

CLINICAL STUDY

Perchlorate versus other environmental sodium/iodide symporter inhibitors: potential thyroid-related health effects

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Abstract

Objective: Perchlorate is a known competitive inhibitor of the sodium/iodide symporter (NIS). Possible thyroid-related effects of environmental perchlorate have created great health concerns, especially in the US, resulting in a debated reference dose (RfD) of 0.0007 mg/kg per day in drinking water recommended by the National Academy of Sciences (NAS). However, the impact of other environmental NIS inhibitors and the role of iodine seem to have received little attention in the whole debate.

Methods: We performed a PubMed search for articles published up to February 2006, using the key terms perchlorate, nitrate, thiocyanate, iodine, NIS, RfD, thyroid (alone or in combinations), with particular attention for human studies. In parallel, we critically analysed the January 2005 NAS' report, entitled 'Health implications of perchlorate ingestion'.

Results: The relative potencies of prevalent environmental NIS inhibitors (nitrate, thiocyanate and perchlorate) to inhibit iodine uptake have been estimated repeatedly with robust results. Our calculations show that nitrate and thiocyanate, acquired through drinking water or food, account for a much larger proportion of iodine uptake inhibition than perchlorate. Furthermore, the iodine uptake inhibitory effects of nitrate and thiocyanate – as defined by their legally accepted maximal contaminant levels in drinking water – exceed the potential effect of the proposed RfD for perchlorate by far.

Conclusions: Iodine uptake inhibition and any potential downstream effect by perchlorate are highly dependent on the presence of other environmental NIS inhibitors and iodine intake itself. These potential confounders should therefore be considered in future studies and calculations for risk assessment.

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Introduction

Since the availability of iodine in the terrestrial environment is limited, the thyroidal follicular cells have adopted a mechanism to concentrate and conserve this important trace element. Accumulation of iodide (I^-) by the thyroid was already known to be an energy-requiring mechanism when its Na^+ dependence was recognized. In 1996, a sodium/iodide symporter (NIS) was cloned from the human thyroid (1). The expression of the NIS is not confined to the thyroidal follicular cells, as the transporter is also present in lactating mammary glands, salivary glands and gastric mucosa.

The ability of perchlorate to inhibit thyroidal iodine uptake, thereby potentially inducing hypothyroidism, has been recognized since many decades, even resulting in therapeutic applications for hyperthyroidism (2, 3). Also the mechanism of action, i.e. competitive inhibition of

thyroidal iodine uptake at the level of the NIS, has been elucidated. Based on clinical studies, the perchlorate dose required to cause hypothyroidism in adults is estimated to be more than 0.4 mg/kg per day for months or longer (4). Because of its potential health effect, exposure to environmental perchlorate has recently generated serious debates in the endocrinological, toxicological and ecological community, and resulted in a series of papers in medical journals and in a report of the National Academy of Sciences (NAS), proposing a reference dose (RfD) (see Table 1 for ecotoxicological terminology) for perchlorate of 0.0007 mg/kg per day (4), which in turn is subject to criticism.

Studies on perchlorate and possible thyroid-related health effects have paid only little attention to other environmental NIS inhibitors such as nitrate and thiocyanate. However, these anions constitute ubiquitously present goitrogenic anions, for which maximum

Table 1 US Environmental Protection Agency (EPA) definitions of terminology used in environmental toxicology and regulation (URL source: http://www.epa.gov/NCEA/bmds_training/appendices/glossary.htm).**Adverse effect**

A biochemical change, functional impairment, or pathological lesion that either singly or in combination adversely affects the performance of the whole organism or reduces an organism's ability to respond to an additional environmental challenge

Lowest-observed-adverse-effect level (LOAEL)

The lowest dose or exposure level of a chemical in a study at which there is a statistically or biologically significant increase in the frequency or severity of an adverse effect in the exposed population as compared with an appropriate, unexposed control group

No-observed-adverse-effect level (NOAEL)

An exposure level at which there are no statistically or biologically significant increases in the frequency or severity of adverse effects between the exposed population and its appropriate control; some effects may be produced at this level, but they are not considered as adverse or precursors to adverse effects. In an experiment with several NOAELs, the regulatory focus is primarily on the highest one, leading to the common usage of the term NOAEL as the highest exposure without adverse effect

No-observed-effect level (NOEL)

An exposure level at which there are no statistically or biologically significant increases in the frequency of any effects between the exposed population and its appropriate control

Point of departure (POD)

The point on a dose–response curve established from experimental data, e.g. the benchmark dose (BMD), generally corresponding to an estimated low effect level (e.g. 1 to 10% incidence of an effect). Depending on the mode of action and available data, some form of extrapolation below the POD may be employed for low-dose risk assessment or the POD may be divided by a series of uncertainty factors (UFs) to arrive at a reference dose

Reference dose (RfD)

An estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without appreciable risk of deleterious non-cancer effects during a lifetime

Uncertainty factor (UF)

The value (often a default value of 10) used as a divisor of an NOAEL, LOAEL, or BMD to calculate the RfD. UFs are applied as needed for extrapolation of results in experimental animals to humans, interindividual variability including sensitive subgroups, extrapolation from a LOAEL to an NOAEL, extrapolation of results from subchronic exposures to chronic exposures and database inadequacies

Benchmark dose

An exposure due to a dose of a substance associated with a specified low incidence of risk, generally in the range of 1–10%, of a health effect; or the dose associated with a specified measure or change of a biological effect

Maximum contaminant level (MCL)

The highest level of a contaminant that is allowed in drinking water. MCLs are set as close to maximum contaminant level goals (MCLGs – the level of a contaminant in drinking water below which there is no known or expected risk to health) as feasible using the best available treatment technology and taking cost into consideration. MCLs are enforceable standards

contaminant levels (MCLs) in drinking water have been established. To gain more insight into the large amount of old and recent experimental data and evaluate whether the proposed RfD for perchlorate by the NAS is in line with the presently available scientific evidence, especially with regard to the role of other environmental NIS inhibitors, we performed a literature study along with a critical analysis of the NAS report.

Competitive NIS inhibitors and their relative potencies

The electrophysiological properties of the NIS have been examined in *Xenopus* oocytes, Chinese hamster ovary (CHO) cells, and the cloned rat thyroid cell line FRTL-5 (5–7). A wide variety of monovalent anions with a similar ionic radius, including perchlorate (ClO_4^-), thiocyanate (SCN^-) and nitrate (NO_3^-), were found to generate similar steady-state inward currents as does I^- , suggesting that these anions are also transported by the NIS and act as competitive inhibitors for iodine uptake by the thyroid. Whether or not ClO_4^- is actually transported into the follicular cells by the NIS is still a matter of debate, but using stably transfected COS-7 and FRTL-5 cells, Van Sande

et al. provided evidence for such a translocation (8). The relative potencies of ClO_4^- , SCN^- , NO_3^- and I^- to inhibit radioactive $^{125}\text{I}^-$ uptake by the human NIS stably transfected in CHO cells were recently investigated by Tonacchera *et al.* (9). These authors showed that the relative potency of ClO_4^- to inhibit radioactive I^- uptake at the NIS is 15, 30 and 240 times that of SCN^- , I^- , and NO_3^- respectively on a molar concentration basis. As shown in Table 2, these data are in agreement with earlier studies in rats (10–12). Importantly, the data of Tonacchera *et al.* indicate a mode of action by these anions of simple competitive interaction with no evidence of synergism or antagonism (9). Consequently, the I^- uptake inhibitory effect of a mixture of NIS inhibitors can be expressed as a perchlorate equivalent concentration (PEC), defined as the sum of the molar concentrations of each individual inhibitor, multiplied by the inhibition potency relative to ClO_4^- (7):

$$\text{PEC} = [\text{ClO}_4^-] + [\text{SCN}^-]/15 + [\text{I}^-]/30 + [\text{NO}_3^-]/240.$$

The concentration of a NIS inhibitor expressed as PEC can be interpreted as the concentration of perchlorate needed to inhibit NIS I^- uptake to the same extent as the given concentration of NIS inhibitor.

Table 2 Molar concentration-based potencies of thiocyanate and nitrate to inhibit NIS function relative to perchlorate as determined in different studies.

Study (Refs.)	Perchlorate	Thiocyanate	Nitrate
Inhibition of radioactive $^{125}\text{I}^-$ uptake by human NIS expressed by CHO cells (9)	1	15	240
<i>In vivo</i> inhibition of radioactive $^{125}\text{I}^-$ uptake in rat (10)	1	10	300
<i>In vivo</i> 50% thyroid weight increase in rats (11)	1	20	550
<i>In vitro</i> uptake of radiolabelled I^- in rat thyroid slices (11)	1	20	400
<i>In vitro</i> uptake of radiolabelled I^- in isolated rat thyroids (12)	1	20	240

Environmental perchlorate

Partially due to improved detection methods (ion chromatography and tandem mass spectrometry) (13), low concentrations of perchlorate have been detected in surface- and ground-water in various regions of the US. Perchlorate is used as an oxidant in solid propellants for rockets, missiles, fireworks and certain munitions, but some of the contamination probably has a 'natural' origin (4, 14). Following press reports on the contamination of drinking-water wells and especially of breast milk, research programs have been developed to assess the risk from these exposures. In January 2005, the NAS recommended a perchlorate RfD of 0.0007 mg/kg per day (4), mainly based on a study by Greer *et al.*, the only published prospective study in which multiple doses of perchlorate were given to human subjects (14). In this study, 37 adults were given different doses of perchlorate in drinking water for 14 days, and 8- and 24-h measurements of radioactive I^- uptake before exposure,

on exposure days 2 and 14, and 15 days post-exposure were performed. The researchers determined 7 $\mu\text{g}/\text{kg}$ per day as the no-observed-effect level (NOEL), for this was the dose producing no statistically significant inhibition of radioactive I^- uptake (Fig. 1). From regression relationships for 8- and 24-h relative uptakes on exposure day 14, the true no-effect level was estimated to be 5.2 and 6.4 $\mu\text{g}/\text{kg}$ per day respectively (0% inhibition of I^- uptake) (14). Dose-response relations from other human studies are in agreement with the Greer *et al.* study (14–17). Furthermore, benchmark dose (BMD) calculations from occupational studies (16, 17) were within the range of the Greer *et al.* study, and in agreement with the no-observed-adverse-effect level (NOAEL), as judged by hypothyroidism, of 0.4 mg/kg per day (18). Taking into account the NOEL determined by Greer *et al.* and a total uncertainty factor (UF) of 10, an intraspecies factor to protect the most sensitive population, the NAS recommends an RfD of 0.0007 mg/kg per day, which is equivalent to a 24.5-p.p.b. concentration of perchlorate in drinking water

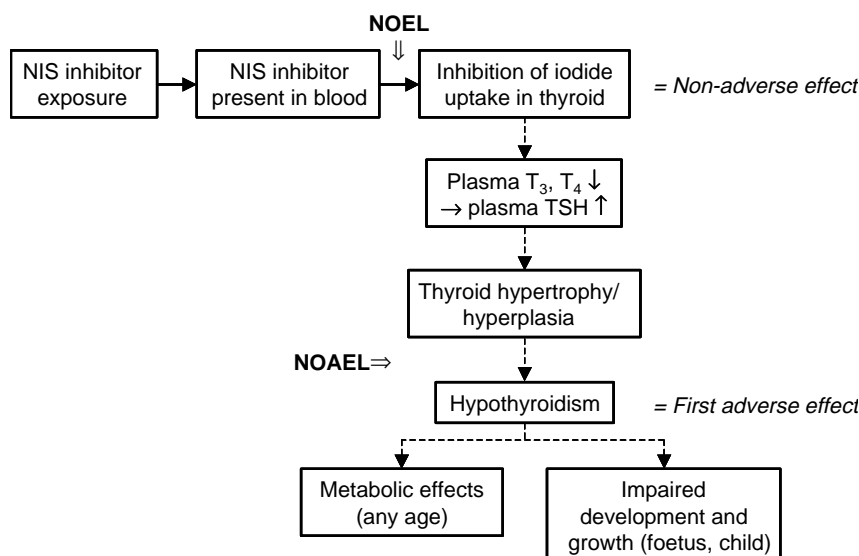


Figure 1 Model for the mode-of-action of NIS inhibitor toxicity in humans, based on the model of the Committee to Assess the Health Implications of Perchlorate Ingestion, which prepared the January 2005 NAS report 'Health implications of perchlorate ingestion'. Dashed arrows represent effects not clearly demonstrated in humans exposed to perchlorate, but biologically plausible in absence of adequate compensation. Hypothyroidism is considered by the NAS as the first adverse effect; inhibition of thyroidal iodide uptake is, therefore, a non-adverse effect. NIS, Na^+/I^- symporter; NOAEL, no-observed-adverse-effect level; NOEL, no-observed-effect level; TSH, thyroid-stimulating hormone (thyrotrophin).

when assuming a body weight of 70 kg, a daily consumption of 2 l drinking water and the drinking water to be the only dietary source of perchlorate. This RfD was adopted by the US Environmental Protection Agency (EPA) in February 2005, although so far no MCLs for drinking water have been established (URL source: <http://www.epa.gov/fedfac/documents/perchlorate.htm>). Despite a suggested MCL of 24.5 p.p.b. based on the NAS report, some states have proposed even lower public health goals for perchlorate (e.g. California, 6 p.p.b.; Massachusetts, 1 p.p.b.).

Analysis of the proposed perchlorate reference dose

NOEL as point of departure

Determining its RfD, the NAS used an NOEL (0.0007 mg/kg per day) rather than an NOAEL (0.4 mg/kg per day), which serves traditionally as the point of departure (POD) for risk assessment (4). The NAS concludes that, given their mode of action model (Fig. 1), “the first adverse effect in the continuum would be hypothyroidism. Any effects that follow and result from hypothyroidism clearly would be adverse”, and elsewhere; “For adverse health effects to occur in healthy adults, thyroid hormone production must fall substantially and, more importantly, must remain low for at least several weeks”. The NAS argues that using a non-adverse effect (thyroidal I^- uptake) instead of an adverse effect (hypothyroidism) as the POD is a “conservative, health-protective approach”, since inhibition of I^- uptake constitutes a biological effect and not necessarily a health effect. This recommendation is probably also based on the fact that only a limited number of studies had been performed on the most sensitive populations to iodine deficiency, being foetuses, neonates and pregnant women. The most feared complication of insufficient supply of thyroid hormones (tri-iodothyronine (T_3)) to the developing brain consists of neurointellectual deficit (reviewed in Ref. 19). Though congenital hypothyroidism is not necessarily associated with impaired intellectual development (20), intellectual deficit was shown in the offspring of mothers with overt or even subclinical hypothyroidism (21, 22). A relation with attention deficit and hyperactivity disorders has also been suggested (23). Thyroid hormone (in particular T_3) bound to nuclear thyroid hormone receptors regulates the expression of specific genes in different brain regions following a precise developmental schedule. Foetal thyroid hormone secretion starts only around mid-gestation, underlining the importance of maternal thyroid hormones during the first trimester (24). This also implies that brain damage in regions of iodine deficiency is much more severe than brain damage caused by sporadic congenital hypothyroidism. The hypersensitivity of

neonates to the effects of iodine deficiency is explained by their small intrathyroidal iodine pool, which requires increased thyrotrophin (TSH) stimulation and a fast turnover rate in order to maintain normal secretion of thyroid hormones. Hypothyroidism occurring around birth, even transient, can affect brain development and intellectual potential. However, when neonatal hypothyroidism is rapidly diagnosed and appropriately treated, later childhood intelligence is normal (25).

Although the majority of studies in newborns and children show no effects of perchlorate intake on thyroidal function (i.e. unaltered thyroid hormone and TSH levels) or neurodevelopmental state (26–28), these data are incomplete and subject to criticism, often because of the lack of measurement of perchlorate or iodine intake (25, 29). An interesting epidemiological study on thyroid function in school-age children born in an area in Chile, where perchlorate is naturally present in drinking water (100–120 p.p.b.) showed that there was no difference in TSH, free thyroxine (T_4) or goitre prevalence compared to children of two other regions with low (5–7 p.p.b.) or no (<4 p.p.b.) perchlorate in the water (30). Moreover, no elevated TSH levels were observed in neonates of the same regions. Considering thyroid hormone change in the pregnant women of this study as the critical effect (POD) would result in an estimated NOAEL-based RfD for perchlorate of 0.002 mg/kg per day; the POD was a no-observed-adverse level of 0.006 mg/kg per day and an UF of 3 was applied, owing to the sufficient amount of available data (31). However, the method used by these authors, calculating the RfD by so-called ‘benchmarking’, remains subject to criticism. Moreover, the use of the Chilean study as a basis of an NOAEL-based RfD has been questioned due to the very high iodine intake of the subjects, which could render substantial competition with perchlorate undetectable. In a following, recently published epidemiological study (32), the effects of chronic perchlorate exposure through drinking water on thyroid function have been studied in pregnant women and neonates in the same areas in Chile, but this time, total iodine intake of the women was similar to that of US pregnant women. Even in the area with the highest dose of perchlorate in the drinking water (114 $\mu\text{g/l}$, equivalent to an uptake of 93.5 $\mu\text{g/day}$), no effects were found on the serum concentrations of TSH, thyroglobulin or T_4 , neither in the pregnant women nor in the neonates. Moreover, neonatal birth weight, length and head circumference were consistent with the US standards. This is the first human study indicating that exposure to environmental perchlorate exceeding the NAS’ RfD several-fold from preconception until neonatal age interferes neither with neonatal thyroid function nor with neurodevelopment. However, to further exclude any effect on brain development, a follow-up study of these neonates is needed with specific psychoneurological endpoints. As long as our knowledge of perchlorate effects in sensitive

populations is only fragmentary and toxicological studies with specific neurodevelopmental endpoints are lacking, the NAS' decision to choose an NOEL rather than an NOAEL as a POD for the risk assessment seems to be justifiable.

Of more concern is whether the NAS' tenfold UF is still needed for interindividual variability. Some authors, like Ginsberg and Rice (33), have proposed even higher UFs, but the recent studies of Braverman *et al.* (17) and Téllez and colleagues (32) contradict this. Ginsberg and Rice argue that an additional UF of 3–10 is needed to account for the potential for greater toxicity to newborns from lactational exposure and for the uncertain relationship between short-term and chronic perchlorate toxicity (33). Braverman *et al.* showed that in workers of a perchlorate production plant, long-term, intermittent, high exposure to perchlorate did not affect plasma TSH and thyroglobulin levels or thyroid volume. These results suggest that, as predicted by the NAS committee, the inhibition of I^- uptake is duration-dependent, and compensatory mechanisms exist to counteract the perchlorate-induced NIS inhibition. Concerning the lactational transfer of perchlorate and the inhibition of I^- uptake by the mammary glands as mentioned earlier, Téllez *et al.* found no perchlorate-induced thyroid effects in neither the Chilean pregnant women nor the neonates exposed to the highest concentration of perchlorate in the drinking water, and moreover, they did not find a decrease in breast milk iodine as feared by Ginsberg and Rice. According to Hershman (34), the vulnerability of pregnant women is far less than would be accounted for by a factor of 2. Moreover, when already 'playing safe' by determining an NOEL instead of an NOAEL as a POD for risk assessment, an UF of 10 might be greatly overstating human health risks from perchlorate.

Impact of other environmental NIS competitors: nitrate and thiocyanate

In the whole debate about perchlorate-induced NIS I^- uptake inhibition, other NIS competitors have been largely ignored. Nevertheless, nitrate is ubiquitous in food, occurring naturally (as in green leafy vegetables), added as a preservative (in meat and fish) or as a contaminant resulting from organic and mineral fertilizers. For instance, winter iceberg lettuce samples purchased in Santa Cruz County, CA, in 1998, contained an average of 973 mg nitrate per kg fresh weight (35). Nitrate is also common in surface and ground sources of drinking water, primarily due to the agricultural use of nitrogen-containing organic and mineral fertilizers. A Slovak study has shown that school children living in a community with drinking-water wells containing high nitrate levels (> 50 mg/l and some even > 100 mg/l) have larger thyroid glands and show signs of subclinical thyroid disorder (thyroid hypoechogenicity by ultrasound, increased TSH levels, positive thyroperoxidase antibodies) with an increased

frequency (36). No iodine deficiency was observed in humans exposed to nitrate in drinking water, but hypertrophy of the thyroid gland was noted at nitrate levels exceeding 50 mg/l (37). Long-term exposure to high perchlorate levels on the other hand, did not cause goitre or hypothyroidism in adults (17).

Furthermore, the presence of dietary thiocyanate should be taken into account. Thiocyanate is a goitrogen interfering in two different steps of thyroid hormone synthesis; it inhibits both thyroidal I^- uptake by the NIS, as well as – in higher concentrations – the thyroid peroxidase-catalysed organification of iodine, prior to the iodination of tyrosyl groups of thyroglobulin. Certain cyanogenic glucosides (found in cassava, sweet potatoes, corn, apricots, cherries and almonds) are hydrolysed by glucosidases in the gut and release free cyanide that is converted to thiocyanate. Thioglucosides found in certain plants, especially plants belonging to the Brassicaceae family (cabbage, Brussels sprouts, cauliflower and broccoli), are metabolized within the body to thiocyanates and isothiocyanates. For example, thiocyanate in raw cauliflower can be as high as 9 million p.p.b. and 250 000 p.p.b. when cooked (38). Endemic cretinism in regions of Central Africa, where cassava represents a major food staple, has been associated with thiocyanate overload and aggravation of iodine deficiency resulting in foetal hypothyroidism (39). Interestingly, goitres were reported to develop when the critical urinary I^-/SCN^- ratio decreases below $3 \mu\text{g } I^-$ per mg thiocyanate, but the goitrogenic effect of cassava is completely reversed by adequate iodine supplementation (40). A very recent epidemiologic study in Germany confirmed the relevance of thiocyanate levels with respect to goitre prevalence, and also demonstrated that a urinary I^-/SCN^- ratio was more predictive than urinary I^- secretion alone (41). Also cigarette smoking is an important source of thiocyanate in the body. In the US, the breast milk thiocyanate concentrations of smokers were fourfold higher than those of non-smokers (42). Laurberg and colleagues also demonstrated in a recent study that iodine content in breast milk of smoking mothers was twofold reduced (43), most probably due to the competitive inhibition for NIS in the mammary gland. Téllez *et al.*, however, did not find a decrease in the breast milk iodine concentrations of women living in a Chilean area with high perchlorate levels in drinking water (32), a finding that was very recently confirmed in lactating cows receiving high doses of perchlorate (44), suggesting that the effect of perchlorate at these levels is negligible compared to that of thiocyanate from cigarette smoking or cassava consumption.

Thus, nitrate and thiocyanate are both important dietary anti-thyroid agents acting at the level of the NIS. In the Greer *et al.* study (14), however, data on nitrate and thiocyanate measurements – which might highly vary due to the kind of recently ingested food – were lacking, but could importantly influence iodine uptake

results. Bruce *et al.* (38) point out correctly that not all the thiocyanate or nitrate in ingested food is bioavailable and refer to studies showing that about 50% thiocyanate and 90–100% nitrate in vegetables is bioavailable following consumption. Furthermore, cooking has also been shown to reduce both thiocyanate and nitrate content of ingested vegetables (38). On the other hand, one should also take into account the half-lives of the I^- uptake inhibitors. Thiocyanate, having a half-life of approximately 6 days as compared to 8 and 5 h for perchlorate and nitrate respectively, remains in the serum 18–29 times longer than the other anions. Based on these differences in half-lives, the perchlorate equivalency ratios of thiocyanate and nitrate in terms of ingested-weight basis have been recalculated to be 0.5 and 240 respectively (9).

The EPA-imposed drinking water MCL for nitrate is 10 mg nitrogen/l or 45 mg NO_3^- /l based on the risk of infant methaemoglobinaemia. The MCL for cyanide is 0.2 mg/l; this criterion is based on the US Public Health Service drinking water standards of 1962 for the protection of human health from the toxic properties of cyanide in drinking water. (High-dose of cyanide inhibits cellular enzymes resulting in histotoxic hypoxia. The central nervous system effects include demyelinating lesions of the brain and a Parkinson-like encephalopathy. The heart is also sensitive to cyanide-induced hypoxia.) Using the perchlorate equivalent ratios of Tonacchera *et al.* (1:15:240 or 1:8.8:150 for $ClO_4^-:SCN^-:NO_3^-$ on the molar- and weight-based ratios respectively, ref. 9), the MCL of nitrate would have the same effect at the level of the NIS as 300 p.p.b. perchlorate, whereas the MCL of thiocyanate is equal to 22.7 p.p.b. perchlorate (Table 3). In other words, the concentration of nitrate allowed in drinking water would cause an I^- uptake inhibitory effect that is 12 times greater than that of perchlorate. For comparison, the Belgian MCLs for nitrate and cyanide are 50 mg/l (equivalent to 333 p.p.b. perchlorate) and 50 μ g/l (equivalent to 5.7 p.p.b. perchlorate) respectively, but actually measured concentrations in drinking water in Belgium are 12.3 mg NO_3^- /l (equivalent to 82 p.p.b. perchlorate) and <2 μ g CN^- /l (equivalent to <0.23 p.p.b. perchlorate) respectively (URL source: <http://www.aww.be/inhoud/waterkwaliteit/watersamenstelling.index.htm>). Without any perchlorate in it, this water would cause

NIS I^- uptake inhibition that is 3 times greater than the effect of the proposed maximum perchlorate dose of 24.5 p.p.b. An average adult drinking 2 l of this water per day, if it contained the maximum perchlorate dose of 24.5 p.p.b. (which is not the case), would be exposed to a PEC of 164 p.p.b. (NO_3^-) + 0.46 p.p.b. (SCN^-) + 49 p.p.b. (ClO_4^-) = 213.5 p.p.b. PEC, of which 23% is derived directly from perchlorate, meaning that about 77% of the resulting NIS inhibition would be caused by the nitrates in the water. The actual average perchlorate level in contaminated active drinking-water sources in California is 8.77 p.p.b. (source: Environmental Working Group analysis of California Department of Health Services drinking water quality database from June 2002), reducing the share of perchlorate in NIS inhibition by drinking water even further to <10% (when assuming the same nitrate content).

Taken together, these data indicate that (i) the thyrotoxic potential of perchlorate cannot be seen separately from the effects of other NIS inhibitors such as nitrate and thiocyanate, (ii) when considering the potential thyroid-related health risks posed by monovalent anions, perchlorate only accounts for a small portion (<10%) of the exposure from drinking water and (iii) MCLs (recommended for perchlorate, legal for nitrate and thiocyanate) for different NIS inhibitors are largely discrepant.

As mentioned before, in addition to drinking water, food also contains considerable amounts of nitrate and thiocyanate. Using milk and lettuce as examples, it becomes evident that the contamination of monovalent anions in food expressed as PECs is much higher than what is actually present in the contaminated drinking water sources. As shown in Table 4, food contamination is predominantly due to the high levels of nitrate and thiocyanate that would account for 98.83 and 99.98% of the total NIS I^- uptake inhibition caused by milk and lettuce respectively. These numbers hardly change when a 50% absorption of thiocyanate in the gut is taken into account; 97.84 and 99.97% for milk and lettuce respectively. Eating just 50 g lettuce would cause an I^- uptake inhibition exceeding the effect of the maximum allowed concentration of 24.5 p.p.b. perchlorate in drinking water by 140 times. By drinking just 100 ml milk, an average 70-kg adult has already been exposed to an equivalent of the perchlorate RfD.

Table 3 MCLs for nitrate and thiocyanate in US drinking water, in comparison to the reference dose (RfD) for perchlorate (weight-based concentrations).

	MCL* or RfD† (mg/l)	MCL or RfD (p.p.b.)	Perchlorate dose (p.p.b.) needed to mimic the I^- uptake inhibition by the MCLs‡
Nitrate	45	45 000	300
Thiocyanate	0.2	200	22.7
Perchlorate	0.0245	24.5	24.5

*Data from EPA, URL: <http://www.epa.gov/safewater/mcl.html#mcls>.

†EPA-adopted RfD for perchlorate.

‡Ratios according to Tonacchera *et al.* (9).

Table 4 Indicative nitrate, thiocyanate and perchlorate levels in milk and green leaf lettuce. Nitrate and thiocyanate data are taken from Bruce *et al.* (38), perchlorate data are taken from the US Food and Drug Administration (URL source: <http://www.cfsan.fda.gov/~dms/clo4data.html>).

	Milk		Lettuce	
	Concentration (p.p.b.)	Perchlorate dose (p.p.b.) needed to mimic the I ⁻ uptake inhibition	Concentration (p.p.b.)	Perchlorate dose (p.p.b.) needed to mimic the I ⁻ uptake inhibition
Nitrate	5000	33.33	1 000 000	6666
Thiocyanate	4000	454.55	545 000	61 932
Perchlorate	5.76	5.76	10.7	10.7

Nevertheless, these levels of nitrate and thiocyanate are considered to pose no health threats and fall well below the legally imposed maximum levels (45, 46).

The actual average daily intake of an adult on a US Department of Agriculture-recommended diet is calculated at 33 mg/day perchlorate equivalents of nitrate (244 mg/day) and thiocyanate (277 mg/day) combined (38). The EPA-adopted RfD for perchlorate in drinking water (24.5 p.p.b.) corresponds to an uptake of 0.049 mg perchlorate per day for a 70-kg adult. These data clearly indicate that the daily PEC intake based on nitrate and thiocyanate alone is about 670 times higher than the amount allowed for perchlorate using the NAS/EPA-proposed RfD. A recent study of Sanchez *et al.* in the Lower Colorado River region confirmed that perchlorate contributes to less than 1–2% of the PEC for consumption of lettuce, thereby showing that the relative iodine uptake inhibition potential due to lettuce nitrate was 2 orders of magnitude higher than that due to the trace levels of lettuce perchlorate (47). Considering all these data, it is obvious that either the RfD for perchlorate is unrealistically strict, or the MCLs for nitrate and thiocyanate are far too high and should be revised accordingly. The latter is hardly possible, as the natural nitrate content of a crop like lettuce or spinach – even grown without fertilizer – would already exceed such an RfD.

Dietary iodine

An important issue in environmental exposure to NIS inhibitors is the modulation of inhibition by dietary iodine. The model of competitive inhibition developed by Tonacchera *et al.* (9) predicts that thyroidal I⁻ uptake is approximately proportional to iodide nutrition for any fixed underlying goitrogen load. Unfortunately, little or no data are available on the daily-required dose of dietary I⁻ to withstand inhibition of NIS I⁻ uptake by perchlorate, nitrate and thiocyanate present in drinking water and food. The model of Tonacchera *et al.* does not predict the levels of dietary I⁻ and goitrogen load at which effects on thyroid economy will occur (9). In their key article, Greer *et al.* mention a separate report on the evaluation of the extent to which population variability in the magnitude of the response to perchlorate is related to iodine nutrition (14), but these data have not been

published yet. Dr Goodman, co-author, stated that “iodine nutrition was not controlled and varied widely (several-fold) within individual subjects during the course of the study and even more widely (an order of magnitude) among subjects at the beginning of the study” (48). Braverman and colleagues suggest that the thyroid may be concentrating less of the dietary iodide during intermittent, high perchlorate exposure in employees of an ammonium perchlorate plant (17). Either way, iodine supplementation should be given to any person showing iodine deficiency, irrespective of whether there is significant exposure to environmental perchlorate, nitrate, thiocyanate or foods that naturally contain these anti-thyroid compounds or their precursors. Public health measures should also include mandatory adequate I⁻ intake during pregnancy. Several other authors share this opinion (14, 24, 34, 49). The recommended daily iodine intake during pregnancy from the WHO/UNICEF/ICCIDD (2001) is 200 µg, while the US Institute of Medicine even suggests a recommended dietary allowance during pregnancy of 220 µg. A recent – though small – American study of Pearce and colleagues revealed that of 100 pregnant women in the Boston area (with the most strict proposed RfD for perchlorate), almost half (49%) had iodine levels below the recommended dietary allowance and 9% were even iodine deficient (50). In Europe, harbouring mild iodine deficiency in nearly two-thirds of the population of Western and Central Europe, adequate iodine supply for pregnant women and women planning a pregnancy is even more important (49, 51).

Conclusions

Considering the lack of sufficient information about the effects of perchlorate in sensitive populations such as fetuses, neonates and pregnant women, the recommendation of the NAS to define an NOEL as a (non-classic) departure point for perchlorate risk assessment seems to be defensible. On the other hand, the UF for the intraspecies variability suggested by the NAS (a full factor of 10) and in particular, the additional safety margins posed by some states, is probably overstating the health effects of perchlorate for several reasons; not only the POD is already an NOEL, but also other more prevalent NIS competitors such as nitrate

and thiocyanate are not acknowledged, while the effect of perchlorate cannot be seen separately from the effects of these anions. Taking into account their individual I^- uptake inhibition potential at the level of NIS, which can be considered as robust since several studies agree on the perchlorate equivalence ratios, our calculations show that perchlorate accounts for less than 10% of possible thyroidal effects resulting from the exposure from drinking water; in food, its share is even negligible. The enormous discrepancy between the safe NOEL-based RfD for perchlorate (in the context of iodine uptake inhibition) and the disproportionately high – legally binding – LOEL-based RfD for nitrate (in the context of acquired methaemoglobinaemia) is not logical, and measures should be taken accordingly. Due to heterogeneity at several levels (inclusion of prevalent NIS inhibitors, type of exposure, populations exposed and measures of exposure) a meta-analysis of available human data – if at all possible – would probably not reveal new conclusions, underlining the need for scientific evidence from carefully designed human clinical studies evaluating the effects of nitrate and thiocyanate (along with dietary iodine) on I^- uptake inhibition, in order to determine RfDs for all prevalent NIS inhibitors in drinking water and food. Moreover, further animal studies are needed, investigating not only thyroidal, but also neurodevelopmental endpoints in circumstances of controlled intake of iodine, perchlorate, nitrate and thiocyanate.

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