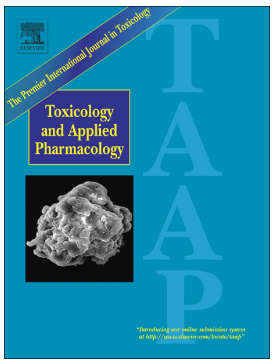

Environmental exposure to perchlorate: A review of toxicology and human health

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Environmental Exposure to Perchlorate: A Review of Toxicology and Human Health

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Abstract

Perchlorate pharmacology and toxicology studies date back at least 65 years in the peer-reviewed literature. Repeated studies in animals and humans have demonstrated perchlorate's mechanism of action, dose-response, and adverse effects over a range of doses. The first measurable effect of perchlorate is inhibition of iodine uptake to the thyroid gland. Adequate levels of thyroid hormones are critical for the development of the fetal nervous system. With sufficient dose and exposure duration, perchlorate can reduce thyroid hormones in the pregnant or non-pregnant woman via this mechanism. The developing fetus is the most sensitive life stage for chemical agents that affect iodide uptake to the thyroid.

Perchlorate has a half-life of eight hours, is not metabolized, does not bioaccumulate, is not a mutagen or carcinogen, and is not reprotoxic or immunotoxic. More recently, epidemiological and biomonitoring studies have been published in the peer-reviewed literature characterizing the thyroidal effects of perchlorate and other goitrogens. While the results from most

populations report no consistent association, a few studies report thyroidal effects at environmentally relevant levels of perchlorate.

We reviewed the literature on health effects of perchlorate at environmental exposure levels, with a focus on exposures during pregnancy and neurodevelopmental effects. Based on the studies we reviewed, health effects are expected to only occur at doses substantially higher than environmental levels.

Keywords

Perchlorate, Thyroid, Goitrogen, Environment

Highlights

- Sufficient doses of perchlorate are necessary to cause adverse effects on the thyroid system.
- The mechanism of action for a non-adverse effect is well characterized and a NOEL is identified.
- A non-adverse effect is a conservative point of departure to use to protect the public health.
- Without adverse effects on the thyroid system, no adverse effects on neurodevelopment can occur.

Introduction

The perchlorate anion is composed of one chlorine and four oxygens, and it readily bonds with ammonium, sodium, and potassium, among other cations, to form salts. We use the term "perchlorate" to identify the dissociated salt.

The scientific database for perchlorate is robust for an environmental chemical. It covers over 65 years of studies with a wide dose-response range spanning low, daily environmental doses up to high, therapeutic doses given singly or for years at a time. Rarely does an environmental chemical have such robust data to make a toxicological assessment.

Perchlorate is a naturally occurring and manmade salt. Anthropogenic perchlorate is used in pharmaceuticals, as an oxidizer in solid fuel rocket engines, fireworks, flares, and is also detected as a byproduct in hypochlorite bleach. Naturally-formed perchlorate is detectable in the environment world-wide at levels up to 325 mg/kg, especially in arid regions, such as the southwestern United States (US; Jackson *et al.*, 2010). Natural perchlorate is a contaminant of some organic fertilizers.

Perchlorate is an environmental contaminant from its use in industry. Past environmental practices were not sufficient at managing release to the soil and water at industrial and agricultural sites. For example, a number of industrial and governmental sites, particularly in the western US, were the first reported sources of perchlorate contamination of water sources. The application of Chilean nitrate fertilizers are also sources of natural perchlorate contamination of agricultural land and contain sizeable amounts of perchlorate, averaging about 1500-1800 mg/kg (Urbansky *et al.,* 2001).

The primary route of human exposure to perchlorate is via ingestion of food and water. Perchlorate concentrations in surface and ground water in the US are usually less than 4 µg/L, but some of the highest levels have registered 400 mg/L at industrial sites (Brandhuber *et al.*, 2009). Perchlorate is taken up by edible plants and consumed by humans or livestock. In the early 2000's, perchlorate was detected in lettuce grown in Southern California, with concentrations ranging from 0.03 to 0.121 mg/kg fresh plant material (Sharp and Lunder, 2003).

When perchlorate was detected in ground water, starting the 1990's, EPA sought to understand the possible risks of perchlorate. A consortium of government, industry, state, and tribal entities developed a toxicology testing strategy for which many experimental studies were conducted (EPA, 2002). We review some of the key toxicology studies herein.

Since the 2000's, many authoritative bodies have reviewed the science of perchlorate, conducted toxicological assessments, and developed levels of acceptable exposure. The National Research Council (NRC), which conducted an objective key assessment of perchlorate, confirmed previous assessments of perchlorate's mechanism of action (MOA): reversible iodide uptake inhibition (IUI) in the thyroid (NRC, 2005). The NRC summarized the MOA for perchlorate (Figure 1). Iodide is actively transported by the sodium iodide symporter (NIS) and stored in the thyroid follicular cell. Perchlorate is also transported by the NIS; hence the two ions, iodide and perchlorate, compete for the same binding sites. IUI must occur with sufficient inhibition to decrease thyroid hormone secretion. When thyroid hormone levels decrease, the anterior pituitary releases thyroid stimulating hormone (TSH) and increases the bioavailability of thyroid hormone through several mechanisms of homeostasis. Clinically, thyroid function is

measured using TSH initially, followed by confirmatory testing of free T4 (fT4) or T3. Without sufficient IUI, no downstream adverse effects can occur.

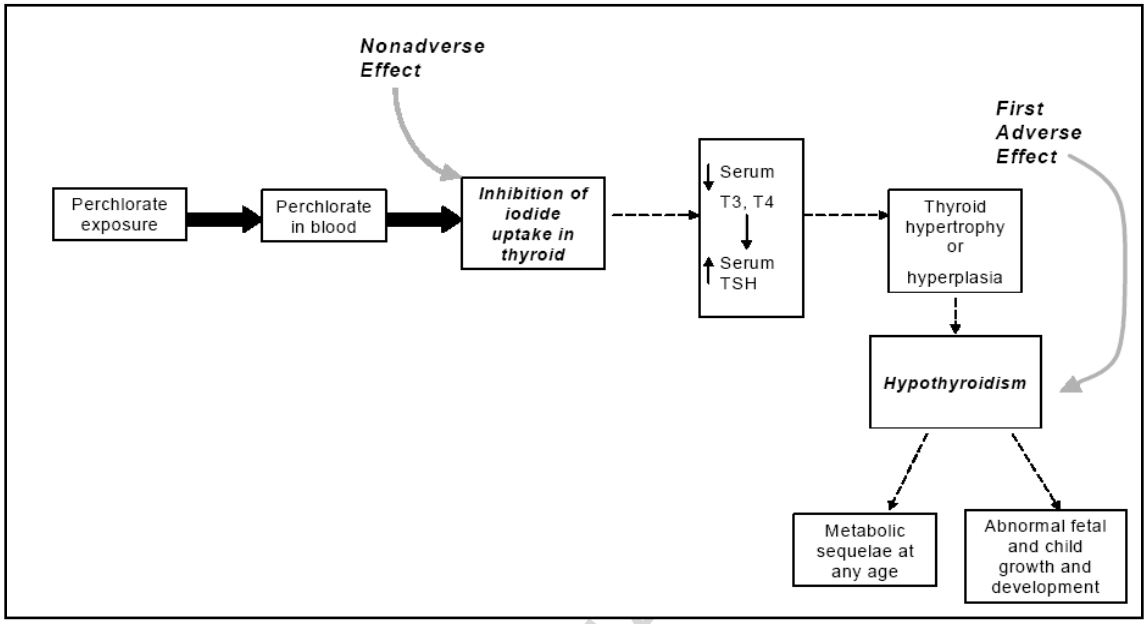

Figure 1. Mechanism of action for perchlorate as summarized in the National Research Council report (2005) NRC's suggested mode-of-action model of perchlorate toxicity in humans. Solid arrows represent outcomes that have been observed in humans with perchlorate doses greater than 0.007 mg/kg-d. Dashed arrows represent outcomes that have not been clearly demonstrated in humans exposed to perchlorate but that are biologically possible in the absence of adequate compensation. The thyroid response to increased serum TSH and an independent increase in thyroid iodide uptake would raise T3 and T4 production to normal and therefore usually prevent the later steps from occurring (NRC, 2005).

NRC did "not view transient changes in serum thyroid hormone and [TSH] concentrations as adverse health effects," and stated that "inhibition of iodide uptake by the thyroid clearly is not an adverse effect…" NRC proposed, and US EPA adopted, a Reference Dose of 0.7 µg/kg based on a No Observed Effect Level (NOEL) for IUI, several steps "upstream" from the first adverse effect (NRC, 2005).

In the later 2000's, EPA began to assess whether perchlorate should be regulated in drinking water. Most recently, EPA enacted a Scientific Advisory Board (SAB) and two peer reviews to evaluate EPA's proposed methods for developing a Maximum Contaminant Level Goal (MCLG). The SAB recommended EPA use a physiologically-based pharmacokinetic (PBPK) model based

on the mechanism of action. EPA followed this advice and released a model which was subsequently peer reviewed and approved for its use in developing an MCLG (EPA, 2018).

Materials and Methods

Our literature review was conducted using a protocol based on the National Toxicology Program and the University of California San Francisco's Program on Reproductive Health and the Environment (OHAT, 2013; Johnson *et al.*, 2014). We reviewed PubMed and Google Scholar to identify studies assessing the possible effect of perchlorate on human health at environmental levels. Search terms included "perchlorate" alone and in conjunction with "health," "human," "animal," "thyroid," "goitrogen," and "neurodevelopment." We focused on studies that provide information on the toxicology or pharmacology of perchlorate. Our literature review is a narrative review of the animal and human health effects resulting from perchlorate exposure.

Results

Perchlorate Pharmacology

Perchlorate is not metabolized, has a short biological half-life of 8 hours, does not bioaccumulate in the body, and is excreted unchanged from the kidneys (NRC, 2005). The most sensitive organ from a human health perspective is the thyroid, an endocrine gland residing in the neck. The animal and human literature is consistent regarding the MOA, absorption, distribution, metabolism, and excretion of the compound. The data cover a range of doses that include environmental and therapeutic exposure concentrations and several species of laboratory animals.

Thyroid hormone homeostasis is necessary for many organ systems and metabolism, including the development of the fetal central nervous system. Iodine is an essential nutrient and it is required in the production of T4 (3,5,3′,5′-tetra-iodo-L-thyronine; also called thyroxine) and T3 (3,5,3′-tri-iodo-L-thyronine). Iodide is transported into the thyroid follicular cell by NIS that are located in the basolateral membrane.

The NIS is a member of solute carrier family 5A, designated as SLC5A5. In humans, the NIS is composed of 643 amino acids (Smanik *et al.*, 1996). The apical membrane of the thyroid follicular cell contains an anion transporter—identified as the protein pendrin (Royaux *et al.*, 2000). Both structures actively transport iodide into the cell, where organification of iodide to thyroglobulin occurs (NRC, 2005). The NIS is found in the salivary glands, stomach, and mammary gland.

At lower therapeutic and environmental doses, the predominant MOA is for perchlorate to competitively and reversibly cause IUI (Wolff, 1998; Hildebrandt and Halmi, 1981; Goldstein *et al.*, 1992; Greer *et al.*, 1966). The NIS actively transports perchlorate into the cell; this action prevents the uptake of iodide while perchlorate is transported in the NIS (Dohán *et al.*, 2007). The NIS transports iodine, thiocyanate, and chlorate with a 2 Na^+:1 anion electrogenic stoichiometry. In contrast, NIS transports perrhenate (ReO_4^-) and perchlorate with a 1 Na^+:1 anion electroneutral stoichiometry (Dohán *et al.*, 2007). Perchlorate's ability to cause IUI is more potent than iodide by 30-fold (Tonacchera *et al.*, 2004). Several other goitrogens share the same MOA with varying potencies for the NIS.

Nitrate and thiocyanate are goitrogens commonly found in food items such as vegetables, dairy, and some meat products. Nitrate in soil and water is taken up by plants and incorporated into plant material and consumed. Thiocyanate is produced by many plants, particularly those of the *Brassica* genus. Perchlorate only accounts for about 1-2% of the total IUI on a daily basis (Tarone *et al.*, 2010; US EPA OIG, 2010; Bruce *et al*., 2013). Several types of *in vivo* and *in vitro* studies have examined perchlorate, nitrate, and thiocyanate for their relative potential to cause IUI (Wyngaarden *et al.* 1952, 1953; Greer *et al.* 1966; de Groef *et al.* 2006; Tonacchera *et al.* 2004; US EPA OIG, 2010). Tonacchera *et al.* (2004) investigated the effects of perchlorate and other competitive inhibitors of iodide uptake by inhibiting radioactive iodide uptake (RAIU) in an *in vitro* test system with human NIS stably transfected into a Chinese hamster ovary (CHO) cell line. They show that perchlorate, thiocyanate, nitrate, and iodide act on the NIS in a simple additive fashion. In this system, the relative molar potency of perchlorate to inhibit ^{125}I uptake at the NIS was 15 and 240 times that of free thiocyanate and nitrate, respectively, or 8.8 and 150 on a weight basis (for extrapolating from ingested doses rather than internal serum concentrations). The relative potencies for IUI of several goitrogens were summarized by Wolff (1998): $TcO_4^- \geq ClO_4^- > ReO_4^- > SCN^- > BF_4^- > I^- > NO_2^- > Br^- > Cl^-$.

The MOA makes perchlorate useful as a therapeutic agent. Medicinal use of perchlorate compounds began in the early 1950s and continued in the 1960s as a drug to treat hyperthyroidism (Stanbury and Wyngaarden, 1952; Wolff, 1998). Therapeutic doses ranged from the hundreds of milligrams (mgs) to 2,000 mg of potassium perchlorate orally per day (equivalent to 20.5 mg/kg-d perchlorate, assuming a 70-kg adult) for weeks, months, and

sometimes longer depending on the treatment (NRC, 2005). In contrast to therapeutic doses, environmental doses are rarely above 0.000234 mg/kg-d (Blount *et al.*, 2007).

In the late 1950s to early 1960s, seven cases of fatal aplastic anemia and nonfatal agranulocytosis were reported in patients with Graves' disease taking 400 to 1000 mg/d of potassium perchlorate (equivalent to 4.1 to 10.3 mg/kg-d of perchlorate) for two to 20 weeks. This was the first and only time aplastic anemia was reported with therapeutic levels of perchlorate and it has been suggested that the drug was contaminated (Wolff, 1998).

Side effects (*i.e.*, adverse effects) from therapeutic levels of perchlorate included nausea, rash, gastrointestinal disturbances, fever, lymph node enlargement, and kidney dysfunction. The percentage of patients reporting these symptoms ranged from 3-4% of patients taking 400-600 mg/d to 16-18% of patients taking 1000-2000 mg/day potassium perchlorate (Wolff, 1998).

Perchlorate is still used in Europe for the treatment of amiodarone-induced thyrotoxicosis (600-1000 mg/d of potassium perchlorate) and in thyroid discharge tests (600 mg IV) around the world, including in the US (Gopolan, 2017;

Perchlorate Toxicology

Many animal and human studies focused on filling data gaps relevant to low dose and human exposures were conducted in the late 1990's and early 2000's. Toxicity studies were conducted in rats with either 14 or 90 days of daily exposure of up to 30 mg/kg-d. Changes in thyroid hormones and TSH were observed with exposures to the lowest dose of 0.01 mg/kg-d and changes in thyroid gland weight and histopathology with 10 mg/kg-d (Siglin *et al.*, 2000; York *et al.*, 2001b). Animal studies demonstrate that perchlorate is not immunotoxic, not a

reproductive toxicant at doses below 30 mg/kg-d in rats (reduced sperm and spermatid density and count in a 2-generation study noted at the highest dose); and not carcinogenic (Keil *et al.*, 1998, 1999; Siglin *et al.*, 2000; York *et al.*, 2001a, 2001b; Kessler and Krüskemper 1996 (as summarized by NRC, 2005); US EPA, 2002; Pajer and Kališnik, 1991). Treated rats developed follicular-cell tumors, however, the NRC (2005) concluded that follicular-cell tumors in rats are expected at goitrogenic doses because of species sensitivity.

Regarding fetal brain development and perchlorate exposure, two key animal studies were performed in the early 2000s. The studies followed US EPA developmental neurotoxicity standardized testing guidelines (OPPTS 870.6300) to assess the potential for perchlorate to cause behavioral effects in the offspring of mothers exposed to perchlorate during pregnancy. York *et al.* (2003) evaluated 15 different neurobehavioral measures in four behavioral tests (passive avoidance testing, water maze testing, auditory startle habituation, and motor activity). Bekkedal *et al.* (2000) measured nine different measures of motor activity. Both studies concluded there were no statistically significant neurobehavioral effects in any of the 24 neurobehavioral indices. Studies showed no adverse effects from doses of 0, 0.1, 1.0, 3.0, or 10.0 mg/kg-day. Exposure periods started at gestational day (GD) 0 and continued through postnatal day (PND) 10 or two weeks prior to conception and continuing through PND 10, for York *et al.* (2003) and Bekkedal *et al.* (2000), respectively.

Since 2005, few animal studies relevant to environmental exposures have been published. After 2005, some animal studies used high doses of perchlorate, at times with a diet deficient in iodine, to induce frank hypothyroidism (Gilbert and Sui, 2008; Kunisue *et al.*, 2011; Van Wijk *et al.*, 2008; Wu *et al.*, 2012). These treatments produced hypothyroidism with classical increase in

TSH, a decrease in T4, and as well as other expected thyroidal changes. Gilbert and Sui (2008) reported that male offspring of hypothyroidic dams showed hippocampal synaptic function deficits, but showed no impairment of motor activity, spatial learning, and fear conditioning. Pregnant rats were exposed to 0, 30, 300, or 1,000 mg/L (up to ~140 mg/kg-d) perchlorate in drinking water from gestational day six until weaning.

van Wijk *et al.* (2008) examined the effects of perinatal and chronic maternal hypothyroidism on neurological function in rat offspring using the grip test, balance beam test, open field test, and Morris water maze test. Dams were fed a diet poor in iodide with 7500 mg/L perchlorate two weeks prior to mating in their drinking water to PND 14, where upon some of those exposed to the low iodine + perchlorate were given to control dams (perinatal group) and others continued the diet to PND 29 (chronic hypothyroid group) when they were all weaned and presumably treatment stopped. There were transient differences at the initial postnatal day 14 of the grip test and balance beam test with both the perinatal and chronic offspring and at PND 28 for only the chronically exposed. All groups resolved their differences in the next testing date (PND 28/29) for the balance beam test with only the chronic hypothyroid pups producing similar results as controls on the following testing session, PND 41. Offspring were tested in open field (PND 40-41) and water maze tests (PND 61-65). For open field testing, chronically-exposed male offspring were initially more hyperactive at the beginning of the trials and not different as trials progressed. A similar pattern was evident for water maze testing. van Wijk and colleagues concluded that, given a normal diet at PND 14, offspring that were exposed to low iodine + perchlorate and then given normal diet were less affected than those chronically hypothyroid to weaning.

Five clinical studies were published where oral doses of perchlorate, from 0.007 mg/kg-d to 0.5 mg/kg-d, were given to volunteers (Brabant *et al.*, 1992; Lawrence *et al.*, 2000, 2001; Greer *et al.*, 2002; Braverman *et al.*, 2006). All of these doses were higher than levels provided in US drinking water. All these studies repeatedly demonstrated changes in IUI without changes in thyroid or pituitary hormones. There was no change from baseline IUI at the 0.007 md/kg-d dose to approximately 70 percent IUI at the 0.5 mg/kg-d dose. An occupational study reported no adverse changes to thyroid hormones with doses up to an estimated 0.167 mg/kg-d (calculated from serum concentrations) in exposures to workers with months to years of cumulative exposure (Braverman *et al.*, 2005).

Questions on the population size and exposure period have been raised about some of the clinical studies. Two studies attempted to address these issues (Braverman *et al.*, 2006; Bruce *et al.*, 2017). Braverman *et al.* (2006) provided 13 healthy volunteers either a placebo, 0.5 mg, or 3.0 mg; potassium perchlorate daily, orally for three or six months. Thyroid function tests were given and 24-h RAIU was measured. Urinary iodide, urinary perchlorate, and serum perchlorate were measured. There was no significant change in RAIU during perchlorate administration. There were no significant changes in serum T3, free T4 index, TSH, or thyroglobulin (Tg) concentrations during the exposure period, compared with baseline or post-exposure values. Nearly half (7/15, 47%) of the dosed subjects in Braverman *et al.* (2006) had higher iodide uptake after three months than they had at baseline, and overall, there was no significant change in RAIU compared with baseline. The reason RAIU is higher is not known but thought to be a homeostatic response such as upregulation of the NIS.

Bruce *et al.* (2017) pooled the IUI data from four similar clinical studies (Lawrence *et al.*, 2000, 2001; Greer *et al.*, 2002; and Braverman *et al.*, 2006). When the data were pooled, the estimate for a dose threshold for a biologically-significant change of IUI from baseline ranged from 1.6 to 3.0 mg/d (0.021 to 0.038 mg/kg-d) depending on the statistical model used. This value is similar, but greater than the LOEL from Greer *et al*. (2002) of 0.02 mg/kg-d.

Population-based Studies of Perchlorate

It is clear from population-based studies that perchlorate, whether made-made or natural, is consistently detected in human urine at low microgram levels. When studies begin assessing possible impacts of perchlorate, the experimental design becomes a key aspect of the study's reliability. For example, most studies before 2005 were ecologic in study design, without individual measures of perchlorate exposures; exposures were based on imprecise surrogate measures of exposure such as geographic area (*e.g.*, residence in a zip code). Since 2005, most published studies in human populations report results of biomonitoring or explore associations between perchlorate exposure and thyroid endpoints, although several studies have tested non-thyroid endpoints.

Biomonitoring Studies of Perchlorate Exposure

Perchlorate is commonly found in the urine of adults and infants and can be found in breast milk (Blount *et al.*, 2006; Cao *et al.*, 2010). Studies based on NHANES datasets report detectable perchlorate in the urine of all individuals tested, highlighting the ubiquity of perchlorate exposure (Blount *et al.*, 2006); however, the concentration of urinary perchlorate has been decreasing in recent years (Jain, 2016; Corey *et al.*, 2017). The reason for the decrease is not certain, however, it could be due to the industrial clean-up activities or the reduction of Chilean

nitrate used in key agricultural areas or both. Perchlorate urinary values are indicative of exposures from both food and water sources, although food sources have been estimated to contribute a greater proportion of overall dose (Huber *et al.*, 2010).

A recent study using the analysis of chlorine and oxygen isotopes demonstrated that perchlorate in urine from people in Atlanta is isotopically similar to perchlorate naturally found in the southwest US, and perchlorate in urine from Chile is isotopically similar to natural Chilean perchlorate. The researchers found that the isotopic fingerprint of urinary perchlorate from Atlanta and Chilean populations is distinct from industrial forms of perchlorate (Poghosyan *et al.*, 2016).

Regarding sources of perchlorate to infants, studies in breast milk and formulae have been conducted showing that perchlorate is detectable but not correlated to urinary iodine or drinking water levels. Perchlorate and iodine were detectable in all samples of infant formulae and in nearly all samples of breast milk in Boston-area and Texas women (Pearce *et al.*, 2007; Kirk *et al.*, 2007). Neither breast milk nor urinary perchlorate levels were significantly correlated with breast milk iodine concentrations (Pearce *et al.*, 2007). There was no correlation between levels of perchlorate in breast milk and perchlorate in drinking water (Kirk *et al.*, 2005). Similarly, perchlorate was detectable in 43 of 46 colostrum samples with no association between concentrations of perchlorate, iodide, and cotinine (Leung *et al*., 2009).

NHANES data are often used to explore relationships between perchlorate exposure and other biological measures. Using NHANES 2001-2002, among non-pregnant females 12 years of age and older, increasing urinary perchlorate was significantly associated with decreasing levels of

total T4 (Blount *et al.*, 2006). This relationship was stronger in women who smoked (Steinmaus *et al.*, 2007) or who had urinary iodide less than 100 µg/L (Blount *et al.*, 2006). There was no effect on functional thyroid measures when perchlorate, nitrate, and thiocyanate were converted to perchlorate equivalence (Bruce *et al.*, 2013).

The relationship between non-pregnant-female- and male-urinary perchlorate and thyroid hormone values is mixed, with reported results ranging from no association to biologically-plausible associations. Using NHANES 2007-2008, there was no association between urinary perchlorate and thyroid hormone endpoints using a regression model. However, when a subset of this data was reevaluated by comparing the combined exposure to perchlorate, thiocyanate, and iodine, the group with the highest perchlorate, highest thiocyanate, and lowest iodide, the high exposure group had total T4 levels 5% lower than the low exposure group (Steinmaus *et al.*, 2013). A different analysis using the 2007-2008 NHANES dataset reported an association between urinary perchlorate and urinary phthalates with fT3 and TT4 in male and females; fT4 was associated in males only (Mendez and Eftim, 2012).

Studies of Perchlorate Exposure in Pregnant Women

Studies of exposure in pregnant women generally evaluate associations of perchlorate and two or more maternal thyroid variables, fT4 and TSH. Two studies have evaluated maternal exposures and neurodevelopmental effects in their offspring.

Ten studies in different populations report no statistical association between maternal urinary perchlorate levels and a maternal thyroid effect (Table 1). The most highly-exposed population of these studies reported no effects of perchlorate on any of the thyroid clinical values tested (Tg, TSH, T3, TPO, and fT4; Tellez Tellez *et al.*, 2005). Tellez Tellez *et al.* (2005) conducted a

longitudinal epidemiologic study in three northern Chilean cities. Each city had different levels of perchlorate in their drinking water (Taltal, 114 µg/L; Chañaral, 6 µg/L; and Antofagasta, 0.5 µg/L). The researchers reported no association between urinary perchlorate exposure and Tg, TSH, and fT4 for women during early pregnancy (~16 ± 4 weeks), late pregnancy (~32 ± 3 weeks), or the neonates at birth. Review and further analysis of their data confirmed their initial conclusions regarding the effect of perchlorate on fT4 or TSH in pregnant women with urinary iodide concentrations generally above 100 µg/L. The portion of the study population with urinary iodide ≤ 100 µg/L was too small to conduct for statistical analysis, however, they report no trend for the data that was available (Gibbs and van Landingham, 2008).

TABLE 1 STUDIES OF MATERNAL URINARY PERCHLORATE AND SERUM THYROID HORMONES

Study	N	Sample Collection for fT4 (median)	Urinary Iodine, μg/L (median)	Urinary Perchlorate, μg/L (median)	Results Related to Maternal Perchlorate Exposure
Steinmaus *et al.*, 2016 San Diego, CA	1,476	17.1 +/- 1.5 w 2nd trimester	154.5 sufficient	6.5	Association with TT4, fT4, and TSH*
Charatcharoenwitthaya *et al.*, 2014; Thailand	200	9.6 +/- 2 w (mean) 1st trimester	153.5 sufficient	1.9	Statistically significant association for fT4 and TSH; no association with fT3
Gibbs and van Landingham, 2008 Chile	149 (fT4), 155 (TSH)	16 w 2nd trimester	277	34	No associations with fT4 and TSH
Horton *et al.*, 2015 New York, NY	284	12 +/- 2.8 w 1st trimester	138.5 marginally deficient	2.57	No associations with fT4 and TSH using multiple regression; statistically significant association for TSH using weighted quantile sum regression
Knight et al., 2018	308	37 w 3rd trimester	88	2.1	Statistically significant association for fT4. No association for TSH.
Mortensen *et al.*, 2016 (NCS cohort)	329	3rd trimester	Not given	4.0	No associations fT4 and TSH
Mortensen *et al.*, 2016 (NHANES cohort)	533	1st, 2nd, and 3rd trimester	Not given	3.3	No associations fT4 and TSH
Pearce *et al.*, 2010 (Cardiff hypo-thy/thyrox cohort)	374	12.5 (12.8 +/- 1.6 given in Lazarus *et al.*, 2012)	98	2.1	No associations fT4 and TSH
Pearce *et al.*, 2010 (Cardiff euthyroid cohort)	480	12.8 +/- 1.6 1st and 2nd trimester	117	2.6	No associations fT4 and TSH
Pearce *et al.*, 2010 (Turin hypo-thy/thyrox cohort)	261	12.8 +/- 1.6 1st and 2nd trimester	55	5.04	No associations fT4 and TSH
Pearce *et al.*, 2010 (Turin euthyroid cohort)	526	12.8 +/- 1.6 1st and 2nd trimester	50	5.2	No associations fT4 and TSH
Pearce *et al.*, 2011 (LA cohort)	134	9.1 +/- 2 1st trimester	144	7.8	No associations fT4 index, total T3, and TSH
Pearce *et al.*, 2011 (Argentina cohort)	107	10.0 +/- 2 1st trimester	130	13.5	No associations fT4 index, total T3, and TSH
Pearce *et al.*, 2012 Greece	134	10.9 +/- 2.3 1st trimester	120 marginally deficient	4.1	Using multivariate regression, there were no associations fT4, fT3, and TSH

* No statistics are presented to determine statistical significance of this association.

In contrast to those studies not reporting a urinary perchlorate and thyroid variable association, four studies report some type of association. Steinmaus *et al.* (2016) report associations identified between increasing maternal $\log_{10}$ urinary perchlorate and decreasing free T4 and increasing TSH; however, the authors do not demonstrate that the association is statistically significant (Steinmaus *et al.*, 2016). Although there was no association between thyroid measures and urinary perchlorate, nitrate, or thiocyanate using linear regression in Horton *et al.* (2015), statistical analysis using a weighted quantile sum regression reported a positive association between all three anions and TSH.

Cross-sectional studies conducted in 200 pregnant Thai women with a gestational age ≤14 weeks and 308 English women with an average gestation week 37, report that environmental exposure to perchlorate is negatively associated with FT4. In the iodine-sufficient Thai population, urinary perchlorate was also positively associated with TSH. The authors report a median urinary perchlorate of 1.9 µg/L, which conflicts with results of other studies that have higher urinary perchlorate levels and show no association. They report several possible confounders, including higher urinary thiocyanate levels compared with studies of women in other countries, and early pregnancy increases in human chorionic gonadotropin which can interfere with serum thyroid hormone and TSH values. In the English population (Knight et al., 2018), the median urinary perchlorate was 2.1 µg/L, but the population was iodine insufficient (median urinary iodide of 88 µg/L <100 ug/L) based on WHO levels for iodine, and all were presenting for breech pregnancy in the third trimester (Knight *et al*., 2018).

Taylor *et al.* (2014) and Amitai *et al.* (2007) are the only population-based studies to attempt to directly examine perchlorate and cognitive effects in offspring. Taylor *et al.* (2014) reported an

association between the highest 10% of urinary perchlorate collected in 487 women ≤16 weeks of pregnancy and offspring IQ (assessed using the Wechsler Preschool and Primary Scale of Intelligence) at 3 years of age. The data were collected from 2002-2006 in the United Kingdom and Italy. Median urinary perchlorate levels measured in the UK and Italian participants were 2.20 and 3.43 µg/L, respectively. No evidence of association was observed between tertiles of maternal urinary iodine status and low IQ in offspring; between the lowest 10% maternal urinary iodide/perchlorate ratio and offspring IQ; or the lowest tertile in maternal urinary iodide/perchlorate ratio and offspring IQ in the lowest 10%. This study was part of a larger study which aimed to evaluate the effect of intervention with thyroxine on offspring development (Lazarus *et al*., 2012). In the larger study, there was no effect of treatment; the treated group and control group had full scale IQ scores of ~99±13 and 100±13 (Lazarus *et al*., 2012).

Amitai *et al.* (2007) reported neonatal T4, measured during the first 48 hours after birth, in three Israeli groups exposed to perchlorate at different drinking water levels: ≥340 µg/L, 42-94 µg/L; or <3 µg/L. The authors reported no statistical differences between neonatal T4 levels among the groups. In a subsequent abstract, a subset of the original 2007 study children was evaluated using the Bayley Scales of Infant Development to assess the motor (fine and gross), language (receptive and expressive), and cognitive development of infants and children ages 0 to 3. The authors reported no difference between the exposure groups on the neurodevelopment of the children (Amitai *et al.*, 2008).

Studies of Perchlorate in Children and Infants

Several studies evaluate various non-thyroidal endpoints with exposures in infants or children.

Cao *et al.* (2010) collected urinary perchlorate, nitrate, and thiocyanate from diapers and compared them with urinary TSH and fT4 values from diaper urine. Testing diapers for these constituents is not a standard medium for measurement of thyroid hormones. All of the measured goitrogens were positively associated (the opposite direction of what would be expected) with both TSH and fT4. The diapers were found to contain perchlorate, which may have affected the results (Cao *et al.*, 2010). In serum from infants, Cao *et al.* (2010) found no association between perchlorate, nitrate, or thiocyanate and TSH and free T4 in blood. Blount *et al.* (2009) found no association between perchlorate, nitrate, and thiocyanate in cord blood and fetal birth weight, head circumference, and birth length. No association was found between infant urinary perchlorate and serum thyroid function tests in Boston-area women and their 1-3 month old infants (Leung *et al.*, 2012).

Conclusions

This review aimed to evaluate whether environmental levels of perchlorate are sufficient to cause the most sensitive adverse health effects. The endpoint most significant to public health is an adverse impact on the developing fetal brain. We have reviewed animal studies, clinical studies, and population-based studies of children and pregnant women for evidence of perchlorate's impact predominately on the thyroid gland. The thyroid gland has a key role in neurological development during gestation and post-natal development. We find that the animal and human clinical data, and the majority of the population-based data to be consistent regarding low environmental doses. The extensive research supports the well-established toxicological principle that, for most chemical agents, an exposure threshold for toxicological

effects exists, and before harm can result from an exposure to an agent, exposure must be of sufficient concentration and duration to produce the necessary internal dose that exceeds this threshold. That said, there are several population-based studies that report associations between perchlorate and some trend related to thyroid function.

Perchlorate has a robust pharmacological and toxicological database spanning over six decades of peer-reviewed research characterizing the MOA and threshold dose for IUI in both laboratory animals and humans. The lowest reported NOEL for a non-adverse effect (IUI) is 7 µg/kg-d (0.007 mg/kg-d; Greer et al., 2002; NRC, 2005). Currently, the MOA is the foundation of the PBPK model the US EPA has proposed to derive a MCLG for perchlorate.

Based on evaluation using classical pharmacology and toxicology principles of the dose-response relationships, the environmental doses of perchlorate are below IUI. Perchlorate exposure through dietary sources in the US is common with perchlorate detectable in the urine of all NHANES participants (Blount *et al*., 2006). The 95th percentile urinary perchlorate concentration in NHANES participants was 14 µg/L, corresponding to a 95th percentile dose of 0.234 µg/kg-d (Blount *et al*., 2007). This dose is approximately 30-fold lower than the NOEL for IUI of 7 µg/kg-d, the most conservative NOEL value obtained from clinical studies (Greer *et al*., 2002; Bruce *et al.*, 2017). The population-based studies on maternal exposures and effects on thyroid hormones report urinary perchlorate levels ranging from 1.9 to 13.5 µg/L, with one study at 34 µg/L, and thus, except for one study, all studies are below the 95^{th} percentile of the NHANES study population study. Furthermore, these doses are based on exposures of this population during the 2000-2001 NHANES (Blount *et al.*, 2007). Since then, the concentration of urinary perchlorate has been decreasing (Jain, 2016; Corey *et al.*, 2017).

Most human studies report no effect or association of environmental perchlorate exposures and thyroid hormone changes. Men and women of reproductive age given perchlorate for two weeks, six months, or occupationally for 1.7 years reported no thyroid hormone changes (Greer *et al*., 2002; Braverman *et al*., 2005, 2006). The population-based study with the highest maternal urinary perchlorate level of 34 µg/L, a level that would be above the 95th percentile of the 2007 NHANES study, reported no association with fT4 and TSH (Gibbs and van Landingham, 2008) and was reinforced with studies representing nine different populations both in the US and internationally (Pearce *et al.*, 2010, 2011, 2012; Mortensen *et al.*, 2016).

Four studies have reported some type of association between environmental perchlorate exposure and thyroidal endpoints in pregnant women. No study demonstrated an adverse effect. Taylor *et al.* (2014) reported an association between urinary perchlorate collected in first-trimester pregnant women and offspring IQ at 3 years of age. In this study, some women were treated with levothyroxine therapy which had no effect on IQ outcome (Taylor *et al*., 2014). Additionally, Pearce *et al*., (2010) report a lack of an association between urinary perchlorate and maternal fT4 in the same population. Contrary to Taylor et al. (2014), Amitai *et al.* (2008) studied children using the Bayley Scales of Infant Development to assess the motor, language, and cognitive development of infants and children ages 0 to 3 with some of the highest exposures ever recorded in the literature (≥340 µg/L). The authors reported no statistical differences in the children per exposure group.

All of these population-based studies have exposures that are below the threshold for IUI, which suggests that it is unlikely that perchlorate was causally associated via the known MOA. Even with minimal IUI, thyroid hormone changes require significant and sustained IUI. In the

wider literature, only clinical studies (using therapeutic doses) or animal studies (with free access to water containing perchlorate) report administration of sufficiently sustained levels of perchlorate that could result in IUI. Except for animal studies at doses higher than environmental perchlorate levels, no thyroid variable changes were noted.

There are four possible explanations why population-based studies report mixed results compared to the clinical literature. First, is it possible that low environmental levels of perchlorate may operate via another MOA. For example, Brent (2010) proposed that there may be direct effects of perchlorate on the fetal thyroid and different thyroid hormone efficacy but provided no data to demonstrate that this is a viable mechanism. No alternative MOA has been tested at environmental doses and reported in the literature. EPA continues to use the current MOA in its PBPK modeling evaluation of perchlorate.

A second possible explanation is the concern about iodine sufficiency in the population and that perchlorate exposure might be causing further stress on the mother and developing child. Iodine physiology is exceedingly complex and while there is universal agreement that severe iodine deficiency causes severe thyroid effects for the developing fetus and neonate, the literature related to minor iodine deficiency is equivocal. Although small changes within the normal reference range may have individual impacts, the overall iodine status of the population is not clearly associated with perchlorate exposure and thyroid endpoints (e.g., iodine insufficient populations from Pearce *et al.* (2010) are not those that demonstrate an association).

A third possible explanation is that the NIS might have some differing sensitivity during pregnancy or fetal stages. However, we retrieved no evidence that the NIS is any more or less

sensitive or is regulated differently in pregnant and not pregnant women. The NIS is well-conserved among species and animal studies do not report significant differences during any life stage. The NIS can be up-regulated with perchlorate exposure in both animal models and humans; this mechanism would allow the body to adjust to different dietary or physiological conditions. Although IUI was not measured, Crooks and Wayne (1960) treated over 400 patients with thyrotoxicosis with one of three antithyroid agents. Twelve of these patients were pregnant women treated with potassium perchlorate up to 1000 mg/d. There were no adverse effects reported, but transient goiter was reported in one infant which resolved shortly after birth (Crooks and Wayne 1960).

A fourth possible explanation for mixed results is related to study design. Population-based studies are difficult to conduct and study design is critical to the reliability of these studies. Accounting for confounding variables is difficult when a study population was recruited for a different study objective, as many of the studies were (e.g., Steinmaus *et al.*, 2010). Timing of sample collection is also critical in developmental studies (e.g., the changing physiology of the mother and fetus during the course of a pregnancy), however the timing of sample collection was not consistent across studies. Not only does this make comparison between studies difficult, but generally reduces the ability to make conclusions based on these studies collectively. Finally, if perchlorate accounts for less than 1% of IUI, then it appears that the greater impact on thyroidal effects could be related to nitrate and thiocyanate exposures, which were not measured in all studies.

In 2013, EPA's SAB reviewed many of the same studies and concluded that the epidemiological studies published after NRC (2005) were limited by study design, exposure assessment, and

other methodological issues that prevent their use in deriving a causal inference between exposure to perchlorate in the general population and effects on sensitive life stages, including pregnant women and offspring (SAB, 2013).

Clearly all future studies should collect multiple clinical chemistry measures (*e.g.*, multiple 24-hr urine samples rather than a single spot urine sample), with accurate chemical exposure data (*e.g.*, consideration of perchlorate half-life on sampling), and in serial measurements with serial infant outcomes to assess gestational influence while matching many vital variables postnatally (*e.g.*, socio-economic standard, nutrition, exposures to other chemicals). Controlling confounders may further tease out the influence of perchlorate or other possible agents on neurodevelopment.

Animal studies provide additional insight into the potential for neurobehavioral effects from exposure to perchlorate. Doses used in animal studies are tens of thousands of times greater than the dose in the US population, and the rat, the test subject used in neurobehavioral studies, is more sensitive to thyroid influences than the human (NRC, 2005). Offspring in these studies were tested for many neurobehavioral outcomes, including cognitive outcomes such as passive avoidance testing and water maze testing. Studies in rats with anti-thyroid agents such as propylthiouracil and methimazole demonstrate that auditory, motor, and eye-opening effects are some of the more sensitive endpoints of maternal thyroid hormone deficits (Albee *et al*., 1989; Goldey *et al.*, 1995). These effects were not noted in auditory startle habituation or motor testing conducted by York *et al.* (2003) and Bekkedal *et al.* (2004). All laboratory mammals have qualitatively similar thyroidal and regulatory systems, but the quantitative components differ. The rat is more sensitive and responsive to thyroid effects than humans

because of lower protein binding and higher clearance of T4 (NRC, 2005). However, only when perchlorate was used at high doses, as a pharmacologic tool, to induce frank hypothyroidism, were some minor developmental effects noted.

The preponderance of the literature demonstrates that adverse health effects due to perchlorate are not occurring at environmental exposure levels. There are four populations that report an association with a thyroidal endpoint below the doses known to cause IUI. While informative and worth additional exploration, the results of the studies are not consistent with the majority of the studies in humans and animals and the demonstrated MOA; thus, these do not demonstrate a causal relationship with perchlorate. Future epidemiological research must consider experimental design carefully to capture the dynamic changes of maternal and fetal physiology with the pharmacology of perchlorate. Additionally, exquisite care must be taken to ensure confounders and covariates are assessed.

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Conflict of Interest Statement

The Perchlorate Study Group (PSG) funded this work. Funds are based on the work conducted, not outcome of the work. Intertox has conducted independent research for PSG to provide accurate scientific information. This manuscript was not provided to PSG until the manuscript was accepted for final publication. Dr. Pleus has been a toxicology expert in two perchlorate-related litigation cases.