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Letter to the editor

Critical effect of perchlorate on neonates is iodide uptake inhibition

Strawson et al. (2004) calculate a reference dose for perchlorate based on thyroid hormone (TH) change in pregnant women as the critical effect. There are two issues that are not well developed, which renders the overall analysis misleading.

1. *Critical Effect.* Because normal adult humans have a large storage capacity of hormone in the thyroid gland, the 14-day Greer study (Greer et al., 2002), even with high perchlorate exposures, does not inform us about the relationship between perchlorate, iodide inhibition, TH synthesis, and TH levels. Applied to a 3 kg newborn, the Greer findings indicate that ~18–20 μg perchlorate per day will begin to inhibit iodine uptake. Empirical measurements show that neonates do not have TH stored in the thyroid gland (Savin et al., 2003; van den Hove et al., 1999); they must synthesize new hormone daily to meet known requirements. Therefore, any decrease in TH synthesis in a neonate will result in a reduction in serum T_4. Even a short duration (14 days) of TH insufficiency can result in measurable neurological or cognitive deficits in neonates (van Vliet, 1999). But, newborn thyroxine levels do not provide a measure of neonatal thyroid function. A significant proportion of T_4 at birth is derived transplacentally, and the half-life of serum T4 in neonates is approximately 3.5 days (Vulsma et al., 1989). Therefore, data derived from the neonatal screening programs do not measure the impact of perchlorate exposure to neonates and infants directly exposed to perchlorate. These facts are important to incorporate into a risk analysis for perchlorate.
2. *Compensatory or adverse effects*. Capen clearly articulates that direct measures of cell proliferation in the thyroid gland (i.e., hyperplasia versus hypertrophy) are required to determine whether the responsive increase in serum TSH following TH insufficiency is adverse or compensatory within the context of increased risk of thyroid cancer (Capen, 1994, 1997). Similarly, overt measures of neurodevelopment are required to determine whether changes in the HPT axis are adverse or adaptive within the context of neurodevelopment. The unpublished Argus (2001) study found statistically significant changes in measures of neurodevelopment, and these changes were upheld by an independent analysis (TERA, 2001). Although unpublished and controversial, Strawson et al. had no obvious reason to exclude it from their discussion since other unpublished and controversial studies were cited.

The uncertainties surrounding the application of the no observable effect level (NOEL) of Greer et al. to a human neonate seems greater than that described by Strawson et al. Specifically, the establishment of the NOEL was based on seven adults; while useful information, it may not provide a good estimate of the variance in the population for this important "threshold." Moreover, we do not know whether neonates are more or less sensitive than adults to perchlorate. We do not know the degree of iodine uptake inhibition required to inhibit thyroid hormone synthesis. And we do not know specifically the degree, and duration, of thyroid hormone insufficiency in neonates required to produce adverse effects. Finally, there are no clinical data on the effect of perchlorate on neonates that would provide even estimates of these uncertainties.

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Reference dose for perchlorate based on thyroid hormone change in pregnant women as the critical effect

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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 50 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 (Barzilai and Sheinfeld, 1966; Morgans and Trotter, 1960). 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

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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 0.15–0.84% 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 (2002) 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 (Brechner et al., 2000; Crump et al., 2000; Lamm et al., 1999; Li et al., 2000a,b, 2001), 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 RfD methods (Barnes and Dourson, 1988; Dourson, 1994; U.S. EPA, 2002).

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 (Stanbury and Wyngaarden, 1952; Wyngaarden et al., 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 Fig. 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 μg/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 μg/L on gestation day 20 (Argus, 2001); 241 μg/L on postnatal day 5 (Yu et al., 2002), and 886 μg/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 Fig. 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, Fig. 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 μg/L result in a minimal inhibition of iodine uptake of about 2% compared to serum perchlorate levels of 871 μg/L which result in about 70% inhibition of iodine uptake. In contrast, Fig. 2B summarizes several human

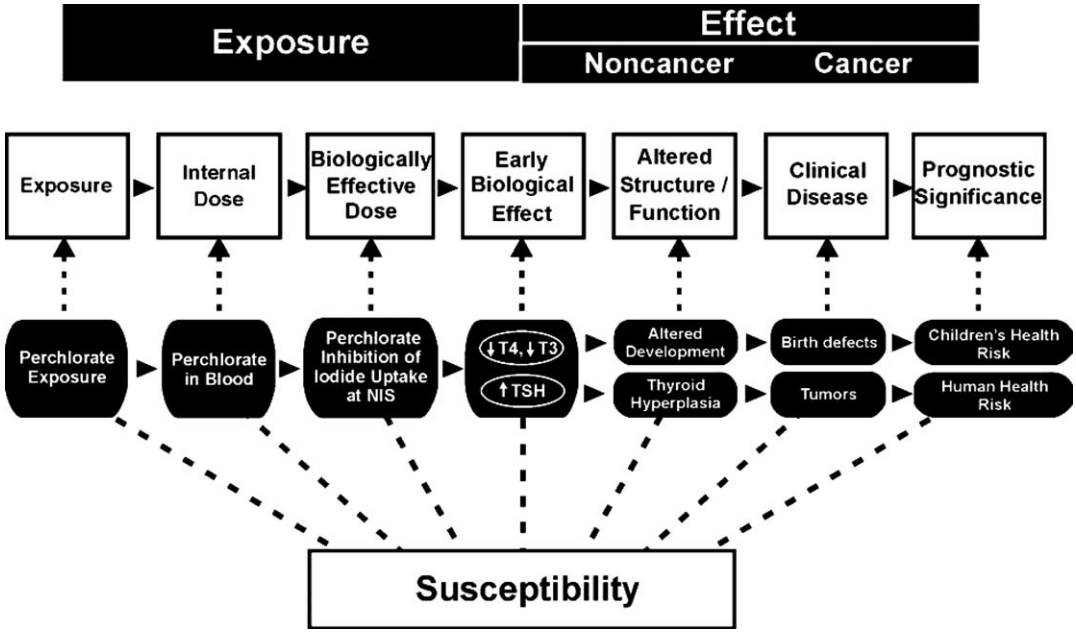

Fig. 1. Mode of action model for perchlorate toxicity proposed by U.S. EPA (2003). Perchlorate interferes with the sodium (Na+)-iodide (I–) symporter (NIS) present in various tissues, particularly thyroid. The model shows the exposure–dose response continuum considered in the context of biomarkers (classified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

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 μg/L. Figs. 2A and B 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 (Gibbs et al., 1998; Greer et al., 2002; Lamm et al., 1999; Lawrence et al., 2000, 2001). 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 Fig. 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 μg/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 μg/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 Fig. 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

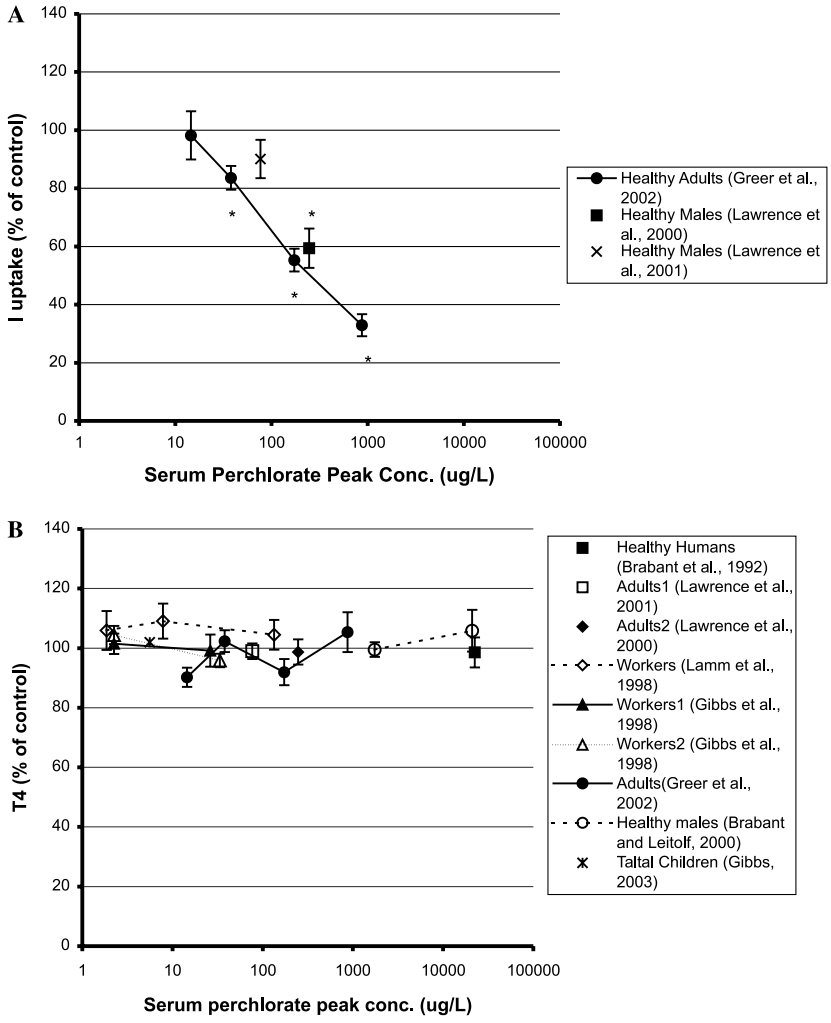

Fig. 2. (A) I uptake in humans as a formation of serum perchlorate peak concentration. (B) Human T4 response to perchlorate dose as a formation of serum perchlorate peak concentration.

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 (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, and 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, postnatal day 5, postnatal day 10 (Argus, 2001; Yu, 2000; Yu et al., 2002), and male and female pups on postnatal 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. Figs. 3A, B, and C show T4, TSH, and thyroid hyperplasia, respectively, in females following 90 days of exposure.

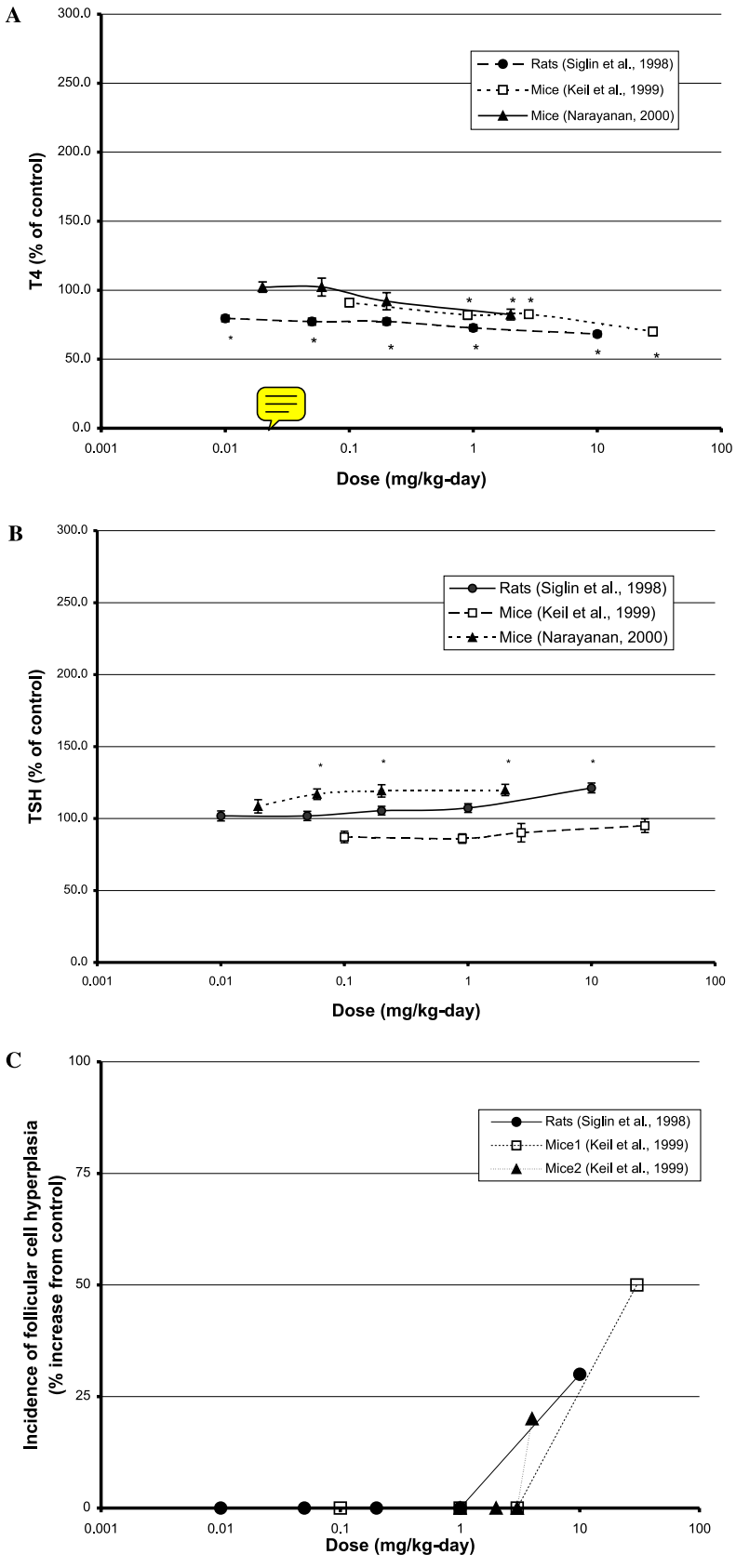

Fig. 3. (A) T4 response in female animals at 90 days. (B) TSH response in female animals at 90 days. (C) Follicular cell hyperplasia in female animals (90 days).

Figs. 4A, B, and C demonstrate the same data in dams, and Figs. 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 Figs. 3–5, 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 Figs. 3–5, 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 Figs. 3–5).

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 Figs. 2A, 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 (Brechner et al., 2000; Crump et al., 2000; Lamm et al., 1999; Li et al., 2000a,b, 2001), 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.

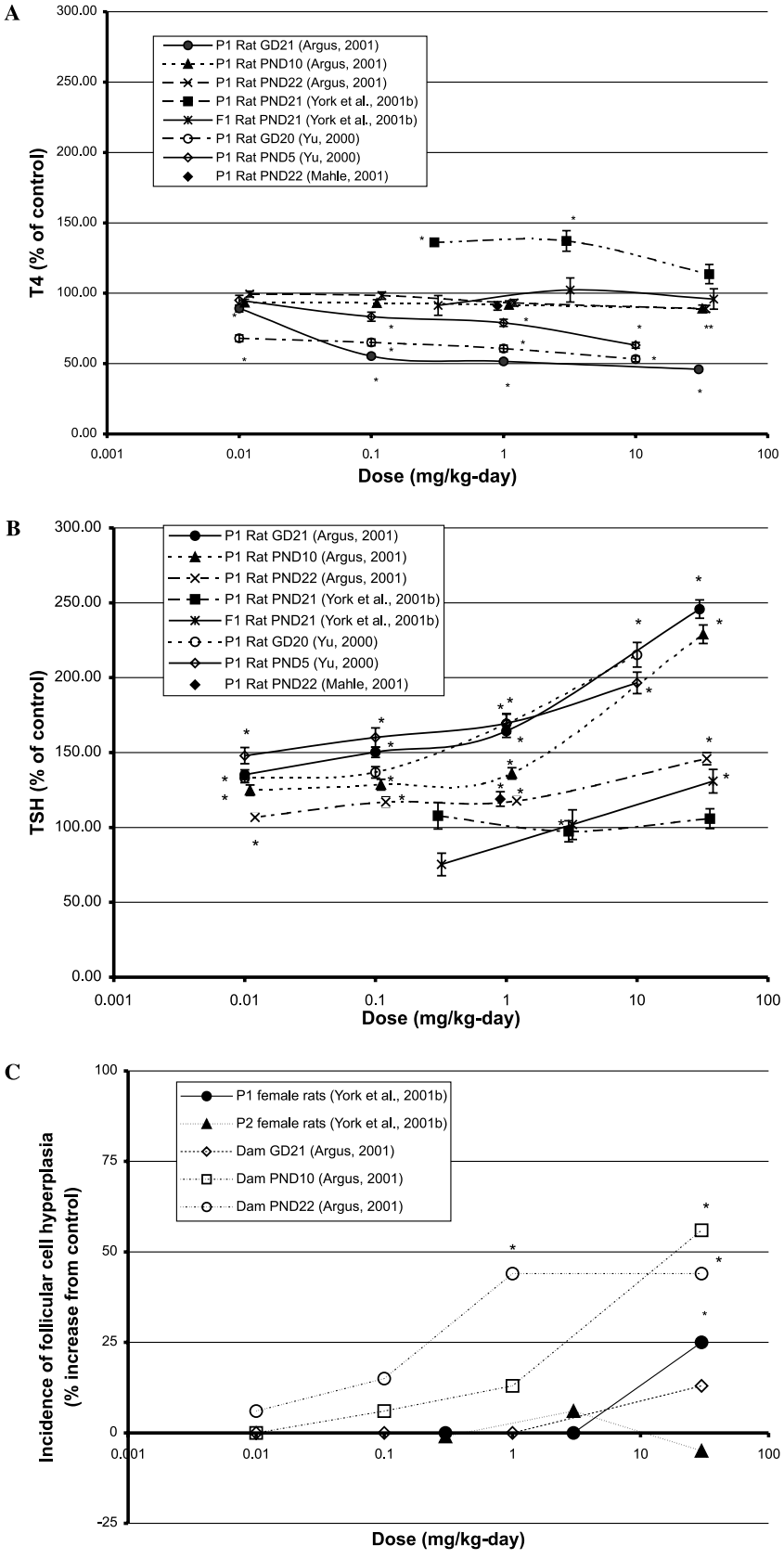

Fig. 4. (A) T4 response in dams. (B) TSH response in dams. (C) Follicular cell hyperplasia in dams.

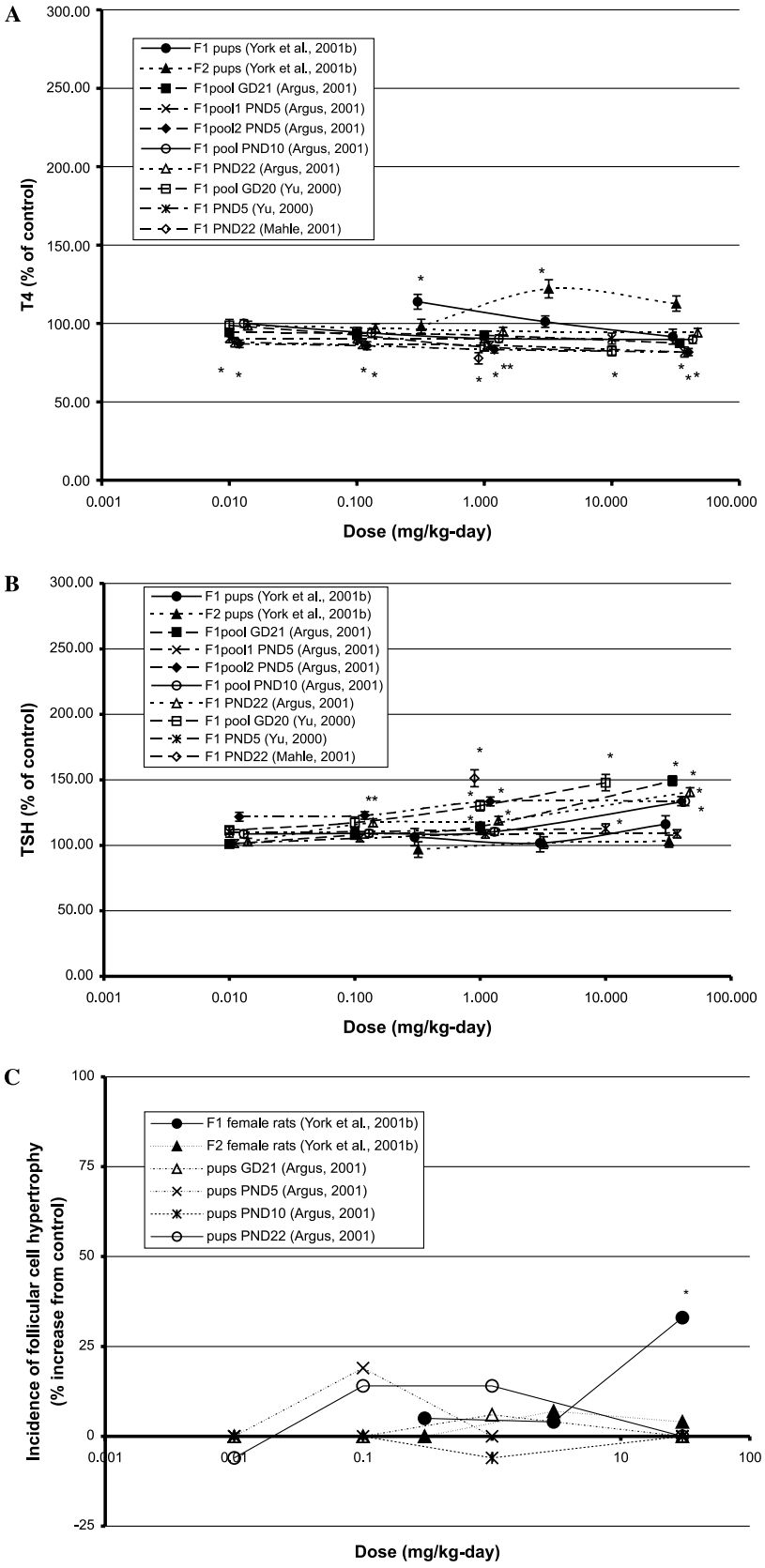

Fig. 5. (A) T4 response in pups. (B) TSH response in pups. (C) Follicular cell hyperplasia in female pups.

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 μg/L, estimated dose of 0.006 mg/kg-day[1]) 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 μg/L) and non-detectable (<4 μg/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 μg/L, with a mean of 5.6 μg/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 μg/L at a dose of 0.007 mg/kg-day (see Fig. 2A).

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

[1] This value is based on average Taltal exposure of 0.112 mg/L (i.e., 112 μg/L) and a drinking water consumption of 1.5 L per day for a 28 kg child (i.e., 0.112 mg/L × 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.

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

	Children in U.S. 1971–1974 (NHANES I)[a]	Children in U.S. 1988–1994 (NHANES III)[a]	Children in Chile[b]		
			Antofagasta (control)	Chanaral (low perchlorate exposure)	Taltal (high perchlorate exposure)
Sample size	1826	3058	53	49	60
Urine iodine (μg/dL)	55.6 ± 3.6 (48.5–62.7)[c]	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 (μg/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 means ± standard error (SE).
[a] The data for the children in U.S. are for the 6–11-year-old age group reported from National Health and Nutrition Examination Surveys I and III (1971–1974 and 1988–1994) (Hollowell et al., 1998).
[b] The data are obtained from Crump et al. (2000).
[c] The values in the parentheses indicate 95% confidence interval.

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[2] 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.

3.3. 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

[2] According to 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.

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 Figs. 2A and B). 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.

Table 2
Benchmark doses and their lower limits for iodine inhibition in adult males and females

	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

Data from Greer et al. (2002) (all values in mg/kg-day).

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

Non-cancer 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 (3.2), 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 Fig. 6A) cause variable, but statistically significant changes in hormone levels (see Figs. 3A and B, 4A and B, and 5A and B). While in humans, doses that cause 70% iodine uptake inhibition have no effect on hormone levels (see Fig. 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)

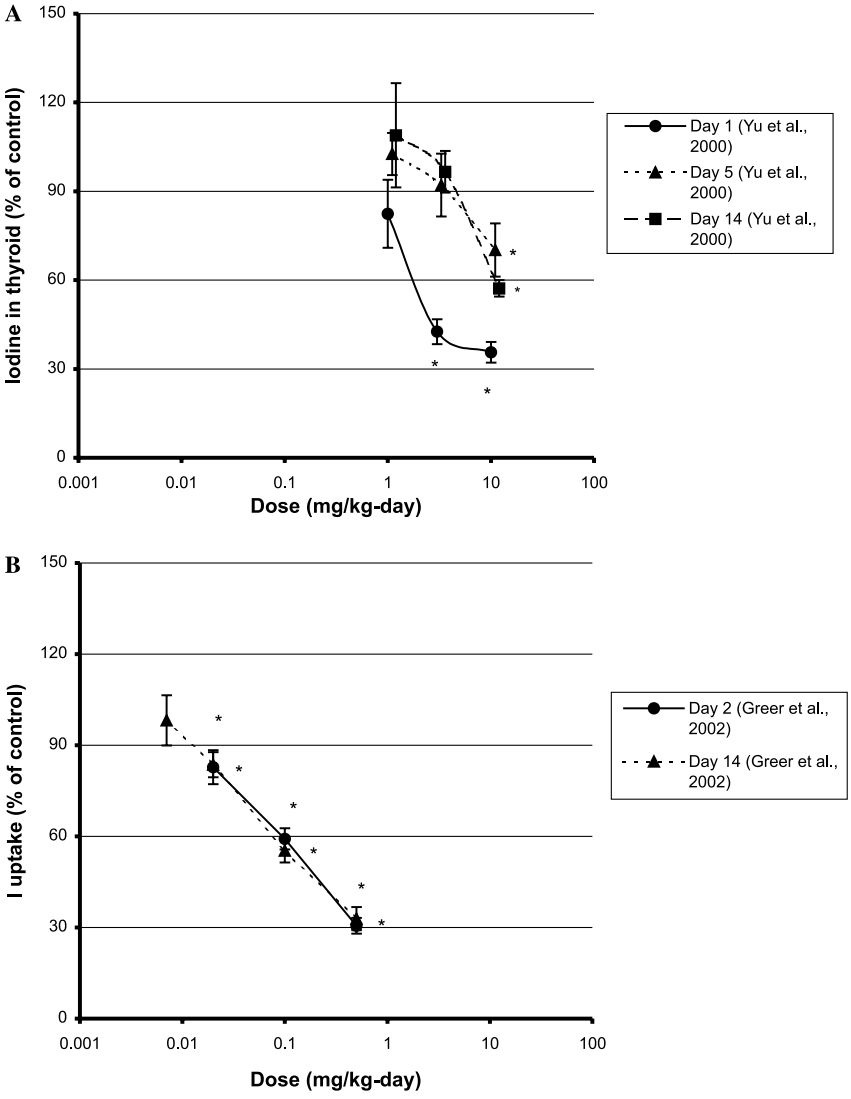

Fig. 6. (A) Iodine uptake in male rats at different times. (B) Iodine uptake in humans at different times.

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.[3] 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 less 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 have been gathered in pregnant women, this uncertainty factor of 3 may no longer be needed.

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, 0.05, 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, 0.025, 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.

[3] 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.

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 (Burleson, 2000; Caldwell et al., 1996; Keil et al., 1999; Siglin et al., 1998) or 90 days (Burleson, 2000; Keil et al., 1999; Siglin et al., 1998). 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 (Figs. 6A and B). For rats, iodine uptake inhibition data were available for days 1, 5, and 14 of drinking water exposure. The Fig. 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). Fig. 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 Fig. 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."[4]

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 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 Krüskemper, 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

[4] 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 (U.S. EPA, 2002).

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 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

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 0.006	Human key event threshold 0.006	Human NOAEL 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		

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, 1994) 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 (2002), 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.

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Response to letter to the editor

Response to "Critical effect of perchlorate on neonates is iodide uptake inhibition" by Zoeller

We thank Drs. Zoeller and Rice for their comments. While we agree with their points concerning uncertainties in the response of neonates to perchlorate, we disagree that these uncertainties prevent the development of a reference dose (RfD) based on human data. Perchlorate has been detected in public water supplies. To regulate perchlorate, an RfD *must* be developed. Given the available perchlorate database, the RfD must be derived from either rat or human studies—and we believe that the rat studies introduce an even greater degree of uncertainty into the risk assessment.

Because our focus was the human studies, we limited our manuscript to primarily discussing these studies. The Argus 2001 developmental toxicity study did find some statistically significant changes in the thickness of some regions of pup brains using a pair-wise comparison. In 2001, TERA asked experts on neurodevelopment to review this study. Far from confirming these findings, this analysis concluded that the statistical methods were inadequate to assess whether any treatment-related effects were observed. In addition, all reviewers concluded that design flaws prevented drawing any conclusions about the effects of perchlorate on neurodevelopment. In 2002, one reviewer conducted a further re-analysis of the Argus 2001 data (Wahlsten, 2002). He did find treatment-related, *very small* increases in the thickness of three brain regions. But the effect was so small that Dr. Wahlsten concluded it was smaller than normal variation in controls and had no biological significance. Because we concluded that the Argus study did not demonstrate neurodevelopmental effects, we did not include it in our paper.

Our RfD is not based on the clinical study by Greer et al. (2002) as our colleagues seem to suggest. We used Crump et al. (2000), which studied thyroid function in 9784 newborns and 162 school-age children in three cities in Northern Chile with perchlorate in public water. We selected this study because it included a large population of neonates—one of the sensitive populations for perchlorate, and it included 127 children approximately age 7 who were likely exposed both in utero and for their entire lifespan. Therefore, use of Crump et al., 2000 as the critical study reduces some of the uncertainties associated with the short-term clinical studies.

Pregnant women are also a sensitive population for perchlorate because metabolic changes that occur during pregnancy require an increased hormonal output by the maternal thyroid (Glinoer, 2001). Therefore, they are sensitive to situations that deplete the availability of iodine. Ongoing studies (Téllez et al., 2004) are examining whether perchlorate affects pregnant women in Chile. Maternal T4, TSH, urinary iodine, and breast milk iodine are comparable among the three cities. Perchlorate was detected in maternal serum, cord serum, and breast milk in women exposed to 114 μg/L perchlorate in water. Therefore, a perchlorate concentration of 114 μg/L appears to be a NOAEL; it is not affecting the ability of pregnant women to maintain an increased output of thyroid hormones.

Next, we address several other uncertainties mentioned in the letter, including (1) relative sensitivity of neonates to adults, (2) degree of iodine uptake inhibition required to inhibit thyroid hormone synthesis, (3) the degree and duration of thyroid hormone insufficiency that produces adverse effects in neonates.

Issue 1. The Chilean studies (Crump et al., 2000; Téllez et al., 2004) provide reasonable data on the response of neonates at doses equivalent to the threshold of iodine uptake inhibition observed in Greer et al. (2002). If neonates were significantly more sensitive than adults to perchlorate, they would respond at lower doses than adults. They do not. Physiologically based pharmacokinetic models demonstrate that the predicted threshold for iodine uptake inhibition in fetuses is approximately 2-fold lower than the predicted threshold in adults (Mattie et al., 2004).

Issue 2. In healthy adults, both a short- and long-term exposure at the highest perchlorate doses resulted in serum perchlorate concentrations that inhibited iodine uptake by 70% without affecting thyroid hormone synthesis. We do not know if this relationship holds true for pregnant women and neonates. However, by basing an RfD on actual measured water concentrations that do not result in the inhibition of thyroid hormones in pregnant women or neonates, we are

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confident that we are protecting these populations. We do not know what perchlorate dose would be required to inhibit hormone synthesis in these populations, but we are confident that it is higher— not lower—than our RfD.

Issue 3. No studies in humans have quantified the degree of T4 suppression that can be tolerated before neurodevelopmental effects are observed. Some data in rats suggest that a >50% decrease of maternal serum T4 would be required before any effect on thyroid hormone levels in pup brains would be observed (Calvo et al., 1990; Pleus personal communication), but the relevance of this to humans is unclear. Nonetheless, no studies of perchlorate in healthy humans have involved doses high enough to result in *any* suppression of T4, much less result in adverse effects from T4 suppression.

In closing, we emphasize that the purpose of developing an RfD is to provide an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily perchlorate exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. The RfD we propose for perchlorate is based on a NOAEL in neonates and young children, is supported by new data in pregnant women, and includes an uncertainty factor to account for the remaining lack of data regarding pregnant women and their fetuses. We may never be able to exactly quantify what perchlorate dose may result in adverse effects in pregnant women and neonates, but we are confident that our RfD is lower than this dose—perhaps by an order of magnitude.

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Letter to the editor

Interspecies differences in susceptibility to perturbation of thyroid hormone homeostasis requires a definition of "sensitivity" that is informative for risk analysis

Lewandowski et al. (2004) develop a case for comparing the sensitivity of various mammalian species to thyroid toxicants on the basis of the lowest dose of perchlorate required to alter circulating levels of thyroid hormones. Two important issues are not addressed in this analysis, which weakens the authors' conclusion that the rat is "more sensitive than humans" to perchlorate.

The authors review the ability of perchlorate to inhibit iodide uptake into the thyroid gland of humans and rats (their Figs. 1 and 2) and its ability to reduce circulating levels of thyroid hormones (their Figs. 3–8). These data indicate that rats and humans are similar in their sensitivity to perchlorate's ability to inhibit iodide uptake into the thyroid gland, but that rats are far "more sensitive" to the ability of perchlorate to decrease serum thyroid hormone levels. In principle, blood levels of a hormone represent a balance between the rates of hormone secretion and clearance. Likewise, the amount of hormone stored in an endocrine gland represents a balance between hormone synthesis and release. Thus, the ability of perchlorate to reduce thyroid hormones in any animal will be determined by its ability to: (1) inhibit thyroidal iodide uptake, (2) inhibit thyroid hormone synthesis, (3) exhaust intrathyroidal stores of hormone, and (4) reduce thyroid hormone secretion.

It is obvious from this sequence that the duration of perchlorate exposure required to cause a reduction in circulating thyroid hormone level will depend on the size of the intrathyroidal store and the serum half-life of thyroid hormones. Because adult euthyroid humans have a serum half-life of T_4 of around 7 days, and intrathyroidal stores of T_4 are estimated to be several month's worth (Greer et al., 2002), it is clear why perchlorate caused a reduction in serum thyroid hormones in rats but not in humans. However, rats and humans may be similarly sensitive to perchlorate's ability to reduce thyroid hormone *synthesis*—a seemingly important issue. Likewise, considering that a human neonate has a serum half-life of T_4 of around 3 days (Vulsma et al., 1989) and intrathyroidal stores of T_4 estimated to be less than one day's worth (van den Hove et al., 1999), it is easily predictable that human neonates will exhibit a decrease in serum thyroid hormone levels within 14 days of exposure to doses of perchlorate that would clearly not affect serum T_4 in normal adults. Thus, if we assume that a human neonate is no more *sensitive* to perchlorate's ability to inhibit thyroid hormone synthesis than are adults, we can still predict that they will be more *vulnerable* to the adverse effects of perchlorate.

The definition of "sensitivity" to thyroid disruption by exogenous chemicals in general should be debated, especially within the context of neurodevelopment. The lowest dose of toxicant that causes a reduction in serum hormone levels is one possible definition, but it does not take into account that animals may differ in their sensitivity to thyroid hormone insufficiency per se, which is likely to be a more significant issue than simply the reduction in hormone levels.

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Interspecies differences in susceptibility to perturbation of thyroid homeostasis: a case study with perchlorate

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Abstract

Despite many physiological similarities, humans and rats exhibit notably different susceptibilities to thyroid perturbation. Considerable research has recently been conducted on the thyroid-active chemical perchlorate, a chemical of emerging environmental and regulatory interest. While the data indicate humans and rats exhibit similar dose–response relationships in terms of acute inhibition of thyroidal iodide uptake, the two species appear to exhibit notable differences in terms of thyroid hormone response, the toxicologically significant consequence of iodide uptake inhibition. We analyzed dose–response data for changes in serum T_3, T_4, and TSH levels from studies in humans, rats, mice, and rabbits. We found that thyroid homeostasis in the rat appears to be strikingly more sensitive to perchlorate than any of the other species. Rats exhibited an increase in serum TSH at 0.1 mg/kg-day whereas other species remained unresponsive even at doses of 10 mg/kg-day. Less pronounced but consistent effects were seen with serum T_3 and T_4. These cross-species comparisons provide strong evidence that data obtained from rat studies should be critically evaluated for their relevance to humans. If rat data are used to develop toxicity criteria for perchlorate, we propose that this is an instance where an inter-species uncertainty factor less than one is supportable.

Disclosure statement: One of the authors (BDB) has been hired by Lockheed Martin Corporation as an expert in litigation involving perchlorate. A portion of the initial research presented in this paper was conducted in conjunction with her role in that matter.

Keywords: Perchlorate; Cross-species; Extrapolation; Thyroid; Uncertainty factors

1. Introduction

Perchlorate (ClO_4^-) has been detected in groundwater in many parts of the U.S., primarily in association with industries involved in rocket, explosives and fireworks manufacturing, and propellant handling (Motzer, 2001). Low concentrations of perchlorate (<30 μg/L) have also been detected in groundwater at locations not associated with industrial use of perchlorate, possibly due to the historical use of nitrate fertilizers that contained small amounts of perchlorate (Urbansky, 2002). Concentrations measured in most public water supplies are below 50 μg/L, although levels as high as several hundred μg/L have been reported in some drinking water wells in certain communities (Motzer, 2001). In a recent draft assessment (USEPA, 2002), the USEPA has proposed a reference dose (RfD) for perchlorate of 3×10^{-5} mg/kg-day, which would be expected to result in a Maximum Contaminant Level Goal (MCLG) in the range of 1–5 μg/L. The proposed RfD is based primarily on data collected in rats coupled with various uncertainty factors. The validity of this approach is questionable given the exceptional sensitivity of the rat model to thyroid perturbation (McClain, 1995; Paynter et al., 1988; Thomas and Williams, 1999; USEPA, 1998). This paper examines the magnitude of inter-species differences in susceptibility to thyroid perturbation, with particular attention to differences between rats and humans. Estimating the magnitude and basis of the inter-species differences in sensitivity is critical to reconciling animal and human data, and using such data appropriately in making risk-based decisions.

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2. Thyroid function

Perchlorate exerts its toxicity via perturbation of the thyroid gland and thyroid hormones (Wolff, 1998). The principal toxicological effect associated with perchlorate exposure in both experimental animals and humans (observed during perchlorate's use as a pharmaceutical) involves the thyroid and alterations in thyroid hormone levels. Dermal, hematological, and immunological effects have been reported sporadically in association with use of perchlorate as a pharmaceutical (Wolff, 1998). However, these effects were most frequently observed at very high perchlorate doses, many hundred of mg/day (Wolff, 1998). This is in contrast to thyroidal effects of perchlorate, which can occur at lower doses (as described below). Studies in laboratory animals also indicate that the thyroidal effect occur at lower doses than effects on other endpoints. Thus, perchlorate's effect on thyroid hormone production is the key endpoint of concern for environmental exposures.

A primary function of the thyroid is production of the thyroid hormones, triiodothyronine (T_3) and thyroxine (T_4). A key component of the thyroid hormone production pathway is the sodium iodide symporter (NIS), a membrane protein that translocates iodide into thyroid follicular cells (Dohan et al., 2003; Eskandari et al., 1997). Iodide (I^-) is transported into the thyroid follicular cell against a concentration gradient by NIS and is subsequently oxidized to iodine (I^0) by the enzyme thyroglobulin peroxidase (TPO), after which iodine is coupled to tyrosine residues on the thyroglobulin (Tg) molecule. Thyroglobulin is stored within a cavity inside the thyroid follicle (called the lumen) in the form of a viscous substance called colloid. In response to signals from the pituitary, Tg is transported back into the follicular cell and is cleaved to yield T_3 and T_4, which are subsequently secreted into the blood. Secretion of thyroid hormones is controlled by a well-known feedback mechanism. When serum T_3 and T_4 levels are too low, thyrotropin releasing hormone (TRH) is secreted by the hypothalamus and thyroid stimulating hormone (TSH) is released by the anterior pituitary to promote thyroidal iodide uptake and thyroid hormone synthesis. The subsequent rise in serum T_3 and T_4 levels results in a negative feedback, causing TRH and TSH levels to fall. Measuring serum levels of these hormones represents the standard approach for assessing thyroid function.

3. Disruption of thyroid hormone homeostasis

Inhibition of iodide uptake and subsequent disruption of thyroid hormone synthesis and increase in serum TSH, if of sufficient magnitude and duration, can result in noticeable symptoms of hypothyroidism (e.g., clinical hypothyroidism) (Wolff, 1998). In both rats and humans, prolonged TSH elevation can lead to thyroid hypertrophy, hyperplasia, and goiter (i.e., thyroid enlargement). Furthermore, in the rat, prolonged increases in TSH are also known to be tumorigenic. However, a similar relationship between prolonged TSH elevation and thyroid tumors has only been observed in humans with congenital defects in TPO or TBG synthesis and only after many years of elevated serum TSH levels (Thomas and Williams, 1999). Effects on the thyroid may also affect other tissues which are influenced by thyroid hormones. Because thyroid hormones are critical during development (Zoeller, 2003), the fetus and neonate may be particularly susceptible to thyroid hormone perturbations, either directly or via the maternal thyroid. However, the level of thyroid hormone perturbation associated with developmental effects is unclear; studies have clearly shown that clinically recognizable maternal hypothyroidism during pregnancy results in adverse developmental outcomes (Bongers-Schokking, 2001) but evidence for developmental effects from slight, subclinical thyroid hormone decrements is conflicting (Haddow et al., 1999; Radetti et al., 2000).

While the effects of thyroid hormone depression are fairly well known, the potency of perchlorate in causing such effects remains a matter of debate and inquiry, particularly in humans. Perchlorate competitively inhibits iodide uptake via NIS, due to similarities in ionic size and charge (Van Sande et al., 2003; Wolff and Maurey, 1963). Similar effects are seen with comparably sized ions such as thiocyanate and, to a lesser extent, nitrate, but not with smaller ions such as bromide (Wolff and Maurey, 1963). Large doses of perchlorate (i.e., hundreds of mg/day) disrupt thyroid hormone homeostasis, as seen in both experimental animals and humans receiving perchlorate to treat thyrotoxicosis.

In rats, the homeostatic disruption caused by high levels of perchlorate can lead to development of thyroid tumors (Capen, 1994; Fernandez Rodriguez et al., 1991; Gauss, 1972; Kessler and Kruskemper, 1966). This high dose effect has not been observed in humans, although the high dose data in humans is limited to individuals receiving perchlorate for a pre-existing hyperthyroidism. Data regarding the carcinogenic potential of perchlorate at lower exposure levels are available from two studies in human populations exposed to perchlorate in drinking water (Li et al., 2001; Morgan and Cassady, 2002). Neither study reported an excess incidence of thyroid tumors in the exposed populations. Overall, the data indicate that rats are more susceptible than humans to thyroid carcinogenesis from thyroid active agents such as perchlorate. This conclusion has been expressed quite widely (Hill et al., 1989; McClain, 1995; Paynter et al., 1988; USEPA, 1998).

A more recent concern has focused on the neurodevelopmental effects of perchlorate, given the importance of thyroid hormones for development in utero. Animal studies of these endpoints (e.g., alterations in the size of various brain structures, altered patterns of myelination, behavioral responses) have not been definitive with negative results in rabbits (York et al., 2001a,b) and contradictory findings in rats (Argus, 2001; York et al., 2001a,b). Ecological studies conducted in human populations (focusing on neonatal hormone levels rather than neurodevelopmental endpoints) have yielded generally negative findings (Crump et al., 2000; Kelsh et al., 2003; Lamm and Doemland, 1999; Li et al., 2000a,b) with one positive published study (Brechner et al., 2000). The animal and human data have been subject to considerable scrutiny and are currently the subject of vigorous debate. When various criteria such as consistency and dose–response are considered, however, the data do not provide convincing evidence that low dose perchlorate exposures (i.e., those less than 1 mg/kg) have an effect on neurological development.

An important component in interpreting the low dose studies with respect to human health risk involves identifying the level of perchlorate exposure that causes an adverse impact on thyroid hormone levels. Plasticity in the thyroid hormone production system compensates for daily variations in dietary iodide intake and the presence of thyroid active compounds (e.g., thiocyanates, isoflavones) in the diet (Divi et al., 1997; Laurberg et al., 2002; Michalkiewicz et al., 1989). This plasticity has many components, including storage of a reserve of Tg as colloid, the ability to upregulate NIS activity and iodide uptake, and temporary hypertrophy of follicular cells. There appear to be quantitative differences in the effectiveness of these compensatory mechanisms among species, which likely affects species differences in sensitivity to thyroid active agents.

4. The unique sensitivity of the rat thyroid

Although the basic process of thyroid hormone synthesis and release is qualitatively similar across species, there are notable quantitative species-specific differences in hormone synthesis and serum binding that lead to remarkably different susceptibilities to thyroid hormone perturbation. These factors are listed in Table 1.

Expression of NIS protein appears to be an important indicator of the increased sensitivity of the rat to thyroid perturbation. In the rat, NIS is expressed at high density in most thyroid follicular cells (Josefsson et al., 2002). In contrast, in humans and other species, NIS is not observed in all follicular cells and, when expressed, is expressed in a ''patchy'' pattern (Josefsson et al., 2002). However, humans with the autoimmune disorder Graves' disease express significantly higher levels of NIS, similar to the pattern observed in the rat (Caillou et al., 1998).

The two thyroid hormones, T_3 and T_4, are released into the bloodstream bound to protein carriers. Binding of thyroid hormones to carrier proteins protects the hormones from metabolic degradation and reduces their elimination via the kidneys. In humans, T_3 and T_4 are primarily bound to thyroxine-binding globulin (TBG), a

Table 1
Species-specific physiological differences in thyroid and thyroid hormone parameters

	Human	Rat	Dog	Rabbit	Monkey
NIS expression	Sporadic[a]	Ubiquitous[a]	nd	nd	nd
Colloid	Plentiful[b]	Limited[b]	Plentiful[c]	nd	Plentiful[d]
Thyroxine binding globulin	Present[e,l]	Absent[e,l,*]	Present[e,l]	Absent[f,l]	Present[g,l]
T_3 half-life (days)	1[b]	0.25[b]	nd	nd	1.1[h]
T_4 half-life (days)	5–6[b]	0.5–1[b]	0. 6[e]	1.3[i]	2.3[h]
Serum T_3 (ng/dL)	147[e]	25–100[j]	48–154[j]	130–143[j]	54–295[j]
Serum T_4 (μg/100 ml)	7.2[e]	3–7[j]	1.5–3. 6[j]	1.7–2.4[j]	1.8–7.6[j]
Serum TSH (ng/ml)	0.05–0.5[k]	0.6–3.4[j]	2.7– 7.9[j]	nd	2.5[j]

nd—No data could be located in the literature.
[a] Josefsson et al. (2002).
[b] USEPA (1998).
[c] Kameda (1984).
[d] Golarz de Bourne and Bourne (1975).
[e] Kaptein et al. (1994).
[f] Larsson et al. (1985).
[g] Seo et al. (1989).
[h] Sawhney et al. (1978).
[i] Kannan et al. (1990).
[j] Loeb and Quimby, 1999.
[k] Kaptein et al. (1994) plus a hormone potency conversion factor of 8 μIU/ng.
[l] Dohler et al. (1979).
[*] Present in neonates and older animals but not found in adults of typical experimental age.

specific, high affinity carrier protein. Approximately 68 percent of the total circulating T_4 in humans is bound to TBG (Kaptein et al., 1994). The remainder is bound to less specific carrier proteins such as albumin and transthyretin, with less than 1 percent existing as the free (biologically active) hormone (Kaptein et al., 1994). Binding of thyroid hormones to TBG is 1000–100,000 times stronger than binding to albumin (McClain, 1995). TBG has been detected in other primates as well as in dogs and certain ungulates (Dohler et al., 1979). Levels of TBG protein in rats vary considerably with age, with TBG levels peaking at about one month, then declining rapidly to virtually non-detectable levels by two months. TBG levels then gradually increase beginning at about seven months, reaching levels that are approximately 25% of the peak post-natal levels by about 20 months of age (Savu et al., 1991). Thus, TBG is not found in rats between the ages of 2 and 7 months, the age range typically used in basic toxicology studies. In adult rats, T_3 and T_4 are bound to the low affinity carriers albumin and transthyretin. As a consequence, the half-life of thyroid hormones in adult rats is substantially shorter than in humans. For example, the T_4 half-life in adult rats is 12 h as compared with 5–9 days in adult humans (McClain, 1995). Similarly, the T_3 half-life is about 6 h in adult rats as compared to 24 h in adult humans (Hill et al., 1989).

In humans, the pool of TBG-bound thyroid hormone functions as a stable reserve that may be used when additional amounts of thyroid hormone are required. Without the high affinity carrier, rats have very little reserve capacity of circulating thyroid hormone. The faster turnover of thyroid hormones in the rat results in an increased need for thyroid hormone production, which is maintained by higher circulating levels of TSH. T_4 production in the rat has been reported to be approximately 10× that in the human (Dohler et al., 1979) and serum TSH levels in rats are 6- to 60-fold higher than those in humans (Hill et al., 1989). The rat thyroid has therefore been described as being under a chronic state of stimulation (Hill et al., 1989).

The greater synthetic demands placed on the rat thyroid are reflected in species-specific differences in thyroid histology. The majority of follicles in the rat are much smaller and contain much less colloid than primate follicles (McClain, 1995). As previously noted, colloid serves as a reserve pool of thyroid hormone precursor which can be rapidly mobilized to maintain serum thyroid hormone levels. With minimal colloid reserve, decreases in thyroid hormone levels in the rat due to changes in thyroidal iodide uptake might be expected to be much sharper than in other species.

These species-specific, physiological differences in the thyroid suggest that the rat would be more susceptible to thyroid perturbation. This appears to be borne out by experimental evidence. Rats have particularly high background rates of thyroid tumor incidence compared to either mice or humans (Ries et al., 2002; USEPA, 2002). Rats have also been shown to be more susceptible than humans or other species to thyroid carcinogenesis after exposures to certain exogenous chemicals (Littlefield et al., 1989, 1990; Steinhoff et al., 1983; Swarm et al., 1973; USEPA, 1998). We accordingly sought to determine whether the unique sensitivity of the rat thyroid was observed with regard to blocking of iodide uptake at the NIS, an effect known to be associated with perchlorate.

5. Interspecies differences in susceptibility to perchlorate

5.1. Approach

We obtained effects data for perchlorate from the published literature and key unpublished studies for humans, rats, mice, and rabbits. The studies used in this comparison involved acute (1–2 days), sub-acute (14–48 days), or sub-chronic (90 days) exposures. We focused on two endpoints: iodide uptake by the thyroid and alterations in serum thyroid hormone and TSH levels. These endpoints represent early events in perchlorate's mode of action. The evaluation did not include changes in thyroid histopathology, thyroid neoplasia, or the neurodevelopmental effects of perchlorate because these have only been studied in a limited number of species and, in any event, are necessarily preceded by thyroid hormone changes. Data for male, female, pregnant female, neonatal, and fetal rats were obtained from studies conducted by the US Air Force (Meyer, 1998; Yu, 2000, 2002; Yu et al., 2000) and recent studies sponsored by USEPA (Argus, 2001; Siglin et al., 2000). Data for pregnant rabbits were obtained from the study of York et al. (2001a) while data for adult female (non-pregnant) mice were obtained from immunological studies conducted by Burleson Research Technologies (BRT, 2000) and Keil et al. (1999). Subacute data for humans (male and female adults) were obtained from the studies by Greer et al. (2002) and Lawrence et al. (2000, 2001). Two occupational studies of perchlorate exposed workers (Gibbs et al., 1998; Lamm et al., 1999) were also considered. Details of the studies used in the analysis are summarized in Table 2.

We are aware of the 2-generation study of perchlorate in rats published by York et al. (2001a,b). Although the data from this study do provide an indication of the effects of lifetime perchlorate exposures, the data from this study were not included in our analysis because the exposure times were not sufficiently described in their publication to allow comparison with other studies. We are also aware of data collected by Brabant and Leitolf under contract to the U.S. Air Force (Mattie, 2000). In this study, adult human volunteers were given

Table 2
Methodological details of studies used in the comparative analysis

Study	Species	Lifestages	Exposure duration (days)	Dose route	Perchlorate doses (mg/kg-day)[a]	N per dose group[b]	Relevant figures in this article
Argus (2001)	Rat	Pregnant dams	34	Drinking water	0, 0.0085, 0.085, 0.85, 25.5	14–16	3A, 4A, 5A
		Fetuses	21			2–6	
		Postnatal dams	46			16[c]	
		Neonates	31			16[c]	
BRT (2000)	Mouse	Adult females	14 and 90 days	Drinking water	0, 0.017, 0.051, 0.17, 1.7	7 for 14 day exposure, 6–8 for 90 day exposure	3B, 4B, 5B, 6, 8
Gibbs et al. (1998)[d]	Human (workers)	Adults (primarily males)	Unknown, avg job tenure–yr	Airborne dust	~0[e], 0.036	18 exposed 83 controls	6, 8
Greer et al. (2002)	Human	Adults males and females	14	Drinking water	0, 0.007, 0.02, 0.1, 0.5	7–10	2, 3B, 4B, 5B
Keil et al. (1999)	Mouse	Adult females	14 and 90 days	Drinking water	0, 0.085, 0.85, 2.55, 25.5	7–22	3B,4B,5B,6–8
Lamm et al. (1999)	Human (workers)	Adults (primarily males)	Unknown but likely chronic	Airborne dust	0.002, 0.005, 0.094, 0.85[f]	6–14	6–8
Lawrence et al. (2001, 2000)[g]	Human	Adults males	14	Water	0, 0.04, 0.14	8–9	1, 2, 3B, 4B, 5B
Meyer (1998)[h]	Rat	Adult males	NA	iv	0, 0.0085, 0.085, 0.85, 2.55	6	1
Siglin et al. (2000)	Rat	Adult males	14 and 90	Drinking water	0, 0.0085, 0.425, 0.17, 0.85, 8.5	20 (10 male/10 female)	3A, 3B, 4A, 4B, 5A, 5B, 6–8
York et al. (2001a)	Rabbit	Pregnant dams	22	Drinking water	0, 0.085, 0.85, 8.5, 25.5	25	3B, 4B, 5B
Yu (2000)	Rat	Pregnant dams	18	Drinking water	0, 0.0085, 0.085, 0.85, 8.5	6	2, 3A, 4A, 5A
		Fetuses				4[i]	
		Postnatal dams	25			6	
		Neonates				6	
Yu et al. (2000)	Rat	Adult males	14	Drinking water	0, 0.085, 0.85, 2.55, 8.5	8	1
Yu (2002)	Rat	Pregnant dams	NA	iv	0.85	6	1
		Fetuses				6	
		Postnatal dams				6	
		Neonates				6	

NA, not applicable for iv dosing.
[a] As perchlorate ion.
[b] Number of animals per dose group for which hormone data were collected. Total animal number in the experiment may differ.
[c] Data for DL (Day of Lactation) 10 dams and neonates were used in this paper. Data were also collected on DL5 and DL22.
[d] Data from single shift portion of study.
[e] Doses were not estimated for controls.
[f] Estimated from the average group dose (mg/day) and an assumed mean body weight of 70 kg.
[g] Doses (mg/d) were divided by an assumed body weight of 70 kg.
[h] These data are also reported in Yu et al. (2002) in graphical form but were actually tabulated in the reference shown here.
[i] Four data points representing fetuses pooled from four litters.

perchlorate for up to two weeks at doses of 1 and 12 mg/kg-day. We chose not to include these data in our analysis as the study has not been published in either the peer reviewed literature or in publicly available government publications (as is the case with rat studies conducted by Yu and colleagues). However, a review of the Brabant and Leitolf data indicates no significant effects of perchlorate exposure on TSH, T_3 or T_4 even at doses of 12 mg/kg-day, which is well above the doses employed by Lawrence et al. (2000, 2001) and Greer et al. (2002).

We used the administered perchlorate dose (in mg/kg-day) as the common metric for comparison. When doses were reported in the animal studies as ammonium perchlorate or potassium perchlorate, the doses were converted to perchlorate ion, the form reported in the human studies. With respect to TSH, we converted the human data reported in μIU/ml to ng/ml (the format used for all animal data) using a conversion factor of 1.5 μIU/ng. This represents the lower end of the range of 1.5–15 μIU/ng reported by USEPA (1998).[1] For the Lawrence et al. (2000, 2001) studies, which reported separate control data for the two exposures (i.e., 3 and 10 mg/day), data for the 10 mg/day group were normalized to those in the 3 mg/day group based on a comparison of control data. When data were presented only in graphical form, the graphs were scanned and digitized (Datathief II, European Design Centre, Eindhoven, the Netherlands).

5.2. Results

We first compared dose–response data for the inhibition of thyroidal iodide uptake in rats and humans. In the rat, thyroidal iodide uptake is markedly depressed after an acute intravenous dose of perchlorate, within the span of several hours (Fig. 1). A similar response is observed in humans within 2 days of exposure (the earliest timepoint identified). The situation is substantially different with longer perchlorate exposures (Fig. 2). After administration of perchlorate in drinking water for 14–23 days, rats at various life stages (male, pregnant female, and postnatal female dam) all have less inhibition, and, in some cases have iodide uptakes which are even higher than pre-dosing baseline levels (indicated as negative inhibition in the figure). Upregulation in the number and action of the sodium iodide symporter molecules has been suggested as the basis for this

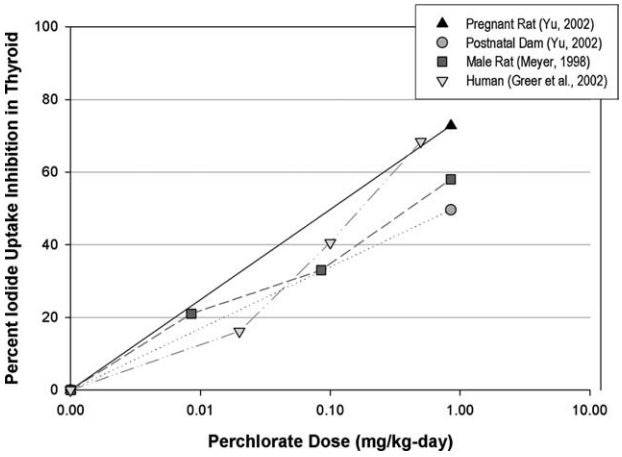

Fig. 1. Effect of acute perchlorate exposure on thyroidal iodide uptake. Rat doses were given intravenously (iv) whereas the human dose was given in drinking water (dw) over the course of 2 days. Details of the studies are provided in Table 2.

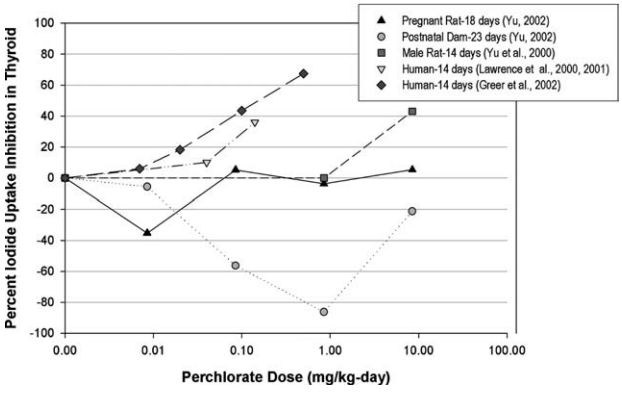

Fig. 2. Effect of subacute perchlorate exposure on thyroidal iodide uptake. Details of the studies are provided in Table 2.

adaptive response (Merrill et al., 2003). In contrast, the human response after 14 days of perchlorate exposure is similar to the acute response after two days. The basis for this difference in response is not clearly known. Perchlorate treatment in the rat may have a sufficiently negative impact on thyroid hormone homeostasis in the rat to trigger a compensatory response (e.g., upregulation of NIS protein). Alternatively, NIS in the rat may be more sensitive to small fluctuations in TSH than NIS in humans, providing a means for compensating for the rat's low reserve of hormone stored as colloid. Additional research regarding potential species differences in the sensitivity of NIS to TSH stimulation is needed.

The effects of subacute perchlorate administration via drinking water on thyroid hormones are shown in Figs. 3A,4A and B,5B. Figs. 3A, 4A, and 5A show data for rats at different life stages, whereas Figs. 3B, 4B, and 5B show data for cross-species comparisons among adult animals. The periods of exposure covered by these

[1] The lower end of the range was chosen to facilitate simultaneous graphing of human and animal data. Use of a higher conversion factor shifted the human data further down the *y*-axis towards zero. Because we are interested in comparing the patterns of responses across species rather than evaluating the magnitude of effect within a species, the use of the lower end of the range to accommodate graphing requirements should not be of concern. Use of the high end or mean of the range would not change our conclusions.

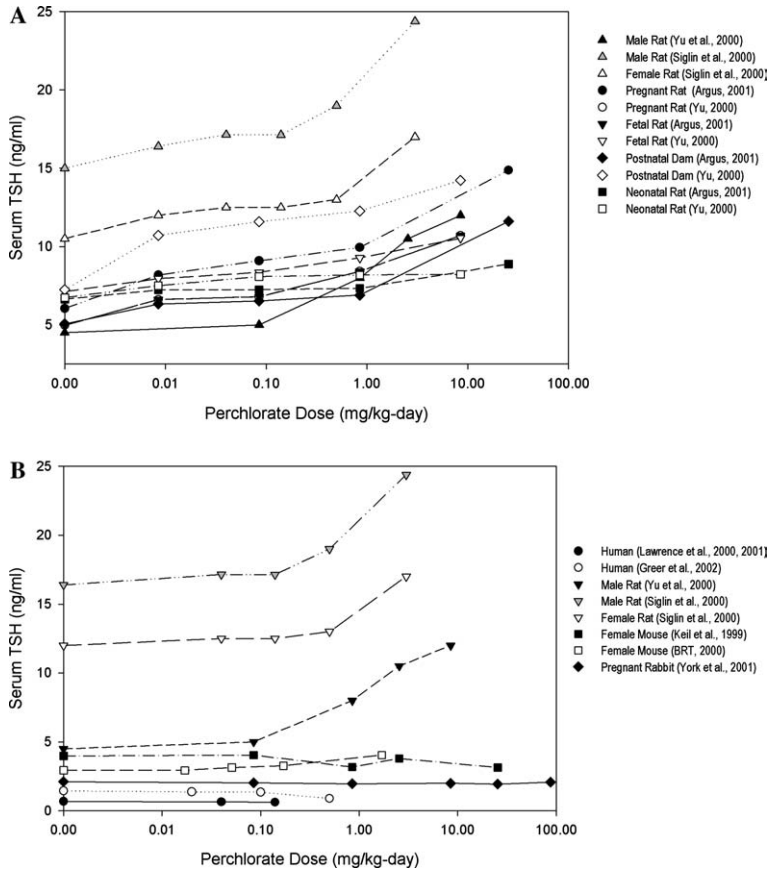

Fig. 3. Effect of subacute perchlorate exposure on serum TSH. (A) Data for the rat at different life stages. (B) Data for adult animals of different species. Due to the large number of datapoints, error bars not shown. Details of the studies are summarized in Table 2.

data ranged from 14 days (non-pregnant adult rat and human) to 46 days (post-natal rat dam). Fig. 3A shows the effect of subacute perchlorate exposure on serum TSH in rats of different life stages. What is striking is the clear dose-dependent increase in serum TSH in the rat at all life stages studied (adult male, adult female, pregnant female, postnatal female, fetus, and neonate). Of these, the most sensitive appears to be the pregnant female (Yu, 2000) and postnatal female (Yu, 2000) with a lowest observed effect level (LOEL) of 0.01 mg/kg-day.[2] Interestingly, although the TSH changes were statistically significant for fetal and neonatal rats (LOEL of 0.1 mg/kg-day in each case), the animals have relatively flat dose–response curves compared to adult animals (e.g., the postnatal animals from the Yu et al., study). The physiological significance of the statistically significant TSH changes seen in the fetus and neonate is therefore uncertain.

Fig. 3B shows the effect of subacute perchlorate exposure on serum TSH in adult rats, adult humans, adult mice, and adult (pregnant) rabbits. Again, the male and female rats evidence a clear upward trend in serum TSH with increasing perchlorate dose. Compared to rats, mice are less responsive with a statistically significant but quantitatively small increase in TSH at 0.2 mg/kg-day (i.e., a LOEL). The mouse data do not appear to show a dose–response relationship as the TSH effect is similar at 0.2 and 30 mg/kg-day (Keil et al., 1999). There was no apparent effect of subacute perchlorate exposure in pregnant rabbits or humans. For example, in the human study of Greer et al. (2002), individuals given perchlorate at 0.48 mg/kg-day for 14 days did not have serum TSH levels significantly different from controls.

The effect of subacute perchlorate exposure on serum T_3 in rats of different life stages is shown in Fig. 4A. Male rats from the Siglin et al. (2000) study show the most pronounced dose–response effect, although dose-dependent decreases are apparent for the other adult rats as well. T_3 decreases in fetal and neonatal rats were less pronounced than in adults, including pregnant dams, in the study reported by Yu (2000), although T_3

[2] These changes are referred to as Lowest Observed Effect Levels (LOELs) rather than Lowest Observed Adverse Effect Levels (LOAELs) because changes in serum levels of thyroid hormones are not necessarily adverse effects. Serum thyroid hormone levels in humans and other species fluctuate in response to normal dietary and environmental factors as well as circadian rhythms (Chan et al., 1978; Zimmermann and Kohrle, 2002; Zoeller et al., 2002). As noted previously, the degree and duration of thyroid hormone alteration required to elicit adverse effects is currently a subject of debate.

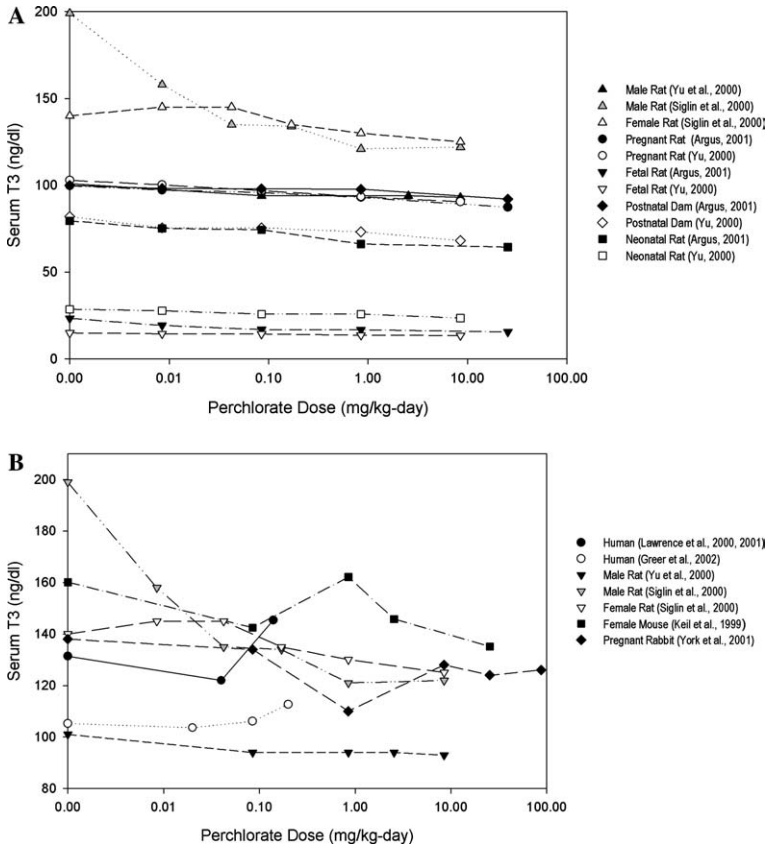

Fig. 4. Effect of subacute perchlorate exposure on serum T_3. (A) Data for the rat at different life stages. (B) Data for adult animals of different species. Due to the large number of datapoints, error bars not shown. Details of the studies are summarized in Table 2.

decreases were similar to the pattern in pregnant dams in the study by Argus (2001). The LOEL in pregnant rats, postnatal dams and neonates was 1.0 mg/kg-day. The LOEL for fetal rats and non-pregnant adult rats was 0.01 mg/kg-day. Thus, the lowest LOEL value is 0.01 mg/kg-day.

The effect of subacute perchlorate exposure on serum T_3 across species is shown in Fig. 4B. The inter-species patterns are less clear than those for TSH, but the rat again appears to be the most sensitive species. No statistically significant effects on serum T_3 were seen in mice and rabbits, with rabbits dosed as high as 100 mg/kg-day or 100× the LOEL in the pregnant rat. Data in humans reported by Greer et al. (2002) and Lawrence et al. (2000, 2001) do not show an effect with doses as high as 0.48 mg/kg-day.

The effect of subacute perchlorate exposure on serum T_4 in rats of different life stages is shown in Fig. 5A. In general, T_4 levels in the rat appear to show only modest decrements with perchlorate until doses exceed 1 mg/kg-day. An exception is the pregnant rat, which appears to be more sensitive than the other life stages. For example, in the Yu (2000) study, pregnant rats evidenced significantly decreased serum T_4 levels at 0.01 mg/kg-day. In the study by Argus (2001), significantly decreased serum T_4 was observed in pregnant rats at a dose of 0.1 mg/kg-day. T_4 levels in neonatal and fetal rats declined slightly with perchlorate dose although, as with TSH, the dose–response curve was quite shallow compared to the pregnant rats.

The effect of subacute perchlorate exposure on serum T_4 across species is shown in Fig. 5B. As suggested from Fig. 5A, non-pregnant adult rats did not evidence statistically significant differences in serum T_4 until perchlorate doses exceeded 1 mg/kg-day. The adult female mouse had a slightly stronger response with a LOEL of 0.2 mg/kg-day according to the data obtained by BRT (2000), although a LOEL was not observed in the mouse data obtained by Keil et al. (1999) at any dose tested. The pregnant rabbit did not experience statistically significant changes in serum T_4 until perchlorate doses reached 30 mg/kg-day, or 3,000 times the dose which lead to statistically significant T_4 decreases in the pregnant rat. In humans exposed to perchlorate for 14 days, no significant changes in serum T_4 were observed up to perchlorate doses of 0.48 mg/kg-day (Greer et al., 2002).

Limited data were also available for mice and rats dosed subchronically (i.e., 90 days) with perchlorate in drinking water (BRT, 2000; Keil et al., 1999; Siglin et al., 2000). Figs. 6–8 provide these data along with

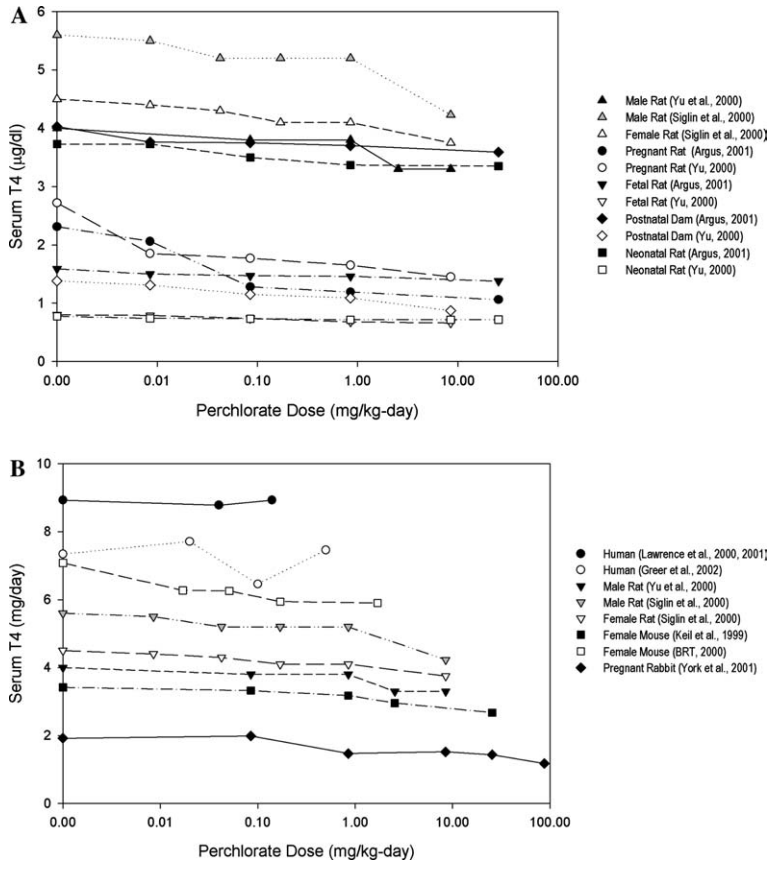

Fig. 5. Effect of subacute perchlorate exposure on serum T_4. (A) Data for the rat at different life stages. (B) Data for adult animals of different species. Due to the large number of datapoints, error bars not shown. Details of the studies are summarized in Table 2.

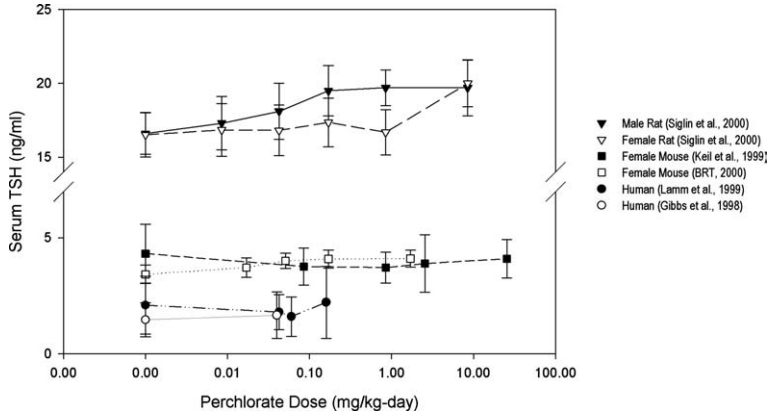

Fig. 6. Effect of subchronic perchlorate exposure on serum TSH. Details of the studies are summarized in Table 2.

data from two studies of perchlorate exposed workers (Gibbs et al., 1998; Lamm et al., 1999). It should be noted that the exposures in the human studies were intermittent (multiple days on, multiple days off), whereas the animals were exposed daily. In the Gibbs et al. (1998), average serum hormone values (TSH and T_4) were taken from the reported post-shift values in the single-shift study (in this study, chronically exposed workers were evaluated for perchlorate exposure and serum hormone levels prior to and after their work shift) and the doses were those estimated by the authors without adjustment for intermittent exposures. In the Lamm et al. study, average serum hormone values (TSH, T_3, and T_4) were taken from the authors' Table 3 with doses per shift (mg/shift) divided by a standard body weight of 70 kg to obtain an estimate of the daily dose in mg/kg-day. No adjustment was made for intermittent exposures. Forty percent of the exposed workers

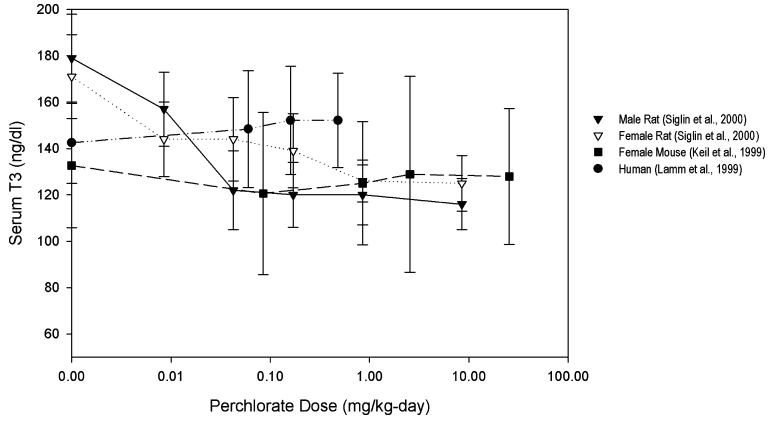

Fig. 7. Effect of subchronic perchlorate exposure on serum T_3. Details of the studies are summarized in Table 2.

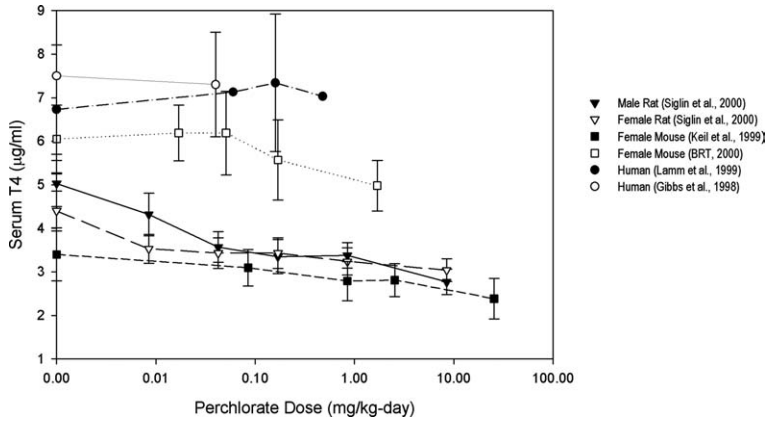

Fig. 8. Effect of subchronic perchlorate exposure on serum T_4. Details of the studies are summarized in Table 2.

Table 3
Lowest Observed Adverse Effect Levels (LOELs) in mg/kg-day for the effect of perchlorate on thyroid hormones as reported in various studies

Thyroid hormone measurement	Adult rat	Pregnant rat	Fetal rat	Neonatal rat	Postnatal rat dam	Female mouse	Pregnant rabbit	Adult human
TSH								
Subacute (14–38 days)	0.1	**0.01**	0.1	0.1	**0.01**	2	None (100)	None (0.5)
Subchronic (90 days)	0.2	na	na	na	na	**0.06**		None (0.48)
T_3								
Subacute (14–38 days)	**0.01**	1.0	**0.01**	1.0	1.0	None (30)	None (100)	None (0.5)
Subchronic (90 days)	**0.01**	na	na	na	na	None (30)		None (0.48)
T_4								
Subacute (14–38 days)	3	**0.01**	1.0	1.0	0.1	0.2	30	None (0.5)
Subchronic (90 days)	**0.01**	na	na	na	na	1.0		None (0.48)

Note. The lowest value reported in all studies addressing a particular species/lifestage is shown. The lowest LOEL value for each hormone measurement is shown in bold text. Sub-chronic human data are from occupational studies which are assumed to involve at least 90 days of exposure. na—Not applicable. This life stage is shorter than the duration of a chronic exposure. none—No LOEL reported. No effect was observed at the highest dose tested, which is identified in parentheses.

in the Lamm et al. study were reported to have been employed at the facility for more than 5 years whereas the mean job tenure of the exposed workers in the Gibbs et al. study was reported to be 9 years.

The results of the cross-species comparison for TSH following subchronic perchlorate exposure are shown in Fig. 6. After 90 days of perchlorate exposure via drinking water, rats, particularly male rats, show a clear trend of increasing serum TSH levels, with a LOEL of 0.2 mg/kg-day (the LOEL for females was 10 mg/kg-day). In the mouse study by BRT (2000), the LOEL was lower (0.06 mg/kg-day) whereas no effect was seen in the

Keil et al. (1999) study at any dose tested (up to 30 mg/kg-day). No significant effect of perchlorate exposure was observed in perchlorate exposed workers (maximum estimated dose = 0.48 mg/kg-day).

The effect of sub-chronic perchlorate exposure on serum T_3 levels is shown in Fig. 7. Serum T_3 levels in both male and female rats decreased in a dose-dependent manner after 90 days of exposure to perchlorate. The LOEL for this response was 0.01 mg/kg-day in both male and female rats (Siglin et al., 2000). In contrast, serum T_3 levels in mice were not significantly affected by 90 days of perchlorate treatment up to a dose of 30 mg/kg-day (Keil et al., 1999). No significant effect of perchlorate exposure was observed in exposed workers (maximum estimated dose = 0.48 mg/kg-day).

The response of serum T_4 levels to sub-chronic perchlorate dosing is shown in Fig. 8. As compared with serum T_3 concentrations, there was a greater decrease in serum concentrations of T_4. Data for male and female rats from the Siglin et al. (2000) study suggest a very sensitive response to perchlorate with a LOEL of 0.01 mg/kg-day. At this dose (the lowest dose tested) serum T_4 levels were decreased more from control values than serum T_3 values. Mice appear to be less responsive, with a LOEL of 1 mg/kg-day based on the data of Keil et al. (1999) and 2 mg/kg-day based on the BRT (1998) study. Again, no statistically significant effects were observed in perchlorate exposed workers (maximum estimated dose = 0.48 mg/kg-day).

The comparisons described above strongly suggest that the rat is exceptionally sensitive to the effects of perchlorate compared to other species. To summarize the effects seen across species, developmental stages and timeframes, LOEL values reported in the studies examined are summarized in Table 3. The lowest reported value for each endpoint across all species is shown in bold. Based on the LOELs alone, among the various life stages evaluated in rats, the non-pregnant adult, pregnant female, postnatal female and fetal rat appear most sensitive based on the short term exposure data. With respect to cross-species differences, a review of the LOEL values listed in Table 3 indicate that the rat is unusually susceptible to perchlorate. The LOEL values listed in Table 3 for other species are 2–3 orders of magnitude higher than those listed for the rat. Although the LOEL listed in Table 3 for sub-chronic effects on TSH in mice is lower than the corresponding value for the rat (0.06 vs 0.2 mg/kg-day), this is based on the immunotoxicity study by BRT (2000); the comparable immunotoxicity mouse study by Keil et al. (1999) did not observe an effect of perchlorate exposure on TSH up to doses of 30 mg/kg-day. Also notable is the comparison between adult humans and adult rats—LOEL values for thyroid hormone changes in adult rats occur at doses as low as 0.01 mg/kg-day at subacute and subchronic exposures, whereas equivalent effects have not been reported in adult humans with subacute doses up to 50× greater (i.e., 0.48 mg/kg-day in the 14-day Greer et al. (2002) study and 0.48 in the Lamm et al. (1999) study).

Although the LOEL values listed in Table 3 reflect only a single point of measurement, unusual sensitivity of the rat to perchlorate is also borne out by a review of the dose–response curves as a whole. A review of Figs. 3A, 4A, and 5A suggests that the steepest dose–response is associated with non-pregnant adult rats, pregnant rats and, to a lesser extent, postnatal dams. The dose–response curves for fetal and neonatal animals suggest a shallower dose–response relationship than those for pregnant and non-pregnant adults (e.g., Fig. 5A). With respect to cross-species differences, the relevant figures (Figs. 3B, 4B, and 5B) indicate that, compared to other species, rats have a much steeper dose–response to the effects of perchlorate on thyroid homeostasis.

6. Discussion

This analysis suggests that the rat is a problematic model to use in estimating human health risks for chemicals that perturb thyroid function. An evaluation of the relevant physiology indicates that the adult rat thyroid appears to be in a state of continuous stimulation such that it is extremely sensitive to the effects of chemicals that affect the pituitary–hypothalamic–thyroidal axis. Examining the TSH, T_3, and T_4 response to perchlorate in other species, including humans, suggests a greater potential for adaptation after exposure to thyroid-active agents, without changes in levels of serum thyroid hormones.

Particularly in regard to TSH, the rat exhibits a significant response at 0.01 mg/kg-day, following subacute exposures to perchlorate, whereas other species remain unresponsive even at doses of 10 mg/kg-day. Serum T_3 and T_4 levels appear to be less affected by perchlorate than TSH, although the rat still experiences decreases in these hormones, which are not observed in other species at equivalent doses.

Although we have grouped our data into the categories of subacute and subchronic exposure, it should be noted that exposure times were not identical across studies and therefore across species. This is to be expected since the goal of the studies was not to facilitate cross-species comparisons. In addition, some experimental designs (e.g., those involving pregnancy) inherently involve different, species-specific durations. We believe that the slight differences in exposure times among the studies considered do not affect our conclusions because (1) the effect of perchlorate on iodide uptake is immediate and perchlorate is rapidly excreted, thus differences in exposure time would not be expected to lead to differences in the dose at the target site; and (2) patterns of response are remarkably similar within a

single species (i.e., the rat) even when exposure times vary from 14 days for the adult male rat to 46 days for the postnatal dam.

Dietary iodide intake, which is a potential confounder in our analysis, was not reported in the animal and human studies listed in Table 2. If the diets of rats in the studies were iodide deficient relative to those of mice, rabbits and humans, this could possibly explain the greater sensitivity of the rat to thyroid perturbation by perchlorate. This does not appear to be the case. The National Research Council (1995) recommends an iodide concentration in feed of 150 μg/kg (0.15 ppm) for laboratory rats. Animal feeds used for rodents generally are formulated to contain approximately 0.8–1 ppm iodide. For example, the Purina Mills International (PMI Certified Rodent Diet #5002, which was used in the Argus (2001) and Siglin et al. (2000) studies, contains 0.77 ppm iodide. At this level of formulation, the diets of most laboratory rats contain 5- to 6-fold more iodide than the NRC's recommended daily intake. In contrast, a recent Centers for Disease Control study has indicated that the U.S. human population has a median urinary iodide profile that suggests adequate to borderline low-dietary iodide intakes (Hollowell et al., 1998). It should be noted that, this study used spot urine samples which may have tended to exaggerate the tails of the intake distribution. A review of the available data therefore suggests that iodide sufficiency in rats was comparable, if not greater than iodide sufficiency in humans and the other species discussed in this review. It is therefore unlikely that the particular sensitivity of the rat to perchlorate which we describe is attributable to differences in dietary iodide.

Physiologically-based pharmacokinetic (PBPK) models for estimating perchlorate concentrations at the target site have recently been developed (Clewell et al., 2003a,b; Fisher et al., 2000; Merrill et al., 2003). We chose not to use these models to develop species-specific estimates of the target organ dose (e.g., perchlorate serum AUC) for two reasons. First, published models are available only for the rat, with a human model only fully described in a non-peer reviewed source (Merrill, 2000). PBPK models have not been developed for mice and rabbits. Thus, use of the PBPK models would have complicated rather than clarified our cross-species comparisons. Second, the rat and human models estimate that equivalent exposures (based on serum perchlorate AUC) differ between rats and humans by a factor of 1–3 (USEPA, 2002). This difference is slight in light of both the logarithmic dosing pattern used in the animal studies and the level of uncertainty embodied in the model predictions.

The available data from controlled human studies are limited to non-pregnant adults. There is speculation that fetal and neonatal humans may be more sensitive to the effects of perchlorate than human adults (CalEPA, 2002; Clewell et al., 2001; USEPA, 2002;). While controlled human studies involving these subpopulations are not available, several ecological studies have dealt with this issue. Data from these studies were not used in our analyses because actual individual doses were not known. Some discussion of the results of these ecological studies is nonetheless appropriate. Crump et al. (2000) examined neonates in 3 communities in Chile with average perchlorate drinking water levels of 111.6, 5.5, and <4 μg/L. This study found no effect of perchlorate on neonatal TSH levels—the only hormone parameter measured. Note that the highest exposure group consumed water with perchlorate concentrations well above proposed health based limits in the U.S. A similar lack of effect was reported by Li et al. (2000a,b) in comparing T_4 and TSH levels in neonatal populations in Las Vegas and Reno NV. Perchlorate levels in Las Vegas ranged between 9 and 15 μg/L during the study period while perchlorate levels in Reno were below the detection limit of 4 μg/L. Kelsh et al. (2003), investigating a perchlorate exposed population in California, also found no increase in the odds ratio for either increased serum TSH or diagnosis of primary congenital hypothyroidism. Finally, Lamm and Doemland (1999) reported no elevation in the incidence of congenital hypothyroidism in seven California and Nevada counties where perchlorate was detected in drinking water wells. In contrast, Brechner et al. (2000) reported a positive association between perchlorate exposure and TSH levels, in a comparison of neonatal TSH values between Yuma and Flagstaff, AZ. The perchlorate level in Yuma was 6 μg/L whereas the perchlorate level in Flagstaff was below detection limits. The reason for the discrepancy between the results of the Brechner et al. and the previously cited studies is not clear. Letters to the editor of the *Journal of Occupational and Environmental Medicine* suggest that other factors, such as iodine nutrition, access to prenatal care, and other social and reproductive factors, may have contributed to the TSH differences between Yuma and Flagstaff observed by Brechner et al. (Crump and Weiss, 2001; Goodman, 2001).

The cross-species comparisons we presented regarding sensitivity to thyroid perturbation provide strong evidence that data collected from experiments conducted in rats need to be carefully evaluated for their relevance to humans. Due to the high susceptibility of the rat to thyroid active agents such as perchlorate, direct application of the rat data to humans will overestimate the potential risk of human exposures. Nonetheless, rat studies of thyroid active agents are of use—particularly when used qualitatively. For example, rat studies can be used to help confirm that the thyroid is the primary target organ, identify any potential extra-thyroidal effects, and evaluate those effects that cannot be readily investigated in humans (e.g., effects on brain

morphometry outcomes). However, use of rat data for quantitative assessments should incorporate cross-species differences in responsiveness. The standard regulatory practice within the United States has been to treat humans as more chemically sensitive than experimental animal species and to use uncertainty factors (generally 3 or 10) to account for potential inter-species differences in susceptibility. In the case of perchlorate, it appears warranted to depart from this general default and make appropriate adjustments for the use of an animal model that is more sensitive than humans. If rat rather than human data are to be used to develop toxicity criteria for perchlorate, we propose that perchlorate is one of the rare chemicals for which an inter-species uncertainty factor of less than 1.0 can be supported.

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Response to letter to the editor

Response to: Interspecies differences in susceptibility to perturbation of thyroid hormone homeostasis requires a definition of "sensitivity" that is informative for risk analysis

In his comments on our article, Dr. Zoeller raises a number of interesting points regarding "sensitive populations" and use of toxicological data to characterize such populations. However, as discussed below, these points do not support the use of data collected in rats for quantitative assessment of the potential effects of perchlorate in humans.

Zoeller's comment that differences in half-life and intrathyroidal stores of T_4 make it "clear why perchlorate caused a reduction in serum thyroid hormones in rats but not in humans" is not quite germane to the appropriateness of the use of the rat for quantifying effects of perchlorate exposure in humans. We presented data for species other than the rat, including pregnant rabbits and occupationally exposed humans. In none of these species were effects of perchlorate on thyroid hormone levels or developmental effects seen, even in pregnant rabbits exposed to doses many orders of magnitude higher than those given to rats (York et al., 2003). We also noted a lack of effects in the human chronic exposure studies by Gibbs et al. (1998) and Lamm et al. (1999). While these studies involved populations with intermittent exposure patterns, such studies nonetheless demonstrated no effect whatsoever on serum thyroid hormones at exposure levels that clearly affected rats. As a whole, these data indicate that, in terms of the thyroidal response to perchlorate, rats differ not only from humans but also from mice and rabbits.

Zoeller also implies that because of issues of thyroid hormone economy that the same effects seen in the rat will eventually be seen in the human once thyroid hormone stores are depleted. U.S. EPA has previously made this point in their "parallelogram" approach for extrapolating between the rat data and humans (U.S. EPA, 2002). We note, however, that this notion does not consider the potential for differences in the *magnitude* of the effect; the greater resilience of the thyroid hormone pool in humans and species other than the rat allows for adaptation or compensation. This is consistent with studies of chronic perchlorate exposures in human occupational (Gibbs et al., 1998; Lamm et al., 1999) and residential (Crump et al., 2000; Gibbs et al., 2004) populations (including children and adults) that have not reported adverse effects even at exposure levels well above those causing such effects in rats.

Zoeller cites studies indicating the child is more vulnerable to disruption of thyroid homeostasis than the adult. Our work examined inter-species differences in thyroid responsivity to perchlorate and did not address the issue of children's increased vulnerability except for a brief summary of some of the epidemiology studies. In the absence of a child-specific model, U.S. EPA's RfD methodology (currently being used to develop regulatory levels for perchlorate) relies upon uncertainty factors to address issues such as children being a particularly sensitive subpopulation (i.e., via the intra-species uncertainty factor). In contrast, the aim of our article was to evaluate the relevance of a particular animal model for predicting human risks, i.e., for purposes of developing an RfD. We do not see that an increased sensitivity of the fetus or neonate relative to adults provides a basis for selecting the rat as an appropriate model for the human. The choice of the animal model should be based on the overall appropriateness of the model and intra-species differences can be addressed subsequently via careful selection of uncertainty factors. Thus the rat model may be appropriate for hazard evaluation or mechanistic studies, but it may not be appropriate to use data collected in rats for direct quantitative dose-response assessment in humans potentially exposed to perchlorate.

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