of 100 µg/L.” Similarly, Zimmerman (2008) concluded that “the median UI is often misinterpreted. Individual iodine intakes and, therefore, spot UI concentrations are highly variable from day-to-day, ... and a common mistake is to assume that all subjects with a spot UI <100mg/l are iodine deficient.” Hollowell and Haddow (2007) analyzed the NHANES III (1988-1994) data for women of reproductive age and women who were pregnant. They concluded that in “the NHANES III survey... we believe that an otherwise normal individual may excrete a concentration of iodine <50 µg/L at the time of study, but this value does not necessarily reflect the long-term pattern for that individual.” Most recently, Caldwell *et al.* (2008) analyzed spot UI levels for 2,526 participants, aged six years and older, participating in NHANES 2003–2004. They reported that the “median UI level for the general U.S. population in 2003–2004 was 160 µg/L (95% confidence interval [CI] 146–172), and 11.3 ± 1.8% of the population had a UI level below 50 µg/L. Children had a higher UI level than adolescents and adults. Among all (pregnant and nonpregnant) women of reproductive age, the median UI level was 139 µg/L (95% CI 117–156), 15.1 ± 3.2% women had a UI level <50 µg/L ... These findings affirm the stabilization of the UI level and the adequate iodine nutrition in the general U.S. population since 2000.”

whereas the binding affinities and dissociation constants were similar” (NRC, 2005).

Harris *et al.* (1998) reported that chloride, bromide and perchlorate were all similarly bound to transferrin, a plasma protein that normally binds iron. Merrill *et al.* (2005) noted “reversible binding of perchlorate to nonspecific human plasma proteins has been qualitatively demonstrated in other studies (Carr, 1952). Additionally, the model [in the Merrill *et al.* study] indicated the existence of plasma binding, as without it the model underestimated serum perchlorate at 0.1 mg/kg-day, while simulations at 0.5 mg/kg-day produced adequate fits. Hence, at the lower level, plasma binding represents a larger proportion of the overall amount of serum perchlorate. Serum levels from the 0.02 mg/kg-day dose group were below the detection limit and thus could not be compared to model predictions. The plasma protein affinity for perchlorate was assumed to be similar to that used in the male rat model, given the same proteins (albumin and prealbumin) appear to be responsible (Carr, 1952; Merrill *et al.*, 2003).” Okabe *et al.* (1993) found the effectiveness of thiocyanate, perchlorate, iodine, and bromide in reducing T4 binding was in the following order: thiocyanate > iodine > perchlorate > bromide.

Taken together, the literature suggests the binding of monovalent anions other than thiocyanate does occur. By including a binding constant for thiocyanate, but not for perchlorate or nitrate, the overall perceived risk from thiocyanate may be artificially low. Binding terms for perchlorate and nitrate should be estimated from the literature and applied to the overall OIG model.

#### 4.6 Up- and Down-regulation of NIS Expression

Over the past decade, research regarding thyroid auto-regulation and the regulation of expression of the NIS has proliferated. Evidence suggests that the NIS is up-regulated in response to iodine deficiency in the thyroid. Up-regulation of the NIS would include the number of NIS increasing; thus, when an individual is iodine deficient, the body increases the number of NIS “pumps” such that the thyroid is more effective at capturing iodide. This is a common adaptive mechanism that many tissues, including the thyroid gland, use to maintain hormone homeostasis. The OIG report appears to consider up-regulation of NIS expression, but doesn’t incorporate it into its risk assessment. The OIG report states that “iodide deficiency is frequently and incorrectly associated with hypothyroidism and increased TSH...” and that “TSH-independent auto-regulation is often overlooked or actually not known to younger Western trained physicians ...”

Likewise, the NRC report also acknowledged the up-regulation of NIS expression, but did not incorporate it into their risk assessment. They state that “rats compensated for the inhibition within 5 days of perchlorate administration, most likely by increasing the expression of NIS in the thyroid. The data suggest that compensation occurs more quickly in rats because rats have a smaller reserve capacity of thyroid hormones than humans.”

Dohán *et al.* (2003) provided perhaps the most comprehensive review of the NIS regulation at the time of the NRC report, stating that “up-regulation of thyroid NIS expression and iodide uptake activity by TSH [thyroid stimulating hormone] has been demonstrated not only in rats *in vivo* but also in the rat thyroid-derived FRTL-5 [Fisher rat thyroid cell line] cell line and in human thyroid primary cultures. TSH up-regulates iodide uptake activity by an increase in NIS transcription. “Both NIS mRNA and NIS protein levels decreased significantly after either 1 or 6 days of iodide administration. NIS mRNA levels were already significantly reduced at 6 hours following the injected single dose of iodide. In contrast, a significant decrease of NIS protein levels was detected

only at 24 hours.” After TSH withdrawal, a reduction of iodide uptake activity is observed in FRTL-5 cells. This is a reversible process, as iodide uptake activity can be restored by TSH. The NIS half-life is approximately 5 days in the presence and approximately 3 days in the absence of TSH” (Dohán *et al.*, 2003). Other studies support up- or down-regulation of NIS during changing levels of iodine (*e.g.*, Eng *et al.* (1999)<sup>10</sup>; Wagner *et al.* (2002)<sup>11</sup>; Merrill *et al.* (2003)<sup>12</sup>; Pedraza *et al.* (2006)<sup>13</sup>; Nordén *et al.* (2007)<sup>14</sup>; and Bizhanova *et al.* (2009).<sup>15</sup>

The OIG report would benefit by the inclusion of this information and the incorporation of it into its assessment. For example, the serum binding could be incorporated into the Tonacchera model using free anions *versus* total anions as such:

$$TIU \propto ([I] \times [NIS \text{ expression}]) / (1.22 + ([\text{free perchlorate}] + [\text{free nitrate}]/240 + [\text{free thiocyanate}]/15))$$

Where the concentration of each anion is expressed in  $\mu\text{mol/L}$  and [NIS expression] is a estimation of the total membrane bound NIS receptors in the thyroid at the point in time.

## 5.0 CONCLUSIONS

The OIG analysis confirms that the U.S. EPA’s current RfD of 0.0007 mg/kg-d is conservative and no adverse effects are expected when exposures are below the RfD. The OIG was conservative in the interpretation of the iodine data, NIS up-regulation, and protein binding. The OIG analysis provides more information regarding the most sensitive populations, pregnant women and their fetuses. Although the OIG used a nontraditional method for a risk assessment, the conclusions they reached are consistent with a large body of evidence that has been previously published and reviewed. This evidence shows that no adverse effects occur to people when exposed to environmental concentrations of perchlorate. The OIG also demonstrates that reducing the concentration of perchlorate in drinking water from 24.5 ppb to 6 ppb would result in no meaningful reduction in risk to the public. Overall, the cumulative risk assessment was scientifically-based and an appropriate method for assessing the risk of perchlorate for regulatory decision making. However, there are several topics which we have discussed where the OIG could usefully provide further support for their model and thereby strengthen their arguments.

<sup>10</sup> Eng *et al.* (1999) found that although “serum TSH levels were unchanged ... both NIS mRNA and protein were decreased at 1 and 6 days after chronic iodide ingestion. NIS mRNA was decreased at 6 and 24 h after acute iodide administration, whereas NIS protein was decreased only at 24 h.”

<sup>11</sup> Wagner *et al.* (2002) found that “stimulation of thyrocytes with TSH (0.1-10 U/ml) ... results in a dose- and time-dependent up-regulation of NIS expression reaching a maximum at 10 mU/ml TSH (2211 +/- 761 copies) ... after 24h.”

<sup>12</sup> Merrill *et al.* (2003) studied the inhibition of thyroid <sup>125</sup>I- uptake after drinking water exposure to perchlorate. TSH-induced up regulation of NIS compensated for competitive inhibition of thyroid I- uptake by perchlorate across doses in the drinking water study” (Merrill *et al.*, 2003).

<sup>13</sup> Pedraza *et al.* (2006) reported that their “present data; therefore, confirm an important role of thyroid autoregulatory responses in the efficient adaptation to a mild degree of ID. A possible role of the sodium/ iodide symporter in ID has hardly been addressed experimentally in rats.”

<sup>14</sup> Nordén *et al.* (2007) reported that “TSH increased the NIS expression >100-fold after 48 h and 5- to 20-fold after prolonged stimulation. It was concluded that down-regulation of NIS is the likely explanation for (<sup>131</sup>I)-induced thyroid stunning.

<sup>15</sup> Bizhanova *et al.* (2009) demonstrated that “Thyroid-stimulating hormone (TSH) and iodide regulate iodide accumulation by modulating NIS activity via transcriptional and post-transcriptional mechanisms.”

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