The Director General

Maisons-Alfort, 8 April 2014

OPINION
of the French Agency for Food, Environmental and Occupational Health & Safety

on the presence of perchlorate in infant formula and in drinking water in France

ANSES undertakes independent and pluralistic scientific expert assessments.
ANSES primarily ensures environmental, occupational and food safety as well as assessing the potential health risks they may entail.
It also contributes to the protection of the health and welfare of animals, the protection of plant health and the evaluation of the nutritional characteristics of food.

It provides the competent authorities with all necessary information concerning these risks as well as the requisite expertise and scientific and technical support for drafting legislative and statutory provisions and implementing risk management strategies (Article L.1313-1 of the French Public Health Code).

Its Opinions are made public. This opinion is a translation of the original French version. In the event of any discrepancy or ambiguity the French language text dates 8 April 2014 shall prevail.

On 1 August and 12 December 2011, ANSES received a formal request from the Directorate General for Health (DGS) firstly, to contribute knowledge on the average perchlorate contamination of infant formulas marketed in France and estimate levels of exposure to perchlorate in infants aged under six months, and secondly, to assess the relevance of taking into account all goitrogenic anions present in the environment when assessing the health risks associated with the presence of perchlorate in drinking water.

1. BACKGROUND AND PURPOSE OF THE REQUEST

Following reports of the presence of perchlorate in drinking water in the Aquitaine and Midi-Pyrénées regions due to industrial pollution upstream from water catchments used in the production of drinking water, ANSES published an opinion on 18 July 2011 on the assessment of health risks associated with the presence of perchlorate in drinking water (Opinion 2011-SA-0024). In its conclusions, ANSES considered that consumption of drinking water with a perchlorate concentration of less than 15 µg/L presented no health risk to the adult consumer under normal conditions of use, but advised not using water contaminated by perchlorate for the preparation of feeding bottles for infants aged up to six months. This latter recommendation had been issued as a precaution, due to the lack of data on perchlorate concentrations in infant formulas marketed in France.

Concentrations of perchlorate higher than the management values proposed by the DGS were identified in source water and water supplied in several distribution units in the Nord-Pas de Calais region, in a different context to that of the pollution observed in Aquitaine and Midi-Pyrénées. The DGS therefore made another formal request to ANSES on 27 April 2012 to assess, on the basis of available epidemiological data, the potential health risks associated with the management limits in drinking water being exceeded, respectively 15 µg/L for adults[1] and 4 µg/L for children[2]. An opinion on the analysis of epidemiological data relating to a possible association between water-related exposure to perchlorate and changes in concentrations of pituitary thyroid-stimulating hormone (TSH) and thyroid hormones in the *a priori* most vulnerable populations (i.e. pregnant women, foetuses and newborns) was then published by ANSES on 20 July 2012 (Opinion 2012-SA-0119).

This current opinion follows two new formal requests by the DGS made to ANSES on 1 August 2011 and 12 December 2011, to:

- examine the relevance of taking into account goitrogenic anions present in the environment other than perchlorate (*e.g.* nitrate, thiocyanate, etc.) when assessing the health risks associated with perchlorate;

- characterise the exposure of children aged from 0 to 6 months based on contamination data provided nationally for infant formula and for drinking water.

2. ORGANISATION OF THE EXPERT APPRAISAL

The expert appraisal was carried out in accordance with French standard NF X 50-110 “Quality in Expert Appraisals – General requirements of Competence for Expert Appraisals (May 2003)”.

This expert appraisal fell within the field of competence of the Expert Committees (CESs) on Water and on Assessment of the physical and chemical risks in foods. ANSES initially entrusted the expert appraisal to the Working Group on Assessment of the health risks associated with situations of water non-compliance, especially in the context of the ANSES opinion of 18 July 2011. The work relating to this current opinion was discussed by the CES on Water (2011-2013 mandate) that met on 4 December 2012 and 3 December 2013, and by the CES on Assessment of the physical and chemical risks in foods that met on 20 November and 19 December 2013. This opinion was also validated by the experts of the CES on Water (2014-2016 mandate) that met on 4 February 2014.

3. DISCUSSION

Origin and sources of perchlorate contamination

Ammonium perchlorate is used in many industrial applications, especially in the military and aerospace fields (ATSDR, 2009).

In particular, it is used:

- as an oxidant for rocket propellants;

- for the manufacture of pyrotechnic devices, flares and explosives for civil or military applications;

- in small quantities, in the powder for some firearms;

- mixed with sulphamic acid to produce a thick smoke or fog for military applications;

[1] management value applicable to the adult population, based on the figure adopted by ANSES of 60% of the TRV being attributed to water-related exposure, and an exposure scenario for a 70 kg bw adult consuming two litres of water per day (see ANSES Opinion of 18 July 2011)

[2] management value adopted by the DGS for children aged under six months

- in systems for triggering airbags;

- for the manufacture of oxygen candles used in confined environments (aircraft cabins, submarines, etc.) for civil and military applications;

- as a component of temporary adhesives for metal plates;

- to adjust the ionic strength of electroplating baths.

Cases of contamination associated with use of Chilean saltpetre (sodium nitrate) containing perchlorate and used in granule form as an agricultural fertiliser have been reported in the USA (ATSDR, 2009).

Perchlorate ions have also been reported as impurities in industrial hypochlorite solutions used to disinfect water (Asami *et al.,* 2009).

A few therapeutic uses of perchlorate have been identified, including its now discontinued use to inhibit iodine uptake by the thyroid in patients with Graves' disease, or its administration (without marketing authorisation) to treat cases of thyrotoxicosis induced by amiodarone, a class III antiarrhythmic drug according to the Vaughan-Williams classification. Perchlorate may also be used when screening for iodine organification disorders (perchlorate test).

The only known natural sources of perchlorate are the trace amounts found in certain arid areas, particularly in Chile where Chilean saltpetre is extracted (Dasgupta, 2005) and in 56 counties in north-western Texas and eastern New Mexico, where no anthropogenic origin has been found for its presence at low concentrations in shallow groundwater, which is attributed to a process of atmospheric deposition (Rajagopalan *et al*., 2006).

In France, the perchlorate contamination of some water in the Aquitaine and Midi-Pyrénées regions (see the ANSES Opinion of 18 July 2011) can be explained by local industrial production of ammonium perchlorate.

Perchlorate was later identified in groundwater in other regions, in particular in the Nord-Pas de Calais. The origin of the contamination here was investigated by the French Geological Survey (BRGM), which concluded that, given:

- the spatial distribution of levels in groundwater in Nord-Pas de Calais;
- the overlaying of this spatial distribution with the mapping of combat zones from the First World War (1914-1918);
- the use of perchlorate in French and German military explosives used extensively during this conflict;
- the lack of widespread modern uses of perchlorate in high volumes in the area after the First World War;

it seems reasonable to establish a link between the presence of chlorine oxyanions (including perchlorate) and the First World War in the Nord-Pas de Calais region, without however ruling out a contribution from certain industrial activities in this region (Hube, 2013).

Physico-chemical properties

Ammonium perchlorate (NH_4ClO_4) is a solid that is highly soluble in water and releases ammonium NH_4^+ and perchlorate ClO_4^- ions after hydrolysis. Table I outlines its main physico-chemical characteristics.

Table I: Main physico-chemical characteristics of ammonium perchlorate (IUCLID, 2000)

CAS number	7790-98-9
Molecular formula	NH_4ClO_4
Molar mass	117.49
Melting point	130°C, begins to decompose at 439°C
Density	1.95 g/cm^3
Solubility in water	200 g/L at 25°C

Ammonium perchlorate is relatively stable at ambient temperature. When heated, it decomposes into chlorine, water and nitrogen oxide, leaving no solid residue.

In the presence of organic products or other oxidants, it leads to highly exothermic reactions.

Treating perchlorates using ion exchange resins or membrane processes may be considered to reduce levels in tap water.

An application for authorisation for a membrane process was recently submitted to the French Ministry of Health.

Mechanisms of action of perchlorate and toxicity reference value

Mechanisms of action of perchlorate in the thyroid

Perchlorate ions ClO_4^-, thiocyanate ions SCN^-, and to a lesser degree nitrate ions NO_3^-, inhibit the uptake of iodine by the thyroid via a mechanism of competitive inhibition in the sodium-iodide symporter (NIS) expressed in the basolateral membrane of the thyrocyte *(*see *Annex 1*).

The uptake of iodine by the thyroid indeed mainly depends on NIS, which ensures the entry into the cell of iodide ions, together with sodium ions. This co-transport (two Na^+ ions for one I^- ion) conforms to a sequential and ordered process with the entry of Na^+ ions preceding that of I^- ions. The energy needed for this active transport of iodine against an ion gradient is generated by the transmembrane concentration gradient for sodium maintained through the Na^+/K^+ ATPase pump. The intracellular concentration of iodide ions is therefore 20 to 40 times higher than the plasma concentration (Plantin-Carrenard *et al.,* 2005).

The transport of iodide ions is therefore blocked by inhibitors of the Na^+/K^+ ATPase pump (*e.g.* ouabain) and by competitive inhibitors of NIS, including perchlorate ClO_4^- ions.

According to Tonacchera *et al.* (2004), who characterised the uptake of the iodide ion in Chinese hamster ovary cell lines transfected with human NIS (CHO-*h*NIS model)*,* the mechanism of inhibition of iodide ion uptake in NIS is a simple competition phenomenon, without synergy or antagonism. According to these authors, the presence of several NIS-inhibiting anions can be modelled by additivity of their respective affinities for NIS. On a molar basis, the perchlorate ion has a greater affinity for NIS than the thiocyanate ion (x 15), iodide ion (x 30) or nitrate ion (x 240).

The uptake of iodide by the thyroid in NIS is one of the first steps in the synthesis of thyroid hormones *(*see *Annex 1).* In the event of variation in iodine intake (exogenous iodine excess or deficiency), several compensation mechanisms act at thyroid, hypothalamic, pituitary or metabolic level to regulate the synthesis of thyroid hormones. The main regulatory mechanisms are the hypothalamic-pituitary-thyroid system and a system of thyroid autoregulation *(*see *Annex 2).* In addition, nutritional status also affects thyroid function, in particular hormone catabolism.

Toxicity reference value

In its opinion of 18 July 2011, ANSES proposed an oral toxicity reference value or tolerable daily intake (TDI) for perchlorate of 0.7 µg.kg $bw^{-1}.d^{-1}$.

This TDI was based on the study by Greer *et al.* (2002) conducted in healthy volunteers (21 women and 16 men) exposed to perchlorate in drinking water at doses of 0.007 – 0.02 – 0.1 and 0.5 mg.kg $bw^{-1}.d^{-1}$ for 14 days and in whom a decrease in radiolabelled iodide uptake by the thyroid was measured. The dose of 7 µg.kg $bw^{-1}.d^{-1}$ caused only a marginal decrease in the uptake of iodide (1.8%), considered not adverse, and was therefore chosen as the no observed effect level (NOEL). The TDI was established by applying an intra-species uncertainty factor (UF) of 10 to take account of the susceptibility of more vulnerable individuals.
Moreover, no significant change in serum thyroid hormone levels was demonstrated even at the highest doses.

This NOEL of 7 µg.kg $bw^{-1}.d^{-1}$ was selected for the development of the TDI in the ANSES opinion of July 2011 for the following reasons:

- uptake of iodine by the thyroid is one of the first steps in the synthesis of thyroid hormones;

- the foetus has a functional thyroid from the end of the first trimester of pregnancy. A deficit in thyroid hormones in the foetus and the child may affect neurological development, particularly in cases of severe maternal hypothyroidism during pregnancy (Haddow *et al.*, 1999; Pop *et al.*, 1999).

- the choice of the critical effect, the pivotal study, the benchmark dose used for calculating the TDI and the intra-species UF of 10, used in the opinion of July 2011, have also been proposed by other organisations including the US National Academy of Sciences and the US EPA in 2005, the ATSDR in 2009 and INERIS in 2011.

The adoption of the TDI of 0.7 µg.kg $bw^{-1}.d^{-1}$ is a conservative choice, in the sense that the selected effect is not based on a clinical observation (hypothyroidism) or biological change (reduction in thyroid hormone levels), but on an early indicator of a change in thyroid function *(see Annex 3).* As with other TDIs calculated using the same type of approach, it is difficult to estimate the health risk of exceeding this TDI in terms of clinically observable effects.

Exposure to perchlorate in drinking water and impaired thyroid function in humans

An examination of the epidemiological studies on associations between water-related exposure to perchlorate and the appearance of changes in thyroid parameters in newborns, children and adults led ANSES to conclude in its opinion of 20 July 2012 (Request SA-2012-0119) that the results of the studies examined could not be used to rule on the existence or absence of an association between levels of perchlorate in drinking water below analytical detection limits (most often 4 µg/L) or up to about 100 µg/L[3] and TSH levels measured in pregnant women or newborns.

Concerning possible impairment of neurological development of the foetus and child following a deficit in thyroid hormone production, ANSES had stated that these effects are observed in situations of severe maternal hypothyroidism during pregnancy (ANSES, 2012).

In this same opinion, ANSES had also indicated that:

i) the absence of information on the iodine status of the studied populations makes it particularly difficult to interpret the published epidemiological data;

ii) it is not possible to quantify the risk associated with exposure to levels higher than the management values set for perchlorate of respectively 15 µg/L for adult consumers under normal conditions (see ANSES Opinion of 18 July 2011) and 4 µg/L for children aged under six months solely on the basis of the available data, and recommended conducting studies on the iodine status of populations living in areas where the public water supply is the most contaminated by perchlorate.

Annexes 4 and 5 summarise the results of epidemiological studies on associations between water-related exposure to perchlorate and changes in thyroid function parameters in newborns, children and adults, including pregnant women. These annexes update the list of studies given in the opinion of 20 July 2012.

The latest results published by Steinmaus *et al.* in 2013 show that, although concomitant exposure to perchlorate and thiocyanate reduces thyroxine levels in the event of low urine iodine levels, this decrease in T4 still remains below the limits associated with hypothyroidism.
In addition, as the pregnant women population has not been investigated in specific epidemiological studies, and the results on the influence of nitrate and thiocyanate are contradictory (Bruce *et al.,* 2013; Steinmaus *et al.,* 2013), there is no new information to modify the conclusions issued in the Opinion of July 2012.

Importance of iodine status in thyroid hormone synthesis and toxicity of perchlorate

Iodine is an essential trace element needed for the production of thyroid hormones. The main source of iodine is *via* food, and iodine requirements vary according to age and physiological circumstances (pregnancy, breastfeeding). They are around 100 µg/d in children (90 µg/d from 0 to 5 years and 120 µg/d from 6 to 12 years) and 150 µg/d in adolescents and adults. In pregnant and breastfeeding women, iodine requirements are greater (200 µg/d) to cover increased iodine clearance and foetal needs (WHO, 2001).

[3] This value of 100 µg/L is also of the order of magnitude of the limit value in drinking water developed on the basis of the TRV proposed by JECFA in 2011 (estimate obtained on the basis of the provisional maximum tolerable daily intake (PMTDI) from JECFA (10 µg/kg bw/d) converted to an equivalent dose in drinking water using a scenario in adults based on 70 kg bw; 2 litres per day; 50% of the PMTDI being attributed to perchlorate intake *via* drinking water).

Sources of iodine intake

The main dietary sources of iodine are seaweed, seafood, saltwater fish, eggs and dairy products. Certain texture or colouring agents and iodine disinfectants used in veterinary practice fortify foods with iodine.
Table salt fortified with iodine is a major source of intake in France. In contrast, animal tissues (meat and poultry) and plants or fruits are naturally low in iodine (AFSSA, 2005).

Some dietary supplements fortified with iodine can also be a source of iodine intake, particularly during pregnancy.

Iodine status of the general adult population in France

According to the National survey of nutrition and health conducted between 2006 and 2007 (ENNS 2006-2007), the adult population residing in France has a satisfactory nutritional status for iodine with regard to the criteria of the World Health Organization (WHO)[4].

Median urine iodine levels were estimated in France at 136 µg/L for all adults aged 18 to 74 years, the 20th percentile was 72 µg/L (*Figure 1*).
This median is significantly lower in women (127 µg/L) than in men (140 µg/L). This difference may be partly explained by the difference in table salt intake between men and women (10.2 g/d in men compared with 7.2 g/d in women).

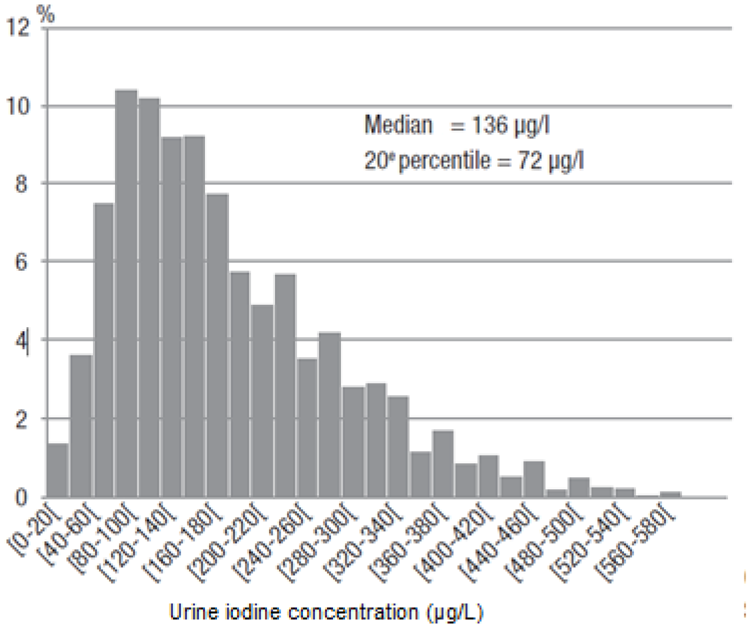


Figure 1: Distribution of urine iodine values in the general adult population in mainland France (ENNS 2006-2007 study *in* DREES, 2010)

The regions in the south west (Aquitaine and Midi-Pyrénées) have the lowest median urine iodine levels (108 µg/L) in France, while the north west (Picardie, Normandie, Nord) have the highest median urine iodine levels (146 µg/L). No significant difference in iodine status has nevertheless been demonstrated between the different geographical areas (*Figure 2*):

[4] According to the WHO, a population is not regarded as having an iodine deficiency when median urine iodine levels exceed 100 µg/L and the 20th percentile is above 50 µg/L.

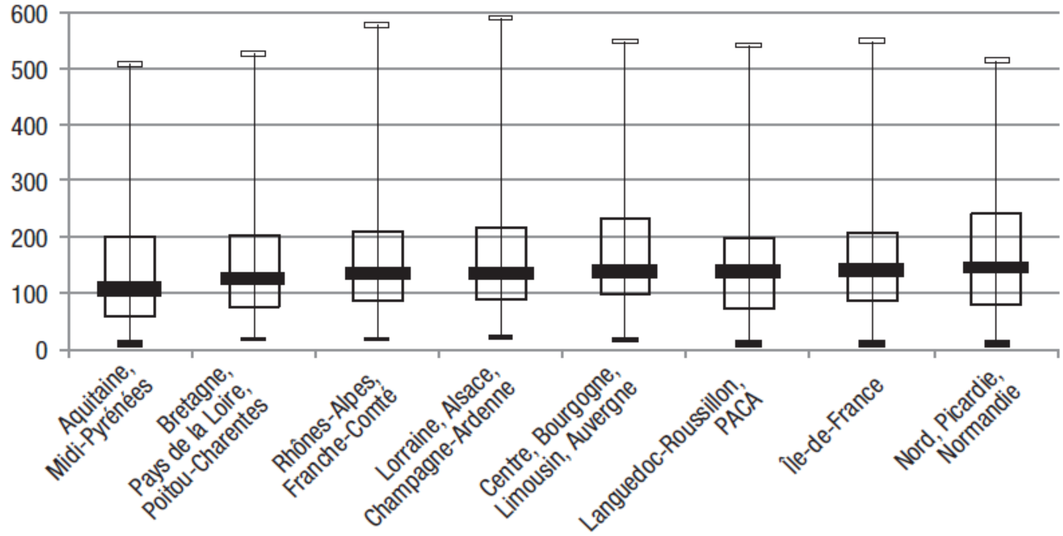


Figure 2: Urine iodine levels measured in the general adult population in mainland France (in µg/L) – minimum, median, maximum value, 25th and 75th percentile according to geographical area (ENNS 2006-2007 study)

Iodine status of pregnant women in France

T4 levels and maternal iodine reserves are two fundamental parameters of foetal thyroid homeostasis. Thyroid function in pregnant women is modified during pregnancy (decreased free T4, increased iodine clearance, transplacental passage of iodine intended for the synthesis of foetal thyroid hormones) and leads to an increase in daily iodine requirements in the mother (Glinoer, 2004).

In three studies conducted in women during pregnancy, iodine intake corresponded to less than 50% of the recommended population reference intakes (PRI) for pregnant women (Caron *et al*. 1997; Pivot, 2003 *in* AFSSA, 2005).

A preliminary study conducted at the Nice University Hospital Centre in 108 women at the end of the second trimester of pregnancy showed a median urine iodine level of 59 µg/L (86% of patients had urine iodine levels lower than 100 µg/L, 51% had levels lower than 50 µg/L and 25% had levels lower than 30 µg/L). In this study, only 6.5% of the women had urine iodine levels greater than 150 µg/L (Brucker-Davis *et al.*, 2004).

Another study conducted by Hiéronimus *et al.* (2009), also at the Nice University Hospital Centre, in 330 women in the third trimester of pregnancy, found median urine iodine levels of 64 µg/L, reflecting a clinical iodine deficiency in pregnant women in this region during the third trimester of pregnancy. Depending on the urine iodine threshold selected (100 or 150 µg/L), the prevalence of this deficiency was 74.3 to 85.8%. In addition 5.4% of patients had excessive urine iodine levels (> 250 µg/L) and only 8.8% of women had adequate urine iodine levels. On the basis of thyroid parameters measured in this same population, the median free T4 was 12.3 pmol/L (8 to 20.1) and that of TSH was 1.93 mIU/L (0.24 to 6.57). Based on various diagnostic thresholds proposed in the literature, the prevalence of hypothyroxinemia ranged from 41.2% (<12 pmol/L) to 10% (10.3 pmol/L) and 1.8% (< 9 pmol/L), and that of subclinical hypothyroidism from 26.3% (TSH > 2.5 mIU/L) to 3.9% (TSH > 4 mIU/L). However, no correlation was found between urine iodine levels and thyroid function or predictive maternal risk factors for clinical iodine deficiency.

In 2011, a study conducted in hospitals in northern Paris (Paris public hospital system - APHP), was used to measure urine iodine and thyroid parameters (free T4, free T3 and TSH) in 110 pregnant women in the 12th and 32nd week of pregnancy. An iodine deficiency characterised by a mean urine iodine level of 49.8 µg/L (standard deviation of 2.11 µg/L) was also identified in this study. This iodine deficiency was not significantly correlated with thyroid parameters. On the contrary, it was negatively correlated with the size of the foetal thyroid (Luton *et al.*, 2011).

In 2012, a cross-sectional study by Raverot *et al.* (2012) in a maternity unit of the Hospices Civils de Lyon was used to measure urine iodine levels in 228 pregnant women and to compare the parameters of thyroid function (TSH, free T4, anti-thyroid peroxidase and anti-thyroglobulin antibodies) with those of a control population (men and non-pregnant women). The median urine iodine level found in pregnant women was 81 µg/L (95% CI [8; 832]) which led the authors to believe that 77% of the subjects were deficient in iodine on the basis of a threshold at 150 µg/L. After adjustment for maternal age and the number of weeks of pregnancy, the urine iodine level was not significantly correlated with parameters of thyroid function. Serum thyroglobulin levels were higher in pregnant women than in the controls.

In conclusion, the results of various studies conducted in France show a widespread prevalence of iodine deficiency among pregnant women.

Iodine status of children aged under 1 year in France

Few studies are available in France on the iodine status of young children.
As part of a descriptive study on iodine status conducted by Pouessel *et al.* (2003) in 160 children (93 boys, 67 girls) aged from 10 days to 6 years examined as part of maternal and child welfare consultations (PMI) in the Lille metropolitan area, iodine concentrations were measured in urine samples collected on the day of consultation.
Median urine iodine levels were 196 µg/L (4 - 1042 µg/L) and 76% of children presented urine iodine levels > 100 µg/L. Among the 24% of children with iodine deficiency, 17% had a slight deficiency (between 50 and 99 µg/L), 5% a moderate deficit (between 20 and 49 µg/L) and 2% had a clinical iodine deficiency (< 20 µg/L).
Although there are no reference values for urine iodine defining a normal iodine status before the age of 3 years, Delange *et al.* (2001) proposed using recommended iodine intake (90 µg/d) and hourly urine output to define a normal iodine status as urine iodine levels between 180 and 200 µg/L in this age group. On this basis, the iodine status of these children was satisfactory, apart from the 6-24 month age group (median urine iodine levels: 159.5 µg/L) (Pouessel *et al.*, 2003).

In 2005, these same authors conducted a prospective study to evaluate urine iodine and TSH levels in all children aged under 1 year examined in the general paediatrics sector at the Lille University Hospital Centre between 1 January and 31 May.
Ninety-five (83%) out of 114 children hospitalised during this period were evaluated for urine iodine and 60% for TSH levels.
The results showed a median urine iodine level equal to 328 µg/L (range: 12 – 1580 µg/L). Of these 95 infants, 24 (25%) had an excess of iodine (urine iodine > 400 µg/L), while 19 (20%) had an iodine deficiency (urine iodine < 100 µg/L). Of the latter, five presented with a serious clinical iodine deficiency (urine iodine < 20 µg/L).
The statistical analysis did not reveal any significant relationship between iodine status and the following parameters: family history of thyroid disease, intake of medication by the mother during pregnancy, pregnancy term, type of delivery, socio-professional category of the parents, age, sex, type of feeding, child nutritional status, and presence of chronic disease. Nor was there an abnormal elevation of TSH levels in the infants with iodine deficiency, and the authors of this study were unable to show any association between iodine deficiency and occurrence of hypothyroidism in children (Pouessel *et al.*, 2008).

In conclusion, according to the ENNS 2006-2007 study, the adult population residing in mainland France has a satisfactory iodine status with regard to the WHO criteria.
However, iodine deficiency is common among pregnant women. In children aged under one year, the results available, although few, suggest that iodine status is adequate for 75 to 80% of infants studied.
Moreover, no association has been demonstrated between iodine deficiencies observed in 20 to 25% of infants studied and the onset of hypothyroidism.

Relevance of taking into account other potentially goitrogenic anions present in the environment (nitrate, thiocyanate, etc.) when assessing the health risks associated with perchlorates

Interaction of goitrogenic anions with the thyrocyte

Due to the existence of several ions other than perchlorate ions that may inhibit the uptake of iodide in NIS, the issue is raised of the relevance of including these other ions, known as goitrogenic ions, when assessing the health risks of perchlorate in drinking water and foods.
This essentially concerns thiocyanate and nitrate ions, whose primary route of exposure for the general population seems to be ingestion of foods naturally high in these compounds. Smoking is also a non-negligible source of exposure to thiocyanate (Schlienger *et al.*, 2003).
Some authors therefore propose taking into account the contribution of various anions that have affinity for NIS in the mechanism of inhibition of iodine uptake by the thyroid (De Groef *et al.*, 2006; Tarone *et al.*, 2010).

Quantification of the relative ability of these different goitrogenic anions to inhibit iodide uptake in NIS is based primarily on the results of the aforementioned study by Tonacchera *et al.* (2004) (see Mechanisms of action of perchlorate in the thyroid).

At equal concentrations, the affinity of anions for NIS seems to be, in descending order of affinity: perchlorate (ClO_4^-) > thiocyanate (SCN^-) > iodide (I^-) >> nitrate (NO_3^-). Perchlorate thus has the greatest affinity for NIS compared to the other ions in the experimental conditions of the study by Tonacchera *et al*. (2004).
Accordingly, in the event of simultaneous presence and equal concentrations of iodide and nitrate, iodide will be transported into the thyrocyte to a greater extent than nitrate. Conversely, in the event of simultaneous presence and equal concentrations of iodide and thiocyanate, the latter will be transported more into the thyrocyte.

The results of this study are presented based on the assumption of an equivalent molar concentration for each ion. However, the concentrations of these various anions are very different depending on the environmental compartments.
Furthermore, although iodide, thiocyanate and nitrate have the same stoichiometry of transport processes in NIS (one anion for two sodium ions), this is not the case with perchlorate (one perchlorate ion for one sodium ion). From a stoichiometric point of view, iodine uptake capacity is actually higher than that of perchlorate. For equivalent concentrations outside the thyrocyte and around the affinity constant for NIS (K_T), the ratio of concentrations between the outside and inside of the thyrocyte ($[anion_{ext}]/[anion_{int}]$) is 1/10 for perchlorate while it is 1/100 for iodide.

Therefore, although the *in vitro* model used by Tonacchera *et al.* (2004) is apparently relevant for studying the affinity of these various anions for NIS, the calculation method that involves estimating an equivalent concentration of perchlorate, leads to the simplistic assumption that an anion's effect on the thyrocyte is limited to its sole affinity for NIS.

However, iodine metabolism involves more than just this transporter, and other effectors that have not been as well characterised as NIS, such as pendrin, may have different selectivities depending on the anions (Twyffels *et al.*, 2011). Moreover, apart from the differences in affinities for NIS, these anions have different redox potentials that may affect the oxidised forms of iodide, and may therefore also modulate thyroid regulation.

In conclusion, calculating an equivalent perchlorate concentration to take into account the overall affinity of perchlorate, iodide, thiocyanate and nitrate ions is based on a paradigm that involves considering that an anion's interaction with the thyrocyte comes down to its interaction with the NIS transporter alone. However, the representativeness of such a model with regard to the biological functioning of the thyroid gland is not known, making the extrapolation to humans of these results observed *in vitro* particularly difficult (Steinmaus *et al.*, 2011).

Environmental exposure to thiocyanate or nitrate ions and impaired thyroid function in humans

With regard to nitrate, the study conducted in 2008 by AFSSA on the basis of national data indicates that the main food contributors to exposure of the general population are vegetables known as "concentrators" (60% of intake): lettuce, radishes, peas, green beans and celery. Potatoes, "non-concentrator" vegetables that are nevertheless heavily consumed, also contribute to exposure *via* ingestion, accounting for 13% of this intake. For drinking water, when the concentration of nitrate is close to 20 mg/L, water-related intake may account for about 10% of intake in the French adult population.
If the concentration in water is 50 mg/L (quality limit in drinking water), water would then contribute 34% of exposure to nitrate (AFSSA, 2008). However, there is no consistency in the results of epidemiological studies that have sought to establish an association between thyroid effects and exposure to nitrate (Pearce and Bravervan, 2009).
Moreover, the results of a study conducted in healthy volunteers exposed *via* drinking water for four weeks to the equivalent of 15 mg.kg $bw^{-1}.d^{-1}$ of sodium nitrate did not show any changes in thyroid parameters (Hunault *et al.*, 2007).
Furthermore, one of the specificities of nitrate, compared to thiocyanate and perchlorate, is that the metabolism of nitrate in humans involves an endogenous synthesis pathway (the L-arginine pathway). This unique feature, as well as the intricacy of nitrate, nitrite and nitric oxide metabolism, makes it especially complicated to provide an overall quantitative estimate of human exposure to these molecules.

With regard to thiocyanate, there are currently no national data on contamination and environmental exposure to such ions. Some studies have shown that diets rich in thiocyanate (*e.g.* vegetables of the Brassicaceae family[5]) contribute to the development of goitre in iodine-deficient areas (Vanderpas, 2006). These functional effects, however, only occur in cases of associated clinical iodine deficiency. Moreover, irrespective of its thiocyanate content, no food seems likely in itself to cause goitre or hypothyroidism (Wemeau, 2010).

An association between exposure to thiocyanate ions present in cigarette smoke and the appearance of thyroid effects (*i.e.* decreased levels of TSH and increased thyroid volume) in humans is also suggested by some authors. From a clinical point of view, tobacco has also been described as a risk factor for relapse of treated Basedow-Graves' disease and worsening or onset of Basedow-Graves' ophthalmopathy (Schlienger *et al.,* 2003). Nevertheless, these observed thyroid effects in conjunction with smoking are probably not only attributable to thiocyanate.

[5] family of plants whose characteristic feature is four petals shaped in a cross (formerly known as Cruciferae and including cabbage)

In conclusion, it is currently difficult to determine the overall affinity of the different goitrogenic anions (especially iodide, perchlorate, nitrate and thiocyanate) with the NIS transporter of the thyrocyte in humans and, therefore, the impact of this co-exposure on impaired thyroid function.

Levels of perchlorate in drinking water in France

The levels of perchlorate in drinking water at national level have been derived from the following studies:

- 1/ the results of the national campaign by ANSES's Nancy Laboratory for Hydrology (LHN) on the analysis of perchlorate in drinking water;
- 2/ the results of analyses conducted by the LHN on samples collected by members of the Professional Federation of Water Companies (FP2E) on *a priori* vulnerable sites with regard to contamination of water by perchlorate;
- 3/ the results of analyses of perchlorate in raw and treated water in the Nord-Pas de Calais region;
- 4/ the results of perchlorate concentrations in samples of bottled water marketed in France, based on analyses carried out by the LHN.

Each section presents the analytical results from a different sampling plan. The results of the national campaign conducted by the LHN come from analyses of raw water and treated water where samples were matched (one sample on the quality of raw water was paired with another on the quality of treated water). This sampling protocol enables the efficacy of water treatment to be analysed where necessary.
Regarding the results of analysis in raw and treated water in the Nord-Pas de Calais region, the sampling protocols were not specified and may differ depending on the origin of the data. It is therefore not possible to compare the results described for raw water and treated water.

Results of the national campaign by the Nancy Laboratory for Hydrology (ANSES)

The campaign generated 703 results for 436 municipalities. Several spot samples of raw water and treated water were taken in each French *département*. The sampling protocol is described in Instruction No. DGS/EA4/2011/229 of 14 June 2011. It involves collecting about 300 pairs of raw water/treated water samples according to a regional stratification, with, for each *département*:

- selection of the catchment providing the largest flow in each *département*; these catchments were selected by the LHN from information available in the Health & Environment Information System on Water (*SISE-Eaux*) database;
- a catchment selected randomly in each *département* in the region;
- a site of interest chosen by the Regional Health Agency (ARS) for each *département* in the region based on the site's vulnerability with regard to other emerging substances (see above-mentioned DGS instruction).

The analyses were carried out by 2D ion chromatography coupled to mass spectrometry. The limit of detection (LD) was 0.15 µg/L and the limit of quantification (LQ) was 0.5 µg/L.

Of the 703 results provided by the LHN, 533 (76%) correspond to non-quantified results.

The breakdown of available results according to the type of facility is given in Table II.

Table II: Breakdown of available results according to the type of facility (CAP: catchment; TTP: treatment plant outlet; UDI: distribution unit)

CAP	TTP	UDI
384	299	20

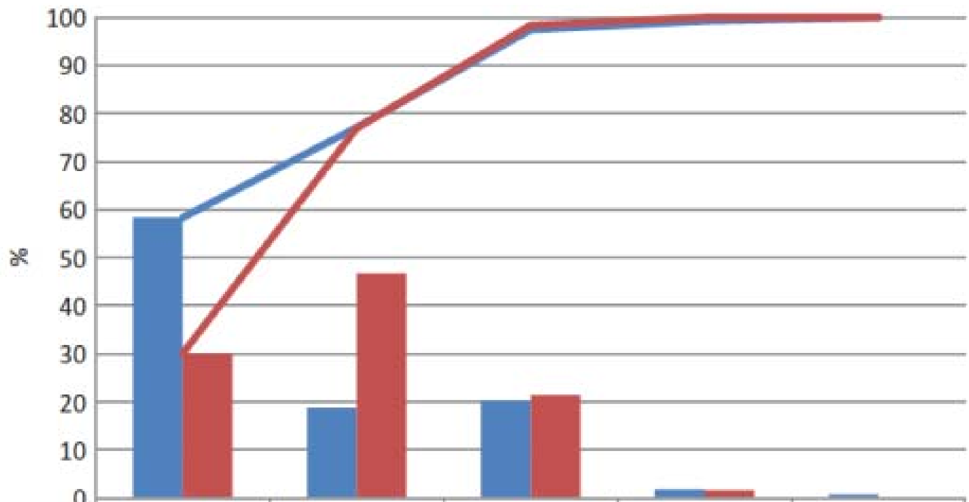
The breakdown of available results in raw water (i.e. type of catchment facility or CAP) according to the type of water is given in Table III.

Table III: Breakdown of available results according to the type of water for raw water (CAP) (ESO: groundwater; ESU: surface water; EMI: mixed water; MER: sea water)

ESO	ESU	EMI	MER
237	144	1	2

Figure 3 shows the breakdown of perchlorate levels in samples from catchments (CAP) and treatment plant outlets (TTP). These results show:

- perchlorate levels below 0.5 µg/L for three quarters of the samples analysed;
- about 2% of the samples had perchlorate levels higher than 4 µg/L;
- none of the treated water samples and three of the catchment water samples (representing 1% of the catchments analysed) had perchlorate levels higher than 15 µg/L;
- the maximum levels observed during this campaign were 22 µg/L for catchments and 13 µg/L for treated water.



	undetected	trace amounts (0.15 - 0.5 µg/L)	0.5 to 4 µg/L	4 to 15 µg/L	> 15 µg/L
CAP	58	19	20	2	1
TTP	30	47	21	2	0
CAP aggregation	58	77	97	99	100
TTP aggregation	30	77	98	100	100

Figure 3: Percentage breakdown of perchlorate levels in samples from catchments (CAP) and drinking water production plant outlets (TTP).

It can be seen that treated water shows traces of perchlorate more frequently than catchment water, illustrating a low level of addition of perchlorate by the treatment system, especially the chlorination step likely to introduce trace amounts of perchlorate from 0.15 to 0.5 µg/L), in agreement with the work described by the SERDP (2005).

Figure 4 shows the breakdown of perchlorate levels according to the origin of the catchment water (surface water/groundwater). It seems that the levels measured in groundwater are slightly higher than those measured in surface water.

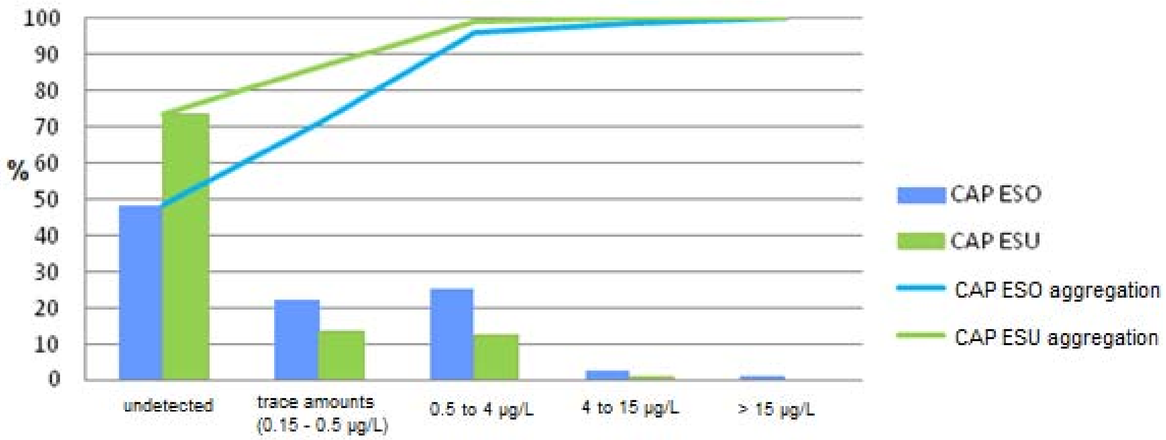


Figure 4: Percentage breakdown of perchlorate levels in samples from catchments according to the origin of the water (groundwater: ESO / surface water: ESU)

Figures 5 and 6 show the spatial distributions in mainland France of perchlorate concentrations in water for both types of facility: catchments and water treatment plant outlets.

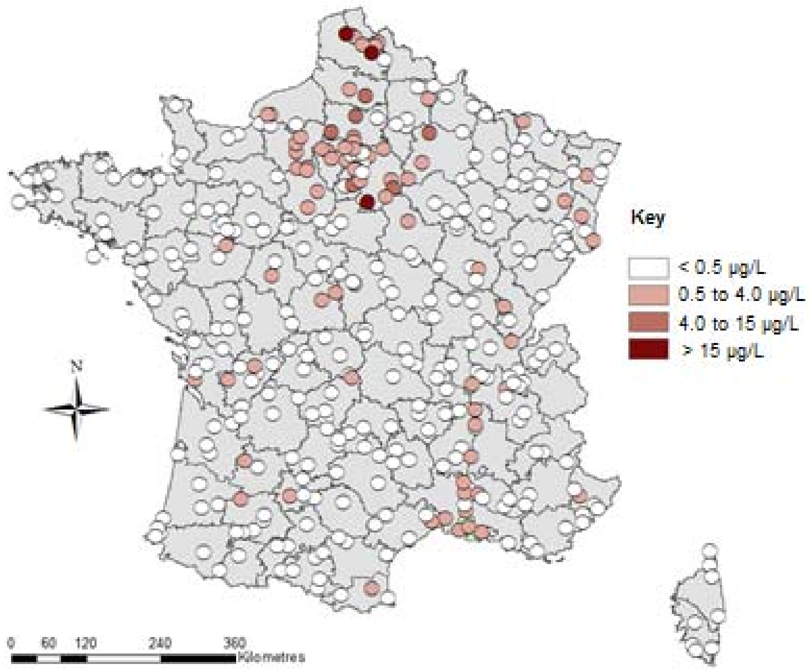


Figure 5: Map of perchlorate levels in mainland France by concentration category (in µg/L) at the catchment point for raw water measured by spot sample during the campaign conducted between October 2011 and May 2012 by ANSES's Nancy Laboratory for Hydrology (LHN)

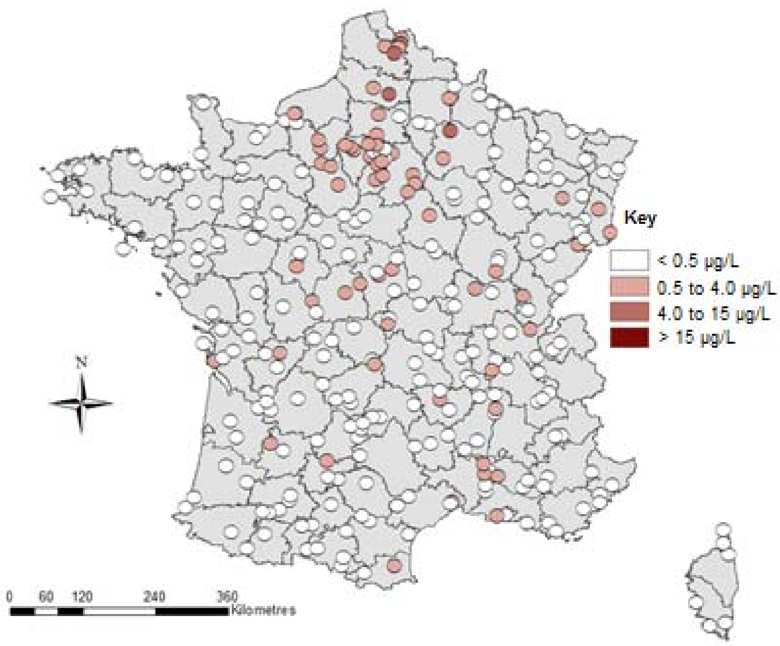


Figure 6: Map of perchlorate levels in mainland France by concentration category (in µg/L) at the water treatment plant outlet measured by spot sample during the campaign conducted between October 2011 and May 2012 by ANSES's Nancy Laboratory for Hydrology (LHN)

Three results out of 703 had a perchlorate concentration higher than 15 µg/L and concerned the Ile-de-France and Nord-Pas de Calais regions.

Results of the campaign conducted in 2012 by the Professional Federation of Water Companies (FP2E)

Via the FP2E, private producers of drinking water conducted sampling of raw and treated water in 33 *départements* of mainland France, in which perchlorate was analysed considering an analysis of perchlorate concentration per water sample taken. The analyses were carried out by the LHN using 2D ion chromatography coupled to mass spectrometry with an LD of 0.15 µg/L and an LQ of 0.5 µg/L.

One hundred and twenty-four usable results were produced (4 samples from the request by the Nord-Pas de Calais ARS, 51 samples taken by Lyonnaise des Eaux, 12 from sampling conducted by the Saur Group, and 57 from sampling conducted by Véolia).

Seventy-three of these 124 results (59%) were below the limit of quantification.

The breakdown of available results according to the type of facility is given in Table IV.

Table IV: Breakdown of available results according to the type of facility (CAP: catchment; TTP: treatment plant outlet; UDI: distribution unit)

CAP	TTP	UDI
66	57	1

Figures 7 and 8 show the breakdown of perchlorate levels for both sampling campaigns (national campaign and FP2E campaign) respectively for catchment water and treatment plant outlet water.

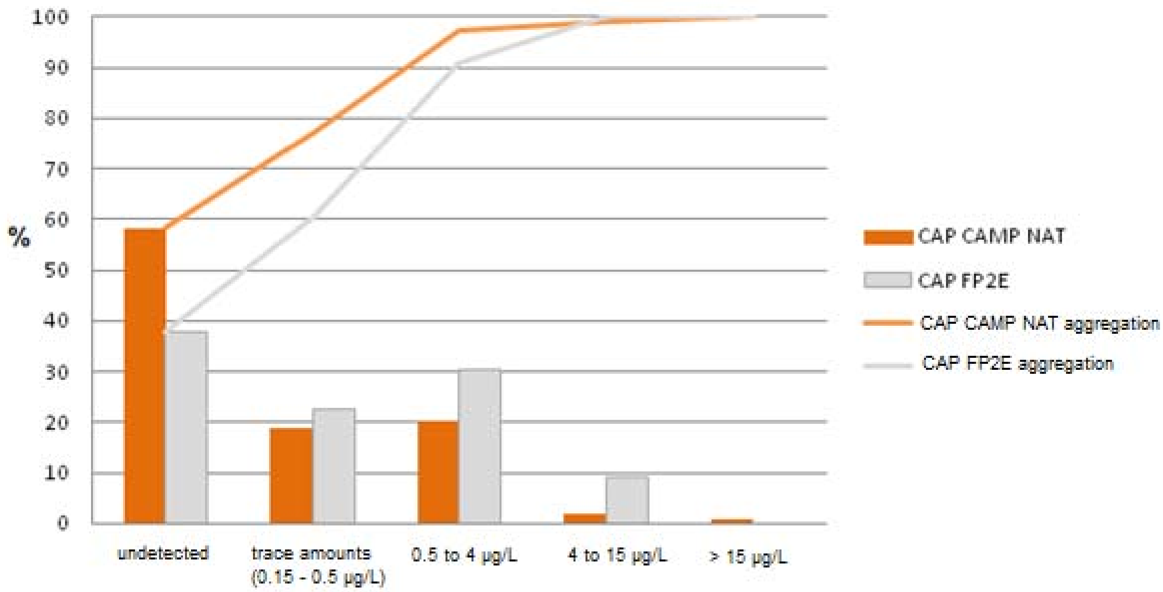


Figure 7: Comparison of occurrences of perchlorate in catchment water (CAP) expressed as a percentage between the national campaign and the FP2E campaign

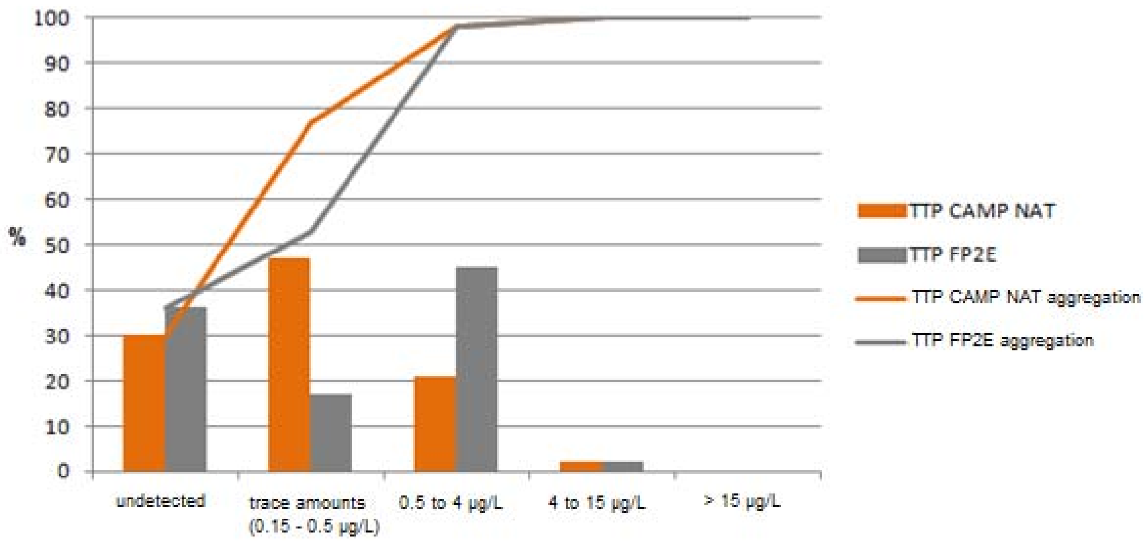


Figure 8: Comparison of occurrences of perchlorate in treatment plant outlet water (TTP) expressed as a percentage between the national campaign and the FP2E campaign

The results on water contamination by perchlorate were provided according to the environmental context, in six categories: bombing areas (A), military sites (B) mining activities (C), industrial activities (D), agricultural activities (E) and other contexts (F).

Table V shows the mean concentrations and standard deviations of concentrations for perchlorate in raw water or the public water supply according to these six categories.

Table V: Breakdown of mean concentrations and standard deviations of concentrations[6] for perchlorate in raw water or the public water supply according to the environmental context.

Category	N	Mean (µg/L)	Standard deviation
A – Bombing	**20**	0.60	0.22
B – Military sites	**12**	1.37	1.32
C – Mining activities	**4**	0.50	0.00
D – Industrial activities	**47**	1.07	1.24
E – Agricultural activities	**27**	1.23	1.37
F - Other	**7**	1.32	1.40

Levels of perchlorate found in at least one group are different to those of the others at the type-I error of 2%.

No result showed a concentration greater than 15 µg/L.

[6] For calculating means and standard deviations, concentrations of perchlorate below the limit of quantification were considered to be equal to this limit.

Results of perchlorate concentrations in the water of Nord-Pas de Calais

In this section, results of the analysis for perchlorate were provided by:
- the Nord-Pas de Calais ARS;
- the Lille urban community;
- Eaux du Nord (part of the Lyonnaise des Eaux Group);
- Véolia.

Methods for analysing perchlorate in water can differ depending on the origin of the data provided. The performance of the analytical methods used for assaying perchlorate in water was assessed through an inter-laboratory test organised by the LHN at the end of 2012. The data on occurrence in drinking water presented in this opinion all come from laboratories that participated in this test.

This inter-laboratory test showed:

- inter-laboratory uncertainties of about 50% at values close to the limit of quantification, and about 30% at a level of 15 µg/L;
- no analytical difficulty, method effect, or matrix effect likely to cause a bias in the results;
- LQs ranging from 0.5 µg/L to 4 µg/L depending on the analytical techniques used, and in most cases less than or equal to 1 µg/L.

The analytical methods used were therefore considered to be appropriate for the objectives set.

The sampling protocols were not documented and may differ depending on the origin of the data. It is therefore not possible to compare the results described for raw water and treated water as was the case with the results of the LHN's national campaign, which included a sample of raw water and a sample of treated water from each treatment plant selected.

Moreover, the reported results concern a period extending from summer 2011 to spring 2012. The information presented in this section relating to water quality with respect to perchlorate in the Nord-Pas de Calais region is therefore not exhaustive.

Lastly, to describe the distributions of perchlorate levels in raw and treated water, levels below the LQ were considered to be equal to half this limit, given a rate of censored data of less than 60% (see the recommendations of the World Health Organization - WHO - GEMS-Food Euro 1995).

Analyses conducted by the Nord-Pas de Calais ARS

Between October 2011 and March 2012, the Nord-Pas de Calais ARS collected water samples in 204 municipalities (225 sampling points). These samples yielded 253 results. Of these, 35 (14%) corresponded to non-quantified results, with an LQ of 0.5 µg/L.

The breakdown of available results according to the type of facility is given in Table VI and the distribution of perchlorate concentrations in raw water and treated water in Table VII.

Table VI: Breakdown of available results according to the type of facility (CAP: catchment; MCA: a mixture of catchments; TTP: treatment plant outlet; UDI: distribution unit)

CAP	TTP	UDI
25	115	113

Table VII: Distribution of the perchlorate concentration in raw water and treated water in the Nord-Pas de Calais region, based on data from analyses conducted by the Nord-Pas de Calais ARS between October 2011 and March 2012 (µg/L)

Water type	n	P5	P25	median	mean	P75	P95	Max
Raw water	25	0.25	0.25	0.6	3.3	5.5	11	17
Treated water	228	0.3	1.3	2.7	7	7.7	30	77

Eight results were higher than 4 µg/L and one result was higher than 15 µg/L for raw water. For drinking water, 85 results exceeded 4 µg/L and 28 results were higher than 15 µg/L.

<u>Analyses carried out between September 2011 and January 2012 by the Lille Urban Community (LMCU)</u>

Following the water samples collected between September 2011 and January 2012 in six municipalities (13 raw water sampling points), 142 results on perchlorate levels were provided by the Lille Urban Community (LMCU). Of these, six results (4%) corresponded to non-quantified results, with an LQ of 0.5 µg/L.

The breakdown of available results according to the type of facility is given in Table VIII and the distribution of concentrations in raw water in Table IX.

Table VIII: Breakdown of available results according to the type of facility
(CAP: catchment; MCA: a mixture of catchments)

CAP	MCA
71	71

Table IX: Distribution of the perchlorate concentration in raw water, based on the results of analyses provided by the Lille Urban Community between September 2011 and January 2012 (µg/L)

Water type	n	P5	P25	median	mean	P75	P95	Max
Raw water	142	1.9	3.3	4	4.4	4.8	8.6	14

Of these 142 analysis results, which only concern samples of raw water, 64 were higher than 4 µg/L and none exceeded 15 µg/L.

<u>Analyses conducted by Eaux du Nord (Lyonnaise des Eaux)</u>

Following the water samples collected between August 2011 and February 2012 in 66 municipalities in Nord-Pas de Calais (211 sampling points), 814 results on perchlorate levels were provided by the company Eaux du Nord (part of the Lyonnaise des Eaux Group). Of these 814 results, 161 (20%) corresponded to non-quantified results.

Among these non-quantified results:
- 131 results were below an LQ of 1 µg/L;
- 30 results were below an LQ of 2 µg/L.

The breakdown of available results according to the type of facility is given in Table X and the distribution of perchlorate concentrations in raw water and treated water in Table XI.

Table X: Breakdown of available results according to the type of facility (CAP: catchment; MCA: a mixture of catchments; TTP: treatment plant outlet; UDI: distribution unit)

CAP	MCA	TTP	UDI
437	40	261	76

Table XI: Distribution of the perchlorate concentration in raw water and treated water in the Nord-Pas de Calais region, based on data from analyses conducted by Lyonnaise des Eaux between August 2011 and February 2012 (µg/L)

Type of water	n	P5	P25	median	mean	P75	P95	Max
Raw water	477	0.5	1	4.3	7.1	11	25	35
Treated water	337	0.5	2.1	3.4	3.7	3.9	8.7	28

251 out of 477 results exceeded 4 µg/L for raw water.
75 out of 337 results exceeded 4 µg/L for treated water.

Sixty results were higher than 15 µg/L for raw water. This concerned three municipalities: Bouchain (8 results higher than 15 µg/L), Wavrin (1 result higher than 15 µg/L) and Flers-en-Escrebieux (51 results higher than 15 µg/L). Two results were above 15 µg/L for treated water, relating to the municipality of Flers-en-Escrebieux.

Analyses conducted by Véolia

Following the samples collected between October 2011 and January 2012 in 67 municipalities in Nord-Pas de Calais (99 sampling points), 180 results on perchlorate levels were provided by the company Véolia. Of these 180 results, 20 (11%) corresponded to non-quantified results.
Among these non-quantified results:
- 11 results were below an LQ of 0.5 µg/L;
- 9 results were below an LQ of 1 µg/L.

The breakdown of available results according to the type of facility is given in Table XII and the distribution of perchlorate concentrations in raw water and treated water in Table XIII.

Table XII: Breakdown of available results according to the type of facility (CAP: catchment; TTP: treatment plant outlet; UDI: distribution unit)

CAP	TTP	UDI
107	29	44

Table XIII: Distribution of the perchlorate concentration in raw water and treated water in the Nord-Pas de Calais region, based on data from analyses conducted by Véolia between October 2011 and January 2012 (µg/L)

Type of water	n	P5	P25	median	mean	P75	P95	Max
Raw water	107	0.3	3	8.9	13.5	26	37	67
Treated water	73	0.5	4.2	14	16.7	27	38	50

Seventy-five results were higher than 4 µg/L for raw water. Fifty-five results exceeded 4 µg/L for treated water.

Thirty-four results were higher than 15 µg/L for raw water. Thirty-three results exceeded 15 µg/L for treated water. Among these results for treated water, 19 concern the town of Douai.

<u>Other information</u>

A map of regional water contamination provided by the Nord-Pas de Calais ARS and including more recent data is shown in *Annex 6*.

Description of results of perchlorate concentrations in samples of bottled water marketed in France

ANSES's Nancy Laboratory for Hydrology (LHN) analysed perchlorates in samples of bottled water marketed in France corresponding to 71 natural mineral waters (EMN), 5 waters made drinkable by treatment (ERPT) and 72 spring waters (ES) (LHN 2013 study). The analyses were carried out using 2D ion chromatography coupled to mass spectrometry. The LD was 0.15 µg/L and the LQ was 0.5 µg/L.

Figure 9 shows the number of water sources used to produce bottled water by perchlorate concentration categories and water types.

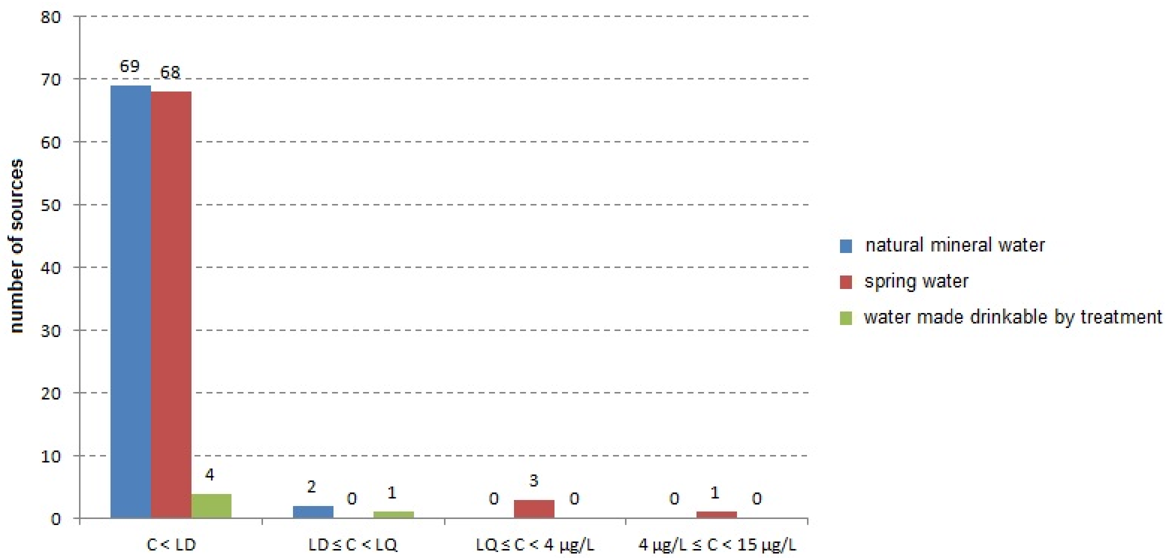


Figure 9: Breakdown of the number of sources of bottled water based on categories of perchlorate concentration in the water and water type

Of the 72 spring waters analysed, the maximum level of perchlorate was 6 µg/L (n = 1), the other quantified values (n = 3) remained below 2 µg/L.

For the other types of bottled water (natural mineral waters and water made drinkable by treatment), no brand presented a perchlorate level higher than the LQ (0.5 µg/L).

Conclusions

The national analysis campaign conducted by the LHN helped to provide a picture of concentration levels of perchlorate covering approximately 25% of all the drinking water produced in France. The results, obtained from analysing each of two samples per treatment plant (raw water and treated water) and with about three sites per *département*, revealed:

- for source water: levels below 4 µg/L in 97% of cases and below 15 µg/L in 99% of cases;
- for treated water: levels below 4 µg/L in 98% of cases and below 15 µg/L in 100% of cases.

Perchlorate contamination of drinking water and source water remains highly localised and concerns mainly the Nord-Pas de Calais[7] and Picardie regions, located near combat zones or ammunition storage areas from the First World War. The origin of this perchlorate contamination of water is most likely explained by these historical uses, although this explanation does not rule out other hypotheses (Chilean saltpetre or industrial activities, for example).

The additional sampling campaign conducted by the FP2E, with the aim of highlighting the influence of different environmental contexts on perchlorate levels in water, did not however, show any concentration greater than 15 µg/L.

Perchlorate levels were less than 4 µg/L for more than 99% of sources analysed by the LHN in 2013 (n = 148) and used to produce bottled water marketed in France.

Levels of perchlorates in infant formulas marketed in France

Results from the national surveillance plan on infant formula

Data on perchlorate contamination of infant formulas marketed in France come from the surveillance plan produced by the Directorate General for Competition, Consumer Affairs and Fraud Control (DGCCRF) in 2012 at the request of ANSES.

A total of 67 samples representative of the formulas marketed in the country[8] was analysed by the Joint Laboratories Service (SCL) in Strasbourg (DGCCRF and General Directorate for Customs and Indirect Taxation (DGDDI)) using HPLC/MS-MS as described by the FDA (revised version of 2005). The expanded measurement uncertainty was around 60% for perchlorate levels close to the LQ of 2 µg/L. The analytical method used by the SCL for assaying perchlorate in infant formula was tried with an inter-laboratory test organised by ANSES in 2013, which was conclusive.

As part of this surveillance plan, analyses were carried out on the product as consumed after reconstitution with perchlorate-free water according to the manufacturer's instructions. The

[7] Region where concentrations in perchlorates of the order of 50 µg/L were measured in drinking water.

[8] The formulas sampled under the surveillance plan (see names and brands) are formulas that according to the TNS-Sofres consumer study in 2005 and KantarWorpanel 2009 purchase data are: 1/ declared to be consumed by more than 5% of children or 2/ contribute 90% of the total diet and 3/ account for 80% of market share.

67 available samples included 24 first-stage infant milk preparations, 29 follow-on milk preparations and 14 growing-up milk preparations.

Thirty-two samples had values higher than or equal to the laboratory's LQ (2 µg/L). Censored data (*i.e.* non-quantified[9]) were processed according to the recommendations of the World Health Organization (WHO-GEMS-Food Euro 1995). In this study, as the rate of censored data was less than 60% (52%), the non-quantified values were considered to be equal to half of the LQ (see middle bound (MB) assumption).

The results of these analyses, presented in Table XIV, show that the mean levels of perchlorate in infant formulas marketed in France are respectively equal to 1.8 and 2.8 µg/L for first-stage infant milk and follow-on milk preparations. These results are consistent with those published by Schier *et al.*, in 2010, on infant formulas based on cow's milk containing lactose (see mean levels estimated at 1.72 µg/L).

The mean level of perchlorate measured in reconstituted growing-up milk marketed in France was 7.0 µg/L. It is of the same order of magnitude as that published by the US Food and Drug Administration (US FDA) between 2004 and 2005 (Murray *et al.*, 2008) in milk intended for adults (see 5.8 µg/kg fresh weight on the basis of 125 analyses) and that was provided to ANSES by the French infant food trade association (Alliance 7) for milk powders for adults (Table XV).

Table XIV: Levels of perchlorate in reconstituted infant formula marketed in France (DGCCRF)

	Number of samples analysed (N)	N < LQ*	N > LQ*	Mean level of perchlorates (µg/L) based on the Middle bound hypothesis (MB**)	Minimum and maximum levels of perchlorates (µg/L) if N > LQ
First-stage milk (0-6 months)	24	19	5	1.8	2.0 – 8.7
Follow-on milk (6 months - 1 year)	29	15	14	2.8	2.0 – 10.2
Growing-up milk (> 1 year)	14	1	13	7.0	3.0 – 12.8

* LQ = 2 µg/L (analytical limit of quantification)

** MB: non-quantified values were considered to be half of the value of the LQ

Results published by the French infant food trade association (Alliance 7)

The results of perchlorate levels measured in 2012 in infant formulas marketed internationally by infant food manufacturers are shown in Table XV.

Of the 33 samples of infant formulas analysed, only seven came from infant formulas marketed in France. In these seven samples, the levels of perchlorate were below the LD.
The highest levels of perchlorate were observed in formulas from Asia with maximum values of respectively 30 and 20 µg/kg in first-stage and follow-on milks. These milks with the highest levels of perchlorate are not marketed in France.

[9] Values below the limit of quantification (LQ) and above the limit of detection (LD)

Moreover, the three results obtained on milk powders for adults marketed in France are similar to those observed for growing-up milks analysed by the Joint Laboratories Service (Table XV).

Table XV: Levels of perchlorate in infant formulas marketed internationally - results described by infant food manufacturers (Alliance 7)

	Number of samples analysed (N)	Number of French datasets	N < LQ*	N > LQ*	Mean level of perchlorates (µg/L) based on the Middle bound hypothesis (MB**)	Minimum and maximum levels of perchlorates (µg/L) if N > LQ
First-stage milk (0-6 months)	18	2	8	10	5.1	3.8 – 30
Follow-on milk (6 months - 1 year)	15	5	6	9	6.4	2.5 – 20
Milk powder for adults	3	3	0	3	8.9	6.1 – 13

* LQ = analytical limit of quantification

** MB: non-quantified values were considered to be half of the value of the LQ

Assessment of levels of exposure to perchlorate in children aged from 0 to 6 months based on contamination data provided at national level for infant formula and the public water supply

Exposure levels described in the literature

Food seems to be the main route of exposure to perchlorate, with water being considered a minor route (Borjan *et al.*, 2011).

According to the results obtained as part of a diet study, dairy products seem to be the foods contributing most to perchlorate exposure (51% of exposure for children aged 1 to 2 years and about 20% for adults), followed by fruit and vegetables (about 25% for children aged 1 to 2 years and 40% for adults) (Murray *et al.*, 2008). The estimated quantities ingested are around 0.35 to 0.39 µg.kg $bw^{-1}.d^{-1}$ for children aged 1 to 2 years, and from 0.08 to 0.11 µg/kg $bw^{-1}.d^{-1}$.for adults (Borjan *et al.*, 2011).

The results published in a recent Korean study indicate similar levels of exposure: from 0.33 to 0.34 µg.kg $bw^{-1}.d^{-1}$ for children aged 1 to 2 years, and from 0.005 to 0.015 µg.kg $bw^{-1}.d^{-1}$ for adults (Oh *et al.*, 2011).

In addition, exposure to perchlorates in infants aged 1 to 6 months estimated from data on contamination of milk powder show cases in which the TDI of 0.7 µg.kg $bw^{-1}.d^{-1}$ was possibly exceeded (Schier *et al.*, 2010).

These possible cases of exceeded TDI were also shown in the context of internal exposure studies conducted in infants (i.e. measuring urinary perchlorate levels in infants). In 2011, Valentin-Blasini *et al.* indeed found that the average exposure of infants to perchlorate was around 0.255 µg.kg $bw^{-1}.d^{-1}$ and that some infants exceeded the TDI of 0.7 µg.kg $bw^{-1}.d^{-1}$

(Valentin-Blasini *et al.*, 2011). It also appears, through this study, that breastfed children are markedly more exposed than those fed cow's milk formula (mean exposures, respectively, of 0.420 and 0.208 µg.kg $bw^{-1}.d^{-1}$).

In general, it seems that breast milk is more contaminated than cow's milk, which itself is more contaminated than soy milk. However, high intra-and inter-individual variability has been observed for perchlorate levels in breast milk (Kirk *et al.*, 2007).

For vegetables, broad-leaved varieties are reported as being generally more contaminated (e.g. lettuce, spinach). An accumulation of perchlorate in vegetables from irrigation water has been described, especially for spinach (Ha *et al.,* 2011), and the bioconcentration factor has been estimated at from 3 to 9 for some Asian vegetables (Yang and Her, 2011).

The contribution of vegetables to perchlorate exposure was estimated at between 24 and 28 ng.kg $bw^{-1}.d^{-1}$ in Canada for all age groups. That of fruit varies according to age, from 17.1 ng.kg $bw^{-1}.d^{-1}$ for children aged from 5 to 11 years, to 3.8 ng.kg $bw^{-1}.d^{-1}$ for women aged from 18 to 34 years (Wang *et al.*, 2009).

Levels of exposure to perchlorate estimated for children aged from 0 to 6 months residing in France

Exposure to perchlorate of children aged from 0 to 6 months living in France was calculated using firstly, the consumption data from a survey conducted in 2005 by the company TNS-Sofres[10] on behalf of the French infant food trade association (SFAE) (Fantino and Gourmet, 2008) and secondly, data on contamination of infant formula by perchlorate from the surveillance plan implemented by the DGCCRF in 2012 (see above).

<u>Calculation of exposure to perchlorates</u>

Using data on individual consumption and levels of perchlorates measured in infant formula, exposure was calculated with the following equation:

$$E_i = \sum_{k=1}^{n} \frac{C_{i,k} \times L_k}{PC_i}$$

where E_i is the total daily exposure of individual i (µg.kg $bw^{-1}.d^{-1}$), $C_{i,k}$ is the average daily consumption of food k by individual i ($g.d^{-1}$), L_k is the estimated level[11] of perchlorates in food k (mg.kg fw^{-1}), BW_i is the body weight of individual i (kg) and n is the total number of foods consumed by individual i.

<u>Results of exposure to perchlorate in children aged from 0 to 6 months</u>

Exposure to perchlorate in infants aged from 0 to 6 months was estimated in France at 0.15 µg.kg $bw^{-1}.d^{-1}$ on average, based on daily consumption of 415 g/day of first-stage milk reconstituted in perchlorate-free water (*Table XVI*).

[10] These data were collected at the homes of 713 children (aged from 15 days to 36 months), using the food notebook technique for three consecutive days, meal by meal. The study included infants or young children not breastfed (either exclusively or partially) and not attending a collective crèche or school during the three days following recruitment.
[11] Individual contamination of food k if available, or average contamination in its category (first-stage, follow-on milk)

Table XVI: Exposure of children aged from 0 to 6 months to perchlorate solely through consumption of infant formulas marketed in France.

	General population of children aged from 0 to 6 months				
Food	No. of individuals	Average milk consumption in g/day	Exposure to perchlorates in µg/kg/day		Rate of consumers (%)
			mean	P95	
First-stage milk	**251**	**415**	**0.15**	**0.36**	**51**
Follow-on milk	**251**	**289**	**0.10**	**0.42**	**47**

The 95th percentile represents the 5% of infants most exposed to perchlorates.
The rate of consumers is the % of individuals consuming first-stage or follow-on milk within the general population (see N=251)
These estimates do not take into account the possible intake of perchlorates via the public water supply.

For 5% of infants, exposure to perchlorate was higher than 0.36 µg/kg bw/d.

These estimates based on perchlorate concentrations measured in infant formulas marketed in France are of the same order of magnitude as those described in the United States by Valentin-Blasini *et al.* in 2011 (see median = 0.16 µg.kg $bw^{-1}.d^{-1}$ and mean = 0.255 µg.kg $bw^{-1}.d^{-1}$) and those estimated by Schier *et al.* in 2010 (see mean equal to respectively 0.25 and 0.19 µg.kg $bw^{-1}.d^{-1}$ in infants aged 1 and 6 months, and 90th percentile at respectively 0.35 and 0.27 µg.kg $bw^{-1}.d^{-1}$ in infants aged 1 and 6 months).

However, they are lower than those estimated in ANSES's opinion of 18 July 2011 based on consumption data from the recommendations of the French Society of Paediatrics (Bocquet *et al.,* 2003) and perchlorate levels in milk powders reported in the South Korean study, which were 33 µg of perchlorate/kg of milk powder (Her *et al.*, 2010).

<u>Probability of the TDI of 0.7 µg.kg $bw^{-1}.day^{-1}$ being exceeded in children aged from 0 to 6 months</u>

Based on these different exposure estimates, it appears that for 95% of the population of children aged from 0 to 6 months, infant formula consumption alone does not lead to the TDI of 0.7 µg.kg $bw^{-1}.d^{-1}$ being exceeded when feeding bottles are reconstituted with perchlorate-free water (*Figure 10*) or water with 1 µg/L[12] perchlorate concentration.

[12] Average concentration of perchlorate in water calculated based on all the data available nationally from the 299 results in TTPs and the 20 results in UDIs from the study by the LHN, assuming that non-quantified results are equal to the limit of quantification of 0.5 µg/L..

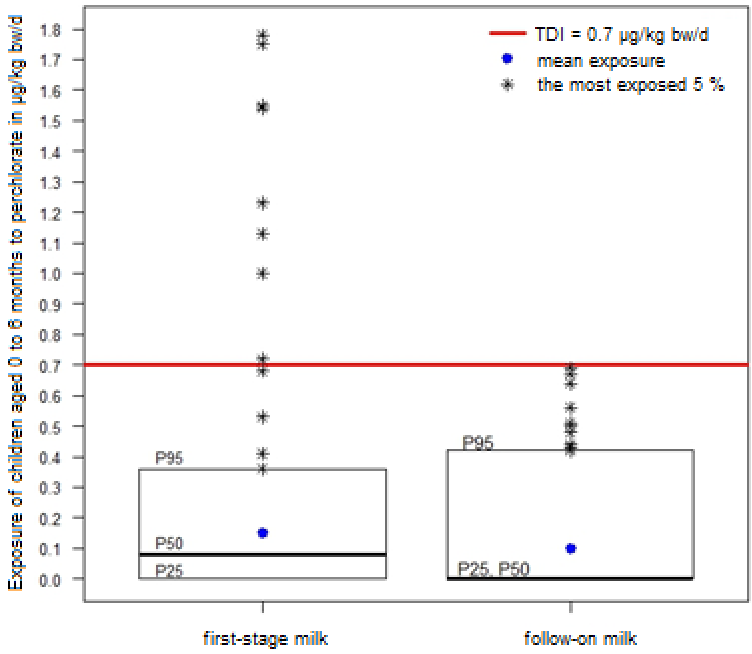


Figure 10: Exposure of children aged from 0 to 6 months to perchlorates solely through consumption of infant formula reconstituted with perchlorate-free water

However, according to data published in the study by TNS-Sofres (2005) on consumption of infant formula in children aged from 0 to 6 months and data from the national surveillance plan on contamination of first-stage and follow-on milk, the TRV may be exceeded in 5% of children in this age group consuming infant formula reconstituted with water containing perchlorate levels equal to 4 µg/L (*Figure 11*).

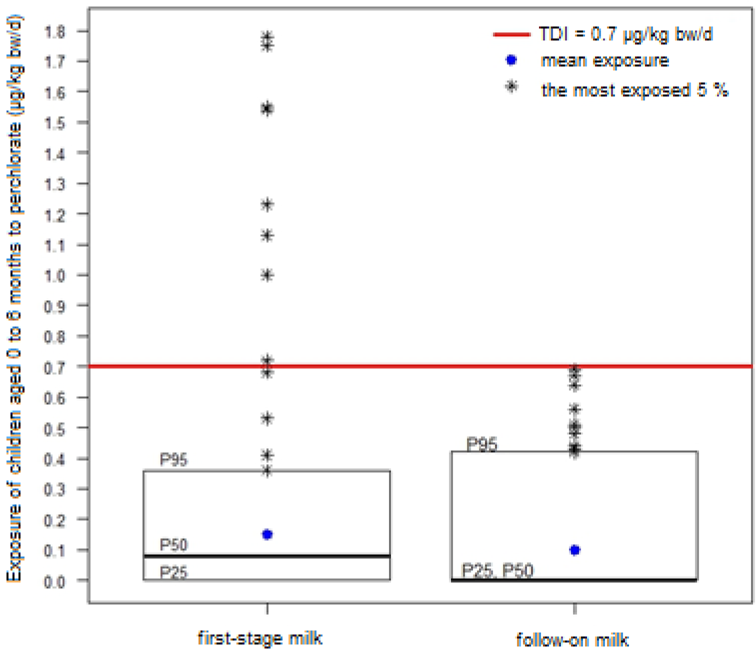


Figure 11: Theoretical exposure of children aged from 0 to 6 months to perchlorates via consumption of infant formula reconstituted with water having perchlorate levels equal to 4 µg/L

Based on the simulations conducted, it was found that reconstituting bottles with water having a perchlorate content higher than 2 µg/L can lead to the TRV of 0.7 µg.kg $bw^{-1}.d^{-1}$ being exceeded in the most exposed children.

The probability of this TRV being exceeded must, however, be balanced with regard to:

- infant formula reconstitution practices reported in France in 2011 (*Figure 12);*
- consumption levels for tap water described in 2005 for children aged from 0 to 3 years (*Table XVII*).

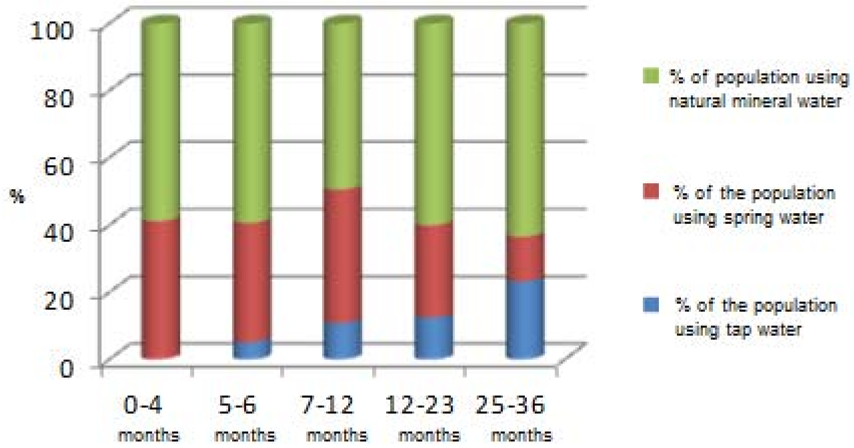


Figure 12: Infant feeding bottle reconstitution practices in France (see *ANSES study conducted in February 2011 among 429 households spread across France and concerning preparation practices for food intended for children aged under 3 years - data being published)*

Table XVII: consumption of tap water (excluding water used to reconstitute feeding bottles) in children aged 0 to 3 years in France *(TNS-Sofres, SFAE 2005*; *Fantino and Gourmet, 2008)*

	Number of children studied	Number of children consuming tap water	% of children consuming tap water	Tap water consumption in mL/day in children consumers	
Age groups				**mean**	**maximum**
0-6 months	251	2	0.8	**148**	260
7-12 months	195	14	7	**73**	237
13-36 months	259	112	43	**244**	1650

4 ANALYSIS AND CONCLUSIONS OF THE CESS ON WATER AND ON ASSESSMENT OF THE PHYSICAL AND CHEMICAL RISKS IN FOODS

Based on all this information, the CESs on Water and on Assessment of the physical and chemical risks in foods note that:

- levels of perchlorate in the public water supply measured nationally (see the Analysis Campaign conducted by the Nancy Laboratory for Hydrology – LHN – in 2012), and accounting for 25% of the water produced in France, show that the management levels of 15 and 4 µg/L[13] defined for drinking water based on the TRV of 0.7 µg.kg $bw^{-1}.d^{-1}$ were possibly exceeded on occasions.

    - The management value of 4 µg/L defined by the DGS for children aged under 6 months was exceeded in about 2% of the water treatment and production units (TTPs) that had been sampled under the national campaign conducted by the LHN;

    - The management value of 15 µg/L applicable to the adult population was exceeded (*see the ANSES Opinion of 18 July 2011*) in none of the water treatment and production units (TTPs) but in around 1% of the catchment water samples taken under the national campaign conducted by the LHN;

- the results of the monitoring plan implemented in 2012 to measure perchlorate levels in infant formulas available on the French market[14] show average levels of respectively 1.8 µg/L and 2.8 µg/L and maximum levels of respectively 8.7 µg/L and 10.2 µg/L in first-stage infant milk and follow-on milk reconstituted with perchlorate-free water and intended for children aged under 6 months. The average levels are of the same order of magnitude as those described in the United States and are lower than those described in some Asian countries (see value of about 30 µg/kg adopted in the ANSES Opinion of 18 July 2011);

- daily intakes of perchlorate, calculated on the basis of perchlorate levels in infant formulas available on the French market, do not exceed the TDI of 0.7 µg.kg $bw^{-1}.d^{-1}$ for 95% of the population of children aged under 6 months consuming infant formula based on an average concentration of perchlorates of 1 µg/L[15] in drinking water for reconstituting infant bottles. However, based on the simulations conducted, it was estimated that reconstituting milk for infant bottles with water having a perchlorate content higher than 2 µg/L could lead to the TDI of 0.7 µg.kg $bw^{-1}.d^{-1}$ being exceeded in 5% of this population of children aged under 6 months;

- the available data from the scientific literature cannot be used to assess the impact in humans of co-exposure to other goitrogenic anions (nitrate and thiocyanate) on changes to thyroid function;

- the data on the iodine status of the French population indicate that:

[13] The value of 15 µg/L corresponds to the management value for water applicable to the adult population, based on the figure of 60% of the TRV being attributed to water-related exposure that was adopted by ANSES, and an exposure scenario for a 70 kg bw adult consuming two litres of water per day (see ANSES Opinion of 18 July 2011). The value of 4 µg/L corresponds to the management value for water selected by the DGS for children aged under six months.

[14] This monitoring plan follows the recommendations issued by ANSES in its Opinion of 18 July 2011.

[15] Average concentration of perchlorates in water calculated on all the data available nationally from the 299 results in TTPs and the 20 results in distribution units from the study by the LHN, assuming the non-quantified results to be equal to the limit of quantification of 0.5 µg/L.

- the general adult population has an adequate iodine status with regard to the WHO criteria (ENNS 2006-2007 study);
- iodine deficiency is common in pregnant women;
- the iodine status of infants is not always satisfactory, although the cases of iodine deficiency reported in the two French studies conducted in 2003 and 2005 in newborns were not associated with cases of clinical hypothyroidism.

All this new information should be considered together with the conclusions and recommendations already made by ANSES in its Opinions of 18 July 2011, 20 July 2012 and 24 October 2013, namely that:

- the results of the available epidemiological studies cannot be used to reach a conclusion as to the existence or absence of an association between TSH levels measured in pregnant women or newborns and perchlorate concentrations in drinking water up to around a hundred µg/L;

- iodine status is a key parameter in assessing the health impact of perchlorate in humans and the lack of information on the iodine status of the studied populations makes it difficult to interpret the published epidemiological data;

- in the absence of more precise information on the iodine status of the studied populations in the available epidemiological studies, the health risk associated with cases in which the above-mentioned management levels were exceeded (*i.e.* perchlorate levels in the water higher than 4 or 15 µg/L) cannot currently be quantified; although cases in which these values were moderately exceeded (less than a hundred µg/L) in newborns and pregnant women do not seem to be associated with clinically detectable effects;

- in addition to first-stage infant milk and follow-on milk intended for children aged under 6 months and drinking water, other possible dietary sources of perchlorate intake have been identified, including in particular fruit, vegetables and dairy products. On this subject, ANSES issued an Opinion on 24 October 2013 on the monitoring of food contamination by perchlorates;

- in children older than 6 months, diversification of the diet leads to an increase in consumption of fruit and vegetables, dairy products and water.

Accordingly, the CESs on Water and on Assessment of the physical and chemical risks in foods recommends:

- acquiring additional data on the iodine status of pregnant and breastfeeding women, as well as of children aged under three years, especially in areas where perchlorate levels in the public water supply are highest;

- updating the data on drinking water consumption among children aged under 6 months, considering in particular infant formula reconstitution practices and possible socio-economic determinants;

- acquiring information on the sources of contamination of infant formula by perchlorate, and continuing investigations to identify the actual origins of water contamination by perchlorate;

Pending such information and evidence relating to the level of contamination of fruit and vegetables marketed in France (see the ANSES Opinion of 24 October 2013), the CESs on Water and on Assessment of the physical and chemical risks in foods recommend:

- informing the population supplied by drinking water with perchlorate content higher than 4 µg/L, advising them to limit consumption among children aged under 6 months;

- conducting a specific study of catchments intended for producing bottled water in the event of perchlorate concentrations greater than 4 µg/L and taking the necessary measures concerning its marketing and information to consumers;

- reducing the presence of perchlorate in infant formulas marketed in France.

They also state that:

- at national level, the French Institute of Health Surveillance (InVS) was mandated by the DGS to carry out an epidemiological study in the Nord-Pas de Calais region to investigate possible associations between perchlorate levels in water and levels of pituitary thyroid-stimulating hormone (TSH) measured in newborns as part of screening for congenital hypothyroidism. Analyses are underway and some results may be published in 2014;

- at EU level, the assessment of the health risks associated with the presence of perchlorate in fruit and vegetables is currently underway and has, on the basis of the toxicity reference value of 10 µg.kg $bw^{-1}.d^{-1}$ proposed in 2011 by JECFA[16], already resulted in the establishment of provisional regulatory limits in fruit and vegetables placed on the EU market.

- at international level, an Opinion by the US EPA on the assessment of the health risks associated with the presence of perchlorate in drinking water should be published in 2014. This Opinion will rule on the relevance of using physiologically-based pharmacokinetic modelling (PBPK) to establish a guideline value in drinking water.

5 AGENCY CONCLUSIONS AND RECOMMENDATIONS

The French Agency for Food, Environmental and Occupational Health & Safety adopts the conclusions and recommendations of the CESs on Water and on Assessment of the physical and chemical risks in foods. This work mainly focused on contamination data generated by the LHN in the context of a national study requested by the Ministry of Health. Cases of exposure exceeding the management limits in drinking water or the TRV for some specific populations have been calculated based on these data. It is important to note that locally, some perchlorate concentrations could be much higher than those measured by the LHN, and could lead to some situations in which the management limits are exceeded. These local

[16] The choice of the benchmark dose used by JECFA in 2011 to calculate the TRV for perchlorate differs from that used by ANSES in its Opinion of 18 July 2011 and that of other agencies, including the US National Academy of Sciences and the US EPA in 2005, the ATSDR in 2009 and INERIS in 2011 (see the ANSES Opinion of 18 July 2011).

situations have been assessed by ANSES and are currently being investigated via an epidemiological study led by the InVS and run in the Nord-Pas de Calais region.

Under ANSES's collaboration with the European Food Safety Authority (EFSA), the Agency has sent it the analytical data published in this opinion so that it can integrate them into its ongoing work on the contamination of food by perchlorate ions.

The Director General

Marc Mortureux

KEY WORDS

Perchlorate, water, drink, foods, infant formula, infants, diet

BIBLIOGRAPHY

French Food Safety Agency (AFSSA; 2005). Evaluation de l'impact nutritionnel de l'introduction de composés iodés dans les produits agroalimentaires [Assessment of the nutritional impact of introducing iodine compounds into food products]. March 2005. 120 p.

French Food Safety Agency (AFSSA; 2008) Avis de l'Agence française de sécurité sanitaire des aliments relatif à l'évaluation des risques sanitaires liés aux situations de dépassement de la limite de qualité des nitrates et des nitrites dans les eaux destinées à la consommation humaine [Opinion of the French Food Safety Agency on the assessment of the health risks associated with non-compliance with quality limits for nitrates and nitrites in water intended for human consumption]. July 2008. 58 p.

French Agency for Food, Environmental and Occupational Health & Safety (ANSES; 2011). Avis de l'ANSES relatif à l'évaluation des risques sanitaires liés à la présence d'ions perchlorate dans les eaux destinées à la consommation humaine [ANSES Opinion on the assessment of the health risks associated with the presence of perchlorate in drinking water]. July 2011. 22 p.

French Agency for Food, Environmental and Occupational Health & Safety (ANSES; 2012) Avis de l'ANSES relatif aux études épidémiologiques portant sur les associations entre une exposition aux ions perchlorate dans l'eau de boisson et la fonction thyroïdienne dans des populations spécifiques [ANSES Opinion concerning epidemiological studies on associations between exposure to perchlorate in drinking water and thyroid function in specific populations]. July 2012. 16 p.

Amitai, Y., Winston, G., Sack, J., Wasser, J., Lewis, M., Blount, B.C., Valentin-Blasini, L., Fisher, N., Israeli, A., Leventhal, A. (2007) Gestational exposure to high perchlorate concentrations in drinking water and neonatal thyroxine levels. *Thyroid*, 17 (9), 843-850.

ATSDR (2009) Toxicological profile for perchlorates. US Department of Health and Human Services. Public Health Service Agency for Toxic Substances and Disease Registry. 299

Asami, M., Kosaka, K., Kunikane, S. (2009) Bromate, chlorate, chlorite and perchlorate in sodium hypochlorite solution used in water supply. *Journal of Water Supply: Research and Technology - AQUA*, 58 (2), 107-115.

Attanasio, R., Scinicariello, F., Blount, B.C., Valentin-Blasini, L., Rogers, K.A., Nguyen, D.C., Murray, H.E. (2011) Pendrin mediates uptake of perchlorate in a mammalian in vitro system. *Chemosphere*, 84 (10), 1484-1488.

Beaufrère, B., Bresson, J.L., Briend, A., Ghisolfi, J., Goulet, O., Navarro, J., Putet, G., Ricour, C., Rieu, D., Turck, D., Vidailhet, M. (2000) Iodine nutrition in the infant. Committee on Nutrition of the French Society of Pediatrics [La nutrition iodée chez l'enfant. Comité de Nutrition de la Société française de pédiatrie.] *Archives de pediatrie: organe officiel de la Societe francaise de pediatrie*, 7 (1), 66-74.

Blount, B.C., Pirkle, J.L., Osterloh, J.D., Valentin-Blasini, L., Caldwell, K.L. (2006) Urinary perchlorate and thyroid hormone levels in adolescent and adult men and women living in the United States. *Environmental Health Perspectives*, 114 (12), 1865-1871.

Bocquet A., Bresson J.L., Briend A., Chouraqui J.P., Darmaun D., Dupont C., Frelut M.L., Ghisolfi J., Putet G., Rieu D., Turck D., Vidaihet M., Merlin J.P., Rives J.J. (2003) Alimentation du nourrisson et de l'enfant en bas âge. Réalisation pratique. Editions scientifiques et médicales Elsevier SAS. 76-81

Borjan, M., Marcella, S., Blount, B., Greenberg, M., Zhang, J., Murphy, E., Valentin-Blasini, L., Robson, M. (2011) Perchlorate exposure in lactating women in an urban community in New Jersey. *Science of the Total Environment*, 409 (3), 460-464.

Brechner, R.J., Parkhurst, G.D., Humble, W.O., Brown, M.B., Herman, W.H. (2000) Ammonium perchlorate contamination of Colorado river drinking water is associated with abnormal thyroid function in newborns in Arizona. *Journal of Occupational and Environmental Medicine*, 42 (8), 777-782.

Bruce, G.M., Corey, L.M., Mandel, J.H., Pleus, R.C. (2013) Urinary nitrate, thiocyanate, and perchlorate and serum thyroid endpoints based on NHANES 2001 to 2002. *Journal of Occupational and Environmental Medicine*, 55 (1), pp. 52-58.

Brucker-Davis F, Hiéronimus S, Ferrari P, Bongain A, Durand-Reville M, Fénichel P. Iodine deficiency in pregnant women in Nice area. In: In Abstracts book, 76th Annual Meeting of the American Thyroid Association. Canada: Vancouver, 2004; 29 September-2 October.

Buffler, P.A., Kelsh, M.A., Lau, E.C., Edinboro, C.H., Barnard, J.C., Rutherford, G.W., Daaboul, J.J., Palmer, L., Lorey, F.W. (2006) Thyroid function and perchlorate in drinking water: An evaluation among California newborns, 1998. *Environmental Health Perspectives*, 114 (5), 798-804.

Cao, Y., Blount, B.C., Valentin-Blasini, L., Bernbaum, J.C., Phillips, T.M., Rogan, W.J. (2010) Goitrogenic anions, thyroid-stimulating hormone, and thyroid hormone in infants. *Environmental Health Perspectives*, 118 (9), 1332-1337.

Caron P., Hoff M., Bazzi S., Dufor A., Faure G., Ghandour I., Lauzu P., Lucas Y., Maraval D., Mignot F., Réssigeac P., Vertongen F., Grangé V. (1997) Urinary iodine excretion during normal pregnancy in healthy women living in the Southwest of France: correlation with maternal thyroid parameters. *Thyroid*; 7: 749-754.

Crump, C., Michaud, P., Téllez, R., Reyes, C., Gonzalez, G., Montgomery, E.L., Crump, K.S., Lobo, G., Becerra, C., Gibbs, J.P. (2000) Does perchlorate in drinking water affect thyroid function in newborns or school-age children? *Journal of Occupational and Environmental Medicine*, 42 (6), pp. 603-612.

Dasgupta, P.K., Martinelango, P.K., Jackson, W.A., Anderson, T.A., Tian, K., Tock, R.W., Rajagopalan, S. (2005) The origin of naturally occurring perchlorate: The role of atmospheric processes. *Environmental Science and Technology*, 39 (6), pp. 1569-1575.

Delange F, Wolff P, Gnat D, Dramaix M, Pilchen M, Vertongen F (2001): Iodine deficiency during infancy and early childhood in Belgium: does it pose a risk to brain development ? Eur J Pediatr. *Apr;160(4):251-4.*

De Groef B, Decallonne BR, Van der Geyten S, Darras VM, Bouillon R (2006) Perchlorate versus other environmental sodium/iodide symporter inhibitors: Potential thyroid-related health effects. *European Journal of Endocrinology* 155(1), 17-25.

Direction générale de la santé (2011) Instruction No. DGS/EA4/2011/229 du 14 juin 2011 relative aux campagnes nationales de mesures du chlorure de vinyle monomère et des alkylphénols, nitrosamines, acides haloacétiques, trihalométhanes iodés dans les eaux destinées à la consommation humaine.

Direction de la recherche, des études, de l'évaluation et des statistiques (2010) L'état de santé de la population en France. Suivi des objectifs annexés à la loi de santé publique. Rapport 2009-2010. 308 p.

DREES 2010, Direction de la recherche, des études, de l'évaluation et des statistiques: l'état de santé de la population en France - Suivi des objectifs annexés à la loi de santé publique - *Rapport 2009-2010.*

ENNS, 2006-2007, Castetbon K, Vernay M, Malon A, Salanave B, Deschamps V, Roudier C, Oleko A, Szego E, Hercberg S: Dietary intake, physical activity and nutritional status in adults: the French nutrition and health survey. *Br J Nutr. 2009 Sep; 102(5):733-43.*

Fantino M. & Gourmet E., 2008. Apports nutritionnels en France en 2005 chez les enfants non allaités âgés de moins de 36 mois. Archives de pédiatrie, 15, 446-455.

FDA – US Food and Drug Administration (2005): Rapid Determination of Perchlorate Anion in Foods by Ion Chromatography-Tandem Mass Spectrometry. *Revision 2: April 12, 2005*

Gibbs, J.P., Van Landingham, C. (2008) Urinary perchlorate excretion does not predict thyroid function among pregnant women. *Thyroid*, 18 (7), 807-808.

Glinoer, D. (2004) The regulation of thyroid function during normal pregnancy: Importance of the iodine nutrition status. *Best Practice and Research: Clinical Endocrinology and Metabolism*, 18 (2), 133-152.

Gold, E.B., Blount, B.C., O'neill Rasor, M., Lee, J.S., Alwis, U., Srivastav, A., Kim, K. (2013) Thyroid hormones and thyroid disease in relation to perchlorate dose and residence near a superfund site. *Journal of Exposure Science and Environmental Epidemiology*, 23 (4), pp. 399-408.

Greer, M.A., Goodman, G., Pleus, R.C., Greer, S.E. (2002) Health effects perchlorate contamination: The dose response for inhibition of thyroidal radioiodine uptake in humans. *Environmental Health Perspectives*, 110 (9), 927-937.

Ha, W., Suarez, D.L., Lesch, S.M. (2011) Perchlorate uptake in spinach as related to perchlorate, nitrate, and chloride concentrations in irrigation water. *Environmental Science and Technology*, 45 (21), 9363-9371.

Haddow, J.E., Palomaki, G.E., Allan, W.C., Williams, J.R., Knight, G.J., Gagnon, J., O'Heir, C.E., Mitchell, M.L., Hermos, R.J., Waisbren, S.E., Faix, J.D., Klein, R.Z. (1999) Maternal thyroid deficiency during pregnancy and subsequent neuropsychological development of the child. *New England Journal of Medicine*, 341 (8), 549-555.

Her, N., Kim, J., Yoon, Y. (2010) Perchlorate in dairy milk and milk-based powdered infant formula in South Korea. *Chemosphere*, 81 (6), 732-737.

Hiéronimus, S., Bec-Roche, M., Ferrari, P., Chevalier, N., Fénichel, P., Brucker-Davis, F. (2009) Iodine status and thyroid function of 330 pregnant women from Nice area assessed during the second part of pregnancy [Statut iodé et fonction thyroïdienne chez 330 femmes de la région niçoise évaluées en deuxième partie de grossesse]. *Annales d'Endocrinologie*, 70 (4), 218-224.

Hube D. (2013) Potentialités d'un marquage des eaux souterraines par des substances pyrotechniques en relation avec les zones de combats de la première guerre mondiale – Le cas des perchlorates. Rapport final. BRGM/RP-62008-FR. 26 p., 3

Hunault CC, Lambers AC, Mensinga TT, van Isselt JW, Koppeschaar HPF, Meulenbelt J (2007) Effects of sub-chronic nitrate exposure on the thyroidal function in humans. *Toxicology Letters* 175(1-3), 64-70.

INERIS (2011) Profil toxicologique et choix de valeur de référence pour le perchlorate lors d'expositions chroniques par voie orale. Rapport d'étude n° DRC-11-119475-02737A. 24

IUCLID. (2000). Ammonium perchlorate. from http://esis.jrc.ec.europa.eu/doc/IUCLID/data_sheets/7790989.pdf.

JECFA (2011) Safety evaluation of certain contaminants in food / prepared by the Seventy-second meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). 792 p. ISBN 978 92 4 166063 1.

Kelsh, M.A., Buffler, P.A., Daaboul, J.J., Rutherford, G.W., Lau, E.C., Barnard, J.C., Exuzides, A.K., Madl, A.K., Palmer, L.G., Lorey, F.W. (2003) Primary Congenital Hypothyroidism, Newborn Thyroid Function, and Environmental Perchlorate Exposure Among Residents of a Southern California Community. *Journal of Occupational and Environmental Medicine*, 45 (10), 1116-1127.

Kirk, A.B., Dyke, J.V., Martin, C.F., Dasgupta, P.K. (2007) Temporal patterns in perchlorate, thiocyanate, and iodide excretion in human milk. *Environmental Health Perspectives*, 115 (2), 182-186.

Lamm, S.H., Doemland, M. (1999) Has perchlorate in drinking water increased the rate of congenital hypothyroidism? *Journal of Occupational and Environmental Medicine*, 41 (5), 409-411.

Leung, A.M., Braverman, L.E., He, X., Schuller, K.E., Roussilhes, A., Jahreis, K.A., Pearce, E.N. (2012) Environmental perchlorate and thiocyanate exposures and infant serum thyroid function. *Thyroid*, 22 (9), 938-943.

Li, Z., Li, F.X., Byrd, D., Deyhle, G.M., Sesser, D.E., Skeels, M.R., Lamm, S.H. (2000a) Neonatal thyroxine level and perchlorate in drinking water. *Journal of Occupational and Environmental Medicine*, 42 (2), 200-205.

Li, F.X., Byrd, D.M., Deyhle, G.M., Sesser, D.E., Skeels, M.R., Katkowsky, S.R., Lamm, S.H. (2000b) Neonatal thyroid stimulating hormone level and perchlorate in drinking water. *Teratology*, 62 (6), pp. 429-431.

Li, F.X., Squartsoff, L., Lamm, S.H. (2001) Prevalence of thyroid diseases in Nevada counties with respect to perchlorate in drinking water. *Journal of Occupational and Environmental Medicine*, 43 (7), 630-634.

Luton, D., Alberti, C., Vuillard, E., Ducarme, G., Oury, J.F., Guibourdenche, J. (2011) Iodine deficiency in northern Paris area: Impact on fetal thyroid mensuration. PLoS ONE, 6 (2), art. no. e14707

Mendez, W., Eftim, S.E. (2012) Biomarkers of perchlorate exposure are correlated with circulating thyroid hormone levels in the 2007-2008 NHANES. *Environmental Research*, 118, 137-144.

Murray, C.W., Egan, S.K., Kim, H., Beru, N., Bolger, P.M. (2008) US Food and Drug Administration's total diet study: Dietary intake of perchlorate and iodine. *Journal of Exposure Science and Environmental Epidemiology*, 18 (6), 571-580.

NAS (2005) Health implications of perchlorate ingestion. Washington, DC: National Academies Press. http://www.nap.edu/books/0309095689/html/. January 31, 2005.

Oh S.H., Lee J.W., *et al.* (2011) Analysis and exposure assessment of perchlorate in Korean dairy products with LC-MS/MS. *Environmental Health and Toxicology*. 26: e2011011.

NRC - National Research Council. (2005):. Health Implications of Perchlorate Ingestion. *Washington, D.C.: National Academies Press.*

WHO (1995) Reliable evaluation of low-level contamination of food - workshop in the frame of GEMS/Food-EURO. Kulmbach, Germany, 26-27 May 1995.

WHO/UNICEF/ICCIDD (2001) Assessment of iodine deficiency disorders and monitoring their elimination. A guide for programme managers, second edition. WHO/NHD/01.1. World Health Organization, Geneva, 107 p.

World Health Organization (2011) Guidelines for drinking-water quality. Fourth edition. ISBN 978-92-4-154845-1

Park, S.M., Chatterjee, V.K.K. (2005) Genetics of congenital hypothyroidism. Journal of Medical Genetics, 42 (5), 379-389.

Pearce, E.N., Braverman, L.E. (2009) Environmental pollutants and the thyroid. Best Practice and Research: *Clinical Endocrinology and Metabolism*, 23 (6), 801-813.

Pearce, E.N., Lazarus, J.H., Smyth, P.P.A., He, X., Dall'Amico, D., Parkes, A.B., Burns, R., Smith, D.F., Maina, A., Bestwick, J.P., Jooman, M., Leung, A.M., Braverman, L.E. (2010) Perchlorate and thiocyanate exposure and thyroid function in first-trimester pregnant women. *Journal of Clinical Endocrinology and Metabolism*, 95 (7), 3207-3215.

Pearce, E.N., Spencer, C.A., Mestman, J.H., Lee, R.H., Bergoglio, L.M., Mereshian, P., He, X., Leung, A.M., Braverman, L.E. (2011) Effect of environmental perchlorate on thyroid function in pregnant women from Córdoba, Argentina, and Los Angeles, California. *Endocrine Practice*, 17 (3), 412-417.

Pearce, E.N., Alexiou, M., Koukkou, E., Braverman, L.E., He, X., Ilias, I., Alevizaki, M., Markou, K.B. (2012) Perchlorate and thiocyanate exposure and thyroid function in first-trimester pregnant women from Greece. Clinical Endocrinology, 77 (3), 471-474.

Plantin-Carrenard E, Foglietti MJ, Beaudeux JL (2005) Sodium/iodide symporter: Physiopathological aspects and therapeutic perspectives. Le symporteur sodium/iodure: Données récentes et perspectives thérapeutiques. *Pathologie Biologie*. 53(3), 174-182.

Pop, V.J., Vulsma, T. (1999) Impact of maternal thyroid function in pregnancy on subsequent infant health. *Current Opinion in Endocrinology and Diabetes*, 6 (4), 301-307.

Pouessel G., Bouarfa K., Soudan B., Sauvage J., Gottrand F. Turck D. (2003) Statut en iode et facteurs de risque de déficit en iode chez des enfants vus en consultation de protection maternelle et infantile dans le département du Nord. *Archives de Pédiatrie*; 10: 96-101.

Pouessel, G., Damie, R., Soudan, B., Weill, J., Gottrand, F., Turck, D. (2008) Status of iodine nutrition of children until 1 year: Consequences on the thyroid function [Statut en iode chez des enfants de moins de 1 an: conséquences sur la fonction thyroïdienne] *Archives de Pediatrie*, 15 (8), 1276-1282.

Rajagopalan, S., Anderson, T.A., Fahlquist, L., Rainwater, K.A., Ridley, M., Jackson, W.A. (2006) Widespread presence of naturally occurring perchlorate in high plains of Texas and New Mexico. *Environmental Science and Technology*, 40 (10), 3156-3162.

Raverot, V., Bournaud, C., Sassolas, G., Orgiazzi, J., Claustrat, F., Gaucherand, P., Mellier, G., Claustrat, B., Borson-Chazot, F., Zimmermann, M. (2012) Pregnant French women living in the Lyon area are iodine deficient and have elevated serum thyroglobulin concentrations. *Thyroid*, 22 (5), 522-528.

Schier, J.G., Wolkin, A.F., Valentin-Blasini, L., Belson, M.G., Kieszak, S.M., Rubin, C.S., Blount, B.C. (2010) Perchlorate exposure from infant formula and comparisons with the perchlorate reference dose. *Journal of Exposure Science and Environmental Epidemiology*, 20 (3), 281-287.

Schlienger, J.L., Grunenberger, F., Vinzio, S., Goichot, B. (2003) Thyroïde et tabac des relations complexes. *Annales d'Endocrinologie*, 64 (4), 309-315.

Schreinemachers, D.M. (2011) Association between perchlorate and indirect indicators of thyroid dysfunction in NHANES 2001-2002, a cross-sectional, hypothesis-generating study. *Biomarker Insights*, 6, 135-146.

Steinmaus, C., Miller, M.D., Howd, R. (2007) Impact of smoking and thiocyanate on perchlorate and thyroid hormone associations in the 2001-2002 National Health and Nutrition Examination Survey. *Environmental Health Perspectives*, 115 (9), 1333-1338.

Steinmaus, C., Miller, M.D., Smith, A.H. (2010) Perchlorate in drinking water during pregnancy and neonatal thyroid hormone levels in California. *Journal of Occupational and Environmental Medicine*, 52 (12), 1217-1224.

Steinmaus C, Miller MD, Smith AH (2011) Authors response. *Journal of Occupational and Environmental Medicine* 53(5), 466-467.

Steinmaus, C., Miller, M.D., Cushing, L., Blount, B.C., Smith, A.H. (2013) Combined effects of perchlorate, thiocyanate, and iodine on thyroid function in the National Health and Nutrition Examination Survey 2007-08. *Environmental Research*, 123, pp. 17-24.

Strategic Environmental Research and Development Program (SERDP), 2005. Alternative causes of widespread, low concentration perchlorate impacts to groundwater 31P.

Tarone, R.E., Lipworth, L., McLaughlin, J.K. (2010) The epidemiology of environmental perchlorate exposure and thyroid function: A comprehensive review. *Journal of Occupational and Environmental Medicine*, 52 (6), 653-660.

Téllez Téllez, R.T., Chacón, P.M., Abarca, C.R., Blount, B.C., Van Landingham, C.B., Crump, K.S., Gibbs, J.P. (2005) Long-term environmental exposure to perchlorate through drinking water and thyroid function during pregnancy and the neonatal period. *Thyroid*, 15 (9), 963-975.

Tonacchera M, Pinchera A, Dimida A, Ferrarini E, Agretti P, Vitti P, Santini F, Crump K, Gibbs J (2004) Relative potencies and additivity of perchlorate, thiocyanate, nitrate, and iodide on the inhibition of radioactive iodide uptake by the human sodium iodide symporter. *Thyroid* 14(12), 1012-1019.

Twyffels, L., Massart, C., Golstein, P.E., Raspe, E., Van Sande, J., Dumont, J.E., Beauwens, R., Kruys, V. (2011) Pendrin: The thyrocyte apical membrane iodide transporter? *Cellular Physiology and Biochemistry*, 28 (3), 491-496.

US Environmental Protection Agency (2005) IRIS. Perchlorate and perchlorate salts. Washington, DC: Integrated Risk Information System. US Environmental Protection Agency. www.epa.gov/iris/subst/. July 11.

Valentín-Blasini, L., Blount, B.C., Otero-Santos, S., Cao, Y., Bernbaum, J.C., Rogan, W.J. (2011) Perchlorate exposure and dose estimates in infants. *Environmental Science and Technology*, 45 (9), 4127-4132.

Vanderpas J (2006) Nutritional epidemiology and thyroid hormone metabolism. *Annual review of nutrition* 26, 293-322.

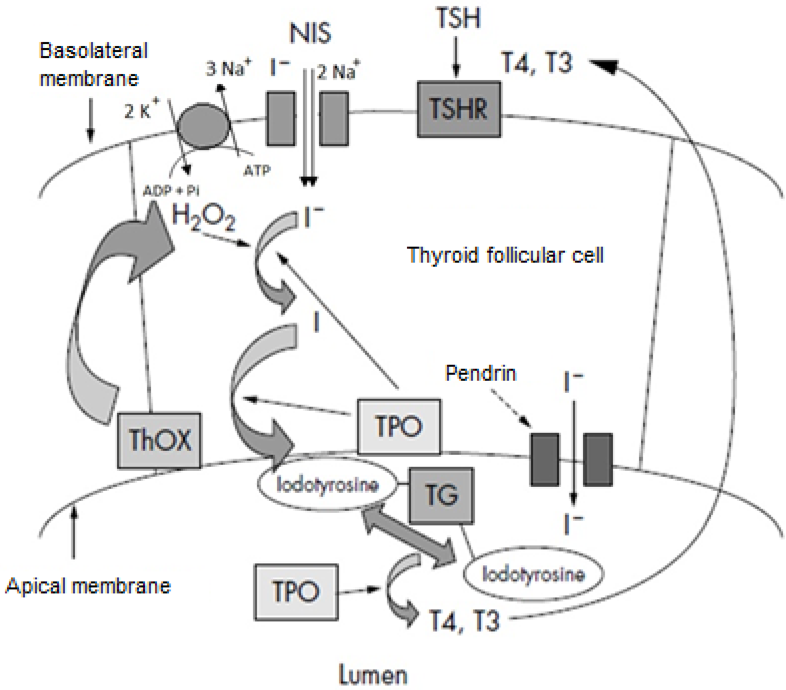
Wang, Z., Forsyth, D., Lau, B.P.-Y., Pelletier, L., Bronson, R., Gaertner, D. (2009) Estimated dietary exposure of Canadians to perchlorate through the consumption of fruits and vegetables available in Ottawa markets. *Journal of Agricultural and Food Chemistry*, 57 (19), 9250-9255.

Wemeau JL. Les maladies de la thyroïde. Masson. ISBN: 978-2-294-07464-6. 7 April 2010. 190 p.

Yang, M., Her, N. (2011) Perchlorate in soybean sprouts (Glycine max L. Merr.), water dropwort (Oenanthe stolonifera DC.), and lotus (Nelumbo nucifera Gaertn.) root in South Korea. *Journal of Agricultural and Food Chemistry*, 59 (13), 7490-7495.

ANNEXES

Annex 1: Description of thyroid hormone synthesis

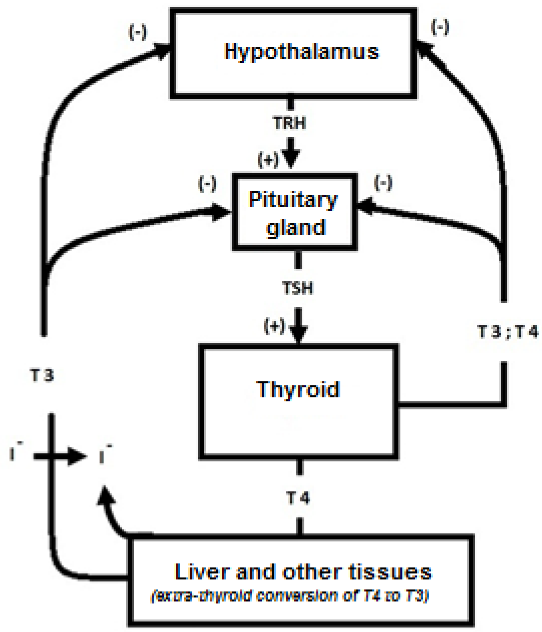


Schematic representation of a follicular cell of the thyroid gland, representing the main steps involved in thyroid hormone synthesis (from Park and Chatterjee, 2005, and Plantin-Carrenard *et al.*, 2005)

Pituitary thyroid-stimulating hormone (TSH) binds to the TSH receptor (TSHR) then stimulates iodine transport into the thyroid follicular cell via the sodium-iodide symporter (NIS). The force needed for the active transport of iodine against an electrical gradient is generated by the transmembrane concentration gradient for sodium maintained through the Na^+/K^+ ATPase pump. The iodide ions are then oxidised by hydrogen peroxide, generated by NADPH oxidase, an enzyme system marked as ThOX in this diagram, and are then bound to tyrosine residues by the action of thyroglobulin (TG) to form iodotyrosine. This step corresponds to the organification of the iodide ions. Some residual forms of iodotyrosine (monoiodotyrosine and diiodotyrosine) are coupled by the action of thyroid peroxidase (TPO) to form active forms of the thyroid hormones (triiodothyronine or T3 and thyroxine or T4). The exact function of pendrin, an iodine transporter of the apical membrane of the thyroid follicular cell, is still being investigated. Some authors have shown that perchlorate and iodine are not only in competition via NIS (basolateral membrane) but also via pendrin (apical membrane) (Attanasio *et al.*, 2011; Twyffels *et al.*, 2011).

The metabolism of iodine in the thyroid follicular cell therefore involves not only the sodium-iodide symporter (NIS), but also other effectors, whose reactivity with perchlorate is less well known (e.g. pendrin).

Annex 2: Description of the hypothalamic-pituitary-thyroid system



Schematic representation of the hypothalamic-pituitary-thyroid system (from NAS, 2005)

TSH acts at different levels and in particular, it controls and stimulates the different stages of hormone synthesis: iodine capture, iodination of thyroglobulin, pinocytosis, hydrolysis of thyroglobulin and hormone secretion. It maintains the phenotype of thyrocytes by regulating the expression and synthesis of thyroglobulin, iodide pumps and thyroid peroxidase. Finally, TSH is a growth factor for the thyroid.

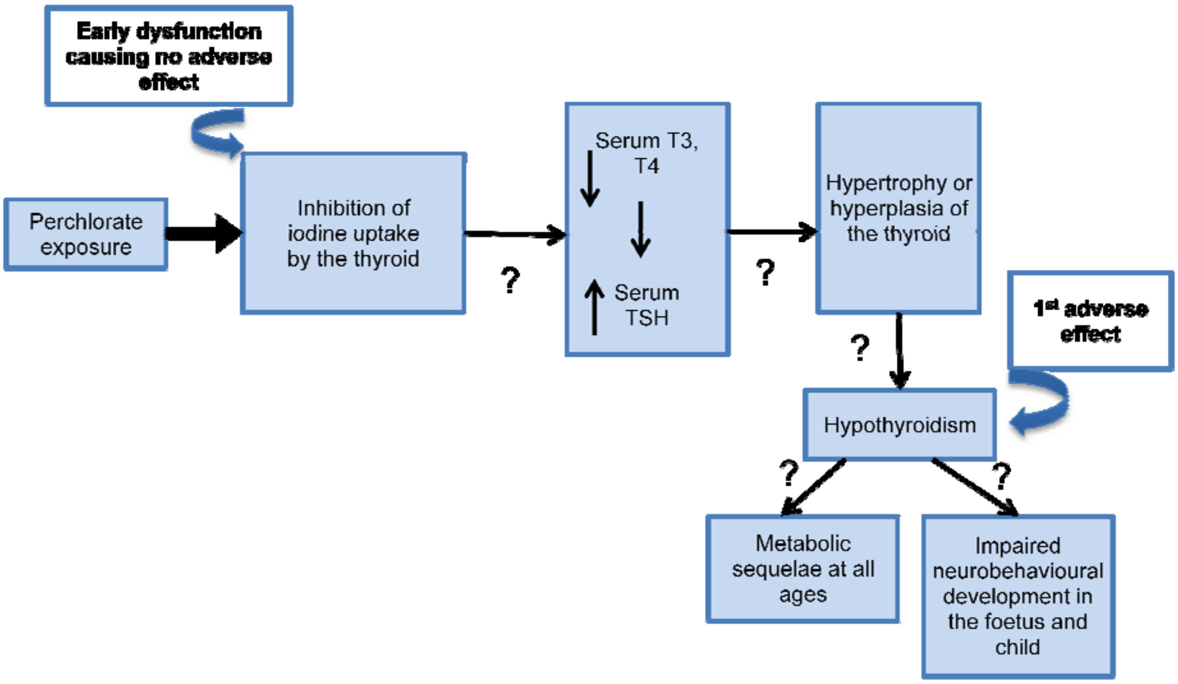
Thyroidal autoregulation takes place through transient mechanisms that provide:

- greater sensitivity in thyrocytes to the action of TSH in the event of iodine deficiency;

- blockage of iodination and secretion in the event of excess iodine (Wolff-Chaikoff effect);

- lastly, the lower the levels of iodine in the gland, the stronger and more prolonged is iodine uptake, and vice versa.

Annex 3: Mechanism of toxic action proposed for perchlorate in humans (from the National Research Council, 2005)



Annex 4: Summary of epidemiological studies relating to associations between water-related exposure to perchlorates and changes to thyroid parameters in newborns and other children (updated from ANSES's Opinion of 20 July 2012)

Authors (year of publication)	Region	Description of exposure groups	Level of perchlorate exposure from drinking water	Duration of study	Confounding factors taken into consideration	Thyroid parameters studied	Main results
Lamm and Doemland (1999)	6 counties in California and one county in Nevada (USA)	700,000 newborns	Perchlorate detected in drinking water in 7 counties	Between 1996 and 1997	-	TSH (neonatal screening for congenital hypothyroidism)	No association between detection of perchlorates in drinking water and prevalence of congenital hypothyroidism
Brechner *et al.* (2000)	2 cities in Arizona (USA): Flagstaff and Yuma	1099 newborns in Yuma (presence of perchlorates) 443 newborns in Flagstaff (absence of perchlorates)	In Yuma, in August 1999: 6 µg/L In Flagstaff: absence of detection	Between October 1994 and December 1997	age of child at time of sampling; ethnic origin	TSH T4	Association between detection of perchlorates in drinking water and increase in mean TSH levels No association between detection of perchlorates in drinking water and T4 levels
Crump *et al.* (2000)	3 towns in Chile (Taltal, Chañaral and Antofagasta)	163 school children between 6 and 8 years old 11,967 newborns	In Taltal: levels between 100 and 120 µg/L (mean = 112 µg/L) In Chañaral: levels between 5.3 and 6.7 µg/L (mean = 6.2 µg/L) In Antofagasta: absence of detection (LoD = 4 µg/L)	Study in Chilean schoolchildren in September 1999 For newborns, data from neonatal screening between February 1996 and January 1999	sex; age; urine iodine levels (for schoolchildren)	TSH Free T4	No association between the detection of perchlorates in drinking water and an increase in mean TSH levels in newborns No association between the detection of perchlorates in drinking water and TSH levels or the prevalence of goitre in schoolchildren Association between detection of perchlorates in drinking water and increase in levels of free T4 considered of no clinical relevance by the authors

Authors (year of publication)	Region	Description of exposure groups	Level of perchlorate exposure from drinking water	Duration of study	Confounding factors taken into consideration	Thyroid parameters studied	Main results
Li *et al.* (2000a)	2 cities in Nevada (USA): Las Vegas and Reno	17,308 newborns in Las Vegas (presence of perchlorates) 5882 newborns in Reno (absence of perchlorates)	In Las Vegas: over period A of 7 months, drinking water had concentrations of between 9 and 15 µg/L; over period B of 8 months, absence of detection (< 4 µg/L) In Reno, absence of detection (LD = 4 µg/L)	Between April 1998 and June 1999	birth weight; age of child at time of sampling	T4	No association between the presence of perchlorates in drinking water and mean T4 levels in newborns
Li *et al.* (2000b)	2 cities in Nevada (USA): Las Vegas and Reno	407 newborns in Las Vegas (presence of perchlorate) 133 newborns in Reno (absence of perchlorates)	*Ditto* Li *et al.* (2000a)	Between December 1998 and October 1999	age; sex	TSH	No association between the presence of perchlorates in drinking water and mean TSH levels in newborns
Li *et al.* (2001)	Towns in Clark County *versus* towns in Washoe County Towns in Clark County *versus* all towns in other counties in Nevada (USA)	176,847 patients in Medicaid programme selected on the basis of thyroid disease	Towns in Clark County: presence of perchlorates at about 8 µg/L between 1997 and 1998 Other counties in Nevada (including Washoe): absence of detection	Between January 1997 and December 1998	none	Thyroid disease (simple non-specific goitre, non-toxic nodular goitre, thyrotoxicosis with or without goitre, congenital hypothyroidism, acquired hypothyroidism, thyroiditis, thyroid cancer, other thyroid disease)	No association between the presence of perchlorates in drinking water and the prevalence of thyroid diseases in patients in the Medicaid programme
Kelsh *et al.* (2003)	Towns in southern California (USA): Redlands *versus* the other towns in San Bernardino and Riverside counties	15,090 newborns in Redlands (presence of perchlorate) 685,161 newborns in the other towns in San Bernardino and Riverside counties (absence of perchlorates)	In Redlands: between 4 and 130 µg/L in raw or treated water. A municipal report for 2001/2002 records concentrations between < 4 µg/L and 9 µg/L Other counties in San Bernardino and Riverside: absence of detection	Between 1983 and 1997	age of child at time of sampling; sex; ethnic origin; birth weight; number of pregnancies of mother; year of birth	Occurrence of congenital hypothyroidism TSH	No association between the presence of perchlorates in drinking water and the occurrence of cases of congenital hypothyroidism No association between the presence of perchlorates in drinking water and TSH levels in newborns

Authors (year of publication)	Region	Description of exposure groups	Level of perchlorate exposure from drinking water	Duration of study	Confounding factors taken into consideration	Thyroid parameters studied	Main results
Buffler *et al.* (2006)	Towns in California (USA)	50,326 newborns in 24 towns exposed to perchlorates *versus* 291,931 newborns in 287 towns not exposed to perchlorates	Towns supplied with drinking water with a perchlorate concentration > 5 µg/L *versus* < 5 µg/L	In 1998	birth weight; ethnic origin; sex; multiple pregnancy status	Occurrence of congenital hypothyroidism TSH	No association between the presence of perchlorates in drinking water and occurrence of congenital hypothyroidism No association between the presence of perchlorates in drinking water and TSH levels in newborns
Amitai *et al.* (2007)	3 towns in Israel (Ramat Hasharon, Hertzlia and Morasha)	97 newborns in Morasha (highly exposed area) 216 newborns in Ramat Hasharon (exposed town) 843 newborns in Hertzlia (non-exposed town)	In Morasha: levels > 340 µg/L In Ramat Hasharon: 42 to 94 µg/L In Hertzlia: levels < 3 µg/L	Between January and September 2004	age; sex; birth weight; duration of pregnancy	T4	No association between the presence of perchlorates in drinking water and T4 levels in Israeli newborns
Steinmaus *et al.* (2010)	Towns in California (USA)	497,458 newborns	Towns supplied with drinking water with a perchlorate concentration of > 5 µg/L *versus* < 5 µg/L	Between January and December 1998	sex; ethnic origin; birth weight; type of feeding; age of mother; income; age of child at time of sampling	TSH	Association between the presence of perchlorates in drinking water at concentrations higher than 5 µg/L and TSH levels in newborns

Authors (year of publication)	Region	Description of exposure groups	Level of perchlorate exposure from drinking water	Duration of study	Confounding factors taken into consideration	Thyroid parameters studied	Main results
Cao *et al.* (2010)	Pennsylvania (USA)	92 children aged less than 1 year	Determination of perchlorates in urine in 206 samples	Second half of 2004	Age, geographical origin, body mass index, sex, type of feeding (breast milk, bottle with reconstituted cow's milk, bottle with reconstituted soy milk)	TSH T4	Using the multivariate linear mixed model taking into account all goitrogenic anions, associations were found between urine thiocyanates or urine nitrates and TSH or T4 but not between urine perchlorates and TSH or T4 Nevertheless, there was an association between urine perchlorates and TSH in children with urine iodine levels below 100 µg/L (however, no association for T4)
Leung *et al.* (2012)	Boston (USA)	64 mothers and their children aged from 1 to 3 months	Determination of perchlorates in maternal urine (median: 3.1 µg/L; range: [0.2-22.4]); in infant urine (median: 4.7 µg/L; range: [0.3-25.3]) and in breast milk (median: 4.4 µg/L; range: [0.5-29.5])	2008-2011	Age of mother, geographic origin, smoking status, use of iodine food supplements and infant food supplementation	TSH Free T4	No association between the levels of perchlorates, iodides and thiocyanates in breast milk, maternal urine and infant urine on the one hand and serum concentrations of TSH and free T4 in children on the other hand

Annex 5: Summary of epidemiological studies relating to associations between water-related exposure to perchlorates and changes to thyroid parameters in pregnant women, women of childbearing age and other adults (updated from ANSES's Opinion of 20 July 2012).

Authors (year of publication)	Region	Description of exposure groups	Level of perchlorate exposure from drinking water	Duration of study	Confounding factors taken into consideration	Thyroid parameters studied	Main results
Téllez Téllez *et al.* (2005)	3 towns in Chile (Taltal, Chañaral and Antofagasta)	60 pregnant women per town seen for three medical consultations (2 before delivery and one after)	In Taltal: levels between 72 and 139 µg/L (mean = 114 µg/L) In Chañaral: levels between 4.7 and 7.3 µg/L (mean = 5.8 µg/L) In Antofagasta: absence of detection (LD = 4 µg/L)	Between November 2002 and April 2004	urine iodine; anti-thyroid peroxidase antibodies; anti-thyroglobulin antibodies	T3 Free T4 TSH Tg	No association between the presence of perchlorates in drinking water and levels of thyroid parameters evaluated in pregnant women No association between the presence of perchlorates in drinking water and birth weight, head circumference or length in newborns
Blount *et al.* (2006)	USA	1188 men and 1111 women aged over 12 years included in the American NHANES 2001-2002 survey	Estimation of exposure by measuring the urine concentration of perchlorates	2001-2002	age, serum albumin; serum cotinine; estimated total caloric intake; pregnancy status; menopausal status; premenstrual status; C-reactive protein; duration of fasting; body mass index; urine creatinine; urine iodine; urine nitrate; urine thiocyanate; ethnic origin; medication intake	T4 TSH	No association between urine perchlorate concentration and serum concentrations of TSH and T4 in men Association between urine perchlorate concentration and serum TSH and T4 concentration in women with urine iodine < 100 µg/L No association between urine perchlorate concentrations and serum T4 concentrations but association with serum TSH in women with urine iodine > 100 µg/L

Authors (year of publication)	Region	Description of exposure groups	Level of perchlorate exposure from drinking water	Duration of study	Confounding factors taken into consideration	Thyroid parameters studied	Main results
Steinmaus *et al.* (2007)	USA	1109 women over the age of 12 included in the American NHANES 2001-2002 survey including 385 with urine iodine levels below 100 µg/L	Estimation of exposure by measuring the urine concentration of perchlorate	2001-2002	Age, geographical origin, iodine status, serum albumin, body mass index, caloric intake, pregnancy status, menopause status, premenstrual status, C-reactive protein, duration of fasting, urine nitrate, breastfeeding status, medication intake, smoking status, urine iodine, urine cotinine, urine thiocyanate	T4 TSH	Association between urine perchlorate and T4 concentration in women with urine iodine below 100 µg/L but no association in women with urine iodine higher than 100 µg/L. This association was stronger in smokers than in non-smokers (coefficient of interaction between perchlorates and tobacco: β = 1.12; p=0.008)
Gibbs *et al.* (2008)	Chile	Observations from the study by Téllez Téllez *et al.* (2005) for three towns carried out during 3 medical examinations were pooled (i.e. 202 measurements of free T4 in 149 pregnant women and 220 measurements of TSH in 155 pregnant women)	Estimation of exposure by measuring urine perchlorate concentration	Between November 2002 and April 2004	age, weeks of pregnancy, tobacco consumption, urine iodine, urine creatinine	Free T4 TSH	No association between urine perchlorate concentration and free T4 or TSH in pregnant women for urine iodine levels lower or higher than 100 µg/L (only 17 TSH results and free T4 results concerned pregnant women with urine iodine levels lower than 100 µg/L)

Authors (year of publication)	Region	Description of exposure groups	Level of perchlorate exposure from drinking water	Duration of study	Confounding factors taken into consideration	Thyroid parameters studied	Main results
Pearce *et al.* (2010)	Cardiff (UK) and Turin (Italy)	1st sub-cohort: 374 pregnant women in Cardiff and 261 women in Turin who all had high TSH serum concentrations and low serum T4 levels at 1st trimester 2nd sub-cohort: 480 euthyroid women in Cardiff and 526 euthyroid women in Turin	Estimation of exposure by measuring urine perchlorate concentration	2002-2006	anti-thyroid peroxidase antibodies; thiocyanate and nitrate urine concentrations	Free T4 TSH	No association in the two sub-cohorts between urine perchlorate concentration and serum free T4 or TSH levels in pregnant women at the 1st trimester of pregnancy, even when urine iodine level was < 100 µg/L
Pearce *et al.* (2011)	Los Angeles (USA) and Cordoba (Argentina)	134 pregnant women in the 1st trimester of pregnancy in Los Angeles 107 pregnant women in the 1st trimester of pregnancy in Cordoba	Estimation of exposure by measuring urine perchlorate concentration (median of 7.8 µg/L in Los Angeles and 13.5 µg/L in Cordoba)	2004-2007	urine iodine; anti-thyroid peroxidase antibodies; duration of pregnancy; urine creatinine	TSH Free T4 index total T3	No association in either city between urine perchlorate concentration and thyroid parameters studied in pregnant women in the 1st trimester of pregnancy, even when urine iodine level was < 100 µg/L
Schreinemachers (2011)	USA	1010 men and 1084 women, aged from 6 to 85 years from the American NHANES 2001-2002 study	Estimation of exposure by measuring urine perchlorate concentration	2001-2002	Urine iodine level, age, urine creatinine, geographic origin, body mass index, urine cotinine, poverty index, duration of fasting, urine thiocyanate, urine nitrate, caloric intake, C-reactive protein, alcohol consumption, medication intake	Indirect indicators according to the author (levels of haemoglobin, haematocrit and high-density lipoproteins)	A decrease in haemoglobin and haematocrit levels was associated with the urine perchlorate concentration in boys, men, women aged 15-49 years and pregnant women. A decrease in high-density lipoprotein levels was observed in men

Authors (year of publication)	Region	Description of exposure groups	Level of perchlorate exposure from drinking water	Duration of study	Confounding factors taken into consideration	Thyroid parameters studied	Main results
Gold *et al.* (2013)	Sacramento (USA)	814 women living for at least 6 months on one of three sites selected according to a history of perchlorate contamination	Estimation of exposure by measuring urine perchlorate concentration in 178 women	2001-2007	Age, geographical origin, economic hardship, health insurance, annual income, employment, marital status, smoking, physical activity, body mass index, family history of health problems	TSH Free T4 Thyroid diseases (hyperthyroidism, hypothyroidism, medication intake)	No association for the 3 sites between urine perchlorate concentration and the thyroid parameters evaluated
Mendez and Eftim (2012)	USA	970 men and 907 women from the American NHANES 2007-2008 study	Estimation of exposure by measuring creatinine-adjusted urine perchlorate concentration	2007-2008	Age, geographical origin, poverty index, smoking, body mass index, serum cotinine, past thyroid disease, anti-thyroglobulin antibodies, anti-thyroid peroxidase antibodies, medication intake, urine iodine levels, urine nitrate, urine thiocyanate, urine phthalate	TSH Free and total T4 Free and total T3	Associations between urine perchlorate concentrations and serum levels of free T4, total T4 and total T3 in men and women

Authors (year of publication)	Region	Description of exposure groups	Level of perchlorate exposure from drinking water	Duration of study	Confounding factors taken into consideration	Thyroid parameters studied	Main results
Pearce *et al.* (2012)	Athens (Greece)	134 pregnant women recruited in a hospital in Athens	Estimation of exposure by measuring urine perchlorate concentration (median: 4.1 µg/L; range: 0.2 – 118.5 µg/L)	2008-2010	Urine thiocyanate, urine iodine levels, anti-thyroid peroxidase antibodies, duration of pregnancy and age of the mother	TSH Free T4 Free T3	No correlation between urine concentration of perchlorates and serum TSH but inverse correlation with free T4 and free T3 by Spearman's correlation test No association between urine perchlorate concentration and thyroid parameters evaluated after taking confounding factors into account by multiple linear regression
Bruce *et al.* (2013)	USA	833 men and 711 women from the American NHANES 2001-2002 study	Estimation of exposure by measuring perchlorate equivalent concentration in urine (PEC approach, taking into account perchlorates, thiocyanates, nitrates and iodides)	2001-2002	Age, duration of fasting, serum albumin, serum thyroglobulin, anti-thyroid peroxidase antibodies in serum, body mass index, caloric intake, serum cotinine, C-reactive protein level, urine creatinine, geographic origin and use of medication	TSH Free and total T4 Free and total T3	Weak association between PEC (perchlorate equivalent concentration) in urine and total T4 but no association between urine PEC and TSH, free T4, total T3 and free T3

Authors (year of publication)	Region	Description of exposure groups	Level of perchlorate exposure from drinking water	Duration of study	Confounding factors taken into consideration	Thyroid parameters studied	Main results
Steinmaus *et al.* (2013)	USA	491 men and 516 women from the American NHANES 2007-2008 study	Group A (n = 390): lower tertile for urine perchlorates, lower tertile for urine thiocyanates and urine iodine ≥ 100 µg/L Group B (n = 553): Middle tertile for urine perchlorates, middle tertile for urine thiocyanates and urine iodine ≥ 100 µg/L Group C (n = 64): Upper tertile for urine perchlorates, upper tertile for urine thiocyanates and urine iodine < 100 µg/L	2007-2008	Age, sex, urine specific gravity, urine thiocyanate, urine iodine levels, urine nitrate, pregnancy status, menopause status, premenstrual status, breastfeeding status, obesity, medication intake, personal history regarding thyroid problems, anti-thyroglobulin antibodies, anti-thyroid peroxidase antibodies, caloric intake, fasting duration, albumin, geographical origin, level of education and professional income	TSH Free and total T4	Significant difference in the levels of free T4 between individuals from groups A and C (mean difference of 0.058 µg/dl; 95% CI [0.012-0.104] or a difference of about 7% Significant difference in the levels of total T4 between individuals from groups A and C (mean difference of 1.07 µg/dl; 95% CI [0.14-0.65] or a difference of about 13%

Annex 6: Map produced by the Nord-Pas de Calais ARS of perchlorate levels in the public water supply in this region