Reference Dose for Perchlorate Based on Thyroid Hormone Change in Pregnant Women as the Critical Effect

Joan Strawson[1], *TERA*

Qiyu Zhao, *TERA*

Michael Dourson, *TERA*

Running head: Human based RfD for perchlorate

[1] Joan Strawson, Toxicology Excellence for Risk Assessment, 1757 Chase Ave., Cincinnati, OH 45223, (513) 542-7475, e-mail strawson@tera.org

Abstract

The most relevant data for developing a Reference Dose (RfD) for perchlorate exposures comes from human epidemiology and clinical studies, supplemented with available and extensive information on experimental animals. Specifically, serum T4 decrease is the critical effect of perchlorate, based on a mode-of-action analysis and the evidence provided by the body of rodent studies on perchlorate. However, no T4 decreases have been observed in human populations following perchlorate exposure at non-therapeutic doses. An RfD of 0.002 mg/kg-day can be derived using an epidemiology study. A freestanding NOAEL of 0.006 mg/kg-day for T4 decrease was identified in children from the epidemiology study. The use of this NOAEL has the advantage of a being identified in a sensitive subgroup, neonates and children. Data are sufficient to estimate an overall uncertainty factor of 3-fold with this NOAEL based on expected differences in toxicokinetics and toxicodynamics between children, and pregnant women and their fetuses, the second identified sensitive subgroup for perchlorate, and concerns about the over-iodination of this population. This RfD is supported by a human clinical study using inhibition of iodine uptake in adults as a measurable surrogate for the critical effect of T4 decrease in humans. However, although this latter study has a well-established dose response curve for inhibition of iodine uptake, even perchlorate doses that result in a 70% inhibition of iodine uptake have no apparent effect on human T4 levels. Thus, the use of this study as the primary basis of the RfD is problematic. Nevertheless, a benchmark dose of 0.01 mg/kg-day was identified in this clinical study, which supports a threshold value of 0.006 mg/kg-day identified by its authors and the RfD of 0.002 mg/kg-day estimated in this paper.

Keywords: perchlorate, reference dose, RfD, human, TSH

1. Introduction

Over fifty years ago, Stanbury and Wyngaarden (1952) and Wyngaarden et al. (1952) reported the inhibitory effect of perchlorate upon the accumulation and retention of iodide by the human thyroid gland. Such an observation had immediate therapeutic application. Treatment of thyrotoxicosis (including Graves' disease) with 600–2000 mg potassium perchlorate (430–1400 mg perchlorate) daily for periods of several months or longer was once common practice, particularly in Europe (Morgans and Trotter, 1960; Barzilai and Sheinfeld, 1966). According to Wolff (1998), seven case reports of fatal aplastic anemia between 1961 and 1966 curtailed the therapeutic use at that time. However, two decades later there were reports of successful treatment of thyrotoxicosis in the absence of adverse effects, using lower maintenance doses of potassium perchlorate (40–200 mg/day) for durations of 2 years or longer (Connell, 1981; Wenzel and Lente, 1984). More recently, perchlorate has been used (alone or in combination with other anti-thyroid drugs) to treat amiodarone-induced thyrotoxicosis, a condition in which thyroid abnormality results from excess iodine when the iodine-containing drug amiodarone is given to control cardiac arrhythmia. Treatment regimens include potassium perchlorate at 500 mg twice per day for 18-40 days (Bartalena et al., 1966) and for mild cases, 250 mg/day for 4-6 weeks (Loh, 2000).

In addition to these therapeutic applications, perchlorate compounds have been widely used as solid rocket propellants and ignitable sources in munitions and fireworks. Currently, and not surprisingly, the ammonium salt of the perchlorate ion is manufactured

for use by the Department of Defense, the National Aeronautics and Space Administration, and the aerospace industry. It is also manufactured for use as an oxidizer in fireworks and matches. Furthermore, perchlorates are laboratory waste by-products of perchloric acid. Perchlorate also occurs naturally in nitrate-rich mineral deposits used in fertilizers. Analysis of nine commercial fertilizers revealed perchlorate in all samples tested ranging for 0.15 to 0.84% by weight (Susarla et al., 1999).

Due in part to improved analytical methods, perchlorate has been detected in surface water and groundwater near various facilities that have manufactured and tested solid rocket fuels, most notably in California, Nevada and Utah. These advances in analytical chemistry have identified perchlorate in the public drinking water supply in several areas in California (http://www.dhs.cahwnet.gov/ps/ddwem/chemicals/perchl/perchl.htm) and in Lake Mead in Nevada (U.S. EPA, 1998). The current detection limit for perchlorate in water is 4 ppb. Perchlorate has been detected in Lake Mead and the Colorado River at levels of 4-16 ppb and has been detected in 38 California public water supply wells at concentrations greater than the provisional action level of 18 ppb (U.S. EPA, 1998). This environmental occurrence coupled with perchlorate's known mobility and persistence has elevated regulatory concern regarding the compound's health effects, particularly those related to the thyroid gland. U.S. EPA (2002a) and California EPA (Ting et al., 2001) have both released draft toxicity assessments on perchlorate in preparation for developing a drinking water standard.

In 1997, Toxicology Excellence for Risk Assessment (*TERA*) convened an independent peer review panel to evaluate the suitability of the perchlorate database for developing a reference dose (RfD) for chronic environmental exposure by the oral route. The panel concluded that the database at that time was insufficient (see http://www.tera.org/Perchlorate/eleven.htm for a report of that meeting). Since that time, an extensive battery of studies has been conducted and either published or submitted to regulatory agencies in order to support risk assessment activities for perchlorate. Available animal studies include developmental neurotoxicity (Argus, 1998), 90-day rat toxicity (Siglin et al., 1998), rabbit developmental toxicity (York et al., 2001a), rat developmental toxicity (Argus, 2001), rat two-generation reproductive toxicity (York et al., 2001b), developmental brain morphometry in rats (Argus, 2001), developmental motor activity in rats (Bekkedal et al., 2000), mutagenicity/genotoxicity (San and Clarke, 1999; Sharma and Gao, 1998), and a variety of predictive immunotoxicity assays in both mice (Keil et al., 1999) and rats (Burleson, 2000) all conducted under current U.S. EPA guidelines. In addition, the kinetics of perchlorate has been extensively studied in male rats, pregnant and lactating rats, and fetal rats, and to a lesser extent in humans, leading to the development of kinetic models in humans (Merrill et al., 2003). Several human studies have been published as well, including occupational studies (Gibbs et al., 1998; Lamm et al., 1999), epidemiology studies in neonates and/or school age children (Crump et al., 2000; Lamm et al., 1999; Li et al., 2000a,b; Li et al., 2001; Brechner et al., 2000), and clinical studies in adults (Greer et al., 2002; Lawrence et al., 2000, 2001).

The database is now sufficient to allow the development of a high-confidence reference dose for perchlorate. This paper will discuss the identification of a critical effect, selection of a critical study, benchmark dose analysis for estimating a point-of-departure, and selecting appropriate uncertainty factors for a perchlorate RfD.

2. Methods

One risk assessment goal is to determine what exposure might be considered "safe." "Safe" or subthreshold doses are defined by a number of health agencies worldwide. Although many of the underlying assumptions, judgments of critical effect, and choices of uncertainty factors are similar among health agencies in estimating these subthreshold doses, this report will follow U.S. EPA's Reference Dose (RfD) methods (Barnes and Dourson, 1988; Dourson, 1994; U.S. EPA, 2002b).

The first step in defining the RfD is to identify the critical effect(s). U.S. EPA (2003a) and Haber et al. (2001) define critical effect(s) as the first adverse effect(s), or its known precursor, that occurs as dose rate or exposure level increases. In the determination of critical effect, it is crucial that distinctions be drawn between adverse effects and adaptive effects. An adaptive effect enhances an organism's performance as a whole and/or its ability to withstand a challenge; an adverse effect is a biochemical change, functional impairment, or pathological lesion that impairs performance and reduces the ability of an organism to respond to additional challenge (Barnes and Dourson, 1988; U.S. EPA, 2003a). Available animal studies as described above clearly suggest that the thyroid is the primary target organ for perchlorate. Thus, distinguishing adaptive from adverse effects in the thyroid and determining the most appropriate adverse effect on which to

base an RfD is the first, and perhaps most important step, in any perchlorate risk assessment.

The second and third steps in the determination of an RfD are the choice of appropriate species and study, and the point of departure. For this evaluation, we also used U.S. EPA methods as cited above, including a review of existing experimental animal and human data and the use of Benchmark Dose (BMD) for endpoints where this modeling was possible.

The fourth step in the determination of an RfD is the judgment of the appropriate uncertainty factor based on a review of the information supporting the choice of critical effect, and issues associated with extrapolation from experimental animals to humans and to sensitive humans. As before, we used U.S. EPA methods describing five potential areas of uncertainty for this judgment.

3. Results

3.1. Step 1: Identification of Critical Effect

Two lines of reasoning contribute to the identification of critical effect. First, a chemical's mode of action can be evaluated to identify key events that are required for toxicity to be expressed. Second, the empirical data can be evaluated to identify those effects that occur at the lowest doses.

3.1.1. Mode of Action Analysis

Perchlorate, like many chemicals and drugs, disrupts one or more steps in the synthesis and secretion of thyroid hormones, resulting in subnormal levels of T4 and T3 and an associated compensatory increase in secretion of TSH (Capen, 1997). Because of its chemical properties, perchlorate is a competitive inhibitor of the process by which iodide, circulating in the blood, is actively transported into thyroid follicular cells (Wyngaarden et al., 1952; Stanbury and Wyngaarden, 1952). The site of this inhibition is the sodium-iodide symporter, a membrane protein located adjacent to the capillaries supplying blood to the thyroid (Carrasco, 1993). The thyroid follicle is the functional unit of the thyroid.

If sufficient inhibition of iodide uptake occurs, formation of thyroid hormones is depressed. Thyroid hormones are essential to the regulation of oxygen consumption and metabolism throughout the body. Thyroid iodine metabolism and the levels of thyroid hormone in serum and tissues are regulated by a number of fairly well understood homeostatic mechanisms (Greenspan, 1997). Thyrotropin (TSH), a hormone synthesized and secreted by the anterior pituitary gland is the primary regulator of thyroidal iodide uptake and other aspects of thyroid function (Scanlon, 1996). There are five steps associated with the synthesis, storage, release and interconversion of thyroid hormones. They are 1) the uptake of iodide by the gland, 2) the oxidation of iodide and the iodination of tyrosyl groups of thyroglobulin, 3) the conversion of iodotyrosyl residues to iodothyronyl residues within the thyroglobulin, 4) the proteolysis of the thyroglobulin and the release of thyroxine (T4) and triiodothyronine (T3) into the blood and 5) the conversion of thyroxine to triiodothyronine in peripheral tissues.

Inhibition of iodine uptake is the basis for the current and former pharmacological uses of perchlorate and the likely precursor of potentially adverse effects. Subsequent events include decreases in serum T4 (and T3), leading to the potential for altered neurodevelopment if observed in either dams or fetuses/neonates, and increases in serum TSH, leading to the potential for thyroid hyperplasia and tumors. The repeated observation of thyroid effects such as alterations of hormones, increased thyroid weight, and alterations of thyroid histopathology (including tumors) from a large number of rat studies on perchlorate (as cited above) provide supporting evidence for the proposed mode-of-action, and confirms that the perturbation of thyroid hormone economy as the primary biological effect of perchlorate.

However, the key decision for any perchlorate risk assessment is distinguishing adaptive from adverse effects. Because so much is now known about the disruption of thyroid physiology by exogenous toxicants, a model for mode-of-action has been proposed (U.S. EPA, 2003b) for the perchlorate relationship with the thyroid gland, which is presented in Figure 1. This figure provides a tool for evaluating and identifying adaptive and adverse effects for developing a perchlorate RfD. Following oral exposure, in drinking water, serum perchlorate levels increase and provide a measure of the perchlorate internal dose. In humans, drinking water exposure to perchlorate at doses of 0.5 mg/kg-day, resulted in serum peak perchlorate levels of 871 ug/L (Greer et al., 2002). In female rats, drinking water exposure to perchlorate doses of approximately 1 mg/kg-day resulted in serum peak perchlorate levels of 953-964 ug/L on gestation day 20 (Argus, 2001; Yu et al.,

2002); 241 ug/L on postnatal day 5 (Yu et al., 2002), and 886 ug/L on postnatal day 10 (Argus, 2001). Serum perchlorate peak concentrations were calculated based on the perchlorate pbpk models developed by Department of Air Force, Air Force Research Laboratory (Merrill, personal communication).

Using Figure 1 as a model, inhibition of iodine uptake in thyroid, the key event in the ultimate disruption of thyroid function, can be considered as a marker of the biologically effective dose for perchlorate. However, inhibition of iodine uptake, itself, cannot be considered an adverse effect because in humans we do not yet know what levels of iodine uptake inhibition would decrease T4 levels. For example, Figure 2a demonstrates that in humans (Greer et al., 2002; Lawrence et al., 2000, 2001), there is a clear and apparently linear relationship between serum perchlorate levels and inhibition of iodine uptake. Serum perchlorate levels of approximately 15 ug/L result in a minimal inhibition of iodine uptake of about 2% compared to serum perchlorate levels of 871 ug/L which result in about 70% inhibition of iodine uptake. In contrast, Figure 2b summarizes several human studies of differing exposure durations in which serum T4 levels do not change after perchlorate exposure resulting in serum perchlorate levels up to 20,000 ug/L. Figures 2a and 2b suggest that even at serum perchlorate levels that result in significant inhibition of iodine uptake, no decreases of serum T4 have been measured in people (Greer et al., 2002; Lawrence et al., 2000, 2001; Gibbs et al., 1998; Lamm et al., 1999). Additional work could be done on this point, however, since only two short term studies monitored both the inhibition of iodine uptake and the status of thyroid hormones within the same experimental protocol.

Following Figure 1, alteration of hormone levels, including decrease of serum T4 and T3 with a corresponding increase of TSH, is considered to be the early biological effect of exposure to perchlorate. Should these hormone effects be considered adaptive or adverse for thyroid hormone function? The human body has a large reserve capacity of circulating thyroid hormone; serum levels of T4 and T3 are highly variable. Normal levels of T4 are 5-12 ug/dL or 65-156 nmol/L (with free T4 being in the range of approximately 2 ng/dL); T3 levels are 0.08-0.22 ug/dL or 1.2-3.3 nmol/L. No clear-cut information is available on how much decrement of circulating serum T4 can be tolerated without resulting in permanent alteration of thyroid function. However, subclinical hypothyroidism is generally considered to be present when circulating TSH levels are elevated by 2-fold, with, or without decreased levels of T4 (University of Nebraska, 2003).

These hormones also affect neurological development. For example, Schwartz (personal communication) indicates that while T4 is the predominant hormone secreted from the thyroid, T3 is the more active hormone at the tissue and nuclear level. T3 in both human and rat is produced locally in the brain by monodeiodination of T4. In brain, the enzyme type II-5' deiodinase (5'D-II) is primarily responsible for this process. The 5'D-II activity is regulated by the intrabrain T4 levels so that a fall in T4 leads to an increase in enzyme activity and compensates for the diminished serum T4 seen in conditions such as hypothyroidism. In the normal adult rat brain, as much as 80% of the receptor-bound T3 in the cerebrum and 70% in cerebellum may be generated by local production of T3.

Therefore, it appears that there can be a significant decrease in serum T4 levels before local production of T3 in the brain is compromised. Calvo et al. (1990) demonstrated that in rat fetuses of dams treated with methimazole (a drug that prevents the organification of iodine, thus inhibiting the synthesis of T4), infusion of T4 to the dam results in fetal brain T3 that is normalized when there is a 60% decrease of plasma T4. These data would suggest that a decrease in serum T4 would not be adverse until there is a 60% decrease from normal.

Following Figure 1, prolonged alteration of hormones will ultimately result in altered structure and function of the thyroid. While intimately linked in the cascade associated with thyroid hormone physiology, sustained increase in TSH and decrease in serum T4 have very different outcomes as they relate to human risk assessment. In examining and ultimately defining which of the two represents the critical effect, it is important to consider which event is most relevant to human public health. Increased TSH results in thyroid hypertrophy, leading to hyperplasia and possibly tumor formation. Decreased serum hormone levels (T4 and T3) have been linked to altered neurodevelopment. A closer examination of both is shown below.

3.1.1.1. Thyroid Hyperplasia – Tumor formation occurs in rats as a result of continuously increased TSH. Capen (1997) has noted that many chemicals and drugs disrupt one or more steps in the synthesis and secretion of thyroid hormones, resulting first in subnormal levels of T4 and T3, and then a subsequent increase in the secretion of pituitary TSH. In rodents, these compounds result in a progression of effects marked by

early follicular cell hypertrophy, follicular cell hyperplasia and increased thyroid weights, which progresses to an increased incidence of thyroid tumors (typically follicular cell adenomas) following long-term elevation of TSH. In its policy on assessing thyroid follicular tumors, U.S. EPA (1998) notes, "that the consequences of long-term antithyroid action [in humans] are harder to interpret and controversy exists whether the enlarged human thyroid gland undergoes conversion to cancer. Thyroid enlargements and nodules have been implicated as possible antecedents to thyroid cancer in humans, but direct evidence of conversion of these lesions to cancer is lacking." Although it is clear that thyroid tumors are a potential health hazard for rodents following perchlorate exposure, it is not clear that this endpoint is relevant to humans. Therefore, we judge that a human health risk assessment should not be based on observation of tumors in rodent studies.

3.1.1.2. Neuropsychological Development – The observation of cretinism in neonates with congenital hypothyroidism has lead to a body of research on the role of thyroid hormones on the proper neurodevelopment of the fetus and neonate. Cretinism is a severe and clinically obvious problem characterized by defective physical and neurological development of children (Cao et al., 1994). Thyroid insufficiency due to the lack of iodine in the diet has lead to cretinism (Cao et al., 1994) spastic motor disorders, deaf mutism, and severe hypothyroidism (Hollowell and Hannon, 1997). Dietary insufficiency can also lead to impaired intellectual development in apparently normal adults (Boyages et al., 1989). Recently, Haddow et al. (1999) suggested that hypothyroidism in pregnant women adversely effects their children's subsequent performance on neuropsychological tests. The Haddow study prompted Morreale de

Escobar et al. (2000) to conduct a comprehensive review of the literature with the primary aim of clarifying whether the principal factor leading to poorer neurodevelopment of the child is maternal hypothyroidism or maternal hypothyroxinemia (decreased T4) *per se* whether or not TSH is increased. The review examined three different types of studies including 1) Reports from human populations featuring severe Iodine Deficiencies (ID), 2) Studies from human populations without severe ID and 3) Studies performed with experimental animals – presumably with relevance for humans. Morreale de Escobar et al. (2000) developed and submitted what they called a unified hypothesis for the three groups examined. This hypothesis stated that despite the mechanism(s) involved, epidemiological and experimental studies strongly support hypothyroxinemia early in gestation (affecting the availability of T4 and consequently T3 to the developing brain) as the main factor relating maternal thyroid function to poor neurodevelopmental outcome of the progeny, whether or not TSH is increased.

Although studies in humans suggest that decreased maternal T4 can result in neurodevelopmental deficit in fetuses, the available animal studies have not confirmed that maternal perchlorate exposure results in neurodevelopmental deficit in neonates. In a neurodevelopmental toxicity study of perchlorate in rats, no statistically significant changes were observed in any measure of neurotoxicity (Argus, 1998). These results were repeated in a follow-up study of similar design that only measured motor activity in rat pups born to dams with perchlorate exposure (Bekkedal et al., 2000). In both studies it appears rat pups from the perchlorate-treated groups may have altered habituation compared to controls (in later periods of the test session the activity in the treated animals

does not decrease to the level that it does in the untreated animals). While both studies observed these effects, they occurred in different genders and at different ages in each study. And, in fact, in male pups at age 14 days, the Argus study found increased habituation, while the Bekkedal study found decreased habituation. Therefore, it is not clear whether the effects were caused by perchlorate exposure. However, the efficacy of these neurotoxicity studies is controversial (University of Nebraska, 2003). Although, mechanistic data support that neurotoxicity is unlikely at exposures that do not result in a reduction of T4, changes in neurobehavior would not be unexpected in rats at high enough perchlorate exposure. In addition, some mechanism of direct perchlorate interaction with the nervous system might be possible, although available data to date do not suggest that this is occurring.

The mode of action analysis suggests that alteration of hormones (T4, T3, TSH) would be the first observed biological effect of perchlorate exposure. Following a prolonged increase in TSH, thyroid hyperplasia progressing to thyroid tumors would be expected to occur in rodents. However, the relevance of these tumors to humans has been questioned, since this progression has not been observed in humans (Hill et al., 1989). In contrast, human data show that decreased T4 levels, both in pregnant women and in neonates, can lead to neurodevelopmental deficit; although this has not been confirmed in animals following perchlorate exposure. Therefore, of the two pathways to altered structure and function proposed by a mode-of-action analysis for perchlorate, decreased T4 leading to potential neurodevelopmental effects is more relevant to an assessment of human health and should be considered the critical effect.

3.1.2. Evaluation of the Empirical Data

The traditional risk assessment approach to identifying the "critical effect(s)" is to examine the body of data to determine which adverse effect, or its precursor, occurs at the lowest dose, and then to determine whether this effect is relevant to humans. In the body of human studies, described in more detail in the next section, the highest doses of perchlorate evaluated had no effect on hormone levels. Therefore, the human data cannot be used to confirm the critical effect proposed by the mode-of-action analysis. However, several studies of perchlorate in rodents have been conducted in which hormone measurements and thyroid histopathology have been evaluated. Data are available in male and female rats following 14 and 90 days of exposure (Caldwell et al., 1996; Siglin et al., 1998), female mice following 90 days of exposure (Keil et al., 1999; Narayanan, 2000), rat dams on gestation day 20, post natal day 5, postnatal day 10 (Argus, 2001; Yu, 2000;Yu et al., 2002), and male and female pups on post natal days 5, 10, and 22 (Argus, 2001; Yu, 2000;Yu et al., 2002). In order to facilitate a comparison of all of the available animal data, we plotted T4, TSH, and thyroid histopathology data from all studies as a function of percent change relative to the control animals in each study. These values are plotted against administered dose. Figures 3a, b, and c show T4, TSH, and thyroid hyperplasia, respectively, in females following 90 days of exposure. Figures 4a, b, and c demonstrate the same data in dams, and Figures 5a, b, and c show the same data in pups. These figures represent the primary differences among animals at different life stages. That is, dams and pups were selected to illustrate the responses of the likely sensitive subpopulations; non-pregnant female rats were selected for comparison purposes. T4 and

TSH were selected to demonstrate the spectrum of hormone responses to perchlorate exposure; thyroid hyperplasia was included for comparison and to illustrate that effects later in the progression occur at higher doses. We invite other risk assessors to look through all of the available data to make their own judgments on comparison of relevant endpoints. These data can be viewed at http://www.tera.org/Perchlorate/welcome.htm#compare.

From Figures 3, 4, and 5, some key conclusions can be drawn. First, alteration of T4 and TSH following perchlorate exposure is highly variable. In some studies, perchlorate doses as low as 0.01 mg/kg-day resulted in significant decreases of T4 or increases in TSH, while in other studies, no effects on T4 or TSH were observed at any dose. It also interesting that even within a single study no consistent pattern of effect was observed – a dose that caused significant decrease of T4 may have no effect on TSH and vice versa. However, in all studies, although hormone levels were altered at doses ranging from 0.01 to 1 mg/kg-day, statistically significant thyroid hyperplasia was not observed until perchlorate doses at or greater than 1 mg/kg-day were achieved.

From Figures 3, 4 and 5, it is also clear that decreased T4 in dams on GD 20 and TSH increase in dams on GD 20 or PND 5 are the most sensitive responses to perchlorate exposure. These hormones respond at lower doses in pregnant rats than other animals, and the dose-response curves are steeper for pregnant rats than other animals. In pregnant dams, a T4 decrease to between 90 and 60% of control occurs at doses between

0.01 and 0.1 mg/kg-day and is near 50% of control at perchlorate doses between 1 and 10 mg/kg-day.

3.1.3. Conclusions of Critical Effect Analysis

Based on a mode-of-action analysis, it is clear that altered hormone levels are an early biological effect of perchlorate exposure. If allowed to persist, increased TSH levels, at least in rodents, will eventually lead to thyroid hyperplasia and possible thyroid tumors. Even if this pathway is not relevant to humans, persistent decreases in T4 levels increase the potential for neurodevelopmental deficits in children. In this case, decreased T4 can be considered to be a precursor to an adverse effect, rather than an adverse effect in itself, however, because changes in T4 are routinely compensated by normal, and well understood, homeostatic processes. Finally, based on data in animals, it appears that pregnant animals respond with decreased T4 levels at lower doses and with larger T4 decrement than other animals (see Figures 3, 4, and 5).

Therefore, decreases in serum T4 in the pregnant population should be considered to be the critical effect most relevant to human health, based on both an analysis of mode of action, and an evaluation of the empirical data that indicates this occurs at the lowest doses. By developing a RfD based on the critical effect of decreased serum T4, all subsequent potential adverse effects, including controversial results from the experimental animal neurotoxicity tests, will be prevented. This choice of endpoint as the critical effect is essentially the same as the recommendations of a recent symposium on perchlorate science (University of Nebraska, 2003).

3.2. Step 2: Choice of Appropriate Species and Study

The available data on the effects of perchlorate in experimental animals consistently points to thyroid disturbance as the sentinel effect. This disturbance may lead to subsequent thyroid and neurological damage. This information in experimental animals is consistent with the available, but more limited, human data. However, these data also demonstrate that rats may respond to perchlorate exposures in a very different manner than humans, as shown by a quick comparison of Figures 2b and 3a, 4a, or 5a. The reason that such comparisons are not definitive is that the human data do not include information on pregnant individuals. In general, using human data as the basis for developing a RfD for perchlorate will reduce the uncertainty inherent in extrapolating from the rat data. Although the rat data set includes the sensitive subgroup (the pregnant animal and its fetus), whereas the human studies only include measurements of TSH and T4 in adults, infants and children (and not pregnant individuals), rats are known to be more sensitive than humans to thyroid hormone replacement therapy, needing 10 times more T4 than humans to achieve a euthyroid condition (Capen, 2001). Because we feel that the overall uncertainty in determining an RfD is greater from the rat data, when compared with the human data, we judge that the human data are the appropriate choice for determining an RfD. This choice follows standard U.S. EPA guidance.

Since perchlorate has become a public health issue, several human studies have been published, including several epidemiological studies (Crump et al., 2000; Lamm et al., 1999; Li et al., 2000a,b; Li et al., 2001; Brechner et al., 2000), two occupational studies

(Gibbs et al., 1998; Lamm et al., 1999), and two clinical studies (Greer et al., 2002; Lawrence et al., 2000, 2001). The epidemiology studies have examined thyroid endpoints, including congenital hypothyroidism and T4 and TSH levels, in neonates born in areas known to have perchlorate in the public water supply compared with infants born in areas without perchlorate in the public water supply. Another study has compared the prevalence of thyroid disease in Medicaid users in counties with perchlorate exposure through drinking water compared to Medicaid users in counties without perchlorate exposure. All studies, except Brechner et al. (2000), showed that perchlorate had no effect on thyroid parameters. Brechner et al. (2000) found that infants in counties with perchlorate in drinking water had elevated TSH levels when measured by an analysis of variance on the log-transformed TSH values (P=0.017), but not when measured by t-tests for each day of birth separately. The occupational studies evaluated the thyroid function of workers in perchlorate production facilities. No effect on thyroid function was observed in workers after a single shift, or after a working lifetime. Lifetime exposures were up to 0.5 mg/kg-day. Clinical studies in human volunteers identified doses of perchlorate that inhibit iodine uptake. However, even the highest doses tested (up to 0.5 mg/kg-day) had no effect on thyroid parameters after 14 days of exposure.

Of the available human studies, one clinical study (Greer et al., 2002) and one epidemiology study (Crump et al., 2000) were considered to yield sufficient information to determine an RfD. In order to assess the health effects of perchlorate in healthy humans, Greer et al. (2002), administered perchlorate in drinking water at doses of 0.007, 0.02, 0.1, and 0.5 mg/kg-day to 37 male and female volunteers for 14 days. Iodine

uptake was measured in test subjects prior to exposure, and on exposure days 2 and 14. Serum levels of T3, T4, and TSH were measured periodically through out the study. Baseline values of hormone levels and iodine uptake were collected before exposure, so each subject served as his own control. This well-conducted study underwent a rigorous quality assurance audit and conforms to the "Common Rule," the Federal Agency Guidelines on the ethical conduct of human studies (*TERA*, 2002).

Even at the highest dose tested, the Greer study observed no statistically significant effects in serum T4, T3, or TSH. Although, when serum T4 and TSH are plotted against serum area under the curve (AUC) values predicted by the human pbpk model (Merrill, 2001), there was a non-significant trend toward decreasing TSH and increasing T4 levels with dose – an observation that has been observed in other human studies, but one that is in the opposite direction to the expected effect of increasing perchlorate exposure. In keeping with the mode-of-action analysis, and the designation of decreased serum T4 as the critical effect leading to the potential for neurodevelopmental effects, this study defines a NOAEL of 0.5 mg/kg-day for the healthy adult human population for short term exposure.

In 2000, Crump et al. reported on a study to test the hypothesis that perchlorate in drinking water suppresses thyroid function in 9784 newborns and 162 school aged children as demonstrated by increased TSH or decreased free thyroxine. The study was conducted in Northern Chile, which has naturally occurring perchlorate in the drinking water. The city of Taltal has high concentrations of perchlorate (100-120 ug/L, estimated

dose of 0.006 mg/kg-day[2]) in drinking water compared with most areas of the United States and it has had a consistent source of water from the same wells since 1970. Chanaral and Antofagasta have low (5-7 ug/L) and non-detectable (<4 ug/L) perchlorate concentrations, respectively. These cities were selected as comparisons populations because of their proximity and similarity to Taltal.

In a currently ongoing follow-up study, serum from the population of school-age children is being evaluated for perchlorate levels, to ensure that the children were, in fact, exposed to perchlorate. Serum of school age children in Taltal had perchlorate levels that ranged from 2.5 to 9.0 ug/L, with a mean of 5.6 ug/L. Perchlorate was not detectable in the serum of school age children from Chanaral and Antofagasta (Gibbs, 2003). These measurements are consistent with that found in adults in the Greer et al. (2002) study, where perchlorate serum concentrations were approximately 10 ug/L at a dose of 0.007 mg/kg-day (see Figure 2a).

The Crump et al. (2000) study found no evidence that perchlorate in drinking water at concentrations as high as 120 ug/L suppressed thyroid function in newborns or school-

[2] This value is based on average Taltal exposure of 0.112 mg/L (i.e., 112 ug/L) and a drinking water consumption of 1.5 L per day for a 28 kg child (i.e., 0.112 mg/L x 1.5 L/day / 27.5 kg = 0.006 mg/kg-day). Body weights were measured by the study authors; the drinking water consumption value is the 95th percentile of drinking water consumption for 7-year old children (U.S. EPA, 1999). Use of other water consumption assumptions, for example the 50th or 90th percentile water consumption, or consumption based on body weight would not change the NOAEL or resulting RfD by more than 3-fold. In addition, ongoing work on part of this population may enable a different, and perhaps more credible, dose to be estimated, using assumptions related to creatinine clearance (Gibbs, 2003). Furthermore, an ongoing study by Tellez et al. (2003) is measuring perchlorate consumption and serum values directly in pregnant women.

aged children. In the school children (mean age 7.3 years), 127 of whom had lifelong residence in their respective cities, mean TSH, T4, and T3, were similar among the three cities. Incidence of goiter in the lifelong residents was similar in all three cities; although the residents in Taltal self-reported a higher incidence of family history of thyroid disease. A variable introduction of iodized salt started in 1982 and may have affected these observations. Free T4 was significantly increased in children living in Taltal and Chanaral, compared with Antofagasta, a change in the opposite direction than expected. Crump et al. (2000) also studied newborns screened for hypothyroidism by a heel-stick blood sample between February 1996 and January 1999 in the same three Chilean cities. TSH levels were significantly lower in Taltal than in the other two cities, a trend opposite to that hypothesized. The authors concluded that the differences did not appear clinically significant.

One issue to address in the use of this study as a basis of an RfD is the apparent iodine excess when compared with other populations, such as the U.S. For example, Table 1 shows a comparison of urinary iodine concentrations[3] between the Chilean school children and 6 to 11 year old children in the U.S. A 1 to 2.5-fold excess in urinary iodine seen in the Chilean school children may serve to protect this population from perchlorate exposure.

[3] According to John Dunn (2003), a comparison of urinary concentrations is more informative than comparisons based on other measures, such as urinary creatinine, since the latter value is dependent on the nutritional status among populations.

3.3. Step 3: Point-of-Departure Analysis

Following accepted risk assessment approaches, a point-of-departure analysis establishes the threshold dose that serves as the starting point for developing the RfD. Traditionally, the point of departure for a RfD has been the No Observed Adverse Effect Level (NOAEL), which is the highest experimental dose that is without adverse effect. More recently, risk assessors have attempted to incorporate more of the data about the dose response curve by using benchmark dose (BMD) modeling. BMD modeling uses quantitative dose response models to estimate the dose that results in a specified change (such as 10%) in the critical effect, or its precursor.

No human study involved exposures high enough to cause a decrease in T4; therefore, all of the human studies can be said to have identified "freestanding NOAELs" for the critical effect. The highest NOAEL identified in the body of human studies is approximately 0.5 mg/kg-day. This dose was achieved in workers exposed for an average of 8 years (Gibbs et al., 1998; Lamm et al., 1999) and in healthy adults exposed for 14 days in a clinical study (Greer et al., 2002). The lowest NOAEL observed in a human study (Crump et al., 2000) is an estimated NOAEL of 0.006 mg/kg-day (actual exposure is an average of 0.112 mg/L) measured in school-age children who had been exposed in utero and for their entire lifetime (about 7 years). Because, these children were exposed in utero and as neonates, the NOAEL from this study is a freestanding NOAEL in a sensitive population. Therefore, a NOAEL of 0.5 mg/kg-day could be a reasonable point-of-departure for the general human population, while 0.006 mg/kg-day could be a reasonable point-of-departure for a sensitive human population.

However, use of a freestanding NOAEL does incorporate some uncertainty into the risk assessment because the true threshold for the critical effect has not been identified. In other words, the true threshold, or true NOAEL, is likely to be higher than the NOAEL used as the point-of-departure. For this reason, we explored the use of BMD modeling and NOAEL surrogates to use for the point-of-departure. The hormone data from the human studies are not amenable to BMD analysis because, at the doses evaluated to date, the hormone levels in human studies do not change in response to increasing dose.

However, the Greer et al. (2002) study adequately characterizes the dose-response curve for inhibition of iodine uptake in humans. This effect of perchlorate is a key event of the mode of action because it is the essential step in the cascade leading to adverse effects. Without inhibition of iodine uptake, there will be no alteration of T4 or TSH or subsequent adverse effects on neurological development and thyroid hyperplasia. Therefore, a point-of-departure based on inhibition of iodine uptake is a health-protective surrogate that can be used to replace a freestanding NOAEL for decreased T4. The lowest dose evaluated by Greer et al. (2002), 0.007 mg/kg-day, did not cause a statistically significant inhibition of iodine uptake. Based on a regression analysis taking into account the variability of the experimental population, the authors predicted that the dose that would result in 0% inhibition of iodine uptake is 0.0064 mg/kg-day; the 95% upper confidence limit on iodine uptake inhibition at this dose is 8.3%. Greer et al. (2002) concluded that an iodine uptake inhibition less than 10% would not be

biologically significant. This threshold of 0.006 mg/kg-day is a reasonable point-of-departure for estimating a RfD.

However, this threshold was also compared to a BMD analysis of the I uptake inhibition data to estimate a conservative point-of-departure. For the data of Greer et al. (2002), three models were used to develop BMDs and their 95% lower limits (BMDLs). (Note, information on BMD model results from experimental animal studies are available at http://www.tera.org/Perchlorate/welcome.htm) Currently, insufficient data exist to adequately define the level of iodine uptake inhibition in humans that can be tolerated for a lifetime without altering serum T4 and TSH levels. Greer et al. (2002) demonstrated that for 14-day exposure, inhibition of iodine uptake up to about 70%, has no effect on serum T4 or TSH. Occupational studies (Gibbs et al., 1998; Lamm et al., 1999) demonstrated that workers exposed to perchlorate for several years demonstrated no altered T4 or TSH serum levels. When the serum hormone levels from these studies are plotted against serum perchlorate AUC predicted by the human pbpk model, it can be seen that chronic exposure in workers had no effect on serum T4 or TSH at serum AUC values that resulted in approximately 50% I uptake inhibition (this is seen by an overlay of Figures 2a and 2b). Thus, it might be reasonable to conclude that an appropriate benchmark response would be the perchlorate dose that resulted in a 50% inhibition of iodine uptake. Nonetheless, benchmark response levels of 10%, 15%, and 20% inhibition of iodine uptake were modeled in order to be public health protective and take into account the uncertainties involved in extrapolating data from healthy adults to potential sensitive populations such as iodine deficient people, pregnant women, and neonates.

Specifically, the 15% and 20% inhibition levels were included as a comparison and in recognition of the fact that humans appear to tolerate a large inhibition of iodine uptake without effect on thyroid hormone levels.

The Hill and Power models successfully modeled the data, whereas the polynomial model failed. The Power model gave a goodness-of-fit value of 0.57, indicating good fit. The Hill model was unable to provide a goodness-of-fit analysis because of too few degrees of freedom; however the Hill model gave a good visual fit. Modeling results are presented in Table 2. At 10% inhibition, there is a slight difference in BMDL values between the two models; at inhibition of 15% or 20%, the BMDLs from both models are almost identical. Since the Hill model is good for modeling the receptor binding response, there is a biological basis for selecting this model over the Power model - assuming the iodine symporter acts like a traditional receptor. However, mathematically either model is acceptable.

The perchlorate dose that is modeled to cause a 10% inhibition of iodine uptake is rounded down to 0.01 mg/kg-day; the BMDL estimate ranges from 0.004 to 0.008 mg/kg-day. These results are consistent with the conclusions of Greer et al. (2002), which indicated that the no effect level for iodine inhibition ranges from 0.006 (predicted) to 0.007 (measured) mg/kg-day.

Therefore, for the purpose of developing a perchlorate RfD, we will carry forward the analysis considering three different points-of-departure: a freestanding NOAEL of 0.5 mg/kg-day for the general, healthy population, a freestanding NOAEL of 0.006 mg/kg-

day for a sensitive subpopulation; and a the threshold for iodine uptake inhibition of 0.006 mg/kg-day used as a health-protective surrogate for the freestanding NOAELs. The following section describes the uncertainty factor analysis for each of these points-of-departure.

3.4. Step 4: Choice of Uncertainty Factors

Noncancer risk assessment by U.S. EPA (2002) incorporates five different uncertainty factors to address issues of variability and uncertainty. Two factors (Interspecies and Intraspecies) are used to address the uncertainty between experimental animals and humans, and the variability within different human populations. Three factors (Subchronic, LOAEL, Database) are used to address lack of information. Typically, the maximum total uncertainty factor that U.S. EPA will apply is 3000. If all five areas of uncertainty/variability are present warranting a total UF of 10,000, then U.S. EPA (2002) generally concludes that the uncertainty is too great to develop an RfD. However, some older RfDs on IRIS do have uncertainty factors of 10,000, and EPA does consider uncertainty factors of this magnitude on a case-by-case basis.

3.4.1. Interspecies Variability (UF_A):

This factor accounts for the differences that occur between animals and humans and is also thought to be composed of subfactors for toxicokinetics and toxicodynamics. If no information is available on the quantitative differences between animals and humans, then a default value of 10 is used. If information is available on one of the two subcomponents, then this information is used along with a default value of 3 for the

remaining subfactor. If data are available to adequately describe variability in both subfactors, then actual data may be used to replace default values. In addition, if a RfD is based on human data, then a value of 1 is appropriate for this factor.

As discussed earlier (Step 2: Choice of critical study), the body of data in experimental animals demonstrates that the rodent response to perchlorate is dramatically different than the human response. In rats, doses that cause only about 10% iodine uptake inhibition (see Figure 6a) cause variable, but statistically significant changes in hormone levels (see Figures 3a and b, 4a and b, 5a and b). While in humans, doses that cause 70% iodine uptake inhibition have no effect on hormone levels (see Figure 1). We conclude that basing the RfD on animal data will introduce greater uncertainty to the RfD than use of human data. Therefore, human data is the best basis for the RfD. Since all three proposed points-of-departure are obtained from human studies, a factor of 1 is appropriate for this area of uncertainty.

3.4.2. Intraspecies Variability (UF_H)

This factor accounts for the natural differences that occur between human subpopulations and for the fact that some individuals may be more sensitive than the average population. This factor is composed of two subfactors – one to account for toxicokinetic differences (how the body distributes and metabolizes the chemical) and one to account for toxicodynamic differences (how the body responds to the chemical). If no information is available on human variability, then a default value of 10 is used. However, if adequate information is available on one or both of the two subcomponents, then this information

is used along with a default value of 3 for the remaining subfactor. If data are available to adequately describe human variability in both subfactors, then actual data may be used to replace default values and generate compound specific adjustment factors (CSAFs; based on a framework developed by the IPCS [Meek et al., 2001]). In addition, if a RfD is based on human data gathered in the known sensitive subpopulation, a value of less than 10, perhaps even 1, may be chosen for this factor.

We considered the data that address specific differences in either kinetic or dynamic parameters of perchlorate that most closely tie into the critical effect and its sensitive population(s) in order to assess whether the data were available to develop a CSAF for this area of uncertainty. Since no studies have examined doses high enough to alter hormones in humans, it is not possible to examine variability of this effect in people. We investigated the variation in perchlorate AUC or peak exposure when individuals are given the same perchlorate dose. However, human studies have only measured half-life of perchlorate in humans (i.e., Greer et al., 2002), and such measurements have been made in too few individuals to give a sense of the expected variability in the sensitive population. We also investigated the variability in inhibition of iodine uptake as a function of different perchlorate doses (Greer et al., 2002; Lawrence et al., 2000, 2001). While the data suggest that there may be an approximately 5-fold variability in individual measurements of iodine uptake inhibition, these data from healthy adults do not reflect the expected variability of sensitive subgroups. Therefore we conclude that the available data are insufficient to develop a CSAF for human variability at this time.

The judgment of appropriate intraspecies uncertainty factor depends in part on the choice of study as the basis of the RfD. A full factor of 10 is appropriate to use when the RfD is based on the freestanding NOAEL of 0.5 mg/kg-day identified in the healthy adult population (Greer et al., 2002) because this NOAEL does not account for the fact that a NOAEL in sensitive subgroups (i.e., children or pregnant mothers with their fetuses) could be lower. In contrast, a lower factor is appropriate for the freestanding NOAEL of 0.006 mg/kg-day identified in children (Crump et al., 2000). In the Crump et al. (2000) study, the presence of perchlorate in the water has been a long-term problem. The mothers of the children evaluated were exposed before pregnancy, so that if perchlorate were affecting thyroid function in these women, they would already be hypothyroid at the start of pregnancy[4]. The children themselves were exposed as fetuses in utero, as neonates, and throughout their lifetimes. Therefore several of the life stages that are considered sensitive have been studied in the Crump et al. (2000) study. Therefore, the observation of a freestanding NOAEL in this study gives greater confidence that fetuses, neonates, and children will be protected by a RfD based on this point-of-departure. However, we conclude that uncertainty factor of 3, rather than 1, is appropriate to use with this point-of-departure because there are no data to suggest how the other sensitive subpopulation, pregnant women, may respond. Once actual data has been gathered in pregnant women, this uncertainty factor of 3 may no longer be needed.

[4] Note that a follow up study (Tellez et al., 2003) is currently in progress to measure serum perchlorate levels and evaluate the thyroid function of pregnant women in the same Chilean cities that were studied in Crump et al. (2000). This study should address the questions about effects of perchlorate in the remaining sensitive subpopulation.

We suggest that if the threshold for iodine uptake inhibition, 0.006 mg/kg-day from Greer et al. (2002) is used as the point of departure, then an uncertainty factor of 1 is sufficient to account for human variability. This point-of-departure represents a dose of perchlorate that has no effect on any biological function. If iodine uptake is not inhibited, then none of the potential adverse effects can follow. Therefore using this point-of-departure is very health protective and has a large uncertainty factor already built in. If high enough doses were tested to identify the actual NOAEL for decreased T4 in humans, and then the appropriate full factor of 10 was applied to this NOAEL, we believe that the resulting RfD would not be less than this point-of-departure.

One could argue that there are no data addressing the variability of iodine uptake inhibition in pregnant women, justifying the use of an uncertainty factor for this area of uncertainty. However, there are data in rodents that can be used to evaluate this area of uncertainty in humans. Mattie et al. (2003) have used physiologically based pharmacokinetic models for both rats and humans to predict perchlorate doses that will result in a 5% iodine uptake inhibition in different life stage animals. In rats, the predicted doses that result in a 5% inhibition are 0.03 mg/kg-day, 0.05 mg/kg-day, and 0.13 mg/kg-day for male rats, pregnant rats, and lactating rats, respectively. In humans, the predicted doses that result in a 5% inhibition are 0.01 mg/kg-day, 0.025 mg/kg-day, and 0.061 mg/kg-day for healthy adult males and females, pregnant women, and lactating women, respectively. This analysis suggests that pregnant women are not more sensitive to iodine uptake inhibition than healthy adults. In addition, it confirms that the

physiology of pregnancy serves to conserve iodine uptake, making pregnant women less sensitive to iodine uptake inhibition than non-pregnant adults.

Therefore, the appropriate choice for this uncertainty factor is either 10-fold with the use of the Greer et al. (2002) NOAEL for T4 decrease in adults, 3-fold with the use of the Crump et al. (2000) NOAEL for T4 decrease in children, or 1-fold with the use of the Greer et al. (2000) threshold for iodine uptake inhibition.

3.4.3. Subchronic to Chronic Extrapolation (UF_S)

Because the RfD protects for a lifetime exposure, this factor is applied when the database lacks information on the health effects of the chemical following a chronic exposure. Two questions are considered when making judgment on the use of this factor – are there data demonstrating that other, more sensitive, health effects are expected following chronic exposure than shorter term exposure, and are there data demonstrating that the critical effect(s) progresses in severity as exposure duration increases or that its NOAEL or other point of departure decrease in value? If the database contains no information on chronic exposure, a default value of 10 is often applied, unless other data suggest a lack of progression with exposure duration. If the database contains adequate chronic bioassays, then a value of 1 is generally appropriate. If there are data addressing only one of the two issues, then a default of 3 may be applied. Thus, the need for a duration UF for perchlorate can be examined by evaluating whether more sensitive effects are expected after increasing duration of exposure, or whether longer durations of exposure increase the severity or decrease the point of departure for perchlorate's critical effect.

These questions can be answered by first looking at the totality of the database for perchlorate. While there are no studies that cover a full lifetime in either animals or humans for the thyroid effects of concern, there are studies that evaluate longer exposures in humans and studies that demonstrate no increase in the severity of effects with increasing duration in animals. Long-term exposures have been evaluated in both workers (Gibbs et al., 1998; Lamm et al., 1999) and children (Crump et al., 2000). In Gibbs et al. (1998), workers' tenure ranged from 1 to 27 years, with an average of 8 years. In Lamm et al. (1999), 40% of the workers had a tenure greater than 5 years. In Crump et al. (2000), children age 6-8 years who had been exposed their entire lives were evaluated. In all three of these studies parameters investigated include general physical exam, tests of kidney and liver function, and blood counts, as well as tests of thyroid function. No effects on any of these parameters were observed in the exposed populations in these studies. When compared to the results of the 14-day clinical studies in humans (Greer et al., 2002; Lawrence et al., 2000, 2001), these longer-term studies show that increasing duration of exposure in humans does not increase the incidence or severity of thyroid effects, nor does it induce effects in other target organs that were not identified by the short-term studies.

The available animal studies also support the conclusion that increasing exposure duration does not result in an increase in incidence or severity of thyroid effects nor does it reveal non-thyroid effects that are not detected by shorter-term studies. Several studies have evaluated perchlorate after either 14 days (Caldwell et al., 1996; Siglin et al., 1998;

Keil et al., 1999; Burleson, 2000) or 90 days (Siglin et al., 1998; Keil et al., 1999; Burleson, 2000). These studies have evaluated systemic and immunotoxic effects in addition to thyroid effects. None of these studies observed any non-thyroid effects after either 14 or 90 days of exposure, suggesting that increased exposure duration will not result in systemic effects that occur at lower doses than thyroid effects. Although the thyroid response is variable, particularly the hormone changes, these studies also show that animals exposed for 90 days do not show a clear pattern of more severe hormone changes nor an accelerated progression of thyroid pathology to hyperplasia compared with animals exposed for 14 days (data not shown here but found at http://www.tera.org/perchlorate/welcome.htm#compare).

We also investigated whether increasing duration of exposure affects the inhibition of iodine uptake by perchlorate. If iodine uptake inhibition were to increase with increasing duration, then an uncertainty factor for duration may be required. In rats (Yu, 2000) and humans (Greer et al., 2002) dose response curves for iodine uptake inhibition were plotted by duration (Figure 6a,b). For rats, iodine uptake inhibition data were available for days 1, 5, and 14 of drinking water exposure. The Figure 6a, shows that rats up-regulate iodine uptake very quickly and that inhibition actually decreases with time. In fact, following perchlorate exposures for durations longer than 14 days, iodine uptake inhibition could not be measured, because iodine uptake by the thyroid had returned to normal levels (Yu, personal communication). For humans, iodine uptake inhibition data were available following 2 and 14 days of perchlorate exposure (Greer et al., 2002). Figure 6b shows, that in contrast to rats, humans do not up-regulate iodine uptake within

the times measured – dose response curves for iodine uptake are identical for the two points evaluated. However, these data do show that iodine uptake inhibition does not increase with increasing duration in either rats or humans.

One concern raised by the animal studies is the appearance of thyroid adenomas at the high dose (30 mg/kg-day) in the F1 generation males of the two-generation study. It is known that thyroid tumors in rats are ultimately caused by constant stimulation of the thyroid by TSH. It is also known that perchlorate at 30 mg/kg-day caused dramatic increases in TSH in these animals. Thus, it is not necessarily surprising that tumors were evoked. The development of thyroid tumors in rats is not a duration effect per se, but rather a threshold phenomenon. If perchlorate doses stay below a level that induce increased TSH levels, then the production of thyroid tumors is not possible according to the proposed mode of action (Hill et al., 1989; and also Figure 1). Increased duration of perchlorate at doses that are below this threshold will not increase the risk of thyroid tumor formation. In addition, while the development of thyroid tumors in rats can be considered to be qualitatively relevant to humans, there are questions about whether humans do, in fact, develop thyroid tumors by the same mechanism.

Therefore, we conclude that a value of 1 is appropriate to address this area of uncertainty. Longer term studies are available in humans. Both the human and animal studies demonstrate that increasing exposure duration does not result in the appearance of non-thyroid effects at doses lower than the thyroid effects. Thyroid effects in humans and rodents do not increase in incidence or severity with increasing exposure duration.

Inhibition of iodine uptake does not increase in humans or rats with increasing exposure duration.

3.4.4. LOAEL to NOAEL Extrapolation (UF_L)

Because the RfD is considered to be a subthreshold value that protects against any adverse health effects, this factor is applied when the database lacks information to identify a NOAEL. If the database does not identify a NOAEL, then a default of 10 is used for this factor. If a NOAEL is used, a value of 1 is appropriate. Often, if the database does not identify a NOAEL, but the adverse effects observed are of minimal severity, then a default of 3 will be considered appropriate for use of a "minimal LOAEL".[5]

Both the Greer et al. (2002) and the Crump et al. (2000) studies identified freestanding NOAELs for the critical effect of decreased T4. When either of these NOAELs are used as the point-of-departure for the development of an RfD, an uncertainty factor of 1 for this area would be appropriate. A point-of-departure at the threshold for iodine uptake inhibition (Greer et al., 2000) is, likewise, not considered to be a LOAEL. First, inhibition of iodine uptake is a key event in the mode of action rather than an adverse

[5] EPA is currently discussing the application of UF_L when using a BMDL. A BMDL value represents the lower limit on the dose that should cause 10% of the experimental animals to respond with the effect that is being modeled. Because animal studies typically cannot detect a response less than 10%, an experimentally derived NOAEL also represents the dose that causes 10% of the animals to respond. For this reason, U.S. EPA has historically considered a BMDL to be a NOAEL surrogate and selected a UF_L value of 1 when a BMDL is used. Although EPA does not have official guidance on this issue, recent discussions in the agency suggest that if the effect being modeled for the BMDL is adverse, then the BMDL should be considered as a LOAEL. Currently, BMDLs are being evaluated on a case-by-case basis, considering the nature of the effect being modeled and the relationship of the estimated BMDL to observed NOAELs (EPA, 2002).

effect (University of Nebraska, 2003). Second, the recommended point of departure represents a dose at which no inhibition of iodine uptake occurs, so that adverse effects cannot occur following exposure to this dose. This conclusion is confirmed by the body of human data, which demonstrate that no effect on serum hormone levels has been observed at doses equal to or higher than this point of departure. Therefore, this point-of-departure should be considered as a NOAEL surrogate, rather than a LOAEL surrogate, and the appropriate value for this factor is 1.

3.4.5. Database (UF_D)

The database for deriving a high confidence RfD includes at a minimum two chronic bioassays by the appropriate route of exposure in different species, one two-generation reproductive toxicity study, and two developmental toxicity studies in different species. The minimal database required for deriving a RfD is a single subchronic bioassay, that includes a full histopathology examination. The database factor is used to account for the fact that a potential health effect may not be identified if the database is missing a particular type of study. This factor may also be used if the existing data indicate the potential for a heath effect that is not fully characterized by the standard bioassays, for example neurotoxicity or immunotoxicity. If the database is complete, a value of 1 is appropriate. If only the minimal database is available, then a default of 10 is used. A value of 3 may be used if the database is missing one or two key studies.

The database for perchlorate includes an large number of experimental animal studies, including chronic (but older) studies that show tumors at high doses (i.e., Kessler and

Kruskemper, 1966), numerous shorter-term bioassays that unequivocally demonstrate that thyroid disturbance occurs at lower doses than other systemic, immunotoxic, genotoxic, or other effects, developmental toxicity studies in two species, a 2 generation reproduction study that also monitored systemic effects in young rats, a developmental neurotoxicity study, a specialized developmental toxicity study to monitor hormone changes in early life and during late pregnancy and lactation, and a specialized neurobehavioral study to confirm earlier findings. The database also includes human clinical, experimental, epidemiology, and occupational studies.

All of this information demonstrates that the thyroid is the most sensitive organ system. In humans, the threshold for iodine uptake inhibition is well characterized and additional studies are not likely to provide different information that would change the risk assessment. In humans, the perchlorate dose that causes a decrease in T4, the critical effect, is not well characterized since no human population has been exposed to a dose high enough to alter hormone levels. However, if these studies could be done, their effect would likely be to raise the NOAEL. The mode of action analysis suggests the potential for adverse effects as a result of serum T4 levels that are consistently depressed by at least 60%. The doses that cause this degree of T4 decrease are not well characterized in either humans or animals. However, by selecting a point-of-departure that is below the threshold for any T4 change, we have confidence that subsequent effects will not develop. Therefore, we conclude that the overall perchlorate database is complete, and any new studies that are done to fine tune our knowledge of the perchlorate mode of

action will not identify lower points-of-departure than can be estimated from the existing database. We conclude that the appropriate value for this factor is 1.

In summary, the only area of uncertainty for a perchlorate RfD that needs to be addressed by the use of uncertainty factors is human variability and the difference in response between pregnant women and the groups for which data are available. A factor of 1 is appropriate to address all other areas of uncertainty. For the NOAEL for T4 changes in adults from the Greer et al. (2002) study, a 10-fold uncertainty factor is judged to be appropriate because no members of potential sensitive populations were included in the study population. For the NOAEL for T4 change in children from the Crump et al. (2000) study, a 3-fold uncertainty factor is judged to be appropriate because children are one of the sensitive populations for perchlorate exposure. This uncertainty factor is not less than 3, however, because another sensitive population, pregnant women, also exists and may in fact have a lower NOAEL (as is the case in experimental animals). For the inhibition of iodine uptake in adults from the Greer et al. (2002) study, the uncertainty factor is judged to be 1 because use of this biological marker is a conservative choice that has a large degree of safety built into it and data from animal studies and PBPK modeling indicates that iodine uptake inhibition does not differ between adults and sensitive subpopulations.

3.5. Step 5: Developing an RfD

As shown by extensive animal studies, the critical effect of perchlorate is T4 serum decrease. Pregnant rats are demonstrated to be the most sensitive subgroup, likely

followed by the young rat. Several human studies exist that monitored for this critical effect. These studies do not include pregnant women, but they do include children. In addition, our review of comparative data between the experimental animal and human clearly indicate that humans are not more sensitive than the experimental animal species tested to T4 serum decrease by perchlorate; in fact based on toxicodynamics parameters they are much less sensitive (Capen, 2001). This supports the use of the human data for development of a RfD.

The most relevant data for developing the RfD for perchlorate exposures comes from human epidemiology and clinical studies, supplemented with available and extensive information on experimental animals. Specifically, we believe that a NOAEL of 0.006 mg/kg-day for T4 changes in children from the Crump et al. (2000) study provides the most appropriate and relevant basis for the perchlorate RfD. The use of the Crump et al. (2000) study in children has the advantage of evaluating response in a sensitive population. This NOAEL is supported by the data from Greer et al. (2002), which demonstrate that the threshold inhibition of iodine uptake in adults is 0.006 mg/kg-day. Furthermore, the NOAEL of 0.5 mg/kg-day from the Greer et al. (2002) can be used to give an upper bracket to this recommended RfD. The choice of the Greer et al. (2002) in adults has the advantage of evaluating both the key event in the perchlorate mode of action and the critical effect with a well-established dosing regimen. Inhibition of iodine uptake has a well characterized dose response curve.

The uncertainty factors selected in this analysis take into account the expected differences in toxicokinetics and toxicodynamics between children, pregnant women, and adults. We also investigated whether compound specific adjustment factors (CSAFs) could be developed for the perchlorate RfD that would allow for the use of specific data on intraspecies and interspecies differences in toxicokinetics and toxicodynamics, following the recent guidelines of the International Programme on Chemical Safety (IPCS) and U.S. EPA's (2002) recommendations. Unfortunately, data were not sufficient to estimate a CSAFs with confidence.

Table 3 summarizes the different points-of-departure, appropriate uncertainty factors, and resulting RfDs from our analysis. RfDs ranging from 0.002 mg/kg-day to 0.05 mg/kg-day can be developed with high confidence from the existing database.

4. Discussion

Perchlorate is now one of the best-studied environmental pollutants, in part due to its prior and current use as a drug. Many human studies have been published, including occupational studies, epidemiology studies in neonates and school age children, and clinical studies in adults. Several, if not all, of the clinical human studies have been conducted under the guidelines of good clinical practice; at least one of them followed the guidelines of the common rule. Available experimental animal studies include rat developmental neurotoxicity, 90-day systemic toxicity, developmental toxicity, two-generation reproductive toxicity that monitored for systemic endpoints in young animals, developmental brain morphometry, developmental motor activity, and predictive

immunotoxicity. Several of these bioassays are also available in rabbits and mice. All of these experimental animal studies have been conducted under current U.S. EPA guidelines. In addition, the kinetics of perchlorate has been extensively studied in male and female rats, pregnant and lactating rats, and fetal rats.

There are several uncertainties in our proposed RfD. Since no effect on T4 was found in either the children in the Crump et al. (2000) study or the adults in the Greer et al. (2002) study, the NOAELs could actually be higher than the ones used as the basis of our proposed RfD. The effect of this uncertainty is to make the proposed RfD lower than the actual threshold for perchlorate effects and increase the margin of safety for perchlorate. This uncertainty is balanced, however, by characteristics of the study population in Crump et al. (2000) that could have the effect of either lowering or raising the actual NOAEL in other populations. For example, the NOAEL might be lower in U.S. children because children in Chile have higher urinary iodine and presumed iodine intake, and thus might be protected from higher perchlorate exposures. In contrast, the NOAEL might be higher in U.S. children because children in Chile had a higher than expected background incidence of goiter, which could be due several factors, including other goitrogens in the diet, such as nitrate, a unique genetic makeup, or sources of perchlorate in the diet other than drinking water. The degree to which the actual NOAEL may increase or decrease in response to these factors is difficult to determine. However, we feel that the uncertainties balance out and are adequately encompassed within our range of RfDs.

At least one additional human study (Tellez et al., 2003) is ongoing that is monitoring thyroid hormones in pregnant women, in the same three populations in Chile as described in Crump et al. (2000). This study has the potential to change the RfD that we describe here, although the magnitude of the potential change is not expected to be great.

The perchlorate database allows the development of a high-confidence Reference Dose (RfD). Based on a mode-of-action analysis developed by U.S. EPA (2003b), altered hormone levels are early biological effects of perchlorate exposure. If allowed to persist, decreased T4 and increased TSH levels, at least in rodents, will eventually lead to thyroid hyperplasia and thyroid tumors. However, negative mutagenicity/genotoxicity data and other evidence suggests that this pathway may not be relevant for humans. Of more importance, if decreased T4 levels are allowed to persist, an increased potential for a neurodevelopmental adverse effect exists in children. Although decrease of T4 and its balance with increasing TSH is a normal part of homeostatic control and therefore not adverse in itself, it nevertheless is a precursor to the first adverse effect and can thus be defined as the critical effect as per U.S. EPA (2003a). Furthermore, based on data in experimental animals, pregnancy is the most sensitive life stage, with larger decreases in T4 levels occurring at lower doses when compared with lactating females, pups, and adult females and males. Therefore, decreases in serum T4 in the pregnant population should be considered to be the critical effect most relevant to human health, both based on an analysis of mode of action, and an evaluation of the empirical data that this is the effect that occurs at the lowest doses. By developing a RfD based on the critical effect of decreased serum T4, a known precursor to neurodevelopmental adverse effects, all

subsequent potential adverse effects will be prevented. The public's health will be adequately protected from this approach.

Acknowledgements

TERA wishes to thank the Perchlorate Study Group for its support over a number of years to study perchlorate's toxicology and assess its risk. However, support for the development of this manuscript was provided only by internal developmental reserve funds of *TERA*, and not by any outside party. Furthermore, our deliberations shown in this paper have not been influenced by discussions with outside parties, other than the reviewers of this journal. We now welcome comments from all interested colleagues.

References

Argus Research Laboratories, Inc. (1998). A neurobehavioral study of ammonium perchlorate administered orally in drinking water to rats [report amendment: July 27]. Horsham, PA: Argus Research Laboratories, Inc., protocol no. 1613-002.

Argus Research Laboratories, Inc. (2001). Hormone, thyroid and neurohistological effects of oral (drinking water) exposure to ammonium perchlorate in pregnant and lactating rats and in fetuses and nursing pups exposed to ammonium perchlorate during gestation or via maternal milk. Horsham, PA: Argus Research Laboratories, Inc., protocol no. 1416-003.

Barnes, D.G. and Dourson, M.L. (1988). Reference dose (RfD): Description and use in health risk assessments. *Reg. Toxicol. Pharm.* **8**, 471-486.

Bartalena, L., Brogioni, S., Grasso, L., Bogazzi, F., Burell,i A. and Martino, E. (1966). Treatment of amiodarone-induced thyrotoxicosis, a difficult challenge: results of a prospective study. *J. Clin. Endocrinol.* **81**, 2930-2933.

Barzilai, D. and Sheinfeld, M. (1966). Fatal complications following use of potassium perchlorate in thyrotoxicosis: report of two cases and a review of the literature. *Israel Journal of Medical Sciences.* **2**, 453-456.

Bekkedal, M.Y.V., Carpenter, T., Smith, J., Ademujohn, C., Maken, D. and Mattie, D. R. (2000). A neurodevelopmental study of the effects of oral ammonium perchlorate exposure on the motor activity of pre-weanling rat pups. Wright-Patterson Air Force Base, OH: Naval Health Research Center Detachment, Neurobehavioral Effects Laboratory. Report no. TOXDET-00-03.

Boyages, S.C., Collins, J.K., Maberly, G.F., Jupp, J.J., Morris, J. and Eastman, C.J. (1989). Iodine deficiency impairs intellectual and neuromotor development in apparently-normal persons. A study of rural inhabitants of north-central China. *Med. J. Austr.* **150**, 676-682.

Brabant, G., Bergmann, P., Kirsch, C.M., Kohrle, J., Hesch, R.D. and Von Zur Muhlen, A. (1992). Early adaptation of thyrotropin and thyroglobulin secretion to experimentally decreased iodine supply in man. *Metabolism.* **41**, 1093-1096.

Brabant, G. and Leitolf, H. (2000). Consultative letter, AFRL-HE-WP-CL-2000-0039, Hormone data from Brabant human perchlorate (1.0 and 12.0 mg/kg-day) Kinetics drinking water study. Memorandum for U.S. EPA from Dave Mattie. June 30.

Brechner, R.J., Parkhurst, G.D., Humble, W.O., Brown, M.B. and Herman, W.H. (2000). Ammonium perchlorate contamination of Colorado River drinking water is associated with abnormal thyroid function in newborns in Arizona. *J. Occ. And Environ. Med.* **42**, 777-782.

Burleson Research Technologies, Inc. (BRT). (2000). Ammonium perchlorate: effect on immune function. Quality assurance audit: study no. BRT 19990524 – plaque forming cell (PFC) assay; study no. BRT 19990525 – local lymph node assay (LLNA) in mice. Raleigh, NC.

Caldwell, D.J., King, J.H., Jr., Kinkead, E.R., Wolfe, R.E., Narayanan, L. and Mattie, D.R. (1996). Results of a fourteen day oral-dosing toxicity study of ammonium perchlorate. In: Proceedings of the 1995 JANNAF safety and environmental protection subcommittee meeting: volume 1, December; Tampa, FL. Columbia, MD: Chemical Propulsion Information Agency; Joint Army, Navy, NASA, Air Force (JANNAF) interagency propulsion committee publication. p. 634.

Calvo R., Obregon, M.J., Ruiz de Ona, C., Escobar del Rey, F. and Morreale de Escobar, G. (1990). Congenital hypothyroidism, as studied in rats. Crucial role of maternal thyroxine but not of 3,5,3'-triiodothyronine in the protection of the fetal brain. *J. Clin. Invest*. **86**, 889-899.

Cao, X.Y., Jiang, X.M., Dou, Z.H., Rakeman, M.A., Zhang, M.L., O'Donnell, K., Ma, T., Annette, K., DeLong, N. and DeLong, G.R. (1994). Timing of vulnerability of the brain to iodine deficiency in endemic crentinism. *New Eng. J. Med.* **331**, 1739-1744.

Capen, C.C. (1997). Mechanistic data and risk assessment of selected toxic end points of the thyroid gland. *Toxicol. Path.* **25**, 39-48.

Capen, C.C. (2001). Toxic responses of the endocrine system. Chapter 21 of Casarett and Doull's Toxicology: The basic science of poisons. 6th ed, McGraw-Hill. New York, NY. p. 724.

Carrasco, N. (1993). Iodide transport in the thyroid gland. *Biochim. Biophys. Acta.* **1154**, 65-82.

Connell, J.M. (1981). Long-term use of potassium perchlorate. *Postgrad. Med. J.* **57**, 516-517.

Crump, C., Michaud, P., Tellez, R., Reyes, C., Gonzalez, G., Montgomery, E.L., Crump, K.S., Lobo, G., Becerra, C. and Gibbs, J.P. (2000). Does perchlorate in drinking water affect thyroid function in newborns or school-age children? *J. Occ. Environ. Med.* **42**, 603-612.

Dourson, M.L. (1994). Methods for establishing oral reference doses (RfDs). In Risk Assessment of Essential Elements. W. Mertz, C.O. Abernathy and S.S. Olin Ed. ILSI Press Washington, DC. p. 51-61.

Dunn, J. (2003). The Extent and Causes of Iodine Deficiency in the USA. Perchlorate State of the Art Symposium. University of Nebraska Medical Center, Omaha, Nebraska. September 23.

Gibbs, J.P., Ahmad, R., Crump, K.S., Houck, D.P., Leveille, T.S., Findley, J.E. and Francis, M. (1998). Evaluation of a population with occupational exposure to airborne ammonium perchlorate for possible acute or chronic effects on thyroid function. *J. Occ. Environ. Med.* **40**, 1072-1082.

Gibbs, J. (2003). A Natural Laboratory: Northern Chile. Perchlorate State of the Art Symposium. University of Nebraska Medical Center, Omaha, Nebraska. September 30.

Greenspan, F.S. (1997). The role of fine-needle aspiration biopsy in the management of palpable thyroid nodules. *Am. J. Clin. Pathol.* **108**(4 Suppl 1), S26-30.

Greer, M.A., Goodman, G., Pleus, R.C., Greer, S.E. (2000). Does environmental perchlorate exposure alter human thyroid function? Determination of the dose-response for inhibition of radioiodine uptake. In: Abstracts of the 12th International Thyroid Congress, October, Kyoto, Japan. *Endocrine Journal.* **47**(suppl.), 146.

Greer, M., Goodman, G., Pleus, R. and Greer, S. (2002). Health effects assessment for environmental perchlorate contamination: The dose response assessment for inhibition of thyroidal radioiodine uptake in humans. *Environ. Health Perspect.* **110**, 927-937.

Haber, L.T., Dollarhide, J.S., Maier, A. and Dourson, M.L. (2001). Noncancer Risk Assessment: Principles and Practice in Environmental and Occupational Settings. In *Patty's Toxicology*. E. Bingham, B. Cohrssen and C.H. Powell Ed. 5th ed, Wiley and Sons, Inc. New York, NY. p. 169-232.

Haddow, J.E., Palomaki, G.E., Allan, W.C., Williams, J.R., Knight, G.J., Gagnon, J., O'Heir, C.E., Mitchell, M.L., Hermos, R J., Waisbren, S.E., Faix, J.D. and Klein, R.Z. (1999). Maternal thyroid deficiency during pregnancy and subsequent neuropsychological development of the child. *New Eng. J. Med.* **341**, 549-555.

Hill, R.N., L.S. Erdreich, O.E. Paynter, P.A. Roberts, S. L. Rosenthal and C.F. Wilkinson. (1989). Thyroid follicular cell carcinogenesis. *Fund. App. Toxicol.* **12,** 629-697.

Hollowell, J.G., Jr. and Hannon, W.H. (1997). Teratogen update: iodine deficiency, a community teratogen. *Teratology.* **55**, 389-405.

Hollowell, J.G., Staehling, N.W., Hannon, W.H., Flanders, D.W., Gunter, E.W., Maberly, G.F., Braverman, L.E., Pino, S., Miller, D.T., Garbe, P.L., DeLozier, D.M. and Jackson, RJ. (1998). Iodine nutrition in the United States. Trends and public health implications: iodine excretion data from National Health and Nutrition Examination Surveys I and III (1971-1974 and 1988-1994). *J. Clin. Endocrinol. Metab.* **83**, 3401-3408.

International Programme on Chemical Safety (IPCS). (1994). Environmental Health Criteria No. 170: Assessing human health risks of chemicals: Derivation of guidance values for health-based exposure limits. World Health Organization, Geneva.

Keil, D., Warren, D. A., Jenny, M., EuDaly, J. and Dillard, R. (1999). Effects of ammonium perchlorate on immunotoxicological, hematological, and thyroid parameters in B6C3F1 female mice. Final report. Medical University of South Carolina, Department of Medical Laboratory Sciences. Charleston, SC. Report no. DSWA01-97-0008.

Kessler, F.J. and Krüskemper, H.J. (1966). Experimentelle Schilddrüsentumoren durch mehrjährige Zufuhr von Kaliumperchlorat [Experimental thyroid tumors caused by long-term potassium perchlorate administration]. *Klin. Wochenschr*. **44**, 1154-1156.

Lamm, S.H., Braverman, L.E., Li, F.X., Richman, K., Pino, S. and Howearth, G. (1999). Thyroid health status of ammonium perchlorate workers: a cross-sectional occupational health study. *J. Occ. Environ. Med.* **41**, 248-260.

Lawrence, J.E., Lamm, S.H., Pino, S., Richman, K. and Braverman, L.E. (2000). The effect of short-term low-dose perchlorate on various aspects of thyroid function. *Thyroid.* **10**, 659-663.

Lawrence, J., Lamm, S. and Braverman, L.E. (2001). Low dose perchlorate (3 mg daily) and thyroid function [letter]. *Thyroid.* **11**, 295.

Li, Z., Li, F.X., Byrd, D., Deyhle, G.M., Sesser, D.E., Skeels, M.R. and Lamm, S.H. (2000a). Neonatal thyroxine level and perchlorate in drinking water. *J. Occ. Environ. Med.* **42**, 200-205.

Li, F.X., Byrd, D.M., Deyhle, G.M., Sesser, D.E., Skeels, M.R., Katkowsky, S.R. and Lamm, S.H. (2000b). Neonatal thyroid-stimulating hormone level and perchlorate in drinking water. *Teratology.* **62**, 429-431.

Li, F.X., Squartsoff, L. and Lamm, S.H. (2001). Prevalence of thyroid diseases in Nevada counties with respect to perchlorate in drinking water. *J. Occ. Environ. Med.* **43**, 630-634.

Loh, K.C. (2000). Amiodarone-induced thyroid disorders: A clinical review. *Postgrad. Med. J.* **76**, 133-140.

Mahle, D.A., Yu, K.O., Narayanan, L., Mattie, D.R., Fisher, J.W. 2003. Changes in cross-fostered Sprague Dawley rat litters exposed to perchlorate. *Int. J. Toxicol*. **22**, 87-94.

Mattie et al. (2003). A Natural Laboratory: Northern Chile. Perchlorate State of the Art Symposium. University of Nebraska Medical Center, Omaha, Nebraska. September 30.

Meek, M., Renwick, A., Ohanian, E., Dourson, M., Lake, B., Naumann, B. and Vu, V. (2001). Guidelines for application of compound specific adjustment factors (CSAF) in dose/concentration response assessment. *Comments. Toxicol.* 7, 575-590.

Merrill, E. (2001). Consultative letter, AFRL-HE-WP-CL-2001-0004, QA/QC audit. Report for the study of perchlorate pharmacokinetics and inhibition of radioactive iodine uptake (RAIU) by the thyroid in humans (CRC protocol #628) [memorandum with attachments to Annie M. Jarabek]. Wright-Patterson AFB, Dayton, OH. Air Force Research Laboratory. May 10.

Merrill, E.A., Clewell, R.A., Robinson, P.J., Jarabek, A.M., Gearhart, J.M., Sterner, T.R. and Fisher, J.W. (2003). PBPK model for iodide and perchlorate kinetics and perchlorate-induced inhibition of radioiodide uptake in humans. (in press)

Morgans, M.E. and Trotter, W.R. (1960). Potassium perchlorate in thyrotoxicosis [letter]. *Brit. Med. J.* October 8, 1086-1087.

Morreale de Escobar, G., Obregón, M.J. and Escobar del Ray, F. (2000). Is neuropsychological development related to maternal hypothyroidism or to maternal hypothyroxinemia? *J.Clin. Endocrinol. Metab.* **85**, 3975-3987.

Narayanan, L. (2000). Consultative letter, AFRL-HE-WP-CL-2000-0034. Thyroid hormone and TSH co-laboratory study report [memorandum with attachments to Annie Jarabek]. Wright-Patterson Air Force Base, Dayton, OH. Air Force Research Laboratory. June 15.

Scanlon. (1996). As cited in Greer et al., 2000.

San, R.C. and Clarke, J.J. (1999). In vitro mammalian cell gene mutation test (L5178Y/TK+/- mouse lymphoma assay). Rockville, MD, BioReliance study no. G98BA06.702. Available online: http://www.tera.org/perchlorate/2nd%20study%20final.pdf.

Sharma, S. and Gao, P. (1998). Genotoxicity assays for ammonium perchlorate. Final Report. Mantech Environmental Technology study no. 6100-001. Available online: http://www.tera.org/perchlorate/mantech%20genotoxicity.pdf.

Siglin, J.C., Mattie, D.R., Dodd, D.E., Hildebrandt, P.K. and Baker, W.H. (1998). A 90-day drinking water toxicity study in rats of the environmental contaminant ammonium perchlorate. *Toxicol. Sci.* **57**, 61-74.

Stanbury, J.B. and Wyngaarden, J.B. (1952). Effect of perchlorate on the human thyroid gland. *Metab. Clin. Exp.* **1**, 533-539.

Susarla, S., Collette, T.W., Garrison, A.W., Wolfe, N.L. and McCutcheon, S.C. (1999). Perchlorate identification in fertilizers. *Environ. Sci. Tech.* **33**, 3469-3472.

Tellez, R.T., Chacon, P.M., Gibbs, J., Crump, C. and C. R. Abarca. (2003). Chronic Environmental Exposure To Perchlorate And Thyroid Function During Pregnancy And The Neonatal Period. Protocol and preliminary results available at http://www.tera.org/perchlorate/welcome.htm#human.

Ting, D., Howard. R. and Fan, A. (2001). Human health risk assessment on perchlorate exposure through drinking water. California Environmental Protection Agency (Cal EPA). Oakland, California. Presentation at the Society of Risk Analysis. December.

Toxicology Excellence for Risk Assessment (*TERA*). (2002). Use of human data in risk assessment. Comments submitted to U.S. EPA. February 19.

University of Nebraska. (2003). Perchlorate State of the Science Symposium. University of Nebraska Medical Center, Omaha, Nebraska. September 29th to October 1. Available online: http://www.unmc.edu/coned.

U.S. Environmental Protection Agency. (1998). Guidelines for Neurotoxicity Risk Assessment. Federal Register. May 14. Vol. 63, 26926-26954.

U.S. Environmental Protection Agency. (1999). Exposure Factors Handbook. Office of Research and Development. Washington, DC. February. EPA/600/C-99/001.

U.S. Environmental Protection Agency. (2002). A Review of the Reference Dose and Reference Concentration Processes. Risk Assessment Forum. Washington, DC. May. EPA/630/P-02/002A.

U.S. Environmental Protection Agency. (2003a). Integrated Risk Information System (IRIS). Glossary of Terms. Office of Research and Development. Washington, DC. Available online: http://www.US EPA.gov/iris.

U.S. EPA (U.S. Environmental Protection Agency). (2003b). Disposition of Comments and Recommendations for Revisions to "Perchlorate Environmental Contamination: Toxicological Review and Risk Characterization External Review Draft (January 16, 2002)." National Center for Environmental Assessment, Washington, DC. October 27.

Wenzel, K.W. and Lente, J.R. (1984). Similar effects of thionamide drugs and perchlorate on thyroid-stimulating immunoglobulins in Graves' disease: evidence against an immunosuppressive action on thionamide drugs. *J. Clin. Endocrinol. Metab.* **58**, 62-69.

Wolff, J. (1998). Perchlorate and the thyroid gland. *Pharmacol. Rev.* **50**, 89-105.

Wyngaarden, J.B., Wright, B.M. and Ways, P. (1952). The effect of certain anions upon the accumulation and retention of iodide by the thyroid gland. *Endocrinology.* **50**, 537-549.

York, R.G. (2000). Protocol 1416-003 - oral (drinking water) developmental toxicity study of ammonium perchlorate in rats [letter to Annie Jarabek]. Primedica, Argus Division, Horshan, PA. November 21.

York, R.G., Brown, W.R., Girard, M.F. and Dollarhide, J.S. (2001a). Oral (Drinking Water) Developmental Toxicity Study of Ammonium Perchlorate in New Zealand White Rabbits. *Int. J. Toxicology.* **20**, 199-207.

York, R.G., Brown, W.R., Girard, M.F. and Dollarhide, J.S. (2001b). Two-Generation Study of Ammonium Perchlorate in Drinking Water in Rats Evaluates Thyroid Toxicity. *Int. J. Toxicology.* **20**, 183-197.

Yu, K.O. (2000). Consultative letter, AFRL-HE-WP-CL-2000-0038. Tissue distribution and inhibition of iodide uptake in the thyroid by perchlorate with corresponding hormonal changes in pregnant and lactating rats (drinking water study) [memorandum with attachment to Annie Jarabek]. Wright-Patterson Air Force Base, Dayton, OH. Air Force Research Laboratory. June 28.

Yu, K.O., Narayanan, L., Mattie, D.R., Godfrey, R.J., Todd, P.N., Sterner, T.R., Mahle, D.A., Lumpkin, M.H., Fisher, J.W. (2002). The pharmacokinetics of perchlorate and its effect on the hypothalamus-pituitary-thyroid axis in the male rat. *Toxicol. Appl. Pharmacol*. **182**, 148-159.

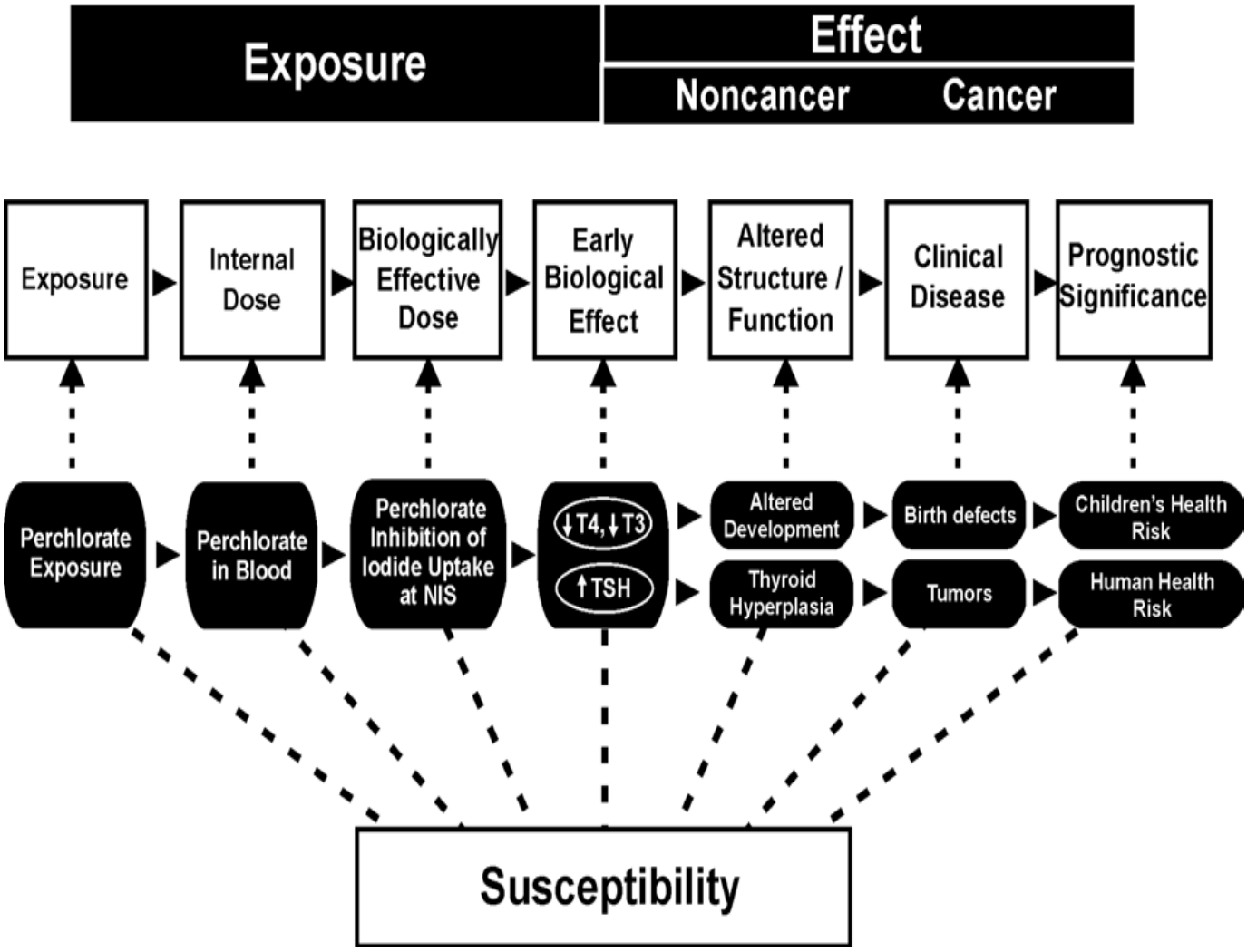

Figure 1. Mode of action model for perchlorate toxicity proposed by U.S. EPA (2003). Perchlorate interferes with the sodium (NA+)-iodide (I-) symporter (NIS) present in various tissues, particularly thyroid. The model shows the exposure-dose response continuum considered in the context of biomarkers (classified as measures of exposure, effect, and susceptibility) and level of organization at which toxicity is observed (adapted directly from U.S. EPA, 2003b)

Figure 2a. I uptake in humans as a formation of serum perchlorate peak concentration.

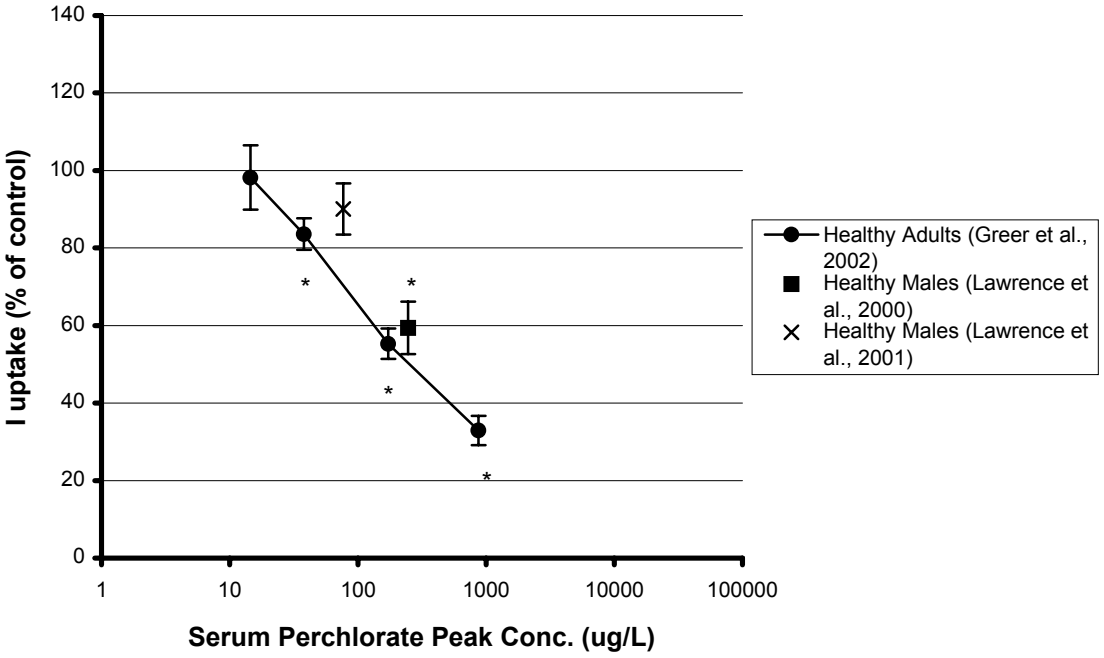

Figure 2b. Human T4 response to perchlorate dose as a formation of serum perchlorate peak concentration.

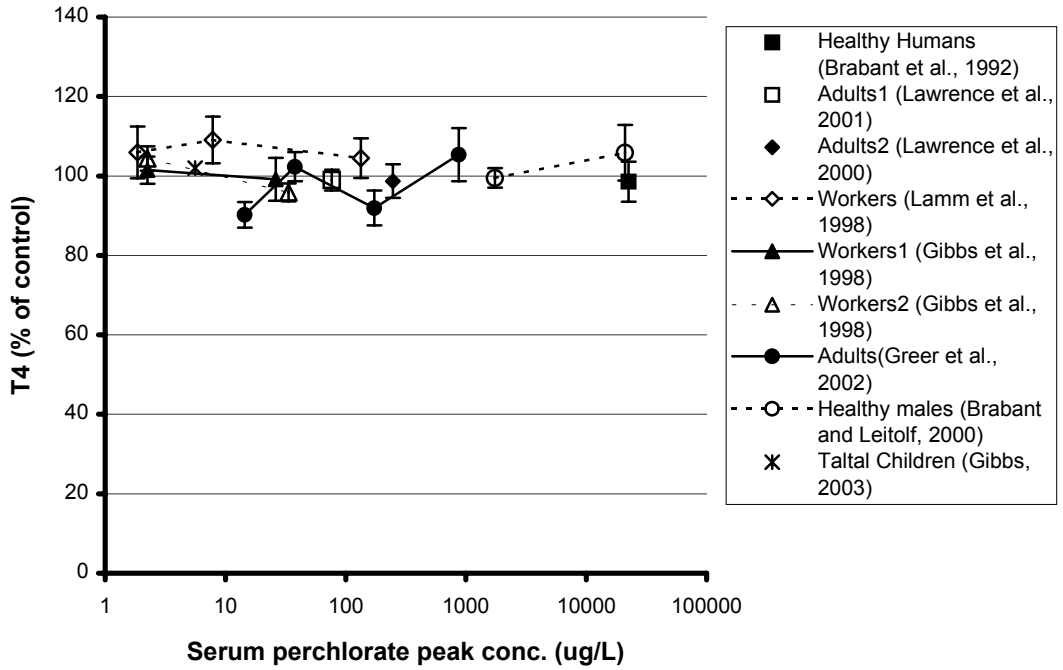

Figure 3a. T4 response in female animals at 90 days.

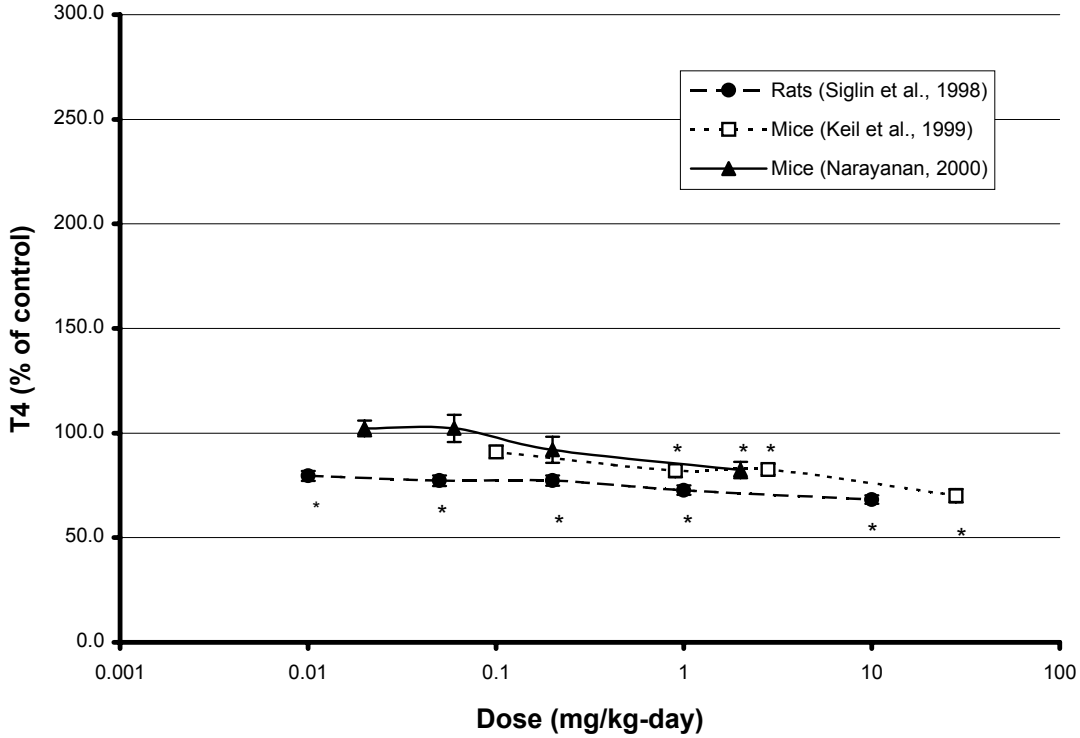

Figure 3b. TSH response in female animals at 90 days.

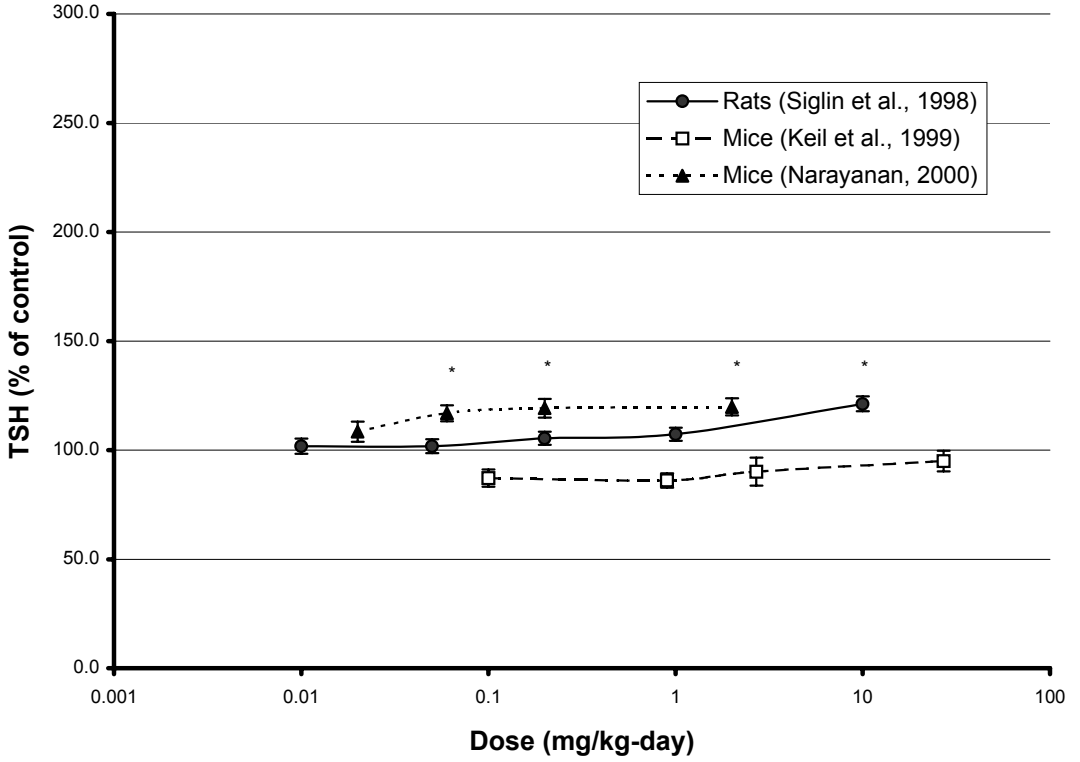

Figure 3c. Follicular Cell Hyperplasia in Female Animals (90 days).

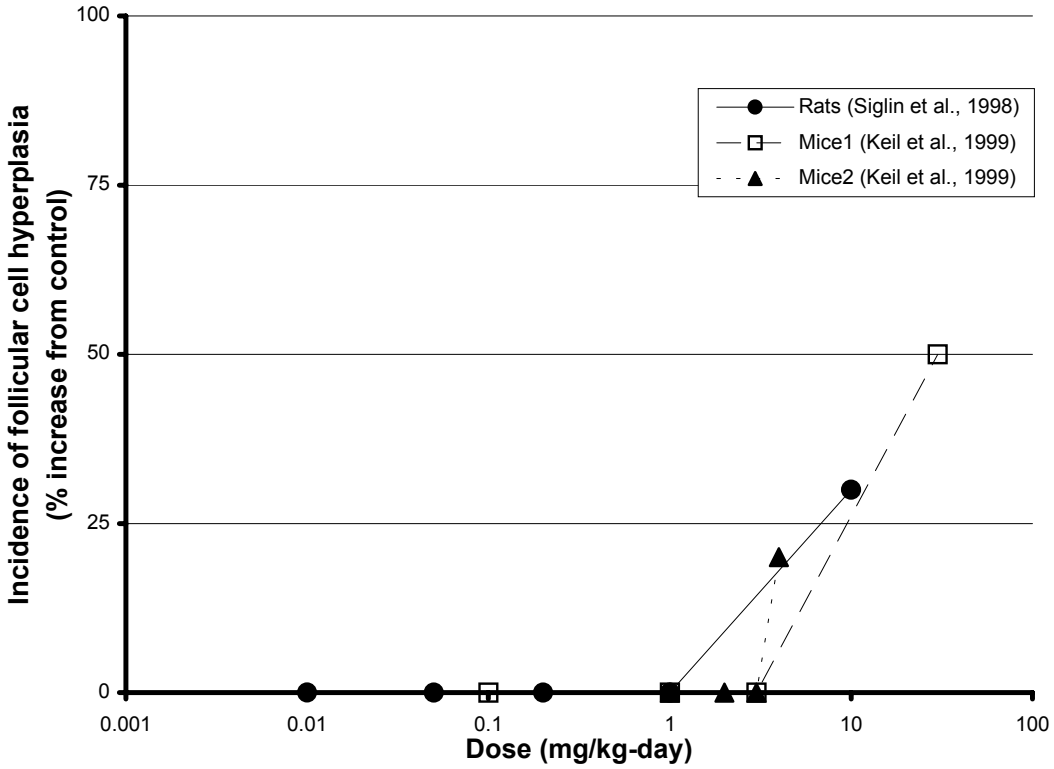

Figure 4a. T4 response in dams.

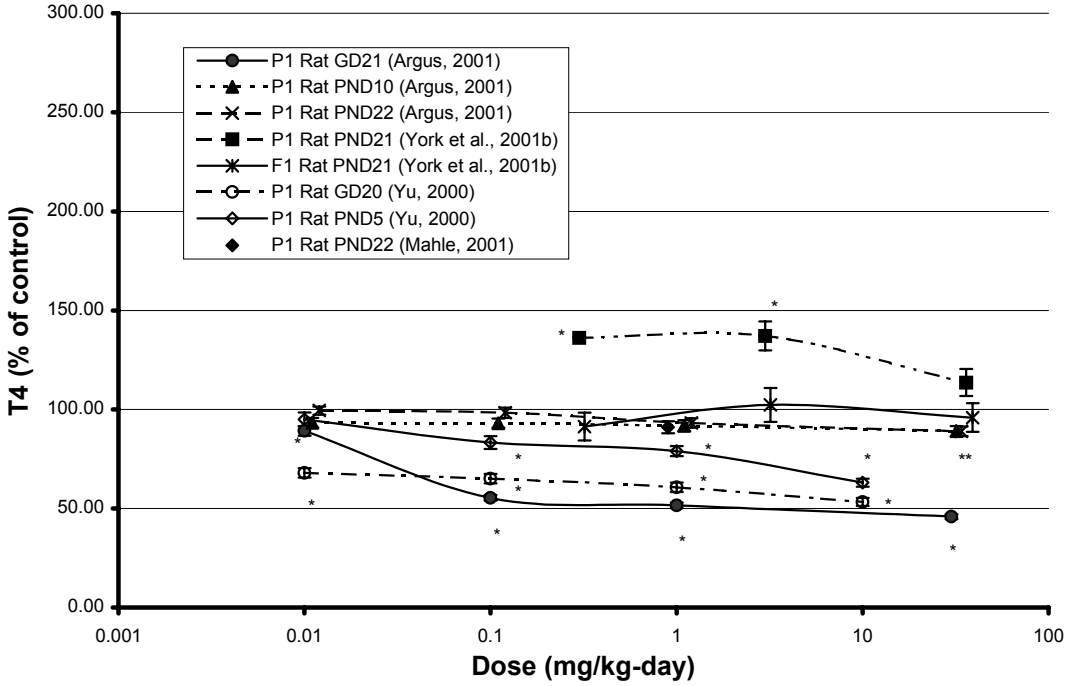

Figure 4b. TSH response in dams.

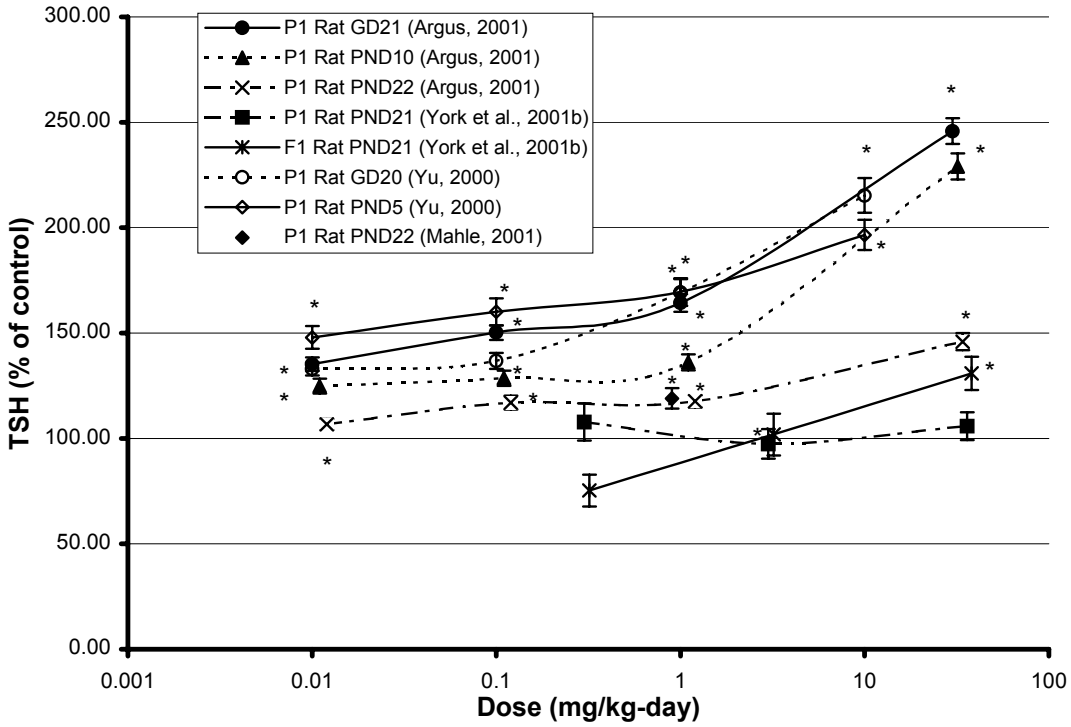

Figure 4c. Follicular cell hyperplasia in dams.

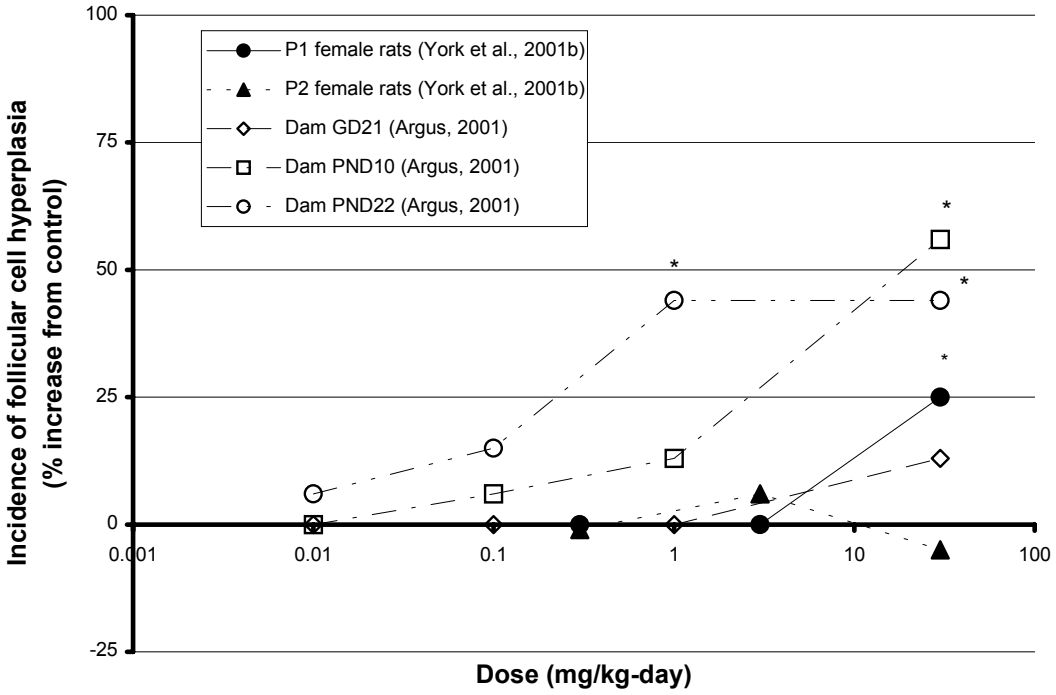

Figure 5a. T4 response in pups.

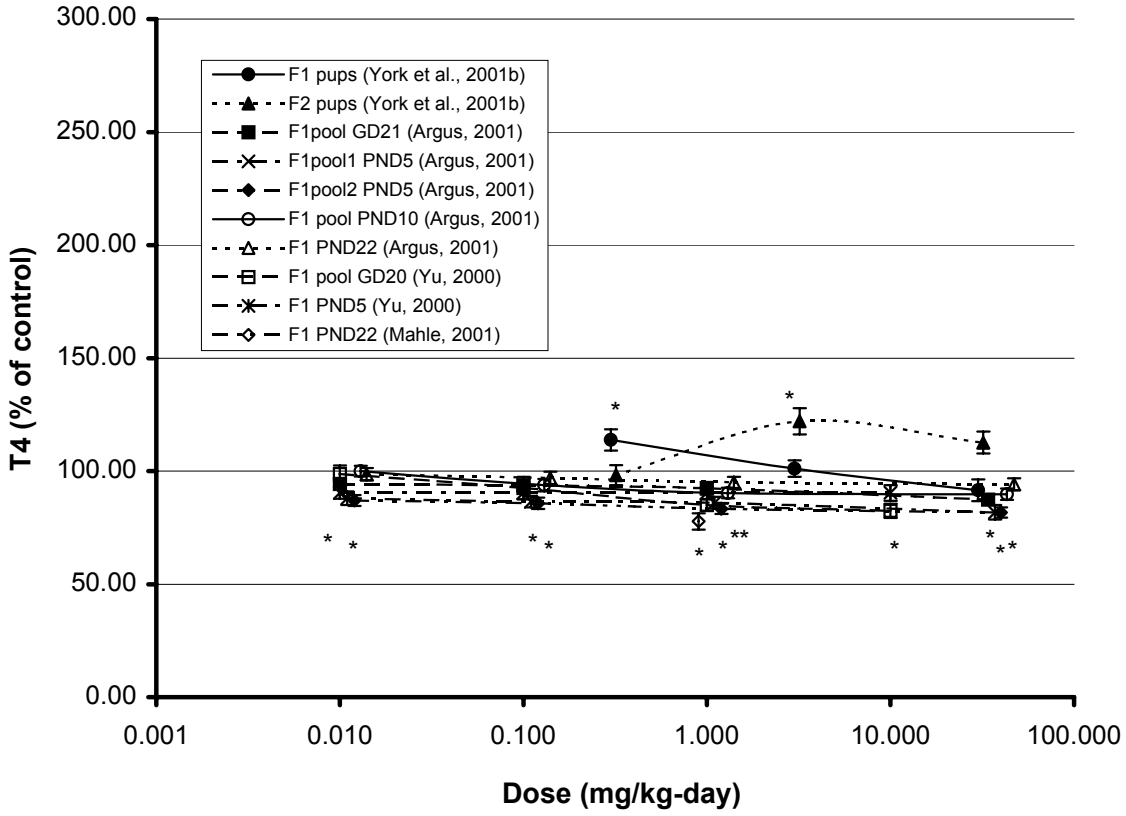

Figure 5b. TSH response in pups.

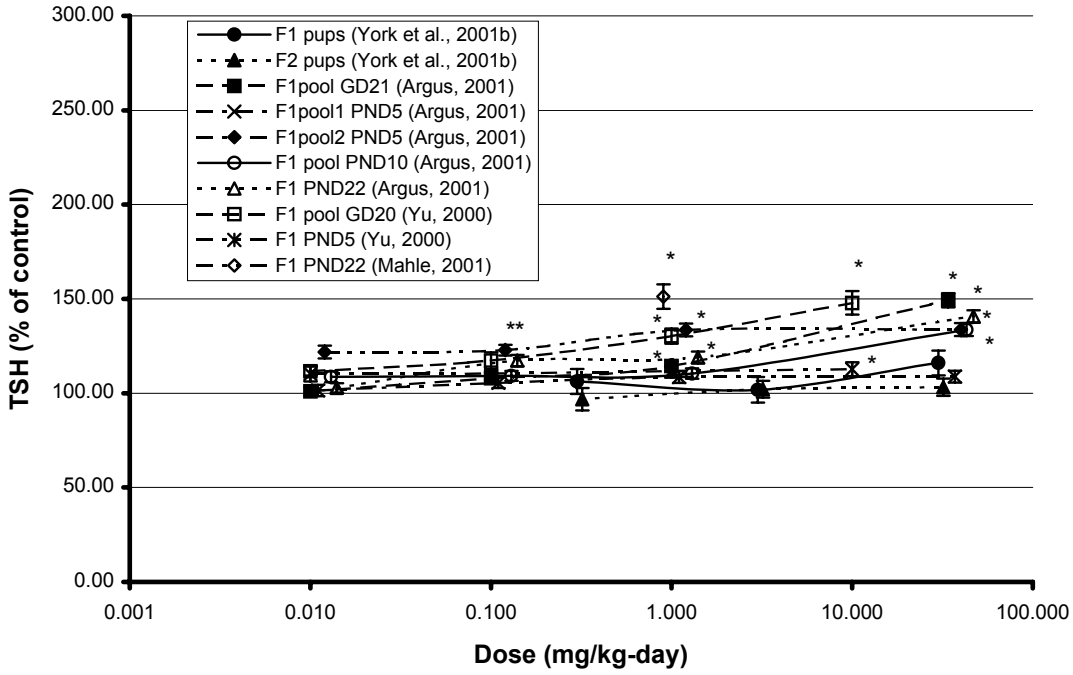

Figure 5c. Follicular cell hyperplasia in female pups.

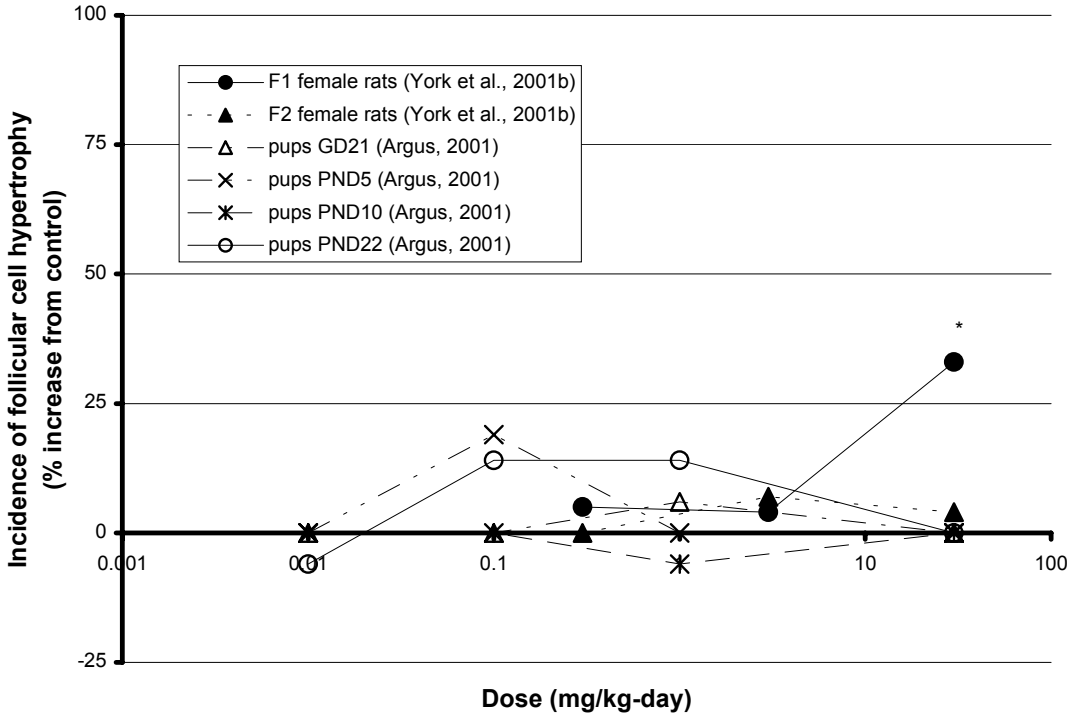

Figure 6a. Iodine uptake in male rats at different times.

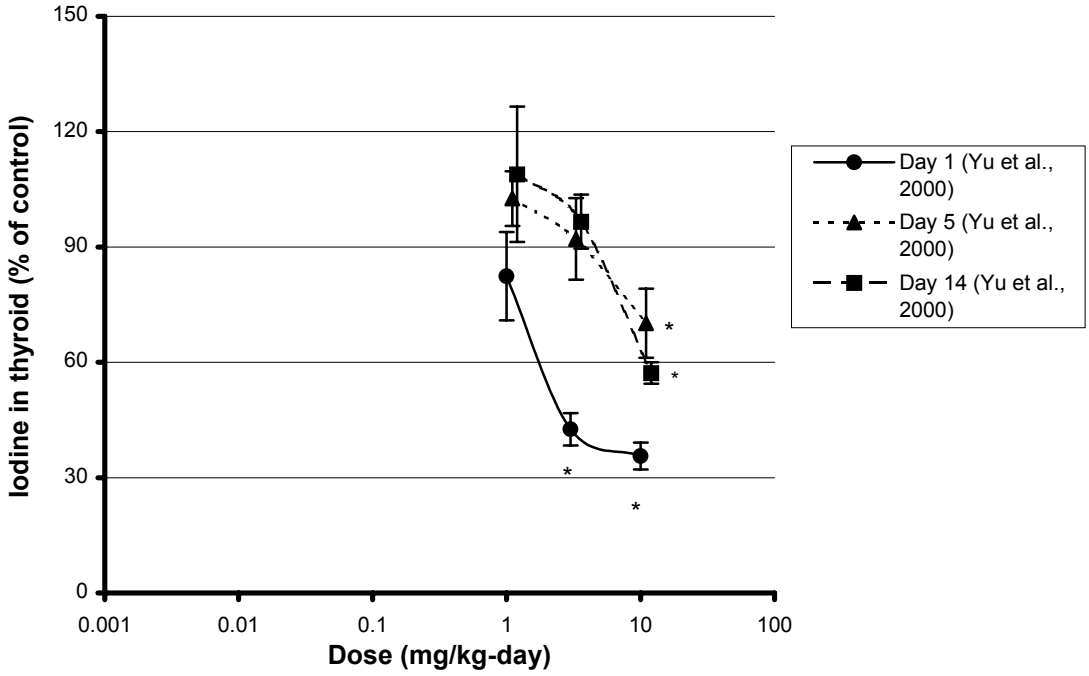

Figure 6b. Iodine uptake in humans at different times.

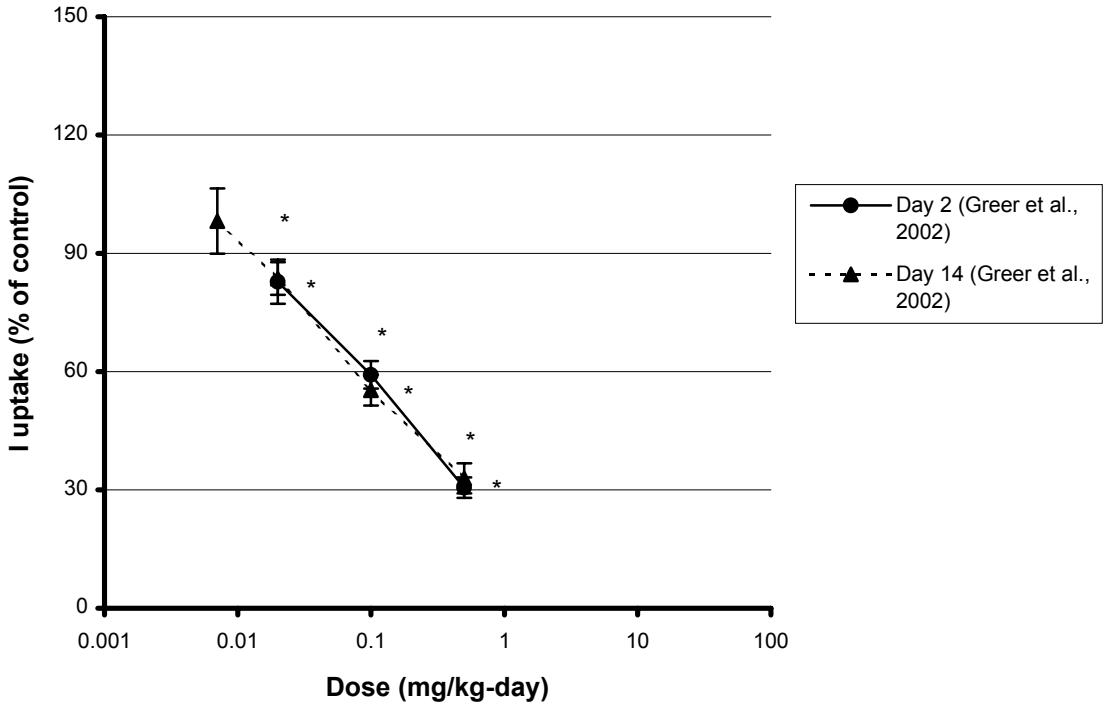

Table 1. Comparison of urinary iodine concentrations between the Chilean school age children and 6 to 11 year old children in the U.S.

	Children in U.S. 1971-1974 (NHANES I[1])	Children in U.S. 1988-1994 (NHANES III[1])	Children in Chile[2]		
			Antofagasta (control)	Chanaral (low perchlorate exposure)	Taltal (high perchlorate exposure)
Sample size	1826	3058	53	49	60
Urine iodine (ug/dL)	55.6±3.6 (48.5-62.7)*	30.5±1.9 (26.8-34.2)	75.6±5.5 (64.5-86.7)	61.4±5.1 (51.1-71.7)	76.6±6.1 (64.4-88.8)
Urine iodine/creatinine (ug/g)	619.3±46.0 (529.1~709.5)	339.6±26.5 (287.7~391.5)	1057.2±51.9 (952.9~1161.5)	827.2±51.3 (724.0~930.4)	947.4±49.6 (848.2~1046.6)

All data are expressed as mean ± standard error (SE).

1. The data for the children in U.S. are for the 6-11 years old age group reported from National Health and Nutrition Examination Surveys I and III (1971-1974 and 1988-1994) (Hollowell et al., 1998)
2. The data are obtained from Crump et al., 2000.

* The values in the parentheses indicate 95% confidence interval.

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

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

Table 3. Perchlorate Reference Doses from Human Studies.

	Recommended RfD	**Supporting RfD**	**Upper Bound RfD**
Critical Effect	**T4 Decrease in Children**	Inhibition of Iodine Uptake	T4 Decrease in Adults
Study	**Crump et al., 2000**	Greer et al., 2002	Greer et al., 2002
Point-of-Departure (mg/kg-day)	**Human NOAEL**	Human Key Event Threshold	Human NOAEL
	0.006	0.006	0.5
Area of Uncertainty:			
within human (UF_H)	**3**	1	10
animal to human (UF_A)	**1**	1	1
subchronic to chronic (UF_S)	**1**	1	1
LOAEL to NOAEL (UF_L)	**1**	1	1
database (UF_D)	**1**	1	1
Total Factor	**3**	1	10
RfD (mg/kg-day)	**0.002**	0.006	0.05
Confidence in RfD	**High**		