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PERCHLORATE

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1. EXPLANATION

The perchlorate ion (ClO_4^-) is very stable in water, and its salts are highly soluble in water. Perchlorate occurs naturally in the environment, in deposits of nitrate and potash, and can be formed in the atmosphere and precipitate into soil and groundwater (Dasgupta et al., 2005; Rao et al., 2007). It also occurs as an environmental contaminant arising from the use of nitrate fertilizers (Susala et al., 1999) and from the manufacture, use and disposal of ammonium perchlorate (Chemical Abstracts Service [CAS] No. 7790-98-9) used in rocket propellants, explosives, fireworks, flares and air-bag inflators and in other industrial processes. Perchlorate can be formed during the degradation of sodium hypochlorite, used for disinfection of drinking-water and water for food processing, if it is stored for long periods and under less than optimal conditions, including high temperature, storage in the light and storage in containers that contain small amounts of old hypochlorite (Greiner et al., 2008). Water, soil and fertilizers are considered to be potential sources of perchlorate contamination in food. Potassium perchlorate (CAS No. 7778-74-7) has been used as a human therapeutic medicine to treat thyroid disease.

Perchlorate has not been previously evaluated by the Committee. It was referred to the Committee for evaluation on request of the Second Session of the Codex Committee on Contaminants in Food (FAO/WHO, 2008).

The health effects of perchlorate salts are due to the perchlorate ion itself and not the other components.

2. BIOLOGICAL DATA

2.1 Biochemical aspects

2.1.1 Absorption, distribution and excretion

(a) Rats

Following oral administration of radiolabelled perchlorate to the rat, peak blood concentrations are reached by 3 h. Half-lives in the blood range from less than 8 h to about 20 h. Perchlorate is actively transported into the thyroid gland and concentrated in the lumen of the gland, with a peak uptake at 4–6 h. Its relative distribution between thyroid follicular cell, stroma and lumen is comparable with that obtained for iodine (Wolff, 1998). Nonspecific binding of perchlorate to albumin and prealbumin in plasma is high, but it does not interfere with the binding of thyroid hormones to plasma thyroxine binding globulin (TBG) (Merrill et al., 2003). Nonspecific binding to plasma proteins appears to be saturated at doses of perchlorate between 1.0 and 10.0 mg/kg body weight (bw) per day (Clewell et al., 2003a).

In the rat, clearance of perchlorate from serum is biphasic, with a slow terminal phase. The proportion of a dose of perchlorate that is sequestered in the thyroid over a 4 h time period is inversely proportional to the dose and ranges from 1% for a dose of 10.0 mg/kg bw per day to 11% for a dose of 0.5 mg/kg bw per day (Yu et al., 2002).

Perchlorate crosses the placenta and has been detected in rat maternal milk and in the serum, gastrointestinal contents and skin of neonatal rats (Clewell et al., 2003a,b). Perchlorate is concentrated not only in the thyroid gland, but also in the gastrointestinal contents, skin, mammary gland and milk (Clewell et al., 2004).

(b) Humans

In a study in human volunteers, the serum half-life of perchlorate given at 0.5 mg/kg bw per day was 6.0–9.3 h (average 8.1 h) (Greer et al., 2002). Later, Crump & Gibbs (2005) recalculated the serum half-life in this study as averaging 7.5 h. A study of two workers occupationally exposed to perchlorate indicated elimination half-lives of 7.9 and 8.2 h from measurements of urinary perchlorate (Lamm et al., 1999).

Perchlorate is found in human serum, plasma, urine, saliva and breast milk (Kirk et al., 2005, 2007; Pearce et al., 2007; Dasgupta et al., 2008; Kannan et al.,

2009; Leung et al., 2009; Oldi & Kannan, 2009a,b). Details are given below in section 2.3.1.

2.1.2 Biotransformation

Very little of the perchlorate ion is metabolized in the rat or human, and more than 90% is excreted unchanged in the urine (see reviews by Wolff, 1998; Fisher et al., 2000). In a rat study, more than 99.5% of radiolabelled perchlorate was recovered in the urine over 48 h (Yu et al., 2002).

2.1.3 Effects on enzymes and other biochemical parameters

As the thyroid is the critical target organ for perchlorate toxicity, the effects of perchlorate on hormones released by the hypothalamic–pituitary–thyroid axis, which can be measured in blood, are described in section 2.2, rather than in this section.

2.2 Toxicological studies

2.2.1 Acute toxicity

A dietary concentration of 3.55% (35 500 mg/kg diet, approximately 3621 mg/kg bw per day as perchlorate) was reported as the median lethal dose (LD_{50}) for potassium perchlorate in mice exposed for up to 30 days. The first deaths occurred within 4 days of the start of treatment (Gauss, 1972).

2.2.2 Short-term studies of toxicity

Four groups of rats, each comprising six females weighing 220–240 g at the start of treatment and 280–295 g at the end, were given 1) perchlorate alone for 8 weeks, 2) perchlorate plus 4 µg of thyroxine (T_4) daily by intraperitoneal injection, 3) perchlorate plus 50 µg of estradiol by intramuscular injection for the last 5 days of treatment or 4) perchlorate plus T_4 by intraperitoneal injection and estradiol by intramuscular injection. Perchlorate was administered in the drinking-water at a concentration of 400 mg/100 ml. Assuming that the rats consumed 33–40 ml of drinking-water per day, the daily exposure to perchlorate was equivalent to about 600–750 mg/kg bw per day. Another four control groups, each with six rats per group, were given 1) tap water only, 2) distilled water in combination with T_4, 3) distilled water in combination with estradiol or 4) distilled water in combination with T_4 plus estradiol, administered as above. At the end of the 8-week treatment period, the rats were injected intraperitoneally with radiolabelled iodine 1 h before sacrifice. The mammary glands were examined for iodine uptake and histology. In the rats given perchlorate only, iodine uptake was reduced by 52% compared with controls; the mammary glands were of normal weight but histologically showed mild atrophy, some atypia of the lobular epithelium and scattered foci of hyperplastic activity. Co-administration of T_4 did not mitigate the effects of perchlorate on iodine uptake; if anything, mammary dysplasia was slightly enhanced. Co-administration of estradiol reduced the effect of perchlorate on iodine uptake by the mammary gland and reduced the extent of atypical changes (Eskin et al., 1975).

In a comprehensive 90-day study, Sprague-Dawley rats (20 or 30 of each sex per group) were given ammonium perchlorate in the drinking-water at doses of 0, 0.01, 0.05, 0.2, 1.0 and 10.0 mg/kg bw per day. The animals were approximately 7 weeks of age at the start of treatment. Animals in each group were killed after either 14 or 90 days of treatment; in the 0.05, 1.0 and 10.0 mg/kg bw per day groups, some were killed after a recovery period of 30 days. Analytical chemistry confirmed the absence of nitrate in the drinking-water, the stability of the perchlorate in drinking-water and that the concentrations were within ±10% of the target concentrations. Ten rats of each sex per group were sacrificed at each time point. Body weights, food consumption and water consumption were measured weekly. Estrous cycles were examined in females by daily vaginal smears taken during the 3 weeks before scheduled sacrifice. Sperm samples were taken from males at sacrifice for measurement of sperm count, motility and morphology. Ophthalmology was conducted just prior to sacrifice. Blood samples were taken at sacrifice for haematology and clinical chemistry, thyroid stimulating hormone (TSH), triiodothyronine (T_3) and T_4. All animals were subjected to gross necropsy, and a complete set of tissues and organs was preserved for histological examination. In the control and highest-dose groups, all tissues and organs were examined microscopically, including bone marrow samples for micronucleus formation. Liver, kidneys, lungs, thyroids and gross lesions were examined microscopically in the other four dose groups. There were no effects of treatment on body weight, food consumption, water consumption, survival, clinical observations, haematology, clinical chemistry, ophthalmology, estrous cycles, sperm parameters or bone marrow micronucleus formation. There were no gross or histopathological changes in any tissues or organs, apart from the thyroid. Absolute and relative thyroid weights were significantly increased in the 10.0 mg/kg bw per day group after 14 or 90 days of treatment in males and after 90 days in females, but did not differ from control values in either sex after the 30-day recovery period. Both sexes in the top dose group also showed minimal- to mild-grade increases in thyroid follicular cell hypertrophy, microfollicle formation and colloid depletion at both time points, but the changes reached moderate grade in the males only after 14 days of treatment, and there were no differences from controls after the 30-day recovery period. The authors noted that the histopathological changes observed in the thyroid were similar to those reported by others in rats following the feeding of iodine-deficient diets or inhibition of iodine organification. There were no effects on thyroid weights or histology in any other treatment group. After 14 days of treatment, dose-related increases in TSH concentrations were observed at all dose levels in both males and females; the increases were statistically significant in males from 0.2 mg/kg bw per day and in females from 0.05 mg/kg bw per day. T_4 was significantly reduced in males and females at the top dose of 10.0 mg/kg bw per day, and T_3 was significantly reduced at all doses in males but not at any dose in females. After 90 days of treatment, TSH was significantly increased from 0.2 mg/kg bw per day in males, but only at 10.0 mg/kg bw per day in females. T_3 and T_4 were significantly decreased in a dose-related manner at all doses in both sexes. At the end of the 30-day recovery period, at the doses examined (0, 0.5, 1.0 and 10.0 mg/kg bw per day), TSH and T_3 were unaffected in males, whereas T_4 was significantly reduced at all doses; in the females, TSH was significantly increased at all doses, T_4 was reduced, but only

at 10.0 mg/kg bw per day, and T_3 was unaffected. The authors concluded that 1.0 mg/kg bw per day represented the overall no-observed-adverse-effect level (NOAEL) for the study; they considered that the effects on TSH and thyroid hormones seen at lower doses were not adverse effects, as they were unaccompanied by any changes in thyroid weight or histology, and the changes seen at 10.0 mg/kg bw per day were reversible (Siglin et al., 2000).

It should be noted that this study did not establish a no-observed-effect level (NOEL) for effects of perchlorate on the hypothalamic–pituitary–thyroid axis, as reductions in thyroid hormone concentrations were observed in both sexes at all dose levels after 90 days of treatment. The dose of 0.01 mg/kg bw per day may be a lowest-observed-effect level (LOEL) for thyroid hormone changes. However, no effects on thyroid growth or histopathology were seen at doses less than or equal to 1.0 mg/kg bw per day, whereas the dose of 10.0 mg/kg bw per day increased thyroid weight and caused follicular hypertrophy, microfollicle formation and colloid depletion.

A study in male Fischer 344 rats investigated the effect of ammonium perchlorate administered in the drinking-water on serum TSH, T_4 and T_3. Groups of 10 rats, aged 7 weeks, were exposed in the drinking-water to the nominal concentrations of ammonium perchlorate of 0, 0.1, 1.0 and 10 mg/l for 7 days. From data on body weight and water consumption, these concentrations were calculated to be equivalent to ammonium perchlorate doses of 0, 0.024, 0.205 and 1.170 mg/kg bw per day. There was no effect at any dose on TSH, T_4 or T_3. Histological examination of the thyroid gland showed colloid depletion, hypertrophy and hyperplasia of follicular epithelial cells in the high dose group only (Khan et al., 2005). This study indicates a NOEL of 1.170 mg/kg bw per day for effects on thyroid hormones and a NOEL of 0.205 mg/kg bw per day for effects on thyroid histology following short-term (7 days) dosing of ammonium perchlorate. The observation of effects on thyroid histology, unaccompanied by changes in thyroid hormone levels, is in contrast to the findings in most other studies on perchlorate.

A study in male Sprague-Dawley rats, with the objective of investigating the effects of a combination of perchlorate and a pentachlorobiphenyl (PCB 126) on serum TSH, free T_4, total T_4 and thyroid histology, included groups given perchlorate alone. In a first experiment, groups of 16 rats, weighing an average of 216 g, were given ammonium perchlorate in the drinking-water for 14 days at concentrations of 0, 0.09, 0.9 and 9.0 mg/l, to achieve target perchlorate doses of 0, 0.01, 0.1 and 1.0 mg/kg bw per day. In a second experiment, groups of eight rats, weighing an average of 250 g, were given perchlorate at 0.01 mg/kg bw per day in the drinking-water for 1 or 4 days. Animals were killed at the end of the dosing period. There were no effects on body weight, food consumption or water consumption in either experiment. In the first experiment, there was a dose-related increase in TSH, which was statistically significant at 0.1 and 1.0 mg/kg bw per day. Free T_4 and total T_4 were both significantly reduced at 1.0 mg/kg bw per day. In the second experiment, there were no effects on TSH or thyroid hormones. There were no effects on thyroid weight or histology at any dose in either experiment (McLanahan et al., 2007). This study indicates a NOEL of 0.01 mg/kg bw per day for effects on thyroid hormones following short-term (14 days) dosing of perchlorate.

2.2.3 Long-term studies of toxicity and carcinogenicity

The information on carcinogenicity has been summarized by ATSDR (2008). The original studies were not reviewed by this Committee.

Potassium and sodium perchlorates have been shown to produce thyroid tumours (papillary and/or follicular adenomas and/or carcinomas) in rats and mice with long-term exposure (1–24 months) to 1–1.2% concentrations in the feed (10 000–12 000 mg/kg) or drinking-water (10 000–12 000 mg/l) (Kessler & Kruskemper, 1966; Gauss, 1972; Florencio Vicente, 1990; Fernandez-Rodriguez et al., 1991; Pajer & Kalisnik, 1991; Toro Guillen, 1991). Estimated perchlorate doses in these studies ranged from 928 to 2573 mg/kg bw per day. The cancer effect levels from these studies were around 1000 mg/kg bw per day.

The incidence of Ki-*ras* oncogene mutations was determined in follicular cell carcinomas of the thyroid induced by administration of radiolabelled iodine and potassium perchlorate (1%, or 10 000 mg/l, in drinking-water) for up to 18 months. Direct sequencing showed no mutations in the amplified gene segment of any of the induced thyroid tumours. The results suggested that Ki-*ras* activation via mutations at codons 12 and 13 is neither a constant event nor an early event in the development of rat thyroid follicular cell carcinoma (Fernández-Santos et al., 2004).

Low-level exposure to potassium perchlorate (0.1%, or 1000 mg/kg, in the feed, corresponding to a perchlorate dose of 64 mg/kg bw per day) for 19 weeks was shown to promote the development of thyroid tumours initiated by *N*-bis-(2-hydroxypropyl)nitrosamine (Hiasa et al., 1987).

2.2.4 Genotoxicity

The limited information on genotoxicity has been summarized by ATSDR (2008). The original reports were not available to the Committee for this review.

Magnesium perchlorate was negative in a test for SOS-inducing activity in *Salmonella typhimurium* strain TA1535 (Nakamura & Kosaka, 1989) and in a test for production of deoxyribonucleic acid (DNA)–protein crosslinks in cultured human lymphocytes (Costa et al., 1996). No evidence of mutagenicity for ammonium perchlorate with or without metabolic activation was found in six different *Salmonella* strains (Zeiger, 1998a). Ammonium perchlorate was not mutagenic in the mouse lymphoma assay with or without metabolic activation (San & Clarke, 1999).

No evidence of bone marrow erythrocyte micronucleus formation was found in male or female rats as a result of exposure to ammonium perchlorate in the drinking-water at a perchlorate dose of 8.5 mg/kg bw per day for 90 days (Siglin et al., 2000). No increase was reported in micronucleus formation in bone marrow from mice injected intraperitoneally with ammonium perchlorate at a dose of 500 mg/kg bw per day for 3 consecutive days; higher doses were lethal to the mice (Zeiger, 1998b).

2.2.5 Reproductive and developmental toxicity

(a) Multigeneration reproductive toxicity

In a two-generation study, Sprague-Dawley rats (30 of each sex per group) were given ammonium perchlorate in their drinking-water at target doses of 0, 0.3, 3.0 and 30.0 mg/kg bw per day. Age at commencement of dosing and period of dosing before cohabitation for the parental generations were not stated. Doses exceeded target doses by 33–34% in female parental generations during gestation due to a higher water consumption. The F_1 generation was given the same target doses of ammonium perchlorate as their respective parental (P_1) generation, beginning at weaning and continuing until the day of sacrifice. Adult F_1 animals selected to form the P_2 generation were mated (age at cohabitation not stated), and the P_2 females were continued on ammonium perchlorate until they were sacrificed at the same time as their F_2 offspring on day 21 of lactation. Standard reproductive parameters were evaluated. Blood samples were taken for determination of serum TSH, T_3 and T_4 levels, and histopathological examination was conducted on major tissues, including the thyroid of P_1, F_1 and F_2 animals. The authors reported significant increases in lactation index at 3.0 and 30.0 mg/kg bw per day in the P_1 animals and significant increases in fertility index in all dosed F_1 (P_2) groups compared with controls. There was an increased percentage of stillborn pups at 30.0 mg/kg bw per day in the litters born to the F_1 (P_2) generation (2.5% compared with 0.3% in controls). In F_1 generation adults, relative thyroid weights were significantly increased in all dose groups for female rats and in the 3.0 and 30.0 mg/kg bw per day dose groups for male rats. Histopathological changes in the thyroid in P_1 adults, F_1 pups, F_1 adults and F_2 pups consisted of follicular cell hypertrophy and hyperplasia that increased in incidence and severity in a dose-related manner, reaching statistical significance at 3.0 and 30.0 mg/kg bw per day. TSH and thyroid hormone levels were variable, with some statistically significant changes, not all of which were dose related. TSH levels were clearly and significantly increased at the top dose of 30.0 mg/kg bw per day in the adult P_1 and F_1 animals. The authors concluded that perchlorate is not a reproductive toxicant in rats when administered in the drinking-water at doses up to 30.0 mg/kg bw per day, but it can affect the thyroid at doses greater than or equal to 3.0 mg/kg bw per day. Based on these findings, 0.3 mg/kg bw per day was identified as the NOAEL (York et al., 2001a). It should be noted that this study reported effects on thyroid histology at a dose lower than that affecting serum TSH levels.

In a single-generation study, pregnant Sprague-Dawley rats were given perchlorate at either 0 or 1 mg/kg bw per day in the drinking-water beginning on gestation day (GD) 2 and continuing until lactation day 10. A proportion of the control and exposed dams were sacrificed on GD 20 (number not stated). The remainder were allowed to litter out. Some litters from control and treated dams were cross-fostered immediately after parturition. Other control and exposed dams were allowed to rear their own litters. This resulted in four offspring treatment groups: 1) true control pups born to and reared by their own mothers given untreated water during both gestation and lactation; 2) true exposed pups born to and reared by their own mothers given perchlorate during both gestation and lactation; 3) in utero

exposed pups born to mothers given perchlorate during gestation and reared by foster mothers given untreated water during lactation; and 4) lactational exposed pups born to mothers given untreated water during gestation and reared by foster mothers given perchlorate during lactation. The aim was to achieve target group sizes of eight dams and their litters per treatment group; in practice, because of non-pregnancies, the number was reduced to six or seven. Litters were culled to eight pups: four males and four females. Dams and pups were sacrificed on postnatal day (PND) 10. In both experiments, serum and thyroids were taken from the dams at sacrifice, and serum was taken from fetuses or pups and pooled within litters for analysis of perchlorate, TSH, T_3 and T_4. The calculated average daily doses of perchlorate consumed by the foster and donor dams were 1.09 and 0.97 mg/kg bw per day, respectively. Maternal body weight was not affected by treatment. Dams and fetuses exposed to perchlorate from GD 2 to GD 20 had serum perchlorate levels of 0.71 µg/ml and 0.38 µg/ml, respectively. TSH was significantly increased in perchlorate-treated dams and fetuses compared with controls; T_3 levels were significantly reduced in treated dams, but not in treated fetuses; T_4 levels were significantly reduced in treated dams and fetuses. In the cross-fostering part of the study, maternal body weight was not affected by treatment. Pup body weights were reduced in both in utero and lactational exposure groups that were cross-fostered, compared with true control or true exposure groups reared by their own mothers. Dams in the true exposure group and in the lactational exposure group had serum perchlorate levels of 0.41 µg/ml and 0.51 µg/ml, respectively. Pups in the true exposure group and in the lactational exposure group had similar serum perchlorate levels, averaging 0.50–0.55 µg/ml. TSH levels were significantly increased in both the dams and pups in the true exposure group and in the lactational exposure group, but T_3 levels were not significantly affected. T_4 levels were significantly reduced in female pups exposed to perchlorate only during lactation compared with true control pups and pups exposed to perchlorate only during gestation. T_4 levels in male pups were not affected by perchlorate (Mahle et al., 2003). It should be noted that this study did not control for the procedure of cross-fostering itself; the influence of fostering, aside from any other treatment parameters, can be seen in the reduced body weights of cross-fostered litters.

A study using 64 male and 66 female adult prairie voles and 39 male and 39 female adult deer mice compared the effects of giving perchlorate in water (PCW) with the effects of giving a similar dose of perchlorate in food (PCF). Controls received no perchlorate. For food exposure, soya bean plant matter grown in perchlorate-contaminated irrigation water was incorporated into laboratory chow. Animals were treated for 21 days before pairing, and treatment continued during the period of pairing, pregnancy and lactation. Females producing pups reared their offspring until PND 21, when adults and offspring were sacrificed. Reproductive success, tissue perchlorate concentrations, plasma T_3 and T_4 levels and thyroid histopathology were assessed. The doses of perchlorate received by prairie voles were calculated as follows: before pairing, 0.71 and 0.55 mg/kg bw per day, and during cohabitation, 0.32 and 0.40 mg/kg bw per day, in PCF and PCW groups, respectively. The doses of perchlorate received by deer mice were calculated as follows: before pairing, 1.120 and 0.876 mg/kg bw per day, and during cohabitation, 1.115 and 1.043 mg/kg bw per day, in PCF and PCW groups, respectively. There

were some deaths in both species, attributed to stress. Parental and offspring group sizes for each end-point measured ranged from 13 to 44. Both prairie voles and deer mice receiving perchlorate in water or food tended to have lower reproductive success, in terms of litters born, than controls, with no difference between PCW and PCF groups. Other reproductive end-points, such as number of pups born, birth weight and pup survival to PND 21, showed no significant differences between treated and control groups. Perchlorate was detected in only a few samples of liver and kidney, and the authors considered that their method of analysis was not sensitive enough to detect the likely tissue concentrations. In prairie voles, there were no significant differences in T_3 concentrations between PCW, PCF and control groups; T_4 concentrations were significantly reduced in the PCF group and significantly increased in the PCW group, compared with controls, but only in paired males. In deer mice, T_3 concentrations were significantly decreased compared with controls in PCF unpaired males but not in PCF unpaired females or paired males or in PCW groups; T_4 concentrations were significantly increased in paired PCF males compared with controls, but not in unpaired PCF males or unpaired females and not in PCW groups. Thyroid epithelial cell heights in prairie voles were significantly increased in PCW and PCF parents, both before pairing and after pairing; the offspring were unaffected. There were no effects on colloidal area. In deer mice, there were no effects on thyroid histology. The authors concluded, on the basis of the thyroid histology results in prairie voles, that the trends observed suggest that perchlorate exposure via water may result in slightly greater effects compared with exposure to perchlorate via food (Smith et al., 2006). It should be noted that the effects were slight, and the overall results do not allow a conclusion with respect to perchlorate given in water compared with food.

The effects of in utero and lactational exposure to ammonium perchlorate on developing deer mice were evaluated. Forty breeding pairs were allowed to mate and produce three litters, which were weaned (first and third litters) or killed (second litter) at PND 21. The breeding pairs were dosed continuously with ammonium perchlorate at 0, 1 nmol/l, 1 µmol/l or 1 mmol/l in drinking-water (10 pairs per treatment group), from initial cohabitation until weaning of the pups in the third litter. These concentrations were equivalent to 117 ng/l, 117 µg/l and 117 mg/l, but as neither the amounts of water consumed nor body weights were stated, the dose in relation to body weight cannot be calculated. Pups from the second litter were used for evaluation. Litter size, offspring survival, body weights and organ weights (ovary, testes, kidney, liver, adrenals and heart) were measured. Litter size and percentage survival were not significantly different among treatments, and no other significant differences were observed in any analysis performed when litter means were used in statistical analysis. The authors described a number of significant findings in body weights, liver weights and heart weights when statistical analysis was performed on individual pup data (Thuett et al., 2002a). The Committee notes that the appropriate units for statistical evaluation of reproduction studies are the breeding pair and litter, not individual pups.

A related publication from the same laboratory reported on examinations of thyroid histology and plasma T_3 and T_4 concentrations in the second-litter pups at PND 21 from the study described above. The 1 nmol/l and 1 mmol/l treatment

groups had significantly fewer active follicles per unit area than did controls. The 1 mmol/l treatment group also had significantly fewer active follicles than did the 1 µmol/l and the 1 nmol/l treatment groups. Total T_4 concentrations were significantly increased in the 1 nmol/l and 1 µmol/l groups compared with the controls. No significant difference was observed in total T_3 concentrations. The authors commented that, in contrast to the situation in adult rodents, it appears that ammonium perchlorate increases thyroid hormone production in developing deer mice and produces variable effects with increasing concentrations (Thuett et al., 2002b). It should be noted that as there was no dose–response in either T_4 or thyroid histology changes, an alternative conclusion is that a treatment-related effect has not been established.

(b) Developmental toxicity

A developmental toxicity study was conducted in Sprague-Dawley rats given ammonium perchlorate in the drinking-water at target doses of 0, 0.01, 0.1, 1.0 and 30.0 mg/kg bw per day, beginning 14 days before cohabitation and continuing through sacrifice on GD 21. There were 25 mated rats per group. The actual consumed doses averaged 0, 0.01, 0.08, 0.70 and 21.69 mg/kg bw per day during the 2-week pre-cohabitation period and 0, 0.01, 0.10, 0.96 and 28.27 mg/kg bw per day during the 3-week gestation period. The rats were sacrificed on GD 21, and the fetuses were examined for visceral and skeletal alterations. An additional 16 litters per group were sacrificed on GD 21 for measurement of maternal and fetal serum TSH, T_3 and T_4 levels and thyroid histopathology. There were no effects on maternal body weight, feed consumption or water consumption. There were no treatment-related effects on reproductive or fetal parameters apart from small, but statistically significant, reductions in mean litter size and in ossification of fetal sternebrae and phalanges in the group given 30.0 mg/kg bw per day. Maternal absolute and relative thyroid weights were significantly increased, with decreased colloid and hypertrophy of the thyroid in the 30.0 mg/kg bw per day group. Maternal TSH was significantly increased and T_4 was significantly decreased in a dose-related manner at all dose levels; T_3 was significantly reduced at 30.0 mg/kg bw per day. In the fetuses, decreased colloid was present in male and female thyroids in the 1.0 and 30.0 mg/kg bw per day groups, but there was no evidence of hypertrophy. Fetal TSH was significantly increased at 1.0 and 30.0 mg/kg bw per day, T_4 was significantly reduced at 30.0 mg/kg bw per day and T_3 was significantly decreased in a dose-related manner at all dose levels. The authors concluded that the maternal NOAEL was 1.0 mg/kg bw per day, as thyroid weights and the incidence of histopathological changes were increased at 30.0 mg/kg bw per day, and the developmental NOAEL was 1.0 mg/kg bw per day, based on delays in ossification at 30.0 mg/kg bw per day. The authors considered that colloid depletion in the thyroids and the effects on TSH, T_3 and T_4 levels at lower exposures were adaptive and not adverse (York et al., 2003). A NOEL was not established for effects on thyroid hormones, as these were affected in both dams and fetuses at the lowest dose tested of 0.01 mg/kg bw per day.

A developmental toxicity study was conducted in New Zealand White rabbits given ammonium perchlorate in drinking-water at target doses of 0, 0.1, 1.0, 10.0,

30.0 and 100.0 mg/kg bw per day on GDs 6–28. The actual consumed doses averaged 0, 0.1, 0.9, 10.4, 30.3 and 102.3 mg/kg bw per day. Twenty-five rabbits per group were sacrificed on GD 29, and the fetuses were examined for visceral and skeletal alterations. In addition, blood was collected from does for determination of serum TSH, T_3 and T_4 levels, and the thyroids were examined for histopathological changes. Maternal body weight was not affected by treatment. There were no treatment-related effects on reproductive or fetal parameters at any dose level. A significantly increased incidence of thyroid follicular cell hypertrophy was observed in does treated with doses greater than or equal to 10.0 mg/kg bw per day, and significantly decreased T_4 was observed in does treated with doses greater than or equal to 30.0 mg/kg bw per day. There was no effect on maternal TSH or T_3 levels. Based on these data, the authors concluded that the maternal NOAEL was 1.0 mg/kg bw per day and the developmental NOAEL was 100.0 mg/kg bw per day (York et al., 2001b).

The effect of prenatal and postnatal chronic hypothyroidism induced by iodide-deficient diet and perchlorate in the drinking-water on the formation of the adult-type Leydig cell population was investigated in Wistar rats. Treated females, aged 10 weeks, were put on a diet consisting of an iodide-poor feed supplemented with 0.75% (7500 mg/l) sodium perchlorate in the drinking-water for 2 weeks before mating, during gestation and during lactation. Pups were weaned on PND 28, and the males from treated dams were maintained on iodide-deficient diet plus perchlorate into adulthood. Groups of 6–20 males per group were killed at intervals up to PND 120. Plasma TSH, T_3 and T_4 levels were measured at intervals in dams and offspring. There was no significant effect on maternal body weight. In the dams during treatment, plasma TSH levels were significantly increased, and T_4 and T_3 levels were significantly reduced. In the offspring, plasma TSH levels were increased by 4- to 20-fold from PND 12 to PND 120; T_3 levels were significantly reduced from PND 16 to PND 120; and T_4 levels were severely reduced from PND 16. From PND 12, offspring body weights in the hypothyroid group were significantly reduced by 30–70% compared with controls, the percentage reduction generally increasing with age. In the hypothyroid offspring, eye opening was delayed by 1 day, and body temperature was significantly reduced. Absolute wet testis weights were significantly reduced in hypothyroid males between PND 49 and PND 71, but had caught up with controls by PND 88; from PND 49, relative testis weights (ratio of testis weight to body weight) were significantly increased in the hypothyroid group. Adult-type progenitor Leydig cell formation and proliferation were reduced by 40–60% in hypothyroid males on PND 16 and PND 28, followed by persistence of increased Leydig cell proliferation at later ages than in controls, suggesting a possible slower developmental onset of the adult-type Leydig cell population under hypothyroid conditions. Total testosterone and free testosterone levels were significantly increased 2- to 10-fold in the hypothyroid animals between PND 21 and PND 42, compared with the age-matched controls. The normal peak in luteinizing hormone (LH) levels that occurs between PND 21 and PND 35 was not seen in the hypothyroid males. Prolactin levels in hypothyroid males were significantly decreased from PND 21 onwards. In 84-day-old rats, after correction for body weight to testis weight ratio, plasma insulin-like factor-3 levels were 35% lower in the hypothyroid animals, indicative of a reduced Leydig cell population. This was

confirmed by a 37% reduction in the Sertoli cell to Leydig cell ratio in hypothyroid rats. The authors concluded that dietary-induced hypothyroidism delays, but does not block, the development of the adult-type Leydig cell population (Rijntjes et al., 2009). This study does not allow the effects of perchlorate to be separated from those of the iodide-deficient diet.

The ability of perchlorate to affect the thyroid in a standard Tier 1 male pubertal protocol, developed for the United States Environmental Protection Agency's (USEPA) Endocrine Disrupter Screening Program, was investigated in male Wistar rats. Ammonium perchlorate was administered daily by oral gavage at doses of 62.5, 125, 250 and 500 mg/kg bw for 31 consecutive days, from PND 23 to PND 53, until the day of necropsy. Control animals received vehicle (corn oil) only. At necropsy, blood was taken for hormone measurements, reproductive organs were weighed and thyroids were weighed and examined histologically. There were no effects on body weight or signs of toxicity in treated males. There were no effects on age of onset of preputial separation or on ventral prostate, lateral prostate, seminal vesicle, epididymal or testis weights. Doses of 125–500 mg/kg bw per day significantly increased TSH and decreased T_4 levels in a dose-dependent manner, whereas the T_3 level was unchanged at all doses. Thyroid histology was significantly altered at all four doses, with a clear dose-dependent decrease in colloid area and increase in follicular cell height (Stoker et al., 2006). This study, using relatively high doses of perchlorate, showed that exposure during the period of sexual maturation in juvenile male rats did not alter any of the reproductive developmental end-points, but had marked effects on thyroid structure and function. A NOAEL for effects on the thyroid was not established.

(c) Developmental neurotoxicity

Groups of 24 Sprague-Dawley rats were exposed to ammonium perchlorate in the drinking-water at a target dose of 0, 0.1, 1.0, 3.0 or 10.0 mg/kg bw per day from GD 0 (day of mating) throughout gestation and the first 10 days of lactation. The actual consumed doses averaged 0, 0.1, 1.1, 3.5 and 11.6 mg/kg bw per day during the 3-week gestation period and 0, 0.2, 1.6, 4.7 and 15.4 mg/kg bw per day during the 10-day lactation exposure period. Litters were culled to eight pups on PND 5 and weaned on PND 21. Offspring examinations were carried out on 20 litters per group. One pup of each sex per litter per group was assigned to one of four examinations: 1) juvenile brain weights, morphometry and neuropathology; 2) passive avoidance and water maze testing; 3) motor activity and auditory startle habituation; and 4) adult regional brain weights, morphometry and neuropathology. Thyroid histopathology and serum TSH, T_3 and T_4 levels were examined in culled pups (PND 5) and dams (day after pups weaned). There were no effects on maternal body weight, feed consumption or water consumption. There were no effects on reproductive or litter parameters, including offspring weights and sexual maturation. There were no behavioural effects in the offspring in any dose group as evaluated by passive avoidance, swimming water maze, motor activity and auditory startle. There was an apparent increase in the thickness of the corpus callosum of the 10.0 mg/kg bw per day group pups on PND 12. There were no effects on maternal thyroid weight or histology in dams killed when their pups were weaned (12 days

after cessation of treatment). In pups culled on PND 5, an increase in hypertrophy of the thyroid follicular epithelium and a decrease in the thyroid follicle size were observed in females in the 3.0 and 10.0 mg/kg bw per day groups and in males in the 10.0 mg/kg bw per day group. The exposure level for effects on T_3, T_4 and TSH levels in pooled serum from pups culled on PND 5 were 1.0, 3.0 and 10.0 mg/kg bw per day, respectively. The NOAEL for maternal toxicity was greater than 10.0 mg/kg bw per day. Based on the thyroid histopathology and reduced thyroid hormones, the authors concluded that the NOAEL for pups was 0.1 mg/kg bw per day (York et al., 2004).

Another study from the same group further investigated the potential effects of maternal exposure to ammonium perchlorate during gestation and lactation on offspring development and thyroid function. Groups of 25 female Sprague-Dawley rats were given continual access to ammonium perchlorate at 0, 0.01, 0.1, 1.0 and 30.0 mg/kg bw per day in drinking-water beginning 2 weeks prior to cohabitation through to PND 10. The actual consumed doses for the female rats averaged as follows: during pre-cohabitation, 0, 0.01, 0.09, 0.77 and 23.80 mg/kg bw per day; during gestation, 0, 0.01, 0.09, 0.97 and 28.34 mg/kg bw per day; and during lactation, 0, 0.01, 0.12, 1.26 and 38.32 mg/kg bw per day. Maternal, fetal and pup serum TSH, T_3 and T_4 levels and thyroid histopathology were evaluated on GD 21, PND 5, PND 10 and PND 22. There were no effects on maternal body weight, feed consumption or water consumption. There were no effects on reproductive or litter parameters. Thyroid weights and histopathological changes (decreased colloid and follicular hypertrophy) were significantly increased in dams sacrificed on GD 21, PND 10 and PND 22 at 30.0 mg/kg bw per day. Increases in follicular hyperplasia were also evident at 1.0 and 30.0 mg/kg bw per day, but significance levels were not indicated. Maternal TSH levels were significantly increased at all doses on GD 21 and PND 10 and still elevated at doses greater than or equal to 0.1 mg/kg bw per day on PND 22, 12 days after cessation of exposure. Maternal T_4 level was significantly reduced at all doses on GD 21 and at 30.0 mg/kg bw per day on PND 10 and PND 22. Maternal T_3 level was significantly reduced only at 30.0 mg/kg bw per day on GD 21. In the pups, significantly increased thyroid weights in males were observed on PND 5 in the 30.0 mg/kg bw per day group, on PND 10 in all dose groups and on PND 22 in the 1.0 and 30.0 mg/kg bw per day groups; in female pups, significantly increased thyroid weights were observed on PND 10 in the 30.0 mg/kg bw per day group and on PND 22 in the 1.0 and 30.0 mg/kg bw per day groups. Histopathological changes in male and female pup thyroids (decreased colloid) were significantly increased at 1.0 mg/kg bw per day. Pup TSH levels were not significantly increased on PND 5, but were significantly increased at 30.0 mg/kg bw per day on PND 10 and at all doses on PND 22. Pup T_4 levels were significantly reduced at 1.0 and 30.0 mg/kg bw per day on PND 5, not significantly altered at PND 10 but significantly reduced at all dose levels at PND 22. Pup T_3 levels were significantly reduced at 30.0 mg/kg bw per day on PND 5 and at 1.0 and 30.0 mg/kg bw per day on PND 10 and PND 22. The authors concluded that the maternal NOEL was 0.1 mg/kg bw per day, based on follicular cell hyperplasia at 1.0 and 30.0 mg/kg bw per day. A developmental NOEL was not established, as thyroid weights in male pups were increased at all doses on PND 10 (York et al., 2005a).

Another study from the same group generated additional data on developmental neurotoxicity in offspring from in utero and lactational exposure to ammonium perchlorate. Groups of 23–25 female Sprague-Dawley rats were given continuous access to ammonium perchlorate at 1) 0, 0.1, 1.0, 3.0 and 10.0 mg/kg bw per day in the drinking-water beginning 2 weeks prior to mating and continuing through to PND 10 for the behavioural function assessment or 2) 0, 0.1, 1.0, 3.0 and 30.0 mg/kg bw per day beginning on GD 0 and continuing through day 10 of lactation for neurodevelopmental assessments. Motor activity was investigated on PND 14, PND 18 and PND 22, and juvenile brain weights were measured, neurohistopathological examinations were conducted and regional brain morphometry was assessed on PND 10 and PND 22. There were no effects on maternal body weight, feed consumption or water consumption. There were no effects on reproductive or litter parameters. The mean values for the linear dimensions of a number of brain regions in male pups on both PND 10 and PND 22 were found to be significantly greater than for the controls, whereas in female pups at the same ages, some regions were significantly smaller than for the controls; for the majority of these measurements, however, there was no clear dose–response. Some brain regions were larger in perchlorate-treated male offspring than in comparable controls, but there was no evidence of any obvious exposure-related effects on male rat brain weights or neuropathology. Behavioural testing did not show any effect on the development of gross motor movements in the pups (York et al., 2005b).

Groups of 30 pregnant Long-Evans rats were exposed to ammonium perchlorate in drinking-water at 0, 30, 300 or 1000 mg/l from GD 6 until the pups were weaned at PND 30. Actual consumed doses averaged 0, 4.5, 44.2 and 140.3 mg/kg bw per day. A proportion of pups were sacrificed at PND 4, PND 14 and PND 21 for hormone measurements. Adult male offspring were evaluated on a series of behavioural tasks and neurophysiological measures of synaptic function in the hippocampus. There were no effects on maternal body weight. In dams sacrificed on PND 30, TSH levels were significantly increased at 1000 mg/l, T_3 levels were unaffected, but T_4 levels were significantly reduced compared with controls by 16%, 28% and 60% at 30, 300 and 1000 mg/l, respectively. In pups, at PND 4, there were no significant effects on hormone levels; at PND 14, T_3 and T_4 levels were unaffected, whereas TSH levels were significantly increased in the 30 and 300 mg/l groups, but not in the 1000 mg/l group; at PND 21, TSH levels were unaffected, but T_3 and T_4 levels were significantly reduced at 300 and 1000 mg/l. There were no effects on offspring body weight, time of eye opening, brain weight or hippocampal weight. Perchlorate did not impair motor activity at 13 months of age, spatial learning in a Morris water maze at 3 months of age or fear conditioning at 7–8 months of age. However, deficits were observed in inhibitory and excitatory synaptic function in the hippocampus of the offspring tested at 5–9 months of age. Significant reductions in baseline synaptic transmission were observed in hippocampal field potentials at all three dose levels. Reductions in inhibitory function were evident at 300 and 1000 mg/l, and augmentations in long-term potentiation were observed in the population spike measure at 1000 mg/l. The authors concluded that dose-dependent deficits in hippocampal synaptic function were detectable following relatively minor perturbations of the thyroid axis, indicative of an irreversible impairment in synaptic transmission in adults in response to

developmental exposure to perchlorate (Gilbert & Sui, 2008). A NOAEL was not established, as effects on the hippocampus were seen at the lowest dose tested, equivalent to 4.5 mg/kg bw per day. It should be noted that the middle and high doses used in this study (44.2 and 140.3 mg/kg bw per day, respectively) had considerably less marked effects on maternal TSH and T_4 levels than have been observed in other studies, reviewed above, that used lower doses of perchlorate administered via drinking-water (e.g. 30.0 mg/kg bw per day). In addition, at the lowest dose, only baseline synaptic transmission in the hippocampus was affected, and no behavioural measures were affected in any dose group in tests that were anticipated to be sensitive to hippocampal disturbances.

The behavioural effects of perinatal and chronic hypothyroidism induced by iodide-deficient diet and perchlorate in the drinking-water were assessed during development in male and female offspring of Wistar rats. To induce hypothyroidism, six dams were fed an iodide-poor diet and drinking-water containing 0.75% (7500 mg/l) sodium perchlorate from 2 weeks prior to mating. Six control dams were given normal diet and untreated drinking-water. Four days after birth, the litters were sexed and divided between six foster mothers (four hypothyroid and two control), with four males and four females allocated per litter. From PND 14, two of the hypothyroid foster mothers received control diet and no further perchlorate ("hypo-normal" group). Pups were weaned from the dams at PND 29. Treatment of pups continued either until the day of killing on PND 76 (chronic hypothyroidism) or only until weaning (perinatal hypothyroidism) to test for reversibility of any effects observed. Neuromotor competence, locomotor activity and cognitive function were monitored in the offspring until PND 71 and were compared with age-matched control rats. Body weights of the hypothyroid offspring were significantly reduced compared with hypo-normal or control offspring and were only 37% of control values by PND 75. Early neuromotor competence, as assessed in the grip test and balance beam test, was impaired by both chronic and perinatal hypothyroidism. The open field test, assessing locomotor activity, revealed hyperactive locomotor behavioural patterns in chronic hypothyroid offspring only. The Morris water maze test, used to assess cognitive performance, showed that chronic hypothyroidism adversely affected spatial memory. In contrast, perinatal hypothyroidism was found to impair spatial memory in female rats only (van Wijk, Rijntjes & van de Heijning, 2008). It should be noted that only small numbers of animals were used in this study, each treatment group comprising two dams and eight pups per dam, and that the statistical analysis is limited, as individual pup numbers were used as the value for *n*, which would not control for litter effects. This study does not allow any firm conclusions to be drawn and does not allow the effects of perchlorate to be separated from those of the iodide-deficient diet.

2.2.6 Special studies

(a) Physiologically based pharmacokinetic modelling

Physiologically based pharmacokinetic (PBPK) models for prediction of perchlorate distribution and its effects on iodide uptake in the thyroid have been developed for the adult male rat, the lactating rat and the neonatal rat (Clewell et

al., 2001, 2003a,b; Merrill et al., 2003). The models describe the simultaneous kinetics for iodide and perchlorate, their interaction at the sodium–iodide symporter (NIS) and the inhibition of iodide uptake by perchlorate. The predictions of the models were checked against experimental data from studies using intravenous infusions of iodide and perchlorate or perchlorate alone or administration of perchlorate in drinking-water, covering doses ranging from 0.01 to 30.0 mg/kg bw per day. The predictions were successful for both acute intravenous kinetics and subchronic oral kinetics. Perchlorate has a higher affinity for the NIS than does iodide, with a Michaelis-Menten constant (K_m) approximately 10 times lower than that of iodide. Tissues with active uptake were the thyroid, stomach, skin, placenta and mammary gland. The compartments of kidney, liver, plasma, fat and milk were described by passive diffusion. The gestational model suggested significant fetal exposure to perchlorate in late gestation (up to 82% of maternal dose). A comparison of model-predicted internal dosimetrics in the adult male, pregnant female and fetal rat indicated that the fetal thyroid is more sensitive to inhibition than that of the adult. Model simulations predicted a transfer to maternal milk of between 50% and 6% of the maternal dose in the dose range 0.01–10.0 mg/kg bw per day. Comparison of predicted dosimetrics across life stages in the rat indicated that inhibition of the uptake of iodine by perchlorate in the neonate (PND 10) is similar to that in the adult and approximately 10-fold less than that for the fetus. The authors concluded that these rat models can be used to extrapolate to humans, provided a longer time course is included to account for the longer lag time in humans for onset of upregulation of activity of the inhibited thyroid following exposure to perchlorate. Whereas humans show inhibition of iodine uptake into the thyroid by perchlorate similar to that in the rat for equal perchlorate doses, upregulation, in terms of number of NIS and activity of NIS, occurs in the rat within 12 h of commencement of treatment, but is still not evident in humans after 2 weeks of exposure. This may reflect the greater thyroid hormone storage capacity and greater serum binding of thyroid hormones to TBG rather than to albumin and transthyretin in the human compared with the rat. The binding of thyroid hormones to TBG is very strong, which protects them from degradation in the circulation. The level or duration of perchlorate exposure required to induce upregulation in the human is not known.

Data on perchlorate from the published literature were used to compare species differences in perturbation of thyroid homeostasis by perchlorate. Dose–response data for changes in serum T_3, T_4 and TSH concentrations following perchlorate administration in humans, rats, rabbits and mice were analysed. Although the data indicated that humans and rats exhibit similar dose–response relationships in terms of acute inhibition of thyroidal iodide uptake by perchlorate, the two species exhibit notable differences in terms of thyroid hormone response, with the rat thyroid being much more sensitive to perchlorate than any of the other species. Rats exhibited an increase in serum TSH level at 0.1 mg/kg bw per day, whereas other species remained unresponsive even at doses of 10 mg/kg bw per day. Less pronounced but consistent effects were seen with serum T_3 and T_4 levels (Lewandowski, Seeley & Beck, 2004).

(b) Mode of action studies

There has been a debate about whether the mode of action of perchlorate is simply to block the NIS or whether perchlorate is actually a substrate for the NIS and is actively transported into the thyroid follicular cell. The proposal that perchlorate is actively transported into the thyroid follicular cell by the NIS was challenged using an indirect electrochemical technique to infer movement of perchlorate. The authors concluded that perchlorate was not taken up into the thyroid gland, because, in contrast to iodide uptake, an electrical gradient was not created (Riedel et al., 2001).

A subsequent study in rats demonstrated transport of perchlorate into the thyroid. The effect of ammonium perchlorate on the uptake of radiolabelled iodide into the thyroid was also studied. Perchlorate was administered orally in the drinking-water to adult male Sprague-Dawley rats at target doses of 0, 1.0, 3.0 and 10.0 mg/kg bw per day for 1, 5 or 14 days, with six rats per dose per time point, or as single intravenous injections, at doses ranging from 0.01 to 3.0 mg/kg bw. There was a dose-dependent inhibition of uptake of radiolabelled iodide at all doses by both routes of administration and at all time points. The inhibition occurred quickly and was closely related to serum perchlorate concentrations. Other groups of eight rats were given perchlorate in drinking-water at 0, 0.1, 1.0, 3.0 or 10.0 mg/kg bw per day, and serum TSH and thyroid hormone responses were measured on days 1, 5 and 14 of perchlorate treatment. TSH was significantly increased in a dose-related manner at all doses and time points. After 1 day of treatment, T_4 levels were decreased in all dose groups except the lowest. By 14 days of perchlorate treatment, the T_4 values in the 0.1 and 1.0 mg/kg bw per day groups had returned to control values, but T_4 values in the 3.0 and 10.0 mg/kg bw per day groups were still reduced. After 1 day of treatment, free T_4 levels were significantly increased in all dose groups. On day 5 of treatment, free T_4 levels were similar to control values, but by day 14, free T_4 levels in all dose groups were increased again, except in the 0.1 mg/kg bw per day group. T_3 levels declined throughout the perchlorate treatment period for all dose groups. However, the T_3 levels in each dose group were not statistically different from controls. There were no effects on body weight at any dose (Yu et al., 2002). A NOEL for effects on iodide uptake or on TSH or serum thyroid hormones was not established.

Active transport of perchlorate into maternal milk by the NIS in the mammary gland has also been demonstrated using both in vivo and in vitro rat models (Dohán et al., 2007). The same group also showed that the process of active transport of perchlorate in vitro is not electrogenic and that perchlorate ion is transported stoichiometrically with sodium ion in a ratio of 1:1, whereas iodide is transported by the NIS in a ratio of two sodium ions to each iodine ion. This would explain the earlier findings of Riedel et al. (2001), mentioned above, and why those authors had misinterpreted their findings. Dose-related competitive inhibition of iodide uptake by perchlorate has also been confirmed in vitro in rat FRTL-5 cells, which maintain functional NIS (Tran et al., 2008).

Using a simpler PBPK model than that of Merrill et al. (2003) and comparison with previously published experimental data on administration of perchlorate in

drinking-water in the rat, the assumption that the mode of action of perchlorate is solely competitive inhibition of iodide uptake by the NIS was tested. The rapid decrease in serum total T_4 levels and the corresponding increase in serum TSH levels after 1 day of treatment were considered to be inconsistent with a mode of action related solely to competitive inhibition at the level of the NIS and appeared consistent with a reduction in thyroid hormone production or secretion. The authors concluded that perchlorate is translocated into the thyroid gland, where it may also act directly or indirectly on thyroid hormone synthesis or secretion in the rat (McLanahan et al., 2009).

These studies, together with other data reviewed by Clewell et al. (2004), provide strong evidence that the mode of action of perchlorate involves competitive inhibition of iodide transport into the thyroid follicle, transport of perchlorate into the thyroid follicle against a concentration gradient, further transport into the thyroid lumen (where it may again interfere with iodide transport) and, finally, passive diffusion back into the blood.

The affinity of the NIS for perchlorate has been shown to be 30-fold greater than its affinity for iodide in Chinese hamster ovary cells stably transfected with human NIS (Tonacchera et al., 2004). However, in contrast to the rat, in which the NIS is expressed in thyroid follicular cells at a high density, in humans, NIS expression in thyroid follicular cells is "patchy" (Josefsson et al., 2002). The NIS is expressed in human placenta, lactating breast, salivary gland and gastric mucosa (Clewell et al., 2007; Tran et al., 2008) and transports perchlorate across these tissues, resulting in exposure of the developing fetus, neonate and breastfed infant to perchlorate (Kirk et al., 2005, 2007; Blount et al., 2006b; Dasgupta et al., 2008).

2.3 Observations in humans

2.3.1 Biomarkers of exposure

Exposure to perchlorate was assessed in a nationally representative population of 2820 residents of the USA, aged 6 years and older, during 2001 and 2002 as part of the National Health and Nutrition Examination Survey (NHANES). Detectable levels of perchlorate (>0.05 µg/l) were found in all 2820 urine samples tested, indicating widespread human exposure. Urinary perchlorate levels were lognormally distributed, with a median level of 3.6 µg/l (3.38 µg/g creatinine) and a 95th-percentile level of 14 µg/l (12.7 µg/g creatinine). When geometric means of urinary perchlorate levels were adjusted for age, fasting, sex and race or ethnicity, significantly higher levels of urinary perchlorate were found in children compared with adolescents and adults. Total daily perchlorate exposures were estimated for each adult aged 20 years and older, based on urinary perchlorate, urinary creatinine concentration and physiological parameters predictive of creatinine excretion rate. The 95th percentile of the distribution of estimated daily perchlorate doses in the adult population was 0.234 µg/kg bw per day (95% confidence interval [CI] 0.202–0.268 µg/kg bw per day), which is below the USEPA reference dose (RfD) of 0.7 µg/kg bw per day (USEPA, 2005b; Blount et al., 2006a).

In the most recent NHANES data set on 2522 residents of the USA from the period 2003–2004, geometric mean values for urinary perchlorate concentrations in all age, sex and race or ethnicity groups were slightly lower than the corresponding values for 2001–2001. The overall geometric means were 3.54 µg/l (95% CI 3.29–3.81 µg/l) for 2001–2002 and 3.22 µg/l (2.93–3.55 µg/l) for 2003–2004. The 95th-percentile geometric mean values were 14 µg/l (11–17 µg/l) for 2001–2002 and 13 µg/l (12–15 µg/l) for 2003–2004 (CDC, 2009).

A number of studies have investigated perchlorate in human breast milk. They demonstrate that breast milk is a significant route of excretion of perchlorate. It should be noted that studies that have attempted to correlate levels of perchlorate in drinking-water with levels in breast milk may be confounded by the contribution from the diet. It should also be noted that there are large temporal variations in urinary iodine levels within individuals, which may also be reflected in breast milk; therefore, studies using spot samples—that is, the majority of those described below, except Kirk et al. (2007) and Dasgupta et al. (2008)—may not be representative of longer-term breast milk iodine levels. There has been concern that exposure of infants via breast milk or possibly infant formula may lead to exceedance of health-based guidance values (Baier-Anderson et al., 2006; Schier et al., 2010).

Thirty-six breast milk samples from women in 18 states in the USA were analysed, and perchlorate was found in all samples at concentrations ranging from 1.4 to 92.2 µg/l, with a mean of 10.5 µg/l. In this study, in the six breast milk samples with a perchlorate content greater than 10 µg/l, the iodide content was linearly correlated with the inverse of the perchlorate concentration, with an r^2 of >0.9 (Kirk et al., 2005).

In a smaller study involving 10 women, from whom about six samples were collected on each of 3 days, making a total of 147 samples, half the women were from Texas, and the others were from five other states in the USA. There was significant variation in all samples, with a concentration range of 0.5–39.5 µg/l and mean and median perchlorate concentrations of 5.8 µg/l and 4.0 µg/l, respectively. Samples collected in the morning before any food or liquid was consumed had significantly lower amounts of perchlorate than matched samples collected in the evening. Three of the 10 subjects had average perchlorate concentrations in the breast milk above 7 µg/l; thus, for the infants of these mothers, the USEPA RfD for perchlorate of 0.7 µg/kg bw per day (USEPA, 2005b) could have been exceeded, assuming the infants were older than 12 months of age and had an average milk consumption of 100 ml/kg bw per day (Kirk et al., 2007).

Analysis of 55 breast milk samples from a study in three cities in Chile with differing perchlorate levels in the drinking-water indicated that perchlorate levels were highly variable, and no significant correlations could be established between breast milk perchlorate concentrations and either urinary perchlorate concentrations or breast milk iodide concentrations for the individuals evaluated in this study (Téllez Téllez et al., 2005).

Measurements of perchlorate in breast milk obtained from 49 healthy volunteers from the Boston area in Massachusetts, USA, between 10 and 250 days

postpartum found perchlorate in all samples at concentrations ranging from 1.3 to 411 µg/l, with a median concentration of 9.1 µg/l and a mean concentration of 33 µg/l. No correlation was found between perchlorate and iodine concentrations in breast milk, including in a subset of 27 women with breast milk perchlorate concentrations above 10 µg/l (Pearce et al., 2007).

Breast milk samples and 24 h urine samples collected over 9 consecutive days from 13 lactating women from Texas in the USA were analysed for perchlorate and iodine. Breast milk perchlorate concentrations ranged from 0.01 to 48 µg/l, with a median concentration of 7.3 µg/l and a mean concentration of 9.3 µg/l (457 samples in total). The authors calculated that 9 out of 13 infants receiving this milk would have received exposures greater than the proposed National Research Council RfD of 0.7 µg/kg bw per day (NRC, 2005). Iodine concentrations in breast milk ranged from 1 to 1200 µg/l, with a median concentration of 43 µg/l and a mean concentration of 120 µg/l (447 samples in total). Urinary perchlorate concentrations ranged from 0.6 to 80 µg/l, with a median concentration of 3.2 µg/l and a mean concentration of 4.0 µg/l (110 samples in total). Iodine concentrations in urine ranged from 26 to 630 µg/l, with a median concentration of 110 µg/l and a mean concentration of 140 µg/l (117 samples in total) (Dasgupta et al., 2008).

Single spot collections of colostrums from 97 healthy women from the Boston area in the USA were obtained within 60 h postpartum. The median colostrum iodine content in 61 samples was 51.4 µmol/l (range 21.3–304.2 µmol/l). The median urinary iodine concentration in 97 samples was 82.2 µmol/l (range 0.3–417.1 µmol/l). Perchlorate was detectable in 43 of 46 colostrum samples (median 2.5 µmol/l; range <0.05–188.9 µmol/l) and in all 97 urine samples (median 2.6 µmol/l; range 0.2–160.6 µmol/l). There were no significant correlations between perchlorate concentrations and iodine concentrations in urine or colostrum (Leung et al., 2009).

Levels of three physiologically relevant NIS inhibitors (perchlorate, nitrate and thiocyanate) and iodide in maternal and fetal fluids collected during caesarean section surgeries on 150 women in the USA have been measured. Geometric means of perchlorate, thiocyanate and nitrate levels in maternal urine (2.90 µg/l, 947 µg/l and 47 900 µg/l, respectively) were similar to previously published results, whereas urinary iodide levels (1420 µg/l) were significantly higher, likely because of prevalent prenatal vitamin use in the study population (74%). Perchlorate was detected in most samples: urine (100%), maternal serum (94%), cord serum (67%) and amniotic fluid (97%). Maternal urinary perchlorate levels were positively correlated with perchlorate levels in amniotic fluid (r = 0.57). Maternal serum perchlorate was generally higher than cord serum perchlorate (median ratio 2.4:1 for paired samples), and maternal urinary perchlorate was always higher than fetal amniotic fluid perchlorate levels (mean ratio 22:1). Iodide levels were typically higher in fetal fluids than in maternal fluids. There was no association between cord blood levels of these anions and newborn weight, length or head circumference (Blount et al., 2009).

2.3.2 Clinical observations and biomarkers of effect

On a population basis, there are wide variations in circulating levels of T_3 and T_4 among humans, and levels also fluctuate considerably within individuals, reflecting short-term pulsatile secretion and diurnal variation. Therefore, TSH measurements are considered to be critical for reliable diagnostic information on thyroid function (Braverman & Utiger, 2005). For studies involving neonatal TSH measurements, it should be noted that there is a postnatal surge of TSH immediately after birth, which peaks around 12 h of age and then declines during the first 24 h of life. Thus, results obtained during the first 18–24 h of life are not necessarily informative about subsequent thyroid function (Braverman & Utiger, 2005; Buffler et al., 2006).

(a) Healthy, non-pregnant adults

A study was carried out in nine healthy male volunteers, aged 22–30 years, to investigate the effects of short-term ingestion of perchlorate on thyroid hormones. Perchlorate providing a daily dose of 10 mg was ingested in spring water consumed throughout the day for 14 days. Assuming a body weight of 70 kg, an exposure of 10 mg/day would equate to a dose of approximately 0.14 mg/kg bw per day. TSH, free T_4, total T_3, uptake of radiolabelled iodide by the thyroid and 24 h urinary iodine excretion were measured at baseline before perchlorate administration, on days 7 and 14 during perchlorate administration and 14 days after cessation of perchlorate administration. Urinary and serum perchlorate concentrations were elevated in all subjects. By day 14, the 4, 8 and 24 h thyroid radioiodide uptakes were reduced in all subjects by an average of 38% below baseline; uptake rebounded to 25% above baseline 14 days after cessation of perchlorate administration. There was no effect at any time point on TSH, thyroid hormones or urinary iodine excretion (Lawrence et al., 2000).

In a follow-up study, the same group investigated the effects of ingestion of a dose of 3 mg of perchlorate per day for 14 days in eight healthy male volunteers. Perchlorate was again given in spring water consumed throughout the day. Assuming a body weight of 70 kg, an exposure of 3 mg/day would equate to a dose of about 0.04 mg/kg bw per day. Uptake of radiolabelled iodide by the thyroid and other thyroid function parameters were measured at baseline, after 14 days of perchlorate administration and 14 days after cessation of perchlorate administration. The 8 and 24 h values for thyroid uptake of radiolabelled iodide were slightly reduced by about 10% during perchlorate administration, but the reductions were not statistically significant compared with baseline values; 8 and 24 h values for uptake rebounded to 22% and 18%, respectively, above baseline at 14 days after cessation of perchlorate administration. TSH and thyroid hormones were unaffected (Lawrence, Lamm & Braverman, 2001). This study suggests that short-term administration of perchlorate at a dose of 3 mg/day, equivalent to about 0.04 mg/kg bw per day, is around the LOEL for effects of perchlorate on iodine uptake in the thyroid.

A key study in 16 male and 21 female volunteers investigated the time course and dose–response for perchlorate inhibition of iodine uptake by the thyroid. The

volunteers were screened to exclude the possibility of thyroid disease. Perchlorate was given in drinking-water at doses of 0.007, 0.02, 0.1 or 0.5 mg/kg bw per day for 14 days. The daily dose was consumed as four divided doses of 100 ml ingested at 08:00, 12:00, 16:00 and 20:00. The uptake of radiolabelled iodine into the thyroid was measured at baseline before administration of perchlorate, on days 2 and 14 of administration and 15 days after cessation of perchlorate administration. The radiolabelled iodine was given at 09:00 on each day of measurement, and uptake was measured at 8 and 24 h after administration. The uptake of iodide was linearly related to the logarithm of the perchlorate dose and did not differ between day 2 and day 14 of perchlorate administration, indicating that more or less steady-state inhibition was achieved by day 2. There was no difference between the sexes. At 15 days after cessation of perchlorate administration, uptake of iodide had returned to baseline values. Inhibition of iodide uptake on day 14 compared with baseline was 1.8%, 17%, 44% and 67% in the 0.007, 0.02, 0.1 and 0.5 mg/kg bw per day groups, respectively. The inhibition was not significantly different from baseline at the lowest dose of 0.007 mg/kg bw per day, but was significantly increased in all other dose groups. The authors estimated, using their terminology, a "true no-effect level" by extrapolation from the regression relationships for relative uptakes at 8 and 24 h on day 14; the true no-effect levels were 5.2 and 6.4 µg/kg bw per day, respectively. Based on the variability observed in the subjects, the authors estimated that there is a 95% probability that thyroidal iodide uptake will be inhibited by no more than 8.3–9.5% at a dose of 5.2–6.4 µg/kg bw per day. Given default body weight and exposure assumptions, the authors calculated that these no-effect level doses would be ingested by an adult if the drinking-water supply contained perchlorate at concentrations of approximately 180 and 220 µg/l, respectively. Serum total T_4, free T_4, total T_3 and TSH levels were measured in 24 of the subjects on 16 occasions throughout the study. There were no effects at any dose on total T_4, free T_4 or total T_3 levels. A significant effect on TSH levels was observed only in the 0.5 mg/kg bw per day group, which showed marginally decreased TSH concentrations in morning blood draws during perchlorate administration, with recovery by 15 days after cessation of perchlorate administration; this was the opposite of what would be expected from exposure to a thyroid inhibitor. The NOEL from this study for effects of perchlorate on uptake of iodine by the thyroid was 0.007 mg/kg bw per day, and the NOEL for effects on levels of thyroid hormones and TSH was the highest dose tested, 0.5 mg/kg bw per day (Greer et al., 2002).

It should be noted that the dose–response relationships from the other human clinical volunteer studies (Lawrence et al., 2000; Lawrence, Lamm & Braverman, 2001) are essentially in agreement with the results of Greer et al. (2002) and that benchmark dose (BMD) calculations (see section 2.3.2(b) below) from two occupational studies (Lamm et al., 1999; Braverman et al., 2005) are within the range of the Greer et al. (2002) study and in agreement with the NOEL from that study.

An occupational study of employees working in perchlorate production at a manufacturing plant in Utah, USA, was conducted to assess whether exposure to ammonium perchlorate affected thyroid function. Thirty-seven employees (35 males and 2 females) were studied, 40% of whom had been employed for more than

5 years. Workers typically worked three 12 h shifts followed by 3 days off. Results were compared with those from 21 employees (19 males and 2 females, 50% employed for more than 5 years) in the same industrial facility but working in sodium azide production. Exposure to perchlorate was assessed by measurement of ambient air concentrations of total and respirable perchlorate particles, and systemic absorption was assessed by measurement of urinary perchlorate excretion pre- and post-shift. Airborne exposures ranged from 0.004 to 167 mg of total particulate perchlorate per day. Urinary perchlorate measurements demonstrated that exposure to the airborne particulate perchlorate resulted in systemic absorption that is related to airborne concentrations. Workers were grouped into four exposure categories, with mean absorbed perchlorate dosages of 1, 4, 11 and 34 mg/day. Thyroid function was assessed by measurement of serum TSH, T_4 and T_3 levels, free T_4 index, thyroid hormone binding ratio and thyroid peroxidase antibodies and by clinical examination. No differences in thyroid function parameters were found between the four groups of workers across approximately 3 orders of magnitude of exposure and of dose. In addition, no clinical evidence of thyroid abnormalities was found in any exposure group. The authors concluded that a mean absorption of 34 mg/day from occupational airborne exposure to perchlorate demonstrated a NOAEL that can assist in the evaluation of human health risks from environmental perchlorate contamination (Lamm et al., 1999).

The effects of occupational exposure to perchlorate on thyroid function were studied in workers employed for at least 1.7 years (50% over 5.9 years) in the same ammonium perchlorate production plant in Utah as studied by Lamm et al. (1999). The following parameters were assessed in 29 workers after 3 days off and during the last of three 12 h night shifts in the plant and in 12 volunteers (controls) not working in the plant: serum perchlorate, thiocyanate and nitrate; serum T_4, free T_4 index, total T_3, thyroglobulin and TSH; 14 h radiolabelled iodine uptake by the thyroid; and urinary iodine and perchlorate. Estimated exposures to perchlorate during the study, from urinary and serum perchlorate concentrations, ranged from 0.01 to 3 mg/kg bw per shift. Over the year preceding this study, the median absorbed dose was estimated to be 0.33 mg/kg bw per shift. Serum thiocyanate and nitrate concentrations were similar in all groups. Serum and urinary perchlorate were not detected in the control group. In workers after 3 days off, urinary perchlorate was not detected in 12 of 29 workers and was a mean value of 0.27 mg/g creatinine in the remaining 17 workers; serum perchlorate was not detected in 27 of 29 workers. Serum and urinary perchlorate were markedly elevated to 868 µg/l and 43 mg/g creatinine, respectively, during perchlorate exposure. Radiolabelled iodine uptakes by the thyroid were significantly decreased by 38% in workers during three 12 night shifts compared with workers after 3 days off (13.5% versus 21.5%) and were associated with a significant increase in urinary iodine excretion (230 µg of iodine per gram creatinine versus 148 µg of iodine per gram creatinine), but were not significantly different from those in the control group (14.4%). Serum TSH and thyroglobulin concentrations were normal and similar in the three groups. Serum T_4 (8.3 µg/dl versus 7.7 µg/dl), free T_4 index (2.4 versus 2.2) and total T_3 (147 ng/dl versus 134 ng/dl) were slightly but significantly increased in the workers during 12 h night shifts compared with the workers after 3 days off. Thyroid volumes and patterns by ultrasound were similar in the 29 workers and 12 community volunteers.

The authors concluded that high perchlorate absorption during 3 nights of work exposure decreased the 14 h radiolabelled iodine uptakes by the thyroid by 38% compared with those in workers after 3 days off. However, serum TSH and thyroglobulin concentrations and thyroid volume by ultrasound were not affected by perchlorate, suggesting that long-term, intermittent, high exposure to perchlorate does not induce hypothyroidism or goitre in adults (Braverman et al., 2005).

The same group conducted a clinical study in volunteers that was designed to assess the effects of long-term exposure to perchlorate and to counter criticism (Braverman, 2007) that the workers in the study described above were not exposed continuously to perchlorate. The effect of 6 months of administration of perchlorate on thyroid function was studied in a prospective, double-blind, randomized trial. The study population consisted of 13 healthy volunteers (9 women, 4 men), and responses in individuals on placebo (4 subjects) were compared with those administered 0.5 mg (5 subjects) or 3.0 mg (4 subjects) of potassium perchlorate daily. Perchlorate and placebo were administered orally once daily by capsule. Assuming an average daily water ingestion of 2 litres, the 3.0 mg dose corresponded to a perchlorate concentration of approximately 1500 µg/l and the 0.5 mg dose to 250 µg/l. Assuming a body weight of 70 kg, these doses are equivalent to 0.04 and 0.007 mg/kg bw per day as potassium perchlorate. Serum thyroid function tests, 24 h radiolabelled iodide uptake, serum thyroglobulin, urinary iodine and perchlorate, and serum perchlorate were measured. Serum perchlorate was not detected in baseline samples or in those subjects receiving placebo. Serum perchlorate was detected in subjects receiving perchlorate, averaging, over the 6 months, 24.5 ± 16 µg/l in the 0.5 mg perchlorate group and 77.9 ± 18.2 µg/l in the 3.0 mg group. Urinary perchlorate was detected at low levels at baseline in all 13 subjects, averaging 9.2 ± 5.7 µg per 24 h or 7.3 ± 5.5 µg/g creatinine. It remained low in the subjects receiving placebo. The mean urinary perchlorate values during ingestion of 0.5 mg perchlorate daily were 332.7 ± 66.1 µg per 24 h or 248.5 ± 64.5 µg/g creatinine; during ingestion of 3.0 mg perchlorate daily, the values were 2079.5 ± 430.0 µg per 24 h or 1941.7 ± 138.5 µg/g creatinine. There was no significant change in the thyroid radioiodide uptakes during perchlorate administration. There were no significant changes in serum T_3, free T_4 index, TSH or thyroglobulin concentrations during the exposure period, compared with baseline or post-exposure values. Urinary iodine values for the 3.0 mg perchlorate group were higher, but not significantly so, at baseline than during perchlorate exposure. The authors acknowledged the small sample size (further recruitment to the study had been prevented by adverse publicity), concluding that a 6-month exposure to perchlorate at doses up to 3.0 mg/day was without any effect on thyroid function (Braverman et al., 2006). It should be noted that the serum perchlorate values in this volunteer clinical study were considerably lower than those obtained in the worker exposure study by the same group described previously (Braverman et al., 2005) and, in contrast to that study, were not sufficient to affect radiolabelled iodine uptake by the thyroid.

The potential relationship between urinary levels of perchlorate and serum levels of TSH and total T_4 in men and women 12 years of age and older participating in NHANES during 2001–2002 was evaluated. Multiple regression models that

included perchlorate and covariates known to be or likely to be associated with T_4 or TSH levels were used (i.e. age, race or ethnicity, body mass index, estrogen use, menopausal status, pregnancy status, premenarche status, serum C-reactive protein, serum albumin, serum cotinine, hours of fasting, urinary thiocyanate, urinary nitrate and selected medication groups). Perchlorate was not a significant predictor of T_4 or TSH levels in men, and the main analysis presented was for the 1111 women in the survey. The geometric mean value for urinary perchlorate concentration in the women was 2.84 µg/l (95% CI 2.54–3.18 µg/l). For women overall, perchlorate was a significant predictor of both T_4 and TSH. Urinary perchlorate was associated with an increased TSH and decreased total T_4 in women 12 years of age and older in the general population in the USA with urinary iodine levels below 100 µg/l (a potentially susceptible subpopulation). For women with urinary iodine levels at or above 100 µg/l, urinary perchlorate was a significant predictor of TSH but not T_4. The authors concluded that these effects of perchlorate on T_4 and TSH were coherent in direction and independent of other variables known to affect thyroid function, but were found at lower perchlorate exposure levels than were anticipated, based on previous studies (Blount et al., 2006b).

Others have pointed out that analysis of women of reproductive age from the same NHANES data set, but using creatinine-adjusted urinary iodide concentrations, found no association between T_4 and urinary perchlorate concentrations (Lamm et al., 2007). Similarly, a survey from Europe also found no correlation between urinary perchlorate concentrations and TSH or free T_4 among pregnant women with urinary iodide concentrations below 100 µg/l (Pearce, Lazarus & Smythe, 2007), whereas analysis of a cohort of women from Chile also found no significant associations between serum free T_4 or TSH concentrations and urinary perchlorate, whether expressed as micrograms per litre or as micrograms per gram of creatinine (Gibbs & Van Landingham, 2008).

In a further analysis of data from the NHANES 2001–2002 population, the impact of smoking and thiocyanate on the relationship between urinary perchlorate and serum T_4 and TSH was assessed. In women with urinary iodine levels below 100 µg/l, the association between the logarithm of urinary perchlorate concentration and decreased T_4 was greater in smokers (regression coefficient = −1.66, $P = 0.0005$) than in non-smokers (−0.54, $P = 0.04$). In subjects with high, medium and low cotinine levels, these regression coefficients were −1.47 ($P = 0.0002$), −0.57 ($P = 0.03$) and −0.16 ($P = 0.59$). For high, medium and low thiocyanate tertiles, they were −1.67 ($P = 0.0009$), −0.68 ($P = 0.09$) and −0.49 ($P = 0.11$). Clear interactions between perchlorate and smoking were not seen with TSH or with T_4 in women with urinary iodine levels at or above 100 µg/l or in men. The authors concluded that these results suggest that thiocyanate in tobacco smoke and perchlorate interact in affecting thyroid function, and this effect can take place at commonly occurring perchlorate exposures (Steinmaus, Miller & Howd, 2007).

The potential effects of environmental inhibitors of iodide uptake by the NIS, other than perchlorate, have also been considered from the basis of a literature search performed on articles published up to early 2006 on perchlorate, iodide, nitrate and thiocyanate. Using robust data from Tonacchera et al. (2004) on the relative potencies of perchlorate, nitrate and thiocyanate with respect to inhibition

of iodide uptake and the maximum contaminant levels allowed in the USA for nitrate and cyanide in drinking-water, the authors concluded that perchlorate levels in drinking-water would account for only 10% of the resulting iodine uptake inhibition from the presence of these three inhibitors in drinking-water and a negligible proportion with respect to exposure from food (De Groef et al., 2006).

(b) Benchmark dose calculations from data on non-pregnant adults

BMDs for perchlorate effects on TSH and T_4 have been calculated from three of the studies described above—the two occupational cohorts with long-term exposure to perchlorate (Lamm et al., 1999; Braverman et al., 2005) and the clinical study of volunteers exposed to perchlorate for 2 weeks (Greer et al., 2002). Two serum indicators, TSH and free T_4 index, were used in the calculation of BMDs. The BMD for each serum indicator was defined as the dose predicted to cause an additional 5% or 10% of persons to have a serum measurement outside of the normal range. Using the data from the clinical study, the half-life of perchlorate in serum was estimated to be 7.5 h and the volume of distribution 0.34 l/kg. Using these estimates and measurements of perchlorate in serum or urine, doses in the occupational cohorts were estimated and used in benchmark calculations using a non-linear model. Because none of the three studies found a significant effect of perchlorate on TSH or free T_4, all of the BMD estimates were indistinguishable from infinity. The lower 95% statistical confidence limit on the benchmark dose (BMDL) estimated from a combined analysis of the two occupational studies ranged from 24 to 50 mg/day, corresponding to 0.21–0.56 mg/kg bw per day, for free T_4 index and from 57 to 83 mg/day, corresponding to 0.36–0.92 mg/kg bw per day, for TSH. Corresponding estimates from the short-term clinical study were within these ranges (Crump & Gibbs, 2005). Crump & Gibbs (2005) argued that although none of the three studies they utilized found evidence of an effect of perchlorate on thyroid function, BMDLs calculated from such negative results nevertheless represent valid statistical lower bounds on the dose that accounts for a potential, but unobserved, effect of perchlorate. They did acknowledge, however, that BMDLs based on negative data could possibly be highly conservative.

Another BMD analysis based on the Greer et al. (2002) study was conducted by ICF Consulting (2004). BMDLs were calculated from data on free T_4, total T_4 and T_3. The thyroidal measurements for an individual at each of three measurement times (morning, midday and afternoon) on day 14 minus the average of his or her measurements on 2 pre-exposure days and 1 post-exposure day were modelled. For each of these nine cases, BMDLs were calculated corresponding to 5%, 10% or 20% change in response. This resulted in 27 BMDLs ranging from 7 to 89 mg/day. The nine BMDLs calculated for free T_4 correspond to a range of 8–76 mg/day, which contains the range of BMDLs for free T_4 obtained in the analysis by Crump & Gibbs (2005). Crump & Gibbs (2005) considered that the differences between the ICF Consulting (2004) results and their analysis for free T_4 were likely due largely to the decisions by ICF Consulting (2004) to segment the data by time of day (compared with their approach of using all the data and controlling for time of day) and to exclude the data from the uptake study. The ICF Consulting (2004) analysis also employed a different definition of the BMD than was used in the Crump

& Gibbs (2005) analysis and employed a linear model as opposed to the non-linear model employed by Crump & Gibbs (2005).

Crump & Gibbs (2005) also discussed other BMD analyses that have been published for perchlorate based on human data, all of which used the Greer et al. (2002) study of perchlorate consumption by volunteers. The analysis performed by the California Environmental Protection Agency's Office of Environmental Health Hazard Assessment (CalEPA, 2004; Ting et al., 2006) modelled the ratio of the radiolabelled iodine uptake after perchlorate exposure to the baseline value and defined the BMD as the dose corresponding to a 5% change in this ratio. CalEPA (2004) calculated a BMDL of 0.0037 mg/kg bw per day, which, in a 70 kg individual, is equivalent to 0.26 mg/day. This value is much lower than the BMDLs obtained in the analysis by Crump & Gibbs (2005), either from the Greer et al. (2002) study or from the occupational studies (Lamm et al., 1999; Braverman et al., 2005); however, this is not surprising, given that the former were based on inhibition of iodine uptake by the thyroid, whereas the latter were based on TSH and T_4. Crump & Gibbs (2005) commented that the main reason for their different approach is that, unlike CalEPA (2004) and the USEPA, they did not consider a reduction in uptake of radiolabelled iodine, without accompanying changes in TSH and T_4, to be an adverse response.

(c) Pregnant adults, fetuses and neonates

In 1997, perchlorate was detected in the drinking-water supplies of six counties in California, USA, and one in Nevada, USA, at concentrations of 4–16 µg/l. In a series of ecological investigations, the incidence of congenital hypothyroidism and thyroid function (blood T_4 and TSH) in newborns and school performance and the incidence of neurobehavioural diseases in children were assessed in exposed or unexposed populations from these two states in the USA. The incidence of thyroid diseases in exposed and unexposed populations in Nevada was also investigated.

In the first part of the investigation, data on nearly 700 000 newborns from the seven counties in California and Nevada in which perchlorate was detected in the drinking-water were obtained from the neonatal screening programmes of the state health departments during 1996 and 1997. They were analysed for any increased incidence of congenital hypothyroidism in those counties compared with that expected for the whole state. In all, 249 cases were identified, compared with 243 expected, giving an overall risk ratio of 1.0 (95% CI 0.9–1.2). The risk ratios for the individual counties ranged between 0.6 and 1.1. The data in this analysis did not indicate an increase in the incidence of congenital hypothyroidism associated with the reported perchlorate levels (Lamm & Doemland, 1999).

In the second part of the investigation, neonatal blood T_4 levels on days 1–4 of life during the period April 1998 through June 1999 were compared for 17 308 newborns from the city of Las Vegas, Nevada, which had perchlorate in its drinking-water, and 5882 newborns from the city of Reno, Nevada, which did not (detection limit 4 µg/l). Perchlorate levels in drinking-water in Las Vegas measured monthly during this study period ranged from non-detectable for 8 months to levels of 9–15 µg/l for 7 months. No significant difference was found in the mean T_4 levels of the

newborns from these two cities (crude analysis: 17.11 µg/dl for Las Vegas versus 17.22 µg/dl for Reno). The authors noted that the study was sufficiently sensitive to detect the effects of sex, birth weight and the day of life on which the blood sample was taken on the neonatal T_4 level, but it detected no effect from environmental exposures to perchlorate that ranged up to 15 µg/l (Z. Li et al., 2000).

In the third part of the investigation, neonatal blood TSH levels were studied in the same two populations of Las Vegas and Reno over a similar time period, from December 1998 to October 1999. The blood samples from neonates within the birth weight range 2500–4500 g were taken during the first month of life, excluding the first day of life. No significant difference was found in TSH levels between 540 newborns from Las Vegas (geometric mean ± standard deviation [SD] 11.5 ± 1.3 micro-international units [IU] per millilitre) and 130 newborns from Reno (12.5 ± 1.3 µIU/ml), analysed by multiple linear regression on log-transformed data, controlling for the potential confounders of sex and age in days. As TSH levels were measured in neonates only in the lowest 10% of the distribution of blood T_4 levels, this analysis was considered to represent a sensitive subpopulation with respect to the possibility of raised TSH levels (F.X. Li et al., 2000).

In the fourth part of the investigation, school performance and the incidence of attention deficit hyperactivity disorder and autism were assessed in children from exposed and unexposed populations in Nevada. The exposed population of 39 084 children from Clark County, Nevada, which includes the city of Las Vegas, was compared with 8471 unexposed urban controls from Washoe County, which includes the city of Reno, and 7859 rural controls from the rest of the state. Drinking-water supplied to Clark County between July 1997 and May 2002 had a mean perchlorate level of 10.9 µg/l (10th to 90th percentile range 7–15 µg/l). Nevada Medicaid records for children under 18 years of age for the years 1996–2000 were accessed. School performance in fourth graders was ascertained from the results of standardized tests that were administered nationwide. There were no differences between the exposed population and the urban or rural control populations in the incidence of neurobehavioural diseases or in school performance (Chang et al., 2003). Assuming a daily ingestion of water of 2 litres for adults and 1.5 litres for schoolchildren, exposures for the exposed populations in the above investigations were estimated to be 10–32 µg/day for adults and 7.5–24 µg/day for schoolchildren.

In the fifth part of the investigation, an analysis of all users of the Medicaid programme in Nevada was undertaken to determine whether there was an increase in the prevalence of any thyroid disease associated with a perchlorate level of 4–24 µg/l in the water supply of Clark County (including Las Vegas), Nevada. The overall prevalence of thyroid disease, of specific thyroid diseases (goitre, nodule, thyrotoxicosis, congenital hypothyroidism, acquired hypothyroidism, thyroiditis and other thyroid disorders) and thyroid cancer among the Medicaid-eligible population of each county was calculated for the 2-year period 1997–1998. Data from residents in Clark County (population 122 519 exposed to perchlorate) were compared with those from residents in Washoe County (population 29 622 not exposed to perchlorate), the second most populous county in the state, and with those for the rest of the state (also not exposed to perchlorate). There was no evidence of any increase in rate for overall thyroid disease or of any specific thyroid disease, including thyroid cancer, associated with perchlorate exposure (Li, Squartsoff & Lamm, 2001).

Studies were conducted using data on congenital hypothyroidism and neonatal blood TSH levels from the California Newborn Screening Program from 1983 to 1997. Neonates from an exposed population in Redlands (15 090 newborns) were compared with those from unexposed populations in San Bernadino and Riverside counties (685 161 newborns). The adjusted prevalence ratio for congenital hypothyroidism in the study community of Redlands compared with San Bernardino and Riverside counties combined was 0.45 (95% CI 0.06–1.64). The odds ratios for elevated TSH concentration for Redlands compared with San Bernardino and Riverside were 1.24 (95% CI 0.89–1.68) among all newborns screened and 0.69 (95% CI 0.27–1.45) for newborns whose age at screening was 18 h or greater. There were no differences between the study population and control populations in congenital hypothyroidism or TSH levels (Kelsh et al., 2003).

The above study was expanded using data from the California Newborn Screening Program for 1998 to examine the prevalence of congenital hypothyroidism and TSH levels for all California newborns whose mothers resided in communities where water supplies were tested for perchlorate in 1997 and 1998. Thyroid function results for newborns from 24 communities with average perchlorate concentrations in drinking-water above 5 µg/l ($n = 50\,326$) were compared with those for newborns from 287 communities with average concentrations at or below 5 µg/l ($n = 291\,931$). All adjusted odds ratios were controlled for sex, ethnicity, birth weight and multiple birth status. Fifteen cases of congenital hypothyroidism from communities with average concentrations above 5 µg/l were observed, with 20.4 expected (adjusted prevalence odds ratio [POR] = 0.71; 95% CI 0.40–1.19). For newborns screened at or after 24 h, the adjusted POR for high TSH was 0.73 (95% CI 0.40–1.23). This study did not find an association between estimated average perchlorate concentrations above 5 µg/l in drinking-water supplies and the prevalence of clinically diagnosed congenital hypothyroidism or high TSH concentrations (Buffler et al., 2006).

Another study examined whether exposure to drinking-water contaminated by perchlorate affected neonatal TSH levels, using data from the Arizona Newborn Screening Program between October 1994 and the end of 1997. The exposed population comprising 1099 newborns was from Yuma, where drinking-water, supplied from the Colorado River below Lake Mead, is contaminated by ammonium perchlorate. The unexposed population comprising 433 newborns was from Flagstaff, which was supplied by drinking-water that had not been contaminated by perchlorate. Data on perchlorate levels in drinking-water during the study period itself were not available, but concentrations of 6 µg/l were measured in water supplied to Yuma in 1999, whereas perchlorate was undetectable in water supplied to Flagstaff. TSH levels in newborns from Yuma, after adjusting for age in days at measurement and for race or ethnicity, were significantly higher than levels in newborns from Flagstaff (Yuma median 19.9 mIU/l, interquartile range 12.5–28.3 mIU/l; Flagstaff median 13.4 mIU/l, interquartile range 8.8–18.9 mIU/l). This ecological study demonstrated a statistically significant association between perchlorate exposure and newborn TSH levels. The authors concluded that it suggests that even low-level perchlorate contamination of drinking-water may be associated with adverse health effects in neonates (Brechner et al., 2000).

This study is notable in that it is the only study to report an effect of low-level exposure to perchlorate on thyroid function in newborns. Others have commented on these findings, pointing out that there were marked imbalances in age at sampling and suggesting that they could have resulted from variation in medical practices, demographic factors or other geographic factors, rather than differences in perchlorate exposures between the two cities of Flagstaff and Yuma (Goodman, 2001; Lamm, 2003).

A study was conducted in three coastal cities in Chile to investigate whether perchlorate in drinking-water affected thyroid function in newborns and school-aged children. Drinking-water in the city of Taltal had a mean perchlorate concentration of 112 µg/l (range 100–120 µg/l). In the city of Chanaral, perchlorate was not detected (limit of detection [LOD] 4 µg/l) in 4 out of 25 samples, and concentrations in the other 21 samples averaged 6.2 µg/l (range 5.3–6.7 µg/l). In the city of Antofagasta, perchlorate was undetectable. Neonatal screening records were obtained for a total of 9784 neonates born in the three cities between February 1996 and January 1999. A total of 162 children 6–8 years of age were studied; of these, 127 had lived in their city since birth, and their mothers had lived in their city for the year preceding their birth. The study found no evidence that perchlorate in drinking-water affected thyroid function in newborns, adjusted for age, or in school-aged children, as assessed by TSH or free T_4 levels. No cases of congenital hypothyroidism were detected in the cities with perchlorate in the drinking-water, and the prevalence of goitre among the school-aged children was similar in the three cities (Crump et al., 2000).

In a subsequent longitudinal study conducted between November 2002 and April 2004 in the same three cities in Chile, thyroglobulin, urinary perchlorate, TSH and free T_4 were measured in women during early pregnancy (16.1 ± 4.1 weeks) and late pregnancy (32.4 ± 3.0 weeks) and in cord serum at birth. The mean perchlorate levels were similar to those reported in their previous study (Crump et al., 2000), with mean values of 113.9 µg/l for Taltal, 5.82 µg/l for Chanaral and non-detectable for Antofagasta. Exposure from tap water was calculated from self-reported tap water consumption, which was around 1 litre per day, and additional exposure from the diet was calculated by subtracting this amount from the measured urinary perchlorate levels in micrograms per gram creatinine and an assumed creatinine excretion for pregnant women of 1.08 g/day. Total calculated median perchlorate excretion values were 22.1, 40.0 and 118.8 µg/day for Antofagasta, Chanaral and Taltal, respectively, indicating that 21.7, 33.8 and 25.3 µg/day in the three respective cities came from the diet. Perchlorate was detectable in maternal and cord serum in subjects from Taltal, but was non-detectable in serum samples from the other two cities. The incidence of goitre was higher, ranging up to 36.4%, than the incidence in the USA, which the authors suggested was possibly due to higher iodine levels in the past in Chile. The incidence of goitre in subjects from Chanaral was higher than that in those from Antofagasta or Taltal, but the authors considered this finding unexplainable. There were no significant differences in any thyroid parameters or in neonatal birth weight, head circumference or length between the three cities (Téllez Téllez et al., 2005).

A study in Ramat Hasharon, Israel, in 2004 assessed the effect of gestational perchlorate exposure through drinking-water on neonatal T_4 by comparing newborns from mothers residing in suburbs where drinking-water contained differing levels of perchlorate: ≤340 µg/l (very high exposure, *n* = 97), 42–94 µg/l (high exposure, *n* = 216) and <3 µg/l (low exposure, *n* = 843). In the very high and high exposure areas, T_4 values in newborns whose mothers drank tap water exclusively (as determined by a telephone interview) were analysed as a subset. Serum perchlorate levels in blood from donors residing in the area in 2003–2004 were used as proxy indicators of exposure. Neonatal T_4 values (mean ± SD) in the very high, high and low exposure groups were 13.9 ± 3.8 µg/dl, 13.9 ± 3.4 µg/dl and 14.0 ± 3.5 µg/dl, respectively. Serum perchlorate concentrations in blood from donors residing in areas corresponding to these groups were 5.99 ± 3.89 µg/l, 1.19 ± 1.37 µg/l and 0.44 ± 0.55 µg/l, respectively. This study found no change in neonatal T_4 levels despite maternal consumption of drinking-water that contained perchlorate at levels in excess of the USEPA drinking-water equivalent level (DWEL) of 24.5 µg/l based on the RfD of 0.7 µg/kg bw per day (USEPA, 2005b; Amitai et al., 2007).

2.3.3 Physiologically based pharmacokinetic modelling

The work of Clewell and co-workers (Clewell, Merrill & Robinson, 2001; Clewell et al., 2003a,b, 2004) in animals on PBPK modelling, described in section 2.2.6, laid the foundation for development of a human PBPK model for different life stages in humans. A model developed for adult humans describes the kinetics and distribution of radiolabelled iodide and perchlorate in healthy subjects and simulates the subsequent inhibition of thyroid uptake of iodide by perchlorate. The model was checked against data from published studies on human serum and urinary levels of perchlorate and perchlorate inhibition of uptake of iodide in the thyroid following administration in drinking-water. The model incorporates uptake of iodide and perchlorate into human skin, which serves as a large pool for storage and release of the anions, and from where it is assumed to slowly diffuse back into the systemic circulation. However, the authors concluded that the model was not yet sufficiently developed to predict effects of perchlorate on thyroid hormones (Merrill et al., 2005).

In a subsequent study, the same group modelled iodide and perchlorate kinetics at various life stages: pregnant and lactating woman, fetus and nursing infant. This would allow estimation of dose delivery to critical targets, such as the developing thyroid. They modelled iodide uptake inhibition levels for external (i.e. ingested) doses of perchlorate of 1, 10, 100 and 1000 µg/kg bw per day. At the two lower modelled doses of 1 and 10 µg/kg bw per day, the pregnant and lactating woman, fetus and nursing infant were predicted to have higher blood perchlorate concentrations (by 4- to 5-fold) and greater inhibition of iodide uptake by the thyroid at a given drinking-water concentration of perchlorate than either the non-pregnant adult or the older child. Smaller relative differences were predicted at external doses of 100 and 1000 µg/kg bw per day. The authors attributed this change to saturation of uptake mechanisms. The fetus was predicted to receive the greatest dose (per kilogram body weight) due to several factors, including placental NIS activity and reduced maternal urinary clearance of perchlorate. The model predicted a minimal effect of perchlorate on iodide uptake inhibition in all groups of 1.1% or less at an

external dose of 1 µg/kg bw per day, which is about 1–1.5 times the RfD of 0.7 µg/kg bw per day (USEPA, 2005b). Inhibition was predicted to be 10% or less in all groups at an external dose of 10 µg/kg bw per day, which is about 14 times the RfD (Clewell et al., 2007).

2.3.4 Existing reference doses and drinking-water standards

(a) United States National Academy of Sciences and Environmental Protection Agency

In 1998, the USEPA issued a preliminary risk assessment for perchlorate (USEPA, 1998) and, after revision, issued guidance in 1999 on perchlorate, recommending continued use of a provisional reference dose range of 7–35 µg/day for a 70 kg adult or 0.1–0.5 µg/kg bw per day (USEPA, 1999a). In 2002, the USEPA published a revised assessment for comment, which proposed an RfD of 0.03 µg/kg bw per day (USEPA, 2002). This was based on a weight of evidence approach, in which data from rat developmental neurobehavioural studies provided the critical effect of alteration of offspring brain morphometry (York et al., 2004, 2005a,b). The RfD was derived by application of an uncertainty factor of 300 to the human exposure equivalent of 0.01 mg/kg bw per day obtained using PBPK modelling of the BMD from the developmental neurobehavioural studies. At that time, the USEPA considered that the observational epidemiological and human clinical studies had scientific and technical limitations that precluded their use for derivation of an RfD (USEPA, 2002).

In 2003, the United States National Academy of Sciences (NAS) reviewed the USEPA's 2002 draft health assessment for perchlorate. The report of the NAS was published in 2005 (NRC, 2005). Concerning specific health outcomes, the NAS concluded the following, on the basis of the then available evidence:

- *Congenital hypothyroidism:* All studies of a causal association with perchlorate exposure were negative.
- *Changes in thyroid function in newborns:* The evidence was not consistent with a causal association between perchlorate exposure during gestation to concentrations of perchlorate in drinking-water up to 120 µg/l and effects on TSH or T_4 in newborns. However, it was noted that no data were available on the association of perchlorate exposure with thyroid dysfunction in groups of greatest concern, low birth weight or preterm newborns, offspring of mothers who had iodide deficiency during gestation or offspring of hypothyroid mothers.
- *Neurodevelopmental outcomes:* The evidence is inadequate.
- *Hypothyroidism and other thyroid disorders in adults:* The evidence is not consistent with a causal association between perchlorate exposure and thyroid diseases.
- *Thyroid cancer in adults:* The evidence is insufficient, but the committee questioned the plausibility of thyroid cancer as a likely outcome of perchlorate exposure.

Concerning the derivation of an RfD, the NAS concluded the following: After reviewing the human and laboratory animal data, the committee found that the

human data provided a more reliable point of departure for the risk assessment than the laboratory animal data. The committee recommended using clinical data collected in a controlled setting with the relevant route of exposure to derive the RfD, as it considered that the epidemiological studies, while informative, were limited for the purposes of quantitative risk assessment. The committee also noted that the epidemiological studies typically focused on changes in serum thyroid hormones and TSH concentrations or clinical manifestations of the changes, not on inhibition of iodide uptake by the thyroid, which the committee considered to be the key precursor event to the adverse effect of hypothyroidism and the only effect that has been consistently demonstrated in humans exposed to perchlorate. The committee recommended using the Greer et al. (2002) study, in which groups of healthy men and women were administered perchlorate at 0.007–0.5 mg/kg bw per day for 14 days, and which identified a NOEL for inhibition of iodide uptake by the thyroid of 0.007 mg/kg bw per day, noting that this NOEL was consistent with other clinical studies that have investigated iodide uptake inhibition by perchlorate. The committee considered that using the NOEL value from Greer et al. (2002) would be a health-protective and conservative point of departure that was supported by the results of a 6-month study of a small group of healthy subjects exposed to 0.007 mg/kg bw per day, a 4-week study of healthy subjects exposed to higher doses, the studies of perchlorate treatment of patients with hyperthyroidism, and extensive human and laboratory animal data that demonstrated that there will be no progression to adverse effects if no inhibition of iodide uptake occurs. The committee recommended using a total uncertainty factor of 10, comprising a full intraspecies factor of 10 to protect the most sensitive population—the fetuses of pregnant women who might have hypothyroidism or iodide deficiency—which would lead to an RfD of 0.0007 mg/kg bw per day (i.e. 0.7 µg/kg bw per day). No additional factors were needed for duration or database uncertainties. If inhibition of iodide uptake by the thyroid was used, chronic exposure would have no greater effect than that resulting from short-term exposure (it may even have less effect because of the capacity of the pituitary–thyroid system to compensate for iodide deficiency by increasing iodide uptake). The committee considered the database sufficient, given that the point of departure selected was based on inhibition of iodide uptake by the thyroid. The committee recognized that the use of a non-adverse effect upstream in the pathway of events following exposure to perchlorate to derive an RfD differed from the traditional approach of using an adverse effect, but that such an approach would be conservative and protective of health (NRC, 2005).

Some subsequently commented that the NAS RfD, based on data from iodine-replete, healthy adults, was not sufficiently conservative, arguing that inhibition of iodine uptake should be considered as adverse and that a higher overall uncertainty factor should have been used because of remaining uncertainties in the database, particularly those relating to iodine-deficient, pregnant women and lactational exposure of newborns (Ginsberg & Rice, 2005a,b; Zoeller, 2006). Conversely, others argued that the RfD was overly conservative (Strawson, Dourson & Zhao, 2005). Members of the NAS committee on perchlorate and others responded to these comments and criticisms, emphasizing that the committee's recommended RfD of 0.0007 mg/kg bw per day provided a wide margin of safety for all subjects of all ages (Gibbs, Engel & Lamm, 2005; Johnston et al., 2005).

Following completion of the NAS review (NRC, 2005), the USEPA adopted the NAS-recommended RfD of 0.0007 mg/kg bw per day for perchlorate (USEPA, 2005b). A DWEL of 24.5 µg/l for perchlorate was then set, based on the RfD (USEPA, 2005a,b). This level corresponds to the amount that would deliver the RfD of 0.0007 mg/kg bw per day for a 70 kg adult ingesting 2 litres of water a day.

This same DWEL was also used as a groundwater preliminary remediation goal (PRG) set by the USEPA's Office of Solid Waste and Emergency Response in 2006 (USEPA, 2006). Crawford-Brown, Raucher & Harrod (2006), after applying probabilistic risk analysis to varying intakes of perchlorate in drinking-water of between 1 and 50 µg/l, concluded that maximum contaminant levels of up to 24.5 µg/l should pose little or no incremental risk to the large majority of individuals in the most sensitive subpopulations (pregnant women) exposed in the USA at current levels of perchlorate in water. Ginsberg et al. (2007), however, commented that this PRG is not necessarily protective for nursing and bottle-fed infants who consume more liquid per unit body weight than adults, and it does not include the relative source contribution, a factor normally used to lower the PRG due to non-water exposures. Monte Carlo analysis was used to simulate nursing infant exposures associated with the Office of Solid Waste and Emergency Response PRG when combined with background perchlorate, and these were compared with the RfD. It was estimated that the PRG could lead to a 7-fold increase in breast milk concentration, causing 90% of nursing infants to exceed the RfD by an average 2.8-fold, and that drinking-water perchlorate must be below 6.9 µg/l and 1.3 µg/l to keep the median and the 90th-percentile nursing infant exposures, respectively, below the RfD. The authors also analysed data on urinary excretion of perchlorate from women in the NHANES 2001–2002 survey (Blount et al., 2006a) to assess the dietary (non-water) contribution to perchlorate exposure, and this suggested a relative source contribution of 0.7 for pregnant women and of 0.2 for nursing infants.

In December 2008, the USEPA revised its advice to take account of the contribution to overall perchlorate intake from food (USEPA, 2008a). Using data on urinary perchlorate excretion generated in the NHANES database and data on perchlorate intake from food generated in the United States Food and Drug Administration's (USFDA) Total Diet Study (TDS) (Murray et al., 2008), the 90th-percentile intake from food was calculated to be 38% for pregnant women, leaving 62% for the contribution from drinking-water. Accordingly, the USEPA issued an interim drinking water health advisory of 15 µg/l, which covers pregnant women, infants and developing children (USEPA, 2008a). At the same time, the USEPA announced it was seeking further advice from the NAS, including an evaluation of the interim health advisory value of 15 µg/l, the use of modelling to evaluate impacts on infants and young children, and the implications of recent biomonitoring studies, before making a final determination on whether to issue a national regulation for perchlorate in drinking-water (USEPA, 2009).

(b) Others

Strawson, Zhao & Dourson (2004) considered that the most relevant data for developing an RfD for perchlorate exposure come from human epidemiological and clinical studies, supplemented with available and extensive information on

experimental animals. Based on mode of action analysis and the evidence provided by rodent studies on perchlorate, these authors considered a decrease in serum T_4 to be the critical effect of perchlorate, but noted that no T_4 decreases have been observed in human populations following perchlorate exposure at non-therapeutic doses. They identified a freestanding NOAEL of 0.006 mg/kg bw per day for T_4 decrease in children from the epidemiological study of Crump et al. (2000), supported by the threshold for inhibition of iodine uptake of 0.006 mg/kg bw per day from the study of Greer et al. (2002) as a measurable surrogate for the critical effect of T_4 decrease in humans. They selected an overall uncertainty factor of 3-fold, based on expected differences in toxicokinetics and toxicodynamics between children and pregnant women and their fetuses, to derive an RfD of 0.002 mg/kg bw per day (Strawson, Zhao & Dourson, 2004).

Zoeller & Rice (2004), in commenting on the RfD derived by Strawson, Zhao & Dourson (2004), noted that there were uncertainties in applying a NOAEL obtained in human adults to human neonates, who might be more sensitive to perchlorate because of much lower levels of stored hormones in the neonatal thyroid gland and their requirement for thyroid hormones for normal neurobehavioural development.

Srinivasan & Viraraghavan (2009) summarized drinking-water guidance values and advisory action levels proposed by bodies other than the USEPA, including 10 individual states in the USA and Health Canada. The values range from 1 to 18 µg/l.

2.3.5 Cancer

In response to concerns about the possibility of cancer stemming from drinking-water contaminated with ammonium perchlorate (and trichloroethene), the observed and expected numbers of new cancer cases for all sites combined and 16 individual cancer types in the California (USA) community of Redlands, San Bernardino County, were assessed from 1988 to 1998. Cancer cases in the area of the city of Redlands, which had a population of 54 500–66 400 during the study period, were identified from one of the regional cancer registries in California. Expected numbers of cases were obtained from the overall data in the California Cancer Registry, which gathers data from the five regional cancer registries, including the region in which Redlands is located. Contamination of well water was considered likely from 1980 onwards, but testing for perchlorate in one of the areas covered by the study (Redlands) accompanied improved technology and began only in 1997. Levels of perchlorate in Redlands wells in 2001 ranged from 5 to 98 µg/l, although the city of Redlands did not deliver water to customers containing perchlorate at concentrations in excess of 18 µg/l since testing began. Cancer rates were expressed as standardized incidence ratios (SIRs: observed cases divided by expected cases) and 99% confidence intervals. There was a total of 3098 cancer cases in Redlands during the decade studied. No significant differences were found between observed and expected numbers for all cancers (SIR 0.97; 99% CI 0.93–1.02), thyroid cancer (SIR 1.00; 99% CI 0.63–1.47) or 11 other cancer types. Significantly fewer cases were observed than expected for cancer of the lung and bronchus (SIR 0.71; 99% CI 0.61–0.81) and the colon and rectum (SIR 0.86; 99%

CI 0.74–0.99), whereas more cases were observed for uterine cancer (SIR 1.35; 99% CI 1.06–1.70) and skin melanoma (SIR 1.42; 99% CI 1.13–1.77). The findings in this community did not identify a generalized cancer excess or thyroid cancer excess (Morgan & Cassady, 2002).

The analysis of the Medicaid database from Nevada, USA, described previously found no evidence of any increased incidence of thyroid cancer associated with perchlorate exposure from drinking-water (Li et al., 2001).

3. ANALYTICAL METHODS

3.1 Chemistry

A combination of human activity and natural sources has led to the widespread presence of perchlorate in the environment. Perchlorate is highly soluble and very stable in water. The order of solubility of the common perchlorate salts is sodium > lithium > ammonium > potassium (Mendiratta et al., 1996). Although perchlorate has excellent oxidizing ability, the activation energy required to initiate the chemical reaction is very high. The high activation energy and solubility of the salts lead to perchlorate's stability and mobility in the environment.

3.2 Description of analytical methods

Perchlorate is soluble in water and polar organic solvents and is easily extracted from foods using either water or water–acetonitrile mixtures. Water samples are analysed directly, whereas food extracts are subjected to solid-phase extraction (SPE) cleanup prior to determination. Analytical methods used for the detection and determination of perchlorate levels in water and foods include ion chromatography (IC) with conductivity detector, IC coupled to mass spectrometry (IC-MS or IC-MS/MS) and liquid chromatography (LC) coupled to mass spectrometry (LC-MS or LC-MS/MS). IC-MS/MS has significantly improved sensitivity, and the LODs are reported to be in the range of 0.5–5 ng/l for water and 1–15 ng/g for various food matrices.

3.2.1 Screening tests

Development of screening tests for the detection of trace levels of perchlorate in foods has been rather limited. Before 1997, the detection methods for perchlorate were mainly gravimetric and spectrophotometric methods. The LOD for these methods was higher than 100 µg/l, and trace amounts of perchlorate in groundwater were undetectable (Zhou, 2007). Other methods, such as capillary electrophoresis (Ellington et al., 2001), colorimetry (Thorne, 2004) and use of biological sensors (Okeke et al., 2007), have sensitivity limitations. The perchlorate-selective electrode developed using a submicrometre-thick plastic membrane coated on a poly(3-octylthiophene)-modified gold electrode gave an LOD in the range of 20–50 ng/l for purified water, bottled water and tap water. Surface-enhanced Raman scattering was used in a method by Gu, Ruan & Wang (2009) to measure perchlorate at an LOD of 0.1 µg/l. When coupled to a portable Raman spectrometer, this technique has the potential to be used as an in situ, rapid

screening tool for perchlorate in the environment. However, method validation has not been carried out.

3.2.2 Quantitative methods

Analysis of perchlorate at trace and ultra-trace levels in complex food matrices has become possible with the development of chromatographic and mass spectrometric techniques. At present, IC with conductivity detector or IC coupled to MS is commonly used for the determination of perchlorate in drinking-water. Tandem mass spectrometers (MS/MS) coupled to either IC or LC offer lower LODs for the analysis of complex food matrices. Stable isotope–labelled perchlorate ([^{18}O]perchlorate) is used as an internal standard to enhance the accuracy of the method (USFDA, 2005; Oldi & Kannan, 2009b).

Water samples are directly analysed after centrifugation or filtration (USFDA, 2005). Fruits and vegetables are chopped and freeze-dried, then extracted with water using an ultrasonic bath or under boiling conditions. A 1% acetic acid solution is also used to prevent perchlorate retention on graphitized carbon during the SPE cleanup. After centrifugation, the supernatant is cleaned on SPE (C8, C18, RP, Oasis HLB or graphitized-carbon black cartridge), and metal ions are removed by ion exchange or silicon, aluminium or silver adsorbents (USFDA, 2005; Li & George, 2006; Zhang et al., 2007a). An accelerated solvent extraction (ASE) method has been developed for extraction of perchlorate. Grains such as wheat and corn are powdered, homogenized and extracted with water, and acetonitrile is added to precipitate protein.

An IC-MS method has been developed for the determination of perchlorate in milk based on precipitation of proteins with acetonitrile, resulting in satisfactory recoveries at different spiking levels (Zhang et al., 2007b). Frozen ethanol is also used in the extraction of perchlorate from milk, followed by SPE cleanup (Kirk et al., 2003, 2005). The interferences in milk can also be removed by centrifuging multiple times without adding any solvent (Dyke et al., 2006); however, the procedure is time-consuming.

Analytical methods for the determination of perchlorate in animal tissues are rather limited. Anderson & Wu (2002) evaluated a method in which homogenized samples are fortified with an internal standard, dried at 65 °C, extracted with water using ASE and recovered after cleanup on different SPE cartridges. Perchlorate in whole fish, fish heads and fillets was detected following this extraction method and using the Alumina-B and Bakerbond silica gel SPE cartridges for the further cleanup (Theodorakis et al., 2006).

An IC method using conductivity detector has been adopted as the standard method for the determination of perchlorate in water (EPA Methods 314.0 and 314.1; USEPA, 1999b, 2005c). The LOD of EPA Method 314.0 is 0.53 µg/l, and the method reporting limit is 4.0 µg/l (USEPA, 1999b). EPA Method 314.1 is suitable for the determination of perchlorate in waters with a wide range of total dissolved solids, and method reporting limits are in the range of 0.5–1 µg/l. Owing to the nonspecific property of conductivity detectors, false positives may occur if perchlorate is not properly separated (Mathew, Gandhi & Hedrick, 2005). Two-dimensional IC enhanced perchlorate separation, and an anion-suppressed conductivity detection gave an LOD of 55 ng/l (EPA Method 314.2; USEPA, 2008b).

In IC-MS (and MS/MS) methods, predominant ions detected are $^{35}ClO_4^-$ and $^{37}ClO_4^-$ (*m/z* 99 and 101, respectively), as well as the ^{18}O isotope–labelled internal standard ($Cl^{18}O_4^-$). Ion suppression is a common problem in electrospray ionization (ESI) or atmospheric pressure ionization (API). Mobile phases consisting of non-volatile buffers are usually avoided, or suppressors unique to IC are used to avoid the problem. LODs are in the range of 50–100 ng/l (Mathew, Gandhi & Hedrick, 2005). To avoid inorganic salt buildup, an anion self-regeneration is employed (Mathew, Gandhi & Hedrick, 2005; Mou, Liu & Ding, 2005). Perchlorate in water samples is determined using IC-MS coupled with suppressor (Tan et al., 2004). Valve switching is also used to reduce interferences introduced into the MS detector. An LOD of 0.1 µg/l has been achieved for milk using IC-MS or IC-MS/MS (ITRC, 2005). Ion transition in the tandem MS (99>83, 101>85, 107>89 and 109>91) significantly improves sensitivity and selectivity. Derivation of perchlorate with 1,12-bis(trimethylammonium) dodecane (DQ) difluoride offers higher molecular weight ions ([$DQ^{35}Cl^{16}O_4^+$, *m/z* 384.8], [$DQ^{37}Cl^{16}O_4^+$, *m/z* 386.8], [$DQ^{35}Cl^{18}O_4^+$, *m/z* 393.0], [$DQ^{37}Cl^{18}O_4^+$, *m/z* 395.0]), which helps in eliminating background interferences and improving sensitivity (Martinelango et al., 2005; Martinelango, Gumus & Dasgupta, 2006).

Currently, high-performance liquid chromatography (HPLC) coupled to tandem MS has become the main method for perchlorate analysis. Specific IC or C18 columns are used to detect perchlorate (Li & George, 2006). A rapid, sensitive and specific LC-MS/MS using an IC column has been developed for the determination of perchlorate in selected foods, such as lettuce, milk and bottled water (Alexander, Richard & David, 2004; USFDA, 2005). Perchlorate was detected under ESI negative mode, while an internal standard ($Cl^{18}O_4^-$) was used to correct the matrix effects. This method has a wide range of LODs in different matrices. LODs of LC-MS (selected ion mode) and LC-MS/MS (multiple ion reaction mode), using IonPac AS21 columns, were found to be 0.005 µg/l and 0.008 µg/l, respectively (EPA Method 331.0; USEPA, 2005d). LC-ESI-MS/MS has also been used for the determination of perchlorate in water (Soukup-Hein et al., 2007).

3.2.3 Summary

IC-MS and IC-MS/MS methods are used in most surveys conducted for the determination of perchlorate levels in foods and drinking-water. Stable isotope–labelled derivatives are used as internal standards. Currently, certified reference materials are not available, and collaborative method validation studies have not been conducted. A proficiency testing programme is available for potable water only (Massachusetts Department of Environmental Protection, 2004). Other methods, such as spectrophotometry, capillary electrophoresis and ion-selective electrode–based potentiometric methods, lack in sensitivity and may not be suitable for detection at lower levels in foods and water. Rapid screening methods have not yet been developed.

4. SAMPLING PROTOCOLS

Parallel experiments conducted in foods with six individual samples and one composite sample have shown a correlation between the mean value for the six

individual food samples and that of the composite samples, which indicates that common sampling protocols using representative samples appear to be adequate (Wang et al., 2009). This approach has been adopted for analysis of perchlorate in the TDSs in the USA and China and for analysis of human breast milk samples.

5. EFFECTS OF PROCESSING

Perchlorate is stable in the environment, for at least 300 days in groundwater and 90 days in surface water samples. Limited data are available on the fate of perchlorate during food processing. However, perchlorate is stable during whipping, grinding, heating and cooking processes.

6. PREVENTION AND CONTROL

Recent studies have shown that plant species are able to absorb perchlorate from soil and irrigation water. Elevated levels of perchlorate have been found in food and crops (Sanchez et al., 2006, 2008), including lettuce (Sanchez et al., 2005a,b), fish (Park et al., 2005), seaweed (Martinelango, Tian & Dasgupta, 2006; Snyder et al., 2006) and some beverages (Aribi et al., 2006). Perchlorate accumulation in leafy vegetables was found to be higher than that in root vegetables (Tan et al., 2004; Jackson et al., 2005). Hutchinson (2004) found perchlorate accumulated in lettuce during early growth stages under glasshouse conditions. Another potential source of perchlorate in water supplies is the degradation of sodium hypochlorite, used for disinfection of water, if it is stored for long periods and under less than optimal conditions, including high temperature, storage in the light and storage in containers that contain small amounts of old hypochlorite (Greiner et al., 2008). Perchlorate in drinking-water cannot be removed by conventional water treatment processes (Richardson, 2007).

Reduction of perchlorate in foods and water relies mainly on the control of contamination in fertilizers, irrigation systems and water used in food processing and preparation. However, washing can reduce surface contamination in vegetables and fruits.

7. LEVELS AND PATTERNS OF CONTAMINATION IN FOOD COMMODITIES

7.1 Surveillance data

The Committee, at its present meeting, reviewed data from six countries on the occurrence of perchlorate in water and various foods analysed between 2004 and 2009 (Tables 1 and 2). To harmonize the national occurrence data, all data below the limit of reporting (LOR) (below the LOD or limit of quantification [LOQ] where the percentage of non-quantified values was less than 60% for major contributing foods) have been assigned as follows: data below LOD = ½ LOD and data below LOQ = ½ LOQ, following the Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme (GEMS/Food) guidelines (GEMS/Food-EURO, 1995).

Table 1. Summary of perchlorate occurrence data from various countries, 2004–2009

Region	Country	Food		Water	
		Number of analytical results	% of values below LOR	Number of analytical results	% of values below LOR
Asia	China[a]	92	0	83	40
	India[b]	—	—	66	24
	Japan[c]	209	7	50	28
	Republic of Korea[d]	—	—	146	0
North America	Canada[e]	500	26	—	—
	USA[f]	1 065	41	34 728	98
Total		**1 866**	**—**	**35 073**	**—**

[a] China submitted data from pooled samples. Human milk data from China have not been included in the table.
[b] Data for India are from the literature; LOR for drinking-water is 0.02 µg/l.
[c] Japan submitted data on food; data on water were both from submitted information and from the literature.
[d] Data for the Republic of Korea are from the literature; LOR for drinking-water is 0.05 µg/l.
[e] Canada submitted 150 data on food, and a further 350 data were taken from the literature.
[f] USA food data are from exploratory surveys (2004–2005) and TDS (2005–2006); LOR for drinking-water is 4 µg/l.

7.2 National occurrence data

7.2.1 Canada

Canada submitted the results of a survey conducted in 2006 to establish the prevalence of perchlorate in vegetables and fruits, including lettuce, carrot and apple (Health Canada, 2009; Wang et al., 2009). Perchlorate concentrations in 150 samples collected from retail outlets as well as fast food outlets in Ottawa were analysed using the IC-MS/MS technique (LOD and LOQ were 0.3 µg/kg and 3 µg/kg, respectively), and 26% of values were below the LOR. Perchlorate levels were found to be in the range from less than the LOD to 536 µg/kg. Perchlorate levels (mean ± SD) were reported in cantaloupes from Guatemala (156 ± 232 µg/kg), spinach from the USA (133 ± 24.9 µg/kg), green grapes from Chile (45.5 ± 13.3 µg/kg) and romaine lettuce from the USA (29.1 ± 10.5 µg/kg). Perchlorate levels appeared to vary with the type of commodity and its country of origin (Wang et al., 2009). In another study, perchlorate levels were found in over 350 samples of foods and beverages, with concentrations ranging from not detected (ND) to 5.098 µg/l in water samples, from 0.047 to 463.5 µg/kg in food products, from 0.013 to 50.25 µg/l in wine samples, from 0.005 to 21.096 µg/l in beer as well as from ND to 4.795 µg/l in beverage products (Aribi et al., 2006).

Table 2. Occurrence of perchlorate in foods and water from different countries

Description	Country, sample	Number of samples	Number of samples with concentrations higher than LOD	Mean (range) (µg/l or µg/g)	Test method	LOD[a]	Reference
Water	Canada			<1	NA	NA	Health Canada (2008)
	China	54	36	0.2–6.8	IC	0.20 µg/l	Liu et al. (2004)
	China, bottled water	29	14	0.25 (0.037–2.013)	IC-MS/MS	2.0 ng/l (MDL)	Shi & Zhang (2007)
	India	66		<0.02–6.9	LC-MS/MS	0.006 µg/l	Kannan et al. (2009)
	Japan	20	6	0.14–0.35	IC-MS/MS	0.03 ng/g	Japan Ministry of Health, Labour and Welfare (2009)
	Japan, tap water samples	30	30	0.06–37	IC-MS/MS	0.05 µg/l (MRL)	Kosaka et al. (2007)
	Republic of Korea, tap water	146	146	0.15–35	IC; LC-MS/MS	5.0 µg/l; 0.05 µg/l (MRL)	Quiñones et al. (2007)
	United Kingdom	20 sites		Low levels	NA	NA	Blake et al. (2009)
Vegetables Lettuce	Canada	18	18	11.5 (1.27–46.7)	IC-MS/MS	~0.5 µg/kg	Health Canada (2009); Wang et al. (2009)
	Japan	29	29	3 (0.3–21.1)	IC-MS/MS	0.1 µg/kg	Japan Ministry of Health, Labour and Welfare (2009)

Table 2 (contd)

Description	Country, sample	Number of samples	Number of samples with concentrations higher than LOD	Mean (range) (µg/l or µg/g)	Test method	LOD[a]	Reference
	USA	137		10.3 (0.3–129)	IC-MS/MS	1 µg/kg	US FDA (2007b)
	USA	63		7.4	NA	NA	Sanchez et al. (2005a)
	USA	24		8	NA	NA	Sanchez et al. (2005b)
	USA	69		16.5	NA	NA	Sanchez et al. (2005a)
	USA	24		33	NA	NA	Sanchez et al. (2005b)
	USA	8		2.1	NA	NA	USFDA (2009)
			Weighted mean	11.6			
Spinach	Canada	18	18	133 (99–175)	IC-MS/MS	~0.5 µg/kg	Wang et al. (2009)
	Japan	13	13	5.3 (0.6–20.5)	IC-MS/MS	0.1 µg/kg	Japan Ministry of Health, Labour and Welfare (2009)
	USA	36		115 (5.94–927)	IC-MS/MS	1 µg/kg	USFDA (2007b)
	USA	10		85.1	IC-MS/MS	1 µg/kg	Sanchez et al. (2005a)
			Weighted mean	111 (<1–927) (Japan not included)			

Table 2 (contd)

Description	Country, sample	Number of samples	Number of samples with concentrations higher than LOD	Mean (range) (µg/l or µg/g)	Test method	LOD[a]	Reference
Potato	Canada	24	7	1 (<0.4–2.2)	IC-MS/MS	0.39 ± 0.28 µg/kg	Health Canada (2009); Wang et al. (2009)
	USA	60		7.52	IC-MS/MS	1 µg/kg	USFDA (2009)
	USA	6		0.15 (½ LOD)	IC-MS/MS	1 µg/kg	USFDA (2007a)
			Weighted mean	4.8			
Squash	USA	30		56	IC-MS/MS	1 µg/kg	USFDA (2009)
Tomato	Canada	18	11	0.3 (0.2–1.6)	IC-MS/MS	0.39 ± 0.28 µg/kg	Health Canada (2009); Wang et al. (2009)
	USA	73	54	13.7 (<0.3–268)	IC-MS/MS	1 µg/kg	USFDA (2007b)
	USA	4		78	IC-MS/MS	1 µg/kg	USFDA (2009)
			Weighted mean	14			
Carrot	Canada	12	10	1.31 (0.2–3.1)	IC-MS/MS	0.39 ± 0.28 µg/kg	Health Canada (2009); Wang et al. (2009)
	USA	59	50	15.8 (0.3–111)	IC-MS/MS	1 µg/kg	USFDA (2007b)
	USA	49		6.6	IC-MS/MS	1 µg/kg	USFDA (2009)
			Weighted mean	10.5			

Table 2 (contd)

Description		Country, sample	Number of samples	Number of samples with concentrations higher than LOD	Mean (range) (μg/l or μg/g)	Test method	LOD[a]	Reference
	Eggplant	USA	60		78	IC-MS/MS	1 μg/kg	USFDA (2009)
	Broccoli	USA	55		23.8 (3.5–107)	IC-MS/MS	2–3 μg/kg	Sanchez et al. (2007)
		USA	60		3.34	IC-MS/MS	1 μg/kg	USFDA (2009)
				Weighted mean	19			
	Cauliflower	USA	38	38	12.8 (4.2–40.7)	IC-MS/MS	2–3 μg/kg	Sanchez et al. (2007)
		USA	30		1.1	IC-MS/MS	1 μg/kg	USFDA (2009)
				Weighted mean	7			
	Cabbage	Japan	12	12	2 (0.5–6.3)	IC-MS/MS	0.1 μg/kg	Japan Ministry of Health, Labour and Welfare (2009)
		USA	19	19	18.5 (4.6–63.2)	IC-MS/MS	2–3 μg/kg	Sanchez et al. (2007)
				Weighted mean	10			
	Komatsunn and 17 other species of vegetables	Japan	40	39	0.1–29.7	IC-MS/MS	0.1 μg/kg	Japan Ministry of Health, Labour and Welfare (2009)

Table 2 (contd)

Description		Country, sample	Number of samples	Number of samples with concentrations higher than LOD	Mean (range) (µg/l or µg/g)	Test method	LOD[a]	Reference
Fruits	Apple	Canada	24	19	0.48 (0.1–1.60)	IC-MS/MS	0.39 ± 0.28 µg/kg	Health Canada (2009); Wang et al. (2009)
		USA	9	0	0.15 (½ LOD)	NA	0.3 µg/kg	USFDA (2007b)
				Weighted mean	0.5	NA	NA	
	Grape	Canada	12	12	27.7 (1.96–62.05)	IC-MS/MS	0.39 ± 0.28 µg/kg	Health Canada (2009); Wang et al. (2009)
		USA	15		3.3	IC-MS/MS	NA	Sanchez et al. (2006)
		USA	12		8.58 (<0.3–38.6)	IC-MS/MS	NA	USFDA (2007b)
				Weighted mean	28	NA	NA	
	Orange	Canada	12	10	0.5 (0.2–2.89)	IC-MS/MS	0.39 ± 0.28 µg/kg	Health Canada (2009); Wang et al. (2009)
		USA	10	5	3.4 (0.3–23.3)	IC-MS/MS	NA	USFDA (2007b)
		USA	28		7.4	IC-MS/MS	0.39 ± 0.28 µg/kg	
				Weighted mean	5			

Table 2 (contd)

Description		Country, sample	Number of samples	Number of samples with concentrations higher than LOD	Mean (range) (μg/l or μg/g)	Test method	LOD[a]	Reference
	Melon	Canada	18	17	56.1 (0.1–536.4)	IC-MS/MS	0.39 ± 0.28 μg/kg	Health Canada (2009); Wang et al. (2009)
		USA	48	48	28.6 (0.52–713)	IC-MS/MS	0.3 μg/kg	USFDA (2007b)
		USA	32		1.7	NA	NA	
				Weighted mean	19			
	Vegetable and fruit juice	Canada	53	53	25.81 (0.047–463.5) Median, 0.43	IC-MS/MS	0.5 ng/l for deionized water	Aribi et al. (2006)
	Honeydew	Canada	6	6	0.3 (0.1–0.66)	IC-MS/MS	0.39 ± 0.28 μg/kg	Health Canada (2009); Wang et al. (2009)
Whole wheat flour		USA	38		3.5	IC-MS/MS	NA	USFDA (2009)
Rice		China	65	65	0.83 (0.16–4.88)	IC-MS/MS	0.04 μg/kg (MDL)	Shi & Zhang (2007)
		Japan	10	6	(>0.3–0.73)	IC-MS/MS	0.3 ng/g	Japan Ministry of Health, Labour and Welfare (2009)
		USA	19		0.50 (average)	IC-MS/MS	NA	USFDA (2007b)
				Weighted mean	1			

Table 2 (contd)

Description	Country, sample	Number of samples	Number of samples with concentrations higher than LOD	Mean (range) (µg/l or µg/g)	Test method	LOD[a]	Reference
Milk	China	17	17	3.9 (0.30–9.1)	IC-MS/MS	10 ng/l (MDL)	Shi & Zhang (2007)
	Japan	54	54	9.39 (5.67–16.4)	IC-MS	NA	Dyke et al. (2007)
	Japan	25		6.5 (2.0–10.6)	IC-MS/MS	0.1 ng/g	Japan Ministry of Health, Labour and Welfare (2009)
	USA	125		5.8 (1.9–11.3)	IC-MS/MS	1.0 µg/l	USFDA (2007a,b)
			Weighted mean	6.8			
Breast milk	China	24 (1200)	24	19.7 (2.12–136.1)	IC-MS/MS	0.4 µg/l	Chinese CDC (2009)
	USA	147	147	5.8 (0.5–39.5)	IC-MS	0.07 µg/l	Kirk et al. (2007)
	USA	49		33 (1.3–411)	IC-MS		Pearce, Lazarus & Smythe (2007)
	USA	457	457	9.3 (0.01–48)	IC-MS/MS	0.005 µg/l	Dasgupta et al. (2008)
			Weighted mean	16.4			
Infant formula (powder)	China	10	10	22.8 (12.3–43.1)	IC-MS/MS	2 µg/l	Chinese CDC (2009)
	Japan	10	10	7.5 (2.5–35.3)	IC-MS/MS	0.7 ng/g	Japan Ministry of Health, Labour and Welfare (2009)
			Weighted mean	10			

Table 2 (contd)

Description	Country, sample	Number of samples	Number of samples with concentrations higher than LOD	Mean (range) (µg/l or µg/g)	Test method	LOD[a]	Reference
Fish and fishery products	Japan	20	1	<0.8 (0.32–71.6)	IC-MS/MS	0.2 ng/g	Japan Ministry of Health, Labour and Welfare (2009)
	USA	166	50	14.2–1593	IC-CD	2.5 µg/l	Theodorakis et al. (2006)
Wine	Canada	77	77	5.00 (0.013–50.25) Median, 1.54	IC-MS/MS	0.5 ng/l for deionized water	Aribi et al. (2006)
	Japan	27	27	9.4 (0.2–56.9)	IC-MS/MS	0.1	Japan Ministry of Health, Labour and Welfare (2009)
			Weighted mean	6			
Beer	Canada	144	144	1.04 (0.005–21.10) Median, 0.34	IC-MS/MS	0.5 ng/l for deionized water	Aribi et al. (2006)

CD, conductivity detector; MDL, method detection limit; MRL, minimum reporting limit; NA, not available

[a] This column presents the LOD of the analytical method unless stated otherwise in parentheses. For example, for tap water in the Republic of Korea, "5.0 µg/l; 0.05 µg/l (MRL)" means that the LOD of the IC method and the MRL of the LC-MS/MS method were 5.0 µg/l and 0.05 µg/l, respectively (line 8 in this table).

7.2.2 China

China submitted data on perchlorate levels in rice, milk, infant formula, human milk and drinking-water (Chinese CDC, 2009). There were 92 data points from composite samples analysed using the IC-ESI-MS/MS technique, with LORs of 0.1–2.2 µg/kg, depending on the food or matrix. The samples of infant formula and of human breast milk were from a Chinese TDS conducted in 2007. Twenty-four composite samples of breast milk were collected from the urban and rural areas of the 12 provincial sampling sites, and each composite sample consisted of milk from 50 mothers (total of about 1200 subjects). The concentration of perchlorate in breast milk from mothers from urban and rural areas ranged from 2.12 to 136 µg/l and from 2.18 to 93 µg/l, respectively. In infant formula, perchlorate levels ranged from 12.3 to 43.1 µg/kg, with an average of 22.8 µg/kg. The levels of perchlorate in rice, bottled water and milk samples were 0.16–4.88 µg/kg, 0.04–2.01 µg/l and 0.30–9.1 µg/l, respectively (Shi & Zhang, 2007). Perchlorate was found in six of nine samples of finished water from drinking-water plants at average concentrations of 0.5–2.4 µg/l (Liu et al., 2004). Perchlorate was detected in tap water (3.16 µg/l) in Macao Special Administrative Region.

7.2.3 India

In India, 66 samples of tap water from restaurants and homes, groundwater (or well water), bottled water, surface water (rivers and lakes) and rainwater were collected from 13 locations in six states in 2008. The samples were analysed using LC-MS/MS (Kannan et al., 2009). Twenty-four per cent of the samples were below the LOQ (0.02 µg/l). Perchlorate levels ranged from less than 0.02 to 6.9 µg/l (mean 0.42 ± 1.1 µg/l, median 0.07 µg/l). Mean concentrations in drinking-water, groundwater, bottled water, surface water and rainwater were 0.1 µg/l, 1.0 µg/l, <0.02 µg/l, 0.05 µg/l and <0.02 µg/l, respectively. Out of a total of 66 water samples analysed, only 3 groundwater samples contained perchlorate levels above 1 µg/l.

7.2.4 Japan

Japan submitted aggregated results on the occurrence of perchlorate in foods obtained from 209 food samples and 20 bottled water samples (Japan Ministry of Health, Labour and Welfare, 2009). Samples were analysed using the IC-ESI-MS/MS technique. The LOQs ranged from 0.1 to 2.2 µg/kg for different food matrices. The perchlorate concentration in 14 samples was below the LOR. Perchlorate was detected at concentrations ranging from less than 0.1 to 29.7 µg/kg in vegetables (n = 82), from 2.0 to 35.3 µg/kg in milk and milk products, including infant formula (n = 50), from less than 0.2 to 71.6 µg/kg in fishery foods (n = 20), from 0.2 to 56.9 µg/kg in wine (n = 27), from less than 0.3 to 0.73 µg/kg in rice (n = 10) and from less than 0.03 to 0.35 µg/kg in bottled water (n = 20). Perchlorate was detected in powdered infant formula in the concentration range of 2.5–35.3 µg/kg, with an average concentration of 7.4 µg/kg. Dyke et al. (2007) reported that the average concentration of perchlorate in 54 Japanese milk samples was above the LOQ, at 9.39 µg/l, with a range of 5.47–16.40 µg/l. Perchlorate levels in 30 tap water samples were up to 37 µg/l (Kosaka et al., 2007).

7.2.5 Republic of Korea

Perchlorate levels in tap water samples from the Nakdong River basin collected in two investigations ranged from 18.7 to 95.6 µg/l and from 4.0 to 25.6 µg/l (Kim, Kim & Lee, 2009). Among other sampling sites, one at Daegu showed the highest perchlorate level, at 22.3 µg/l.

7.2.6 USA

Perchlorate was detected in public water supplies in 26 states as well as Puerto Rico and the Mariana Islands and was typically present at concentrations below 12 µg/l (Brandhuber, Clark & Morley, 2009). In a USEPA drinking-water survey, only 160 of 3870 water supplies (4%) had perchlorate levels above the LOR (4 µg/l), in the range of 4–420 µg/l, with a mean concentration of 9.9 µg/l. The mean was the average of the concentrations in the samples in which perchlorate was detected. Less than 1% of public water supplies had perchlorate levels of 20 µg/l or higher (NRC, 2005).

The USA submitted data on the occurrence of perchlorate in foods from 2004–2005 USFDA exploratory surveys (USFDA, 2007b) and the 2005–2006 TDS survey (USFDA, 2009). In a study on perchlorate levels in freshwater fish, high perchlorate concentrations of 2092 µg/kg, 2740 µg/kg and 1593 µg/kg were found in whole fish, fish heads and fillets, respectively (Theodorakis et al., 2006). In the USFDA exploratory survey (USFDA, 2007b), perchlorate analysis was targeted on 28 types of foods and beverages, consisting of bottled water, milk, fruits and fruit juices, vegetables, grain products and seafood. The TDS covered a broader range of foods. Of the 1065 samples from the 285 kinds of TDS foods, 41% samples were below the LOR, and perchlorate was not detected in 26% of the samples (Murray et al., 2008). Out of the 104 milk samples analysed, 3 samples were found to contain perchlorate at levels below the LOR. The mean perchlorate levels were found to be in the range of 3.16–11.3 µg/l, with a mean concentration of 5.76 µg/l. Perchlorate levels were found in 81% of infant and baby foods on a ready-to-serve basis, with levels ranging from 1.1 to 1.3 µg/l. In a United States Centers for Disease Control and Prevention study on 15 brands of infant formula from the market, perchlorate levels ranged from 0.03 to 5.05 µg/l in reconstituted samples (Schier et al., 2010). In two studies involving 652 mothers, perchlorate levels in breast milk were found to be in the range of 0.01–411 µg/l, with a mean concentration of 9.3 µg/l (Kirk et al., 2005, 2007).

7.3 Summary of national occurrence data

The Committee, at its present meeting, reviewed data from six countries on the occurrence of perchlorate in water and different foods analysed between 2004 and 2009 (Table 1). Analytical data on 35 073 samples of groundwater and drinking-water indicated that perchlorate levels in 98% of samples were below the LOR. In drinking-water, perchlorate levels exceeded 20 µg/l in only 1% of the samples. The USA reported that only 160 out of 3870 water supplies (4%) had perchlorate levels above the LOR (4 µg/l), in the range of 4–420 µg/l, with a mean concentration of 9.9 µg/l. Analytical data from 1866 samples (vegetables, fruits, rice, milk, infant

formula, fish and fish products, beverages such as juices, beer and wine) were reviewed, and perchlorate levels in 33% of samples were found to be below the LOR. Weighted mean (mean of reported means weighted by the number of samples) perchlorate levels in vegetables were in the range 4.8–110 µg/kg (potato, 4.8 µg/kg; carrot, 6.6 µg/kg; spinach, 110 µg/kg; lettuce, 11.6 µg/kg; tomato, 14 µg/kg; squash, 75 µg/kg; eggplant, 78 µg/kg; broccoli, 19 µg/kg; cauliflower, 7 µg/kg; cabbage, 10 µg/kg). In fruits, the weighted means were in the range 0.5–28 µg/kg (oranges, 5 µg/kg; apples, 0.5 µg/kg; grapes, 28 µg/kg; melons, 19 µg/kg). The weighted mean perchlorate levels for other foods were as follows: rice, 1 µg/kg; whole wheat flour, 3.5 µg/kg; milk, 6.8 µg/kg; beer, 1 µg/kg; and wine, 6 µg/kg. The mean perchlorate levels in human milk in China (n = 24 composite samples) and the USA (n = 652) were found to be 19.7 µg/l (range 2.1–136 µg/l) and 9.3 µg/l (range 0.01–411 µg/l), respectively. Limited data show that the weighted mean perchlorate level in infant formula was 10 µg/kg. Sampling and analysis were carried out on targeted foods. However, general surveys involving a broader range of foods in different countries were lacking. The widespread presence of perchlorate in the environment has been shown in a few countries, but in view of its uses and natural occurrence, it is probable that it will be found more widely in drinking-water and in food.

8. FOOD CONSUMPTION AND DIETARY EXPOSURE ESTIMATES

The Committee evaluated occurrence and dietary exposure data for perchlorate from submissions from China, Japan, Canada and the USA and from the literature. The occurrence data have been summarized in section 7.

Dietary exposure to perchlorate is primarily from vegetable and fruit crops that have been irrigated with water containing perchlorate, from milk and other beverages made from crops that may contain perchlorate, and from drinking-water. Infants are exposed to perchlorate in breast milk and infant formula made with contaminated drinking-water, in addition to any foods consumed.

Perchlorate exposure from drinking-water will be considered separately from dietary exposure.

8.1 International estimates of dietary exposure

The Committee used perchlorate values from the literature as well as submitted information from member countries to arrive at “mean” contamination concentrations for use with the per capita estimates of commodity consumption in the GEMS/Food consumption cluster diets (Table 3). The Committee notes that there has not been a broad examination of foods that might be contaminated and that many foods have not been explored at all. Additionally, the foods that have been examined are in most cases those thought most likely to be contaminated. It is thus not clear whether the derived estimates of exposure are complete or representative, and consequently they should be viewed as preliminary. The national estimates presented in the next section will serve as better indicators of perchlorate dietary exposure, at least for those countries.

Table 3. Food consumption from GEMS/Food consumption cluster diets

Food	Per capita food consumption (g/day)												
	A	B	C	D	E	F	G	H	I	J	K	L	M
Rice	91.0	31.6	94.6	33.2	12.7	12.7	376.9	64.3	38.0	74.3	238.4	381.3	34.6
Potato	19.1	160.8	61.2	243.6	230.1	204.7	52.7	57.1	50.1	4.3	54.7	41.0	168.0
Carrot	0.6	15.1	8.1	13.9	27.1	28.4	5.4	7.9	2.5	3.5	4.1	8.6	19.4
Spinach	0.0	5.0	1.1	0.1	2.6	0.1	9.4	0.4	0.0	0.0	0.2	4.3	2.0
Lettuce	0.2	23.8	3.6	0.6	11.9	18.0	7.1	7.0	0.6	1.9	2.0	7.1	30.6
Tomato	11.8	185.0	118.0	60.7	31.6	40.9	23.5	31.7	15.0	16.2	35.6	9.9	103.0
Squash, pumpkin, gourd	16.3	12.3	14.4	21.9	3.2	1.0	7.1	4.6	11.3	3.0	7.0	6.7	7.6
Eggplant	1.7	17.5	12.3	1.7	0.8	0.4	20.1	0.1	0.6	6.3	0.5	6.3	0.7
Orange	4.2	57.9	38.0	14.4	29.4	41.2	7.4	118.9	8.3	2.4	203.0	12.0	102.4
Apple	0.3	60.5	18.5	39.9	50.8	39.4	14.4	10.1	2.2	0.0	9.8	17.9	36.3
Grape	3.7	128.5	27.1	33.1	107.5	44.0	2.6	4.8	11.7	0.3	6.8	10.9	58.8
Melon	3.6	26.7	22.6	11.5	5.6	2.0	7.5	6.1	0.7	1.4	2.5	6.9	12.4
Cauliflower	0.1	5.2	1.2	0.1	1.7	0.1	3.2	0.1	0.3	0.1	0.6	0.4	1.4
Broccoli	0.0	0.7	1.2	0.1	4.2	4.0	3.2	7.8	0.0	0.0	0.3	0.4	6.6
Cabbage	2.1	19.8	8.3	43.9	29.9	28.0	23.6	5.0	12.0	1.9	3.8	55.5	18.9

Table 3 (contd)

Food	Per capita food consumption (g/day)												
	A	B	C	D	E	F	G	H	I	J	K	L	M
Wheat flour	63.4	296.3	327.5	300.0	181.6	166.2	133.0	60.1	52.4	32.2	87.7	79.6	180.1
Milk of cattle, goat, sheep	52.7	189.5	65.7	295.6	179.6	237.9	48.0	121.1	80.8	94.7	207.7	56.1	287.9
Beer	18.3	84.1	4.1	66	243.1	161.3	21.9	102.7	29.5	12.6	100.9	82.2	218.8
Wine	1.3	76.8	1.1	15.4	68.8	25.6	1.0	0.9	6.8	0.1	3.4	3.6	31

For this analysis, the Committee chose the food perchlorate concentrations set out in section 7.3 above. These figures were approximations derived primarily from three sources: exploratory data posted on the USFDA web site (USFDA, 2007b) and submitted to this meeting of the Committee, Canadian data published in the literature (Wang et al., 2009) and Chinese data submitted to this meeting of the Committee. If the food was mentioned in only one of the sources, the highest levels were approximated. If the food was mentioned in more than one source, then a rough average, towards the higher end, was chosen. Weighted average levels were calculated where feasible. There were data available on more vegetable types from the USFDA, but these vegetables had low levels of perchlorate and are not commonly consumed in large amounts (e.g. kale, turnip greens). Additionally, a number of foods where no quantifiable perchlorate was found have been excluded (e.g. peaches, plums). Because of the reporting regimen for the Food and Agriculture Organization of the United Nations balance sheets that feed into the GEMS/Food consumption cluster diets, wine consumption may cause a double counting, as it is also included under "grapes" from which it is produced. Any double counting would be insignificant in this exercise, as wine does not contribute more than 5% of the total perchlorate exposure for any of the consumption cluster diet totals (cluster E, highest).

The Committee noted that the range of estimated perchlorate dietary exposure (Table 4) is relatively narrow, 0.03–0.22 µg/kg bw per day, when milk consumption is included. Milk consumption provides a large proportion of the perchlorate exposure for most clusters, ranging from 7% (clusters C and G) to 42% (cluster J) of the total.

8.2 National estimates of dietary exposure

National dietary exposure estimates from Canada and the USA were evaluated by the Committee. The Canadian estimates were published by Wang et al. (2009), whereas three different published estimates were available from the USA. One estimate was derived from urinary biomarkers of perchlorate exposure (Blount et al., 2007), one was taken from the USA TDA (Murray et al., 2008) and the third is available on the USFDA web site (USFDA, 2007a). Additionally, a supplement to the USA estimate available on the USFDA web site was submitted to the Committee.

The Canadian estimates were made using data taken from foods available in a public market and included imported fruits and vegetables (Wang et al., 2009). A deterministic approach was used. Perchlorate concentrations were combined with food consumption data available from the Nutrition Canada Food Consumption Survey (<18 years old) and the Nova Scotia Provincial Nutrition Survey (adults >18 years old, including women of childbearing age). Mean dietary exposures to perchlorate were higher for children than for adults. The mean dietary exposure was approximately 0.04 µg/kg bw per day for children 1–11 years of age, compared with 0.03 µg/kg bw per day for adults, including women of childbearing age. The researchers noted that these estimates were made with a limited number of data available from only a few foods and should be considered preliminary. They are in general agreement, however, with the international estimates described above and the estimates from the USA discussed below.

Table 4. International estimates of perchlorate dietary exposure using GEMS/Food consumption cluster diets

Food	Dietary exposure (ng/day)												
	A	B	C	D	E	F	G	H	I	J	K	L	M
Rice	91	32	95	33	13	13	377	64	38	74	238	381	35
Potato	76	643	245	974	920	819	211	228	200	17	219	164	672
Carrot	6	151	81	139	271	284	54	79	25	35	41	86	194
Spinach	0	625	138	13	325	13	1175	50	0	0	25	538	250
Lettuce	2	262	40	7	131	198	78	77	7	21	22	78	337
Tomato	165	2590	1652	850	442	573	329	444	210	227	498	139	1442
Squash, pumpkin, gourd	913	689	806	1226	179	56	398	258	633	168	392	375	426
Eggplant	46	473	332	46	22	11	543	3	16	170	14	170	19
Orange	21	290	190	72	147	206	37	595	42	12	1015	60	512
Apple	0	30	9	20	25	20	7	5	1	0	5	9	18
Grape	104	3598	759	927	3010	1232	73	134	328	8	190	305	1646
Melon	68	507	429	219	106	38	143	116	13	27	48	131	236
Cauliflower	1	68	16	1	22	1	42	1	4	1	8	5	18
Broccoli	0	17	29	2	101	96	77	187	0	0	7	10	158
Cabbage	40	376	158	834	568	532	448	95	228	36	72	1055	359
Wheat flour	222	1037	1146	1050	636	582	466	210	183	113	307	279	630

Table 4. International estimates of perchlorate dietary exposure using GEMS/Food consumption cluster diets

Food	Dietary exposure (ng/day)												
	A	B	C	D	E	F	G	H	I	J	K	L	M
Milk of cattle, goat, sheep	369	1327	460	2069	1257	1665	336	848	566	663	1454	393	2015
Beer	18	84	4	66	243	161	22	103	30	13	101	82	219
Wine	7	384	6	77	344	128	5	5	34	1	17	18	155
Total non-milk (μg/day)	1.8	11.9	6.1	6.6	7.5	5.0	4.5	2.7	2.0	0.9	3.2	3.9	7.3
Total with milk (μg/day)	2.1	13.2	6.6	8.6	8.8	6.6	4.8	3.5	2.6	1.6	4.7	4.3	9.3
Total non-milk (μg/kg bw per day)	0.03	0.20	0.10	0.11	0.13	0.08	0.07	0.04	0.03	0.02	0.05	0.06	0.12
Total with milk (μg/kg bw per day)	0.04	0.22	0.11	0.14	0.15	0.11	0.08	0.06	0.04	0.03	0.08	0.07	0.16
% from milk	17	10	7	24	14	25	7	24	22	42	31	9	22

For the USA, an estimate of dietary exposure to perchlorate was made using urinary biomarker concentrations of perchlorate, available from the 2001–2002 NHANES (Blount et al., 2007). The dietary exposure (which would include exposure from water) was estimated using the ratio of the perchlorate to creatinine concentrations in urine, assuming that 100% of perchlorate in the diet is absorbed and excreted unmetabolized. The 50th-percentile estimate for the total adult population was 0.06 µg/kg bw per day, whereas the 95th-percentile estimate was 0.23 µg/kg bw per day. Dietary exposure for males was slightly higher than that for females. These estimates are also in general agreement with the international estimates. Perchlorate was detected in all of the urine samples examined, confirming the widespread nature of environmental perchlorate contamination.

The USA estimate available on the USFDA web site (USFDA, 2007a) is listed as a preliminary estimate for the reasons already noted: too few foods and a limited number of samples for each. It was also made using perchlorate concentrations measured for the raw commodities and the food consumptions for each, available from the 1994–1996, 1998 Continuing Survey of Food Intake by Individuals (http://www.ars.usda.gov/Services/docs.htm?docid=14531). Food consumption based on United States Department of Agriculture (USDA) food commodity codes (ingredient use) was used for those foods primarily consumed as an ingredient in a mixed food dish: lettuce, tomatoes, spinach, carrots, apples, cantaloupes, grapes, cucumbers, onions, potatoes, rice, oatmeal, wheat flour and corn meal. Consumption of fruit juices was also calculated based on ingredient use. Food consumption based on USDA food codes (consumption as a whole food) was used for oranges, strawberries, watermelon, broccoli, cabbage, greens, green beans, sweet potatoes, milk, catfish, salmon and shrimp. The foods were determined to be consumed primarily as an ingredient or as a whole food so as to maximize the reported intake of the food (i.e. the approach that resulted in the higher exposure to perchlorate was used). For example, because spinach is consumed in higher amounts as an ingredient than as a whole food, it was categorized as an ingredient, which resulted in higher perchlorate intake. The mean estimate of dietary perchlorate exposure from food for the total population was 0.05 µg/kg bw per day, whereas the 90th-percentile exposure was 0.12 µg/kg bw per day. The estimates for children 2–5 years old were 0.17 and 0.34 µg/kg bw per day at the mean and 90th percentiles of exposure, respectively.

The final USA estimate is from the USFDA's TDS. This type of survey provides a good estimate of dietary exposure, as all of the foods in the model diets are prepared for consumption prior to analysis for the substance under investigation. USA model diet "market basket" surveys from 2003 to 2008 were used to make estimates. The 2003–2006 period is published (Murray et al., 2008); an additional estimate from the 2005–2008 market basket surveys was submitted to this meeting of the Committee. The mean estimates for age groups 14 and over were all about 0.1 µg/kg bw per day, with 90th-percentile exposures of approximately 0.23 µg/kg bw per day or less. Dietary exposure was greatest for the 2-year-olds examined through the 2005–2008 market basket surveys. The mean exposure for this group was 0.44 µg/kg bw per day, with a 90th-percentile exposure of 0.734 µg/kg bw per day.

The Committee concluded that the estimates from the USA TDS provide the best indication of dietary exposure to perchlorate, as they do not depend on large numbers of analyses of individual foods or commodities and include water and beverages made from water that might be contaminated with perchlorate. Although all of the national estimates are in general agreement, the Committee noted that the highest estimates were from the USFDA's TDS, confirming the suggestion that the other estimates (and the international estimates prepared by the Committee) are preliminary and do not include all food sources of perchlorate.

9. DOSE–RESPONSE ANALYSIS

The pivotal study for dose–response analysis was the human clinical study on 16 male and 21 female healthy adult volunteers published by Greer et al. (2002), in which perchlorate was given in drinking-water at doses of 0.007, 0.02, 0.1 or 0.5 mg/kg bw per day for 14 days. The uptake of iodide into the thyroid was measured at baseline before administration of perchlorate and on days 2 and 14 of administration at both 8 and 24 h after administration of radiolabelled iodine. These data were used for modelling.

The Committee selected a critical effect size of 50% inhibition of iodide uptake as the benchmark response. This choice was made because human clinical data from healthy adults following both short-term and chronic exposure to perchlorate have shown that such a level of inhibition is not associated with any changes in TSH or thyroid hormone levels. The Committee noted that a benchmark response of 50% inhibition was within the observed range of the study.

PROAST software (version 23.0) for analysis of continuous data was used. In the clinical study, each subject served as his or her own control, with baseline values measured 1 day before the start of the 14-day perchlorate exposure period. In the BMD analysis, the baseline value for iodide uptake in each subject was set at 100%. Analysis of the post-treatment values for iodide uptake at 8 and 24 h and the values from exposure days 2 and 14 as covariates showed that these factors did not have any significant impact. Therefore, the two models used for continuous data—the exponential and Hill models—were fitted to all the data combined.

The plots obtained using the two models were very similar. That obtained using the Hill model is shown as illustration in Figure 1.

Similar values for the BMD_{50} and $BMDL_{50}$ (the BMD and BMDL, respectively, for a 50% response) were obtained from the two models, as shown in Table 5. The lower of the two $BMDL_{50}$ values (rounded to two significant figures) of 0.11 mg/kg bw per day was used as the point of departure.

Figure 1. Graphical plot of the data on inhibition of uptake of radiolabelled iodine by the thyroid in humans exposed to perchlorate for 14 days (Greer et al., 2002)

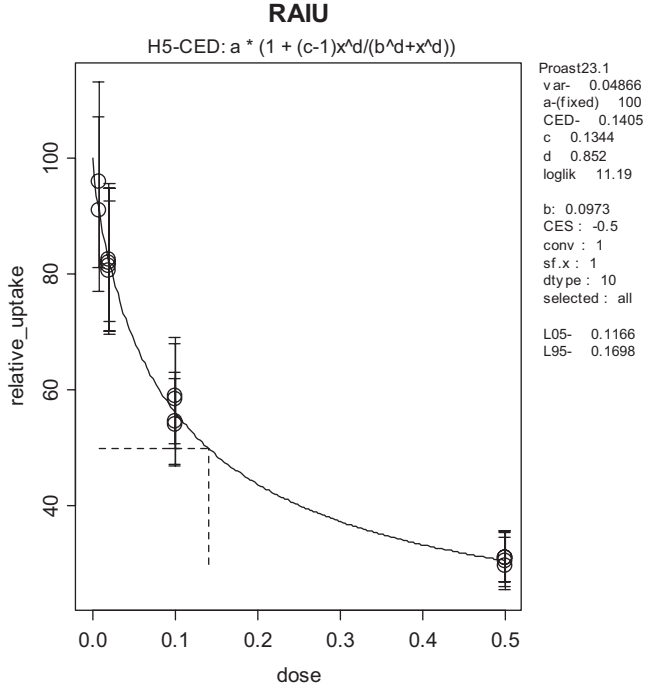


Table 5. BMD and BMDL values for 50% inhibition of uptake of radiolabelled iodine by the thyroid in humans exposed to perchlorate for 14 days

Model	Number of regression parameters	BMD_{50} (mg/kg bw per day)	$BMDL_{50}$ (mg/kg bw per day)
Exponential model	4	0.137	0.114
Hill model	4	0.141	0.117

Source: Greer et al. (2002)

10. COMMENTS

10.1 Absorption, distribution, metabolism and excretion

Perchlorate is rapidly absorbed following ingestion and rapidly excreted unchanged, mostly in the urine. It crosses the placenta and is also found in breast milk.

10.2 Relevance of animal data for human risk assessment

The key toxicological issue for perchlorate is its ability to competitively inhibit uptake of iodide by the thyroid gland, potentially causing hypothyroidism. The inhibition is at the level of the NIS, a glycoprotein expressed in the outer, basolateral membrane of the thyroid follicular cell, which actively transports iodide (and perchlorate) from the blood into the thyroid gland. Inhibition of iodide uptake by perchlorate reduces the amount of iodide available for the synthesis of thyroid hormones, resulting in reductions in the concentrations of circulating T_4 and the more biologically active hormone, T_3. In a negative feedback loop, reductions in the concentrations of T_4 and T_3 reaching the brain trigger the release of thyrotropin-releasing hormone in the hypothalamus, which in turn causes the release of thyrotropin, also known as TSH, from the anterior pituitary gland. TSH initiates the events in the thyroid that result in NIS transport of iodide into the thyroid and synthesis of T_3 and T_4. If there is a sustained reduction in iodide uptake by the thyroid, hypothyroidism may occur. Hypothyroidism has adverse implications for structural and functional brain development in the fetus, infant and child and for metabolism and the functioning of cardiovascular, gastrointestinal, skeletal, neuromuscular and reproductive systems in adults (Wolff, 1998; Braverman & Utiger, 2005; Kirk, 2006).

There is general agreement that for substances known to affect the thyroid and having a mode of action involving inhibition of the uptake of iodide, the rat is not a good model for humans. This applies not only to the likelihood of thyroid cancer (Capen et al., 1999; NRC, 2005; USEPA, 2008a) but also to other perturbations of thyroid physiology and pathology in response to thyroid toxicants (Capen, 1997). The reasons for this are several. There is a difference between rat and human in the proteins to which the thyroid hormones, T_3 and T_4, are bound and their relative binding affinity to those proteins. In humans, thyroid hormones bind mainly to TBG with high affinity, which reduces their rate of hepatic metabolism and hence clearance from blood. In contrast, in the rat, thyroid hormones bind to albumin and transthyretin with affinities that are much lower, by around 100-fold or more, than those for TBG (Connors, 1997). This results in rapid clearance of thyroid hormones in the rat, with serum half-lives of 0.5–1 day and 0.25 day for T_4 and T_3, respectively. This compares with a much slower clearance in humans, with serum half-lives of 5–9 days and 1 day for T_4 and T_3, respectively (Choksi et al., 2003). The rapid clearance in the rat creates a demand for greater synthesis of thyroid hormones, requiring increases in secretion of TSH. The demand for fresh synthesis of thyroid hormones in the rat is further exacerbated because there is much less storage of hormones in thyroidal colloid; the amounts stored in rats are sufficient for a few days, compared with several months in humans (Greer et al., 2002; Braverman & Utiger, 2005). The resulting increases in serum TSH in the rat following exposure to anti-thyroid agents are greater than those seen in humans and, if sustained, will cause increased thyroid growth and pathological changes in the thyroid. For these reasons, the laboratory animal data reviewed by the Committee, which comprised mostly data from rats, while qualitatively supportive of the human data, were not further considered for deriving a health-based guidance value for perchlorate.

10.3 Observations in humans

Human observations include clinical studies on thyroid function in healthy adult volunteers given perchlorate in drinking-water, clinical studies on thyroid function in workers occupationally exposed to perchlorate for several years, population-based epidemiological studies and ecological studies comparing populations living in areas with differing concentrations of perchlorate in the drinking-water. Some of the ecological studies included pregnant women, newborns and children and, in the case of newborns, took advantage of neonatal screening programmes that included measurements of serum TSH and/or thyroid hormones. In the various studies, outcome measures investigated included one or more of the following: radioactive iodide uptake by the thyroid, serum TSH and thyroid hormone concentrations, urinary perchlorate and iodide concentrations, incidence of thyroid diseases, neonatal birth weight, head circumference and length, incidence of congenital hypothyroidism and neurobehavioural measures in children. There are also historical clinical data from past use of perchlorate as a treatment for hyperthyroidism.

None of the ecological studies showed any relationship between perchlorate concentrations in drinking-water and the incidence of thyroid diseases, including congenital hypothyroidism and thyroid cancer. With respect to TSH and thyroid hormones, one study reported a significant association between elevated newborn TSH levels and concentrations of perchlorate in drinking-water. However, it is notable that the concentrations of perchlorate in drinking-water in this study were not as high as in other, negative studies on newborns.

The clinical studies in healthy adult volunteers and workers did not show any significant effects on TSH or thyroid hormone concentrations at daily exposures of up to 0.5 mg/kg bw per day. The NAS in the USA has estimated, from consideration of the human clinical studies in healthy subjects and from studies of long-term treatment of patients with hyperthyroidism, that a sustained exposure to perchlorate of more than 0.4 mg/kg bw per day would probably be necessary in order to trigger hypothyroidism in normal adults. The NAS also commented that in pregnant women, infants, children and people with low iodide intake or pre-existing thyroid dysfunction, the dose of perchlorate required to cause hypothyroidism may be lower (NRC, 2005). Despite lack of effects on TSH and thyroid hormones, the studies in healthy adult volunteers did show clear, dose-related effects on radiolabelled iodide uptake by the thyroid.

A key issue for human risk assessment is whether a point of departure for deriving a health-based guidance value for perchlorate should be based on the end-point of inhibition of thyroidal uptake of iodide or on end-point(s) with clearer implications for adversity, such as increases in TSH, reductions in circulating thyroid hormone levels and clinical hypothyroidism. Inhibition of iodide uptake by the thyroid is clearly a precursor event in the chain of events that ultimately can lead to adverse effects on thyroid function, but by itself cannot be considered adverse, as there will not be adverse effects from iodide uptake inhibition provided circulating thyroid hormone levels remain unchanged. The NAS review also expressed the view that in adults with a normal dietary iodine intake, uptake of iodide by the thyroid would

need to be inhibited by at least 75% for several months or longer in order to cause declines in thyroid hormone production that would have adverse health effects. The available human data do, however, provide some support for such a statement. For example, the key 14-day clinical study by Greer et al. (2002) in healthy adults found no effect on TSH or thyroid hormones at the highest perchlorate dose tested of 0.5 mg/kg bw per day, despite the observation that the highest dose caused a 67% inhibition of iodide uptake by the thyroid. There is also support from the two occupational studies in which there was chronic exposure; in one study (Lamm et al., 1999), perchlorate exposure equivalent to about 0.5 mg/kg bw per day was not associated with any change in TSH or thyroid hormones, and in the other study (Braverman et al., 2005), in which uptake of radiolabelled iodide by the thyroid was measured, a 38% reduction in iodide uptake by the thyroid was not associated with any effect on TSH or thyroid hormones. A further practical constraint in selecting the end-point is that, with the single exception of one study (Blount et al., 2006b), none of the human clinical and epidemiological studies on perchlorate actually identified any significant association between perchlorate exposure and changes in TSH and thyroid hormones that could be used for the point of departure.

A second key issue is to what extent a point of departure derived from studies in healthy adults relates to potentially more vulnerable groups in the population. Given the critical role of thyroid hormones in brain development, it is widely considered that the probability of a permanent adverse effect on neurodevelopment from thyroid disruption, including transient disruption, would be greatest during early life. Consideration needs to be given to the differing thyroid physiology in the fetus and neonate compared with that of children and adults; for example, the amount of thyroid hormone stored in the colloid in late-gestation fetuses and neonates is estimated to be sufficient for less than 1 day only, compared with several months for adults (Scinicariello et al., 2005). The uncertainties surrounding this issue cannot currently be addressed by using data from pregnant women, neonates and young infants, as there are no good quantitative data relating perchlorate intake to thyroidal iodide uptake, TSH or thyroid hormones in these groups. It is also unclear whether nursing infants may be additionally at risk if perchlorate were to reduce the passage of iodine into breast milk. At present, there are very few data on this, and what data are available are contradictory.

As perchlorate competitively inhibits iodide uptake by the thyroid, another issue for consideration is whether populations living in parts of the world where the diet is deficient in iodine might be more susceptible to perchlorate than iodine-replete individuals. WHO (1999) has estimated that 2.2 billion people from 130 countries are at risk from iodine deficiency, living in areas where soil and therefore the crops grown are deficient in iodine. In many countries, the risks from iodine deficiency have been greatly reduced by the provision of iodized salt, but many still remain at risk. The data on this aspect in relation to perchlorate are sparse. In a review, Charnley (2008) pointed out that a study in 398 European women with urinary iodine concentrations below 100 µg/l during the first trimester of pregnancy, who were exposed to perchlorate at levels similar to those in the USA, found no effects on maternal thyroid function (Pearce, Lazarus & Smythe, 2007). However, these findings have been published as an abstract only. WHO (2001) guidance

classifies urinary iodine concentrations in the range 99–50 µg/l as mild deficiency, 49–20 µg/l as moderate deficiency and below 20 µg/l as severe deficiency. NHANES data from 2001–2002 indicate that about 7% of pregnant women in the USA have urinary iodine concentrations below 50 µg/l (Caldwell, Jones & Hollowell, 2005). However, NHANES data from 1988–1994 showed no differences in TSH or T_4 concentrations when women, including pregnant women, with urinary iodine concentrations below 50 µg/l were compared with women with higher urinary iodine concentrations (Soldin, Tractenberg & Pezzullo, 2005). These data collectively suggest that iodine deficiency may not pose an additional risk, at least during gestation. Only one study has indicated a relationship between low urinary iodine (<100 µg/l) and serum TSH increases and T_4 decreases (Blount et al., 2006b). However, a reanalysis of the data on T_4 from that study, adjusting urinary iodine concentrations for creatinine to better reflect 24 h urinary excretion of iodine, showed that the relationship between perchlorate and T_4 disappeared (Lamm et al., 2007). Charnley (2008) also pointed out that the reported effects on TSH in the NHANES data could be due to co-exposure in the diet to other ubiquitous anti-thyroid substances, either with the same mode of action, such as nitrate or thiocyanate, or with differing modes of action, such as organochlorines. Alternatively, the reported effects could be statistical artefacts, because perchlorate naturally co-occurs with iodine, and there is an association between iodine and TSH in the NHANES data.

A further consideration is whether individuals, in particular pregnant women, who already have the condition of hypothyroidism or subclinical hypothyroidism might be additionally affected by low-level perchlorate exposure. These subgroups of the population are numerically significant; in women, including pregnant women, in the general population, estimates of the prevalence of subclinical hypothyroidism range from 2.3% to 10%, and those for overt hypothyroidism, from 0.2% to 5% (Charnley, 2008).

Given the mode of action of perchlorate and the groups most likely to show enhanced susceptibility to perchlorate, the key vulnerable groups are likely to be pregnant women, fetuses, newborns, young infants, those with hypothyroidism and possibly those with iodine-deficient diets. As there are no adequate data on perchlorate exposure and inhibition of thyroidal iodide uptake in these key groups that are comparable to the quantitative data obtained in the study by Greer et al. (2002), these represent significant data gaps and are the major sources of uncertainty in any population-wide risk assessment. The uncertainties discussed above need to be weighed alongside the fact that the point of departure for the risk assessment can be based on inhibition of iodide uptake by the thyroid, a precursor event that, at least at low to moderate levels of inhibition, appears to be non-adverse.

10.4 Analytical methods

Perchlorate is soluble in water and polar organic solvents and is easily extracted from foods using either water or water–acetonitrile mixtures. Water samples are analysed directly, whereas food extracts are subjected to SPE cleanup prior to determination. Analytical methods used for the detection and determination

of perchlorate levels in water and foods include IC with conductivity detector, IC-MS or IC-MS/MS and LC-MS or LC-MS/MS. IC-MS/MS and LC-MS/MS methods offer lower LODs and can be used for the determination of perchlorate in foods. Stable isotope–labelled perchlorates are used as internal standards. Currently, certified reference materials are not available, and collaborative method validation studies have not been conducted. Other methods, such as spectrophotometry, capillary electrophoresis and ion-selective electrode–based potentiometric methods, lack in sensitivity and may not be suitable for detection at lower levels in foods and water. Rapid screening methods have not yet been developed. Most survey data in foods have been obtained using the IC-MS/MS method.

10.5 Effects of processing

Limited data are available on the fate of perchlorate during food processing. However, perchlorate is generally stable at temperatures used in food processing.

10.6 Prevention and control

Reduction of perchlorate in foods and water relies mainly on the control of contamination in fertilizers, irrigation systems and water used in food processing and food preparation. However, washing can reduce surface contamination in vegetables and fruits.

10.7 Levels and patterns of contamination in food commodities

At the present meeting, the Committee reviewed data from six countries on the occurrence of perchlorate in water and different foods analysed between 2004 and 2009 (see Table 1 in section 7.1). Analytical data on 35 073 samples of groundwater and drinking-water indicate that the perchlorate level in 98% of samples was below the LOR. In drinking-water, perchlorate levels exceeded 20 µg/l in 1% of the samples only. The USEPA reported that only 160 out of 3870 water supplies (4%) had perchlorate levels above the LOR (4 µg/l), in the range of 4–420 µg/l, with a mean concentration of 9.9 µg/l; this mean concentration is the average of the detected concentrations. Analytical data in 1866 samples (vegetables, fruits, rice, milk, infant formula, fish and fish products, and beverages, such as juices, beer and wine) were reviewed, and perchlorate levels in 33% of samples were found to be below the LOR. Weighted mean (mean of reported mean levels weighted by number of samples) perchlorate levels in raw vegetables were in the range of 4.8–110 µg/kg (potato, 4.8 µg/kg; carrot, 6.6 µg/kg; spinach, 110 µg/kg; lettuce, 11.6 µg/kg; tomato, 14 µg/kg; squash, 75 µg/kg; eggplant, 78 µg/kg; broccoli, 19 µg/kg; cauliflower, 7 µg/kg; cabbage, 10 µg/kg); in fruits, the weighted mean levels ranged from 0.5 to 28 µg/kg (oranges, 5 µg/kg; apples, 0.5 µg/kg; grapes, 28 µg/kg; melons, 19 µg/kg). Other weighted mean perchlorate levels were as follows: rice, 1 µg/kg; whole wheat flour, 3.5 µg/kg; milk, 6.8 µg/kg; beer, 1 µg/kg; and wine, 6 µg/kg. The mean perchlorate levels in human milk in China (n = 24 composite samples, with each composite sample representing milk from 50 mothers) and the USA (n = 652) were found to be 19.7 µg/l (range 2.1–136 µg/l) and 9.3 µg/l (range 0.01–411 µg/l), respectively. Limited data show that the weighted mean perchlorate level in infant formula was 10 µg/kg. The Committee

noted that sampling and analysis were carried out on targeted foods. However, general surveys involving a broader range of foods in different countries were lacking. In view of the widespread presence of perchlorate in the environment, it is probable that it will be found more widely in drinking-water and food.

10.8 Food consumption and dietary exposure assessment

The Committee evaluated occurrence and dietary exposure data for perchlorate from submissions from China, Japan, Canada and the USA and from the literature. International estimates of dietary exposure were prepared using food consumption information from the 13 GEMS/Food consumption cluster diets and perchlorate concentrations discussed above. The range of estimated dietary exposures to perchlorate is 0.03–0.22 µg/kg bw per day for the 13 GEMS/Food consumption cluster diets. Milk consumption accounts for a large portion of the dietary exposure to perchlorate for most clusters, ranging from 7% (clusters C and G) to 42% (cluster J) of the total. These estimates do not include dietary exposure from drinking-water. Using the WHO default drinking-water consumption for adults of 2 litres per day and the mean concentration from the USEPA data of 9.9 µg/l in samples in which perchlorate was detected, the Committee estimated that additional perchlorate exposure from drinking-water could be 20 µg/day (0.33 µg/kg bw per day).

The Committee also reviewed national dietary exposure estimates from Canada and the USA. The Canadian estimates were made based on data taken from foods available in a public market using a deterministic approach. Perchlorate concentrations were combined with food consumption data available from the Nutrition Canada Food Consumption Survey (<18 years old) and the Nova Scotia Provincial Nutrition Survey (adults >18 years old, including women of childbearing age). Mean dietary exposures to perchlorate were higher for children than for adults. The mean dietary exposure was approximately 0.04 µg/kg bw per day for children 1–11 years of age compared with 0.03 µg/kg bw per day for adults, including women of childbearing age.

For the USA, an estimate of dietary exposure to perchlorate was made using urinary biomarker concentrations of perchlorate, available from the 2001–2002 NHANES. The dietary exposure (which would include exposure from water) was estimated using the ratio of the perchlorate to creatinine concentrations in urine, assuming that 100% of perchlorate in the diet is absorbed and excreted unmetabolized. The 50th-percentile estimate for the total adult population was 0.06 µg/kg bw per day, whereas the 95th-percentile estimate was 0.23 µg/kg bw per day.

An analysis of perchlorate dietary exposures in the USA from the USFDA's TDS from 2003–2008 was submitted to the Committee. The mean estimates for all age subgroups 14 and over were approximately 0.1 µg/kg bw per day, with 90th-percentile estimates less than 0.23 µg/kg bw per day. Dietary exposure was greatest for 2-year-olds; the mean exposure for this group was 0.44 µg/kg bw per day, with a 90th-percentile exposure of 0.73 µg/kg bw per day.

The Committee concluded that the analyses from the USFDA's TDS provide the best estimates of dietary exposure to perchlorate, as they do not depend on

analyses of raw commodities and include water and beverages made from water that might be contaminated with perchlorate. Although all of the national estimates are in general agreement, the Committee noted that the highest estimates were from the TDS, suggesting that the other estimates (and the international estimates prepared by the Committee) may not include all dietary sources of perchlorate.

The highest estimate of dietary exposure from the USFDA's TDS of 0.7 µg/kg bw per day for 2-year-old children and the mean estimate of 0.1 µg/kg bw per day for those 14 years of age and older were chosen for comparison with the health-based guidance value.

10.9 Dose–response analysis

The pivotal study for dose–response analysis was the human clinical study on 16 male and 21 female healthy adult volunteers published by Greer and co-workers in 2002 (Greer et al., 2002), in which perchlorate was given in drinking-water at doses of 0.007, 0.02, 0.1 or 0.5 mg/kg bw per day for 14 days. The uptake of iodide into the thyroid was measured at baseline before administration of perchlorate and on days 2 and 14 of administration at both 8 and 24 h after administration of radiolabelled iodine. These data were used for modelling.

The Committee selected a critical effect size of 50% inhibition of iodide uptake as the benchmark response. This choice was made because human clinical data from healthy adults following both short-term and chronic exposure to perchlorate have shown that such a level of inhibition is not associated with any changes in TSH or thyroid hormone levels. The Committee noted that a benchmark response of 50% inhibition was within the observed range of the study.

PROAST software (version 23.2) for analysis of continuous data was used. In the clinical study, each subject served as his or her own control, with baseline values measured 1 day before the start of the 14-day perchlorate exposure period. In the BMD analysis, the baseline value for iodide uptake in each subject was set at 100%. Analysis of the post-treatment values for iodide uptake at 8 and 24 h and the values from exposure days 2 and 14 as covariates showed that these factors did not have any significant impact. Therefore, the two models used for continuous data—the exponential and Hill models—were fitted to all the data combined. Similar values for the BMD_{50} and $BMDL_{50}$ were obtained from the two models, as shown in Table 5 in section 9. The lower of the two $BMDL_{50}$ values (rounded to two significant figures) of 0.11 mg/kg bw per day was used as the point of departure.

11. EVALUATION

As perchlorate has a very short half-life and is rapidly cleared from the body, it is considered appropriate to derive a provisional maximum tolerable daily intake (PMTDI). The $BMDL_{50}$ of 0.11 mg/kg bw per day for inhibition of uptake of radiolabelled iodide by the thyroid was chosen as the point of departure for derivation of a PMTDI. As it is based on human data, there is no need to apply any interspecies uncertainty factor.

The Committee noted that the $BMDL_{50}$ was derived from a study of relatively short duration but that there are efficient homeostatic mechanisms to cope with short-term and long-term inhibition of iodide uptake, up to (at least) 50%, in healthy children and adults. The Committee also noted that there is at least a 4-fold margin between the value of the $BMDL_{50}$ and the estimate of more than 0.4 mg/kg bw per day that would probably be necessary as a sustained exposure in order to trigger hypothyroidism in normal adults. The Committee therefore concluded that it was not necessary to apply an uncertainty factor to account for the short duration of the pivotal study.

In considering the size of any necessary uncertainty factor for interindividual human differences, the Committee took account of the fact that the effect of perchlorate on inhibition of iodide uptake by the thyroid and on the subsequent synthesis of thyroid hormones in potentially vulnerable groups—such as pregnant women, fetuses, neonates and young infants, those with iodine-deficient diets and those with clinical or subclinical hypothyroidism—may differ from that in healthy adults. The Committee concluded that an uncertainty factor of 10 would be appropriate to cover any differences in the general population, including those in potentially vulnerable subgroups. Applying this 10-fold factor to the $BMDL_{50}$ and rounding to one significant figure, a PMTDI of 0.01 mg/kg bw was established for perchlorate.

The estimated dietary exposures of 0.7 µg/kg bw per day (highest) and 0.1 µg/kg bw per day (mean), including both food and drinking-water, are well below the PMTDI. The Committee considered that these estimated dietary exposures were not of health concern.

12. REFERENCES

Alexander JK, Richard AN, David AN (2004). Determination of perchlorate anion in foods by ion chromatography–tandem mass spectrometry. *Analytical Chemistry*, 76:5518–5522.

Amitai Y et al. (2007). Gestational exposure to high perchlorate concentrations in drinking water and neonatal thyroxine levels. *Thyroid*, 17:843–850.

Anderson TA, Wu TH (2002). Extraction, cleanup, and analysis of the perchlorate anion in tissue samples. *Bulletin of Environmental Contamination and Toxicology*, 68:684–691.

Aribi HE et al. (2006). Analysis of perchlorate in foods and beverages by ion chromatography coupled with tandem mass spectrometry (IC-ESI-MS/MS). *Analytica Chimica Acta*, 567:39–47.

ATSDR (2008). *Toxicological profile for perchlorates*. Atlanta, GA, United States Department of Health and Human Services, Agency for Toxic Substances and Disease Registry (http://www.atsdr.cdc.gov/toxprofiles/tp162.html).

Baier-Anderson C et al. (2006). Estimates of exposure to perchlorate from consumption of human milk, dairy milk, and water, and comparison to current reference dose. *Journal of Toxicology and Environmental Health. Part A*, 69:319–330.

Blake S et al. (2009). *Perchlorate—Risks to UK drinking water sources*. Swindon, Wiltshire, WRc Swindon (WRc Ref: Defra 7845; http://www.dwi.gov.uk/research/completed-research/reports/DWI70_2_218.pdf).

Blount BC et al. (2006a). Perchlorate exposure of the US population, 2001–2002. *Journal of Exposure Science and Environmental Epidemiology*, 17:400–407.

Blount BC et al. (2006b). Urinary perchlorate and thyroid hormone levels in adolescent and adult men and women living in the United States. *Environmental Health Perspectives*, 114:1865–1871.

Blount BC et al. (2007). Perchlorate exposure of the U.S. population, 2001–2002. *Journal of Exposure Science and Environmental Epidemiology*, 17:400–407.

Blount BC et al. (2009). Perinatal exposure to perchlorate, thiocyanate, and nitrate in New Jersey mothers and newborns. *Environmental Science & Technology*, 43:7543–7549.

Brandhuber P, Clark S, Morley K (2009). A review of perchlorate occurrence in public drinking water systems. *Journal of the American Water Works Association*, 101:63–73.

Braverman LE (2007). Clinical studies of exposure to perchlorate in the United States. *Thyroid*, 17:819–822.

Braverman LE, Utiger RD, eds (2005). *The thyroid: a fundamental and clinical text*, 9th ed. Philadelphia, PA, Lippincott Williams & Wilkins.

Braverman LE et al. (2005). The effect of perchlorate, thiocyanate, and nitrate on thyroid function in workers exposed to perchlorate long-term. *Journal of Clinical Endocrinology and Metabolism*, 90:700–706.

Braverman LE et al. (2006). Effects of six months of daily low-dose perchlorate exposure on thyroid function in healthy volunteers. *Journal of Clinical Endocrinology and Metabolism*, 91:2721–2724.

Brechner RJ et al. (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*, 42:777–782.

Buffler PA et al. (2006). Thyroid function and perchlorate in drinking water: an evaluation among California newborns, 1998. *Environmental Health Perspectives*, 114:798–804.

Caldwell KL, Jones R, Hollowell JG (2005). Urinary iodine concentration: United States National Health and Nutrition Examination Survey, 2001–2002. *Thyroid*, 15:692–699.

CalEPA (2004). *Public health goals for chemicals in drinking water: perchlorate*. Sacramento, CA, California Environmental Protection Agency, Office of Environmental Health Hazard Assessment [cited by Crump & Gibbs, 2005].

Capen CC (1997). Mechanistic data and risk assessment of selected toxic end points of the thyroid gland. *Toxicologic Pathology*, 25:39–48.

Capen CC et al., eds (1999). *Species differences in thyroid, kidney and urinary bladder carcinogenesis*. Lyon, International Agency for Research on Cancer (IARC Scientific Publications No. 147).

CDC (2009). Perchlorate. In: *Fourth national report on human exposure to environmental chemicals 2009*. Atlanta, GA, United States Department of Health and Human Services, Centers for Disease Control and Prevention, pp. 243–246.

Chang S et al. (2003). Pediatric neurobehavioral diseases in Nevada counties with respect to perchlorate in drinking water: an ecological inquiry. *Birth Defects Research. Part A, Clinical and Molecular Teratology*, 67:886–892.

Charnley G (2008). Perchlorate: overview of risks and regulation. *Food and Chemical Toxicology*, 46:2307–2315.

Chinese CDC (2009). Perchlorate levels in breast milk, milk powder and infant formula from the 2007 Chinese Total Diet Study. Data submitted to FAO/WHO by the National Institute of Nutrition and Food Safety, Chinese Center for Disease Control and Prevention.

Choksi NY et al. (2003). Role of thyroid hormones in human and laboratory animal reproductive health. *Birth Defects Research. Part B, Developmental and Reproductive Toxicology*, 68:479–491.

Clewell RA, Merrill EA, Robinson PJ (2001). The use of physiologically based models to integrate diverse data sets and reduce uncertainty in the prediction of perchlorate and iodide kinetics across life stages and species. *Toxicology and Industrial Health*, 17: 210–222.

Clewell RA et al. (2003a). Predicting fetal perchlorate dose and inhibition of iodide kinetics during gestation: a physiologically-based pharmacokinetic analysis of perchlorate and iodide kinetics in the rat. *Toxicological Sciences*, 73:235–255.

Clewell RA et al. (2003b). Predicting neonatal perchlorate dose and inhibition of iodide uptake in the rat during lactation using physiologically-based pharmacokinetic modeling. *Toxicological Sciences*, 74:416–436.

Clewell RA et al. (2004). Evidence for competitive inhibition of iodide uptake by perchlorate and translocation of perchlorate into the thyroid. *International Journal of Toxicology*, 23: 17–23.

Clewell RA et al. (2007). Perchlorate and radioiodide kinetics across life stages in the human: using PBPK models to predict dosimetry and thyroid inhibition and sensitive subpopulations based on developmental stage. *Journal of Toxicology and Environmental Health. Part A*, 70:408–428.

Connors T (1997). Physiology of the thyroid gland and agents affecting its secretion. In: Thomas JA, Colby HD, eds. *Endocrine toxicology*, 2nd ed. Washington, DC, Taylor & Francis, pp. 43–68.

Costa M et al. (1996). Interlaboratory validation of a new assay for DNA–protein crosslinks. *Mutation Research*, 369:13–21.

Crawford-Brown D, Raucher B, Harrod M (2006). Intersubject variability of risk from perchlorate in community water supplies. *Environmental Health Perspectives*, 114: 975–979.

Crump C et al. (2000). Does perchlorate in drinking water affect thyroid function in newborns or school-age children? *Journal of Occupational and Environmental Medicine*, 42: 603–612.

Crump KS, Gibbs JP (2005). Benchmark calculations for perchlorate from three human cohorts. *Environmental Health Perspectives*, 113:1001–1008.

Dasgupta PK et al. (2005). The origin of naturally occurring perchlorate: the role of atmospheric processes. *Environmental Science & Technology,* 39:1569–1575.

Dasgupta PK et al. (2008). Intake of iodine and perchlorate and excretion in human milk. *Environmental Science & Technology*, 42:8115–8121.

De Groef B et al. (2006). Perchlorate versus other environmental sodium/iodide symporter inhibitors: potential thyroid-related health effects. *European Journal of Endocrinology*, 155:17–25.

Dohán O et al. (2007). The Na^+/I symporter (NIS) mediates electroneutral active transport of the environmental pollutant perchlorate. *Proceedings of the National Academy of Sciences of the United States of America*, 104:20250–20255.

Dyke JV et al. (2006). Sample processing method for the determination of perchlorate in milk. *Analytica Chimica Acta*, 567:73–78.

Dyke JV et al. (2007). Perchlorate in dairy milk. Comparison of Japan versus the United States. *Environmental Science & Technology*, 41:88–92.

Ellington JJ et al. (2001). Determination of perchlorate in tobacco plants and tobacco products. *Environmental Science & Technology*, 35:3213–3218.

Eskin BA et al. (1975). Rat mammary gland atypia produced by iodine blockade with perchlorate. *Cancer Research*, 35:2332–2339.

FAO/WHO (2008). *Report of the 2nd Session of the Codex Committee on Contaminants in Food. The Hague, The Netherlands, 31 March – 4 April 2008*. Rome, Food and Agriculture Organization of the United Nations and World Health Organization, Codex Alimentarius Commission (ALINORM 08/31/41; http://www.codexalimentarius.net/web/archives.jsp?year=08).

Fernandez-Rodriguez A et al. (1991). Induction of thyroid proliferative changes in rats treated with antithyroid compound. *Anatomia, Histologia, Embryologia*, 20:289–298.

Fernández-Santos JM et al. (2004). Ki-*ras* mutational analysis in rat follicular-cell proliferative lesions of the thyroid gland induced by radioactive iodine and potassium perchlorate. *Journal of Endocrinological Investigation*, 27:12–17.

Fisher J et al. (2000). Preliminary development of a physiological model for perchlorate in the adult male rat: a framework for further studies. *Drug and Chemical Toxicology*, 23: 243–258.

Florencio Vicente MDR (1990). [Proliferative thyroid lesions: An experimental study.] *Dissertation Abstracts International C*, 54:176 (in Spanish).

Gauss W (1972). [Physiological and histological criteria of thyroid gland function during a single or longer treatment of potassium perchlorate in adult mice (*Mus musculus*). I. Long-lasting medication experiments.] *Zeitschrift für mikroskopisch-anatomische Forschung*, 85:469–500 (in German) [cited in ATSDR, 2008].

GEMS/Food-EURO (1995). *Second workshop on reliable evaluation of low-level contamination of food. Report on a workshop in the frame of GEMS/Food-EURO. Kulmbach, Federal Republic of Germany, 26–27 March 1995.* Rome, World Health Organization Regional Office for Europe, Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme (EUR/ICP/EHAZ.94.12/WS04; http://www.who.int/foodsafety/publications/chem/lowlevel_may1995/en/).

Gibbs JP, Van Landingham C (2008). Urinary perchlorate excretion does not predict thyroid function among pregnant women. *Thyroid*, 18:807–808.

Gibbs JP, Engel A, Lamm SH (2005). The NAS perchlorate review: second-guessing the experts. *Environmental Health Perspectives*, 113:A727–A728.

Gilbert ME, Sui L (2008). Developmental exposure to perchlorate alters synaptic transmission in hippocampus of the adult rat. *Environmental Health Perspectives*, 116:752–760.

Ginsberg G, Rice D (2005a). The NAS perchlorate review: questions remain about the perchlorate RfD. *Environmental Health Perspectives*, 113:1117–1119.

Ginsberg G, Rice D (2005b). The NAS perchlorate review: Ginsberg et al. respond. *Environmental Health Perspectives*, 113:A730–A731.

Ginsberg GL et al. (2007). Evaluation of the U.S. EPA/OSWER preliminary remediation goal for perchlorate in groundwater: focus on exposure to nursing infants. *Environmental Health Perspectives*, 115:361–369.

Goodman G (2001). The conclusions of the Arizona perchlorate study require reexamination. *Journal of Occupational and Environmental Medicine*, 43:305–309.

Greer MA et al. (2002). Health effects assessment for environmental perchlorate contamination: the dose response for inhibition of thyroidal radioiodine uptake in humans. *Environmental Health Perspectives*, 110:927–937.

Greiner P et al. (2008). Occurrence of perchlorate in sodium hypochlorite. *Journal of the American Water Works Association*, 100:68–74.

Gu BH, Ruan CM, Wang W (2009). Perchlorate detection at nanomolar concentrations by surface-enhanced Raman scattering. *Applied Spectroscopy*, 63:98–102.

Health Canada (2008). *Perchlorate and human health*. Ottawa, Health Canada (http://www.hc-sc.gc.ca/ewh-semt/pubs/water-eau/perchlorate-eng.php).

Health Canada (2009). Analysis of perchlorate in fruits and vegetables using LC-MS/MS. Data submitted to FAO/WHO by Health Canada, Ottawa.

Hiasa Y et al. (1987). Potassium perchlorate, potassium iodide, and propylthiouracil: promoting effect on the development of thyroid tumors in rats treated with *n*-bis(2-hydroxypropyl)nitrosamine. *Japanese Journal of Cancer Research*, 78:1335–1340.

Hutchinson ST (2004). *A study on the accumulation of perchlorate in young head lettuce. Final report.* Research Triangle Park, NC, United States Environmental Protection Agency, Office of Research and Development (Report EPA/600/R-03/003; http://www.epa.gov/athens/publications/reports/HutchinsonLettuceReport600R03003.pdf).

ICF Consulting (2004). *Recommendation for an oral intake reference dose (RfD) for perchlorate*. Prepared for Environmental Management Division, National Aeronautics and Space Administration. Fairfax, VA, ICF Consulting [cited by Crump & Gibbs, 2005].

ITRC (2005). *Perchlorate: overview of issues, status, and remedial options*. Washington, DC, Interstate Technology & Regulatory Council (http://www.itrcweb.org/Documents/PERC-1.pdf).

Jackson WA et al. (2005). Perchlorate accumulation in forage and edible vegetation. *Journal of Agricultural and Food Chemistry*, 53:369–373.

Japan Ministry of Health, Labour and Welfare (2009). Perchlorate levels in foods and water. Data submitted to FAO/WHO.

Johnston RB Jr et al. (2005). The NAS perchlorate review: adverse effects? *Environmental Health Perspectives*, 113:A728–A729.

Josefsson M et al. (2002). Sodium/iodide-symporter: distribution in different mammals and role in entero-thyroid circulation of iodide. *Acta Physiologica Scandinavica*, 175:129–137.

Kannan K et al. (2009). Occurrence of perchlorate in drinking water, groundwater, surface water and human saliva from India. *Chemosphere*, 76:22–26.

Kelsh MA et al. (2003). Primary congenital hypothyroidism, newborn thyroid function, and environmental perchlorate exposure among residents of a Southern California community. *Journal of Occupational and Environmental Medicine*, 45:1116–1127.

Kessler FJ, Kruskemper HL (1966). [Experimental thyroid tumors caused by many years of potassium perchlorate administration.] *Klinische Wochenschrift*, 44:1154–1156 (in German).

Khan MA et al. (2005). A mixture of ammonium perchlorate and sodium chlorate enhances alterations of the pituitary–thyroid axis caused by the individual chemicals in adult male F344 rats. *Toxicologic Pathology*, 33:776–783.

Kim HK, Kim JH, Lee BC (2009). Occurrence of perchlorate in drinking water sources in Korea. *Water Science and Technology: Water Supply*, 9:133–139.

Kirk AB (2006). Environmental perchlorate: why it matters. *Analytica Chimica Acta*, 567:4–12.

Kirk AB et al. (2003). Perchlorate in milk. *Environmental Science & Technology*, 37: 4979–4981.

Kirk AB et al. (2005). Perchlorate and iodide in dairy and breast milk. *Environmental Science & Technology*, 39:2011–2017.

Kirk AB et al. (2007). Temporal patterns in perchlorate, thiocyanate and iodide excretion in human milk. *Environmental Health Perspectives*, 115:182–186.

Kosaka K et al. (2007). Occurrence of perchlorate in drinking water sources of metropolitan area in Japan. *Water Research*, 41:3474–3482.

Lamm SH (2003). Perchlorate exposure does not explain differences in neonatal thyroid function between Yuma and Flagstaff. *Journal of Occupational and Environmental Medicine*, 45:1131–1132.

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

Lamm SH et al. (1999). Thyroid health status of ammonium perchlorate workers: a cross-sectional occupational health study. *Journal of Occupational and Environmental Medicine*, 41:248–260.

Lamm SH et al. (2007). Perchlorate, thiocyanate and low iodine association not seen with low creatinine-adjusted urine iodine among women of childbearing age. *Thyroid*, 17(Suppl.):S51 (abstract).

Lawrence J, Lamm S, Braverman LE (2001). Low dose perchlorate (3 mg daily) and thyroid function. *Thyroid*, 11:295.

Lawrence JE et al. (2000). The effect of short-term low-dose perchlorate on various aspects of thyroid function. *Thyroid*, 10:659–663.

Leung AM et al. (2009). Colostrum iodine and perchlorate concentrations in Boston-area women: a cross-sectional study. *Clinical Endocrinology*, 70:326–330.

Lewandowski TA, Seeley MR, Beck BD (2004). Interspecies differences in susceptibility to perturbation of thyroid homeostasis: a case study with perchlorate. *Regulatory Toxicology and Pharmacology*, 39:348–362.

Li FX, Squartsoff L, Lamm SH (2001). Prevalence of thyroid diseases in Nevada counties with respect to perchlorate in drinking water. *Journal of Occupational and Environmental Medicine*, 43:630–634.

Li FX et al. (2000). Neonatal thyroid-stimulating hormone level and perchlorate in drinking water. *Teratology*, 62:429–431.

Li YT, George EJ (2006). Reversed-phase liquid chromatography/electrospray ionization tandem mass spectrometry for analysis of perchlorate in water. *Journal of Chromatography A*, 1133:215–220.

Li Z et al. (2000). Neonatal thyroxine level and perchlorate in drinking water. *Journal of Occupational and Environmental Medicine*, 42:200–205.

Liu YJ et al. (2004). Investigation of bromate, haloacetic acids and perchlorate in Beijing's drinking water. *Environmental Science*, 25:51–55.

Mahle DA et al. (2003). Changes in cross-fostered Sprague-Dawley rat litters exposed to perchlorate. *International Journal of Toxicology*, 22:87–94.

Martinelango PK, Gumus G, Dasgupta PK (2006). Matrix interference free determination of perchlorate in urine by ion association–ion chromatography–mass spectrometry. *Analytica Chimica Acta*, 567:9–86.

Martinelango PK, Tian K, Dasgupta PK (2006). Perchlorate in seawater: bioconcentration of iodide and perchlorate by various seaweed species. *Analytica Chimica Acta*, 567:100–107.

Martinelango PK et al. (2005). Gas phase ion association provides increased selectivity and sensitivity for measuring perchlorate by mass spectrometry. *Analytical Chemistry*, 77:4829–4835.

Massachusetts Department of Environmental Protection (2004). *Summary report of the first low-level perchlorate proficiency test study conducted by the Massachusetts Department of Environmental Protection.* Lawrence, MA, Massachusetts Department of Environmental Protection, Division of Environmental Analysis, Senator William X. Wall Experiment Station (http://www.mass.gov/dep/water/priorities/perchpt.pdf).

Mathew J, Gandhi J, Hedrick J (2005). Trace level perchlorate analysis by ion chromatography–mass spectrometry. *Journal of Chromatography A*, 1085:54–59.

McLanahan ED et al. (2007). Low-dose effects of ammonium perchlorate on the hypothalamic–pituitary–thyroid axis of adult male rats pretreated with PCB126. *Toxicological Sciences*, 97:308–317.

McLanahan ED et al. (2009). Competitive inhibition of thyroidal uptake of dietary iodide by perchlorate does not describe perturbations in rat serum total T_4 and TSH. *Environmental Health Perspectives*, 117:731–738.

Mendiratta SK, Dotson RL, Brooker RT (1996). Perchloric acid and perchlorates. In: Kroschwitz JI, Howe-Grant M, eds. *Kirk-Othmer encyclopedia of chemical technology*, 4th ed. *Vol. 18.* New York, NY, John Wiley & Sons, pp. 157–170.

Merrill EA et al. (2003). PBPK predictions of perchlorate distribution and its effect on thyroid uptake of radioiodide in the male rat. *Toxicological Sciences*, 73:256–269.

Merrill EA et al. (2005). PBPK model for radioactive iodide and perchlorate kinetics and perchlorate-induced inhibition of iodide uptake in humans. *Toxicological Sciences*, 83: 25–43.

Morgan JW, Cassady RE (2002). Community cancer assessment in response to long-time exposure to perchlorate and trichloroethylene in drinking water. *Journal of Occupational and Environmental Medicine*, 44:616–621.

Mou SF, Liu KN, Ding XJ (2005). *Ion chromatography and its application*, 2nd ed. Beijing, Chemical Industry Press.

Murray CW et al. (2008). US Food and Drug Administration's Total Diet Study: dietary intake of perchlorate and iodine. *Journal of Exposure Science and Environmental Epidemiology*, 18:571–580.

Nakamura S, Kosaka H (1989). [Genotoxicity of inorganic metal compounds with umu test.] *Japanese Journal of Industrial Health*, 31:430–431 (in Japanese).

NRC (2005). *Health implications of perchlorate ingestion*. Prepared by the Committee to Assess the Health Implications of Perchlorate Ingestion, National Research Council. Washington, DC, The National Academies Press (http://www.nap.edu/catalog/11202.html).

Okeke BC et al. (2007). Development of a perchlorate reductase–based biosensor for real time analysis of perchlorate in water. *Journal of Microbiological Methods*, 68:69–75.

Oldi JF, Kannan K (2009a). Analysis of perchlorate in human saliva by liquid chromatography–tandem mass spectrometry. *Environmental Science and Technology*, 43:142–147.

Oldi JF, Kannan K (2009b). Perchlorate in human blood serum and plasma: relationship to concentrations in saliva. *Chemosphere*, 77:43–47.

Pajer Z, Kalisnik M (1991). The effect of sodium perchlorate and ionizing irradiation on the thyroid parenchymal and pituitary thyrotropic cells. *Oncology*, 48:317–320.

Park JW et al. (2005). Food chain transfer of perchlorate in largemouth bass, *Micropterus salmoides*. *Bulletin of Environmental Contamination and Toxicology*, 74:56–63.

Pearce EN, Lazarus JH, Smythe PP (2007). Thyroid function is not affected by environmental perchlorate exposure in first trimester pregnant women. *Thyroid*, 17(Suppl.):S133–S134 (abstract).

Pearce EN et al. (2007). Breast milk iodine and perchlorate concentrations in lactating Boston-area women. *Journal of Clinical Endocrinology and Metabolism*, 92:1673–1677.

Quiñones O et al. (2007). Perchlorate assessment of the Nakdong and Yeongsan watersheds, Republic of Korea. *Environmental Toxicology and Chemistry*, 26:1349–1354.

Rao B et al. (2007). Widespread natural perchlorate in unsaturated zones of the southwest United States. *Environmental Science & Technology*, 41:4522–4528.

Richardson SD (2007). Water analysis: emerging contaminants and current issues. *Analytical Chemistry*, 79:4295–4324.

Riedel C et al. (2001). Journey of the iodide transporter NIS: from its molecular identification to its clinical role in cancer. *Trends in Biochemical Sciences*, 26:490–496.

Rijntjes E et al. (2009). Prenatal induced chronic dietary hypothyroidism delays but does not block adult-type Leydig cell development. *American Journal of Physiology, Endocrinology and Metabolism*, 296:E305–E314.

San RHC, Clarke JJ (1999). *In vitro mammalian cell gene mutation test (L5178Y/TK+/− mouse lymphoma assay).* Rockville, MD, Perchlorate Study Group (BioReliance Study No. G98BAO6.702) [cited by ATSDR, 2008].

Sanchez CA et al. (2005a). Perchlorate and nitrate in leafy vegetables of North America. *Environmental Science & Technology*, 39:9391–9397.

Sanchez CA et al. (2005b). Accumulation and perchlorate exposure potential of lettuce produced in the lower Colorado River region. *Journal of Agricultural and Food Chemistry*, 53:5479–5486.

Sanchez CA et al. (2006). Potential perchlorate exposure from *Citrus* sp. irrigated with contaminated water. *Analytica Chimica Acta*, 567:33–38.

Sanchez CA et al. (2007). Perchlorate, thiocyanate, and nitrate in edible cole crops (*Brassica* sp.) produced in the lower Colorado River region. *Bulletin of Environmental Contamination and Toxicology,* 79:655–659.

Sanchez CA et al. (2008). Perchlorate exposure from food crops produced in the lower Colorado River region. *Journal of Exposure Science and Environmental Epidemiology*, 1:1–10.

Schier JG et al. (2010). Perchlorate exposure from infant formula and comparisons with the perchlorate reference dose. *Journal of Exposure Science and Environmental Epidemiology*, 20:281–287.

Scinicariello F et al. (2005). Genetic factors that might lead to different responses in individuals exposed to perchlorate. *Environmental Health Perspectives*, 113:1479–1484.

Shi YL, Zhang P (2007). Perchlorate in sewage sludge, rice, bottled water and milk collected from different areas in China. *Environment International*, 33:955–962.

Siglin JC et al. (2000). A 90-day drinking water toxicity study in rats of the environmental contaminant ammonium perchlorate. *Toxicological Sciences*, 57:61–74.

Smith PN et al. (2006). Thyroid function and reproductive success in rodents exposed to perchlorate via food and water. *Environmental Toxicology and Chemistry*, 25:1050–1059.

Snyder SA et al. (2006). Perchlorate and chlorate in dietary supplements and flavor enhancing ingredients. *Analytica Chimica Acta*, 567:26–32.

Soldin OP, Tractenberg RE, Pezzullo JC (2005). Do thyroxine and thyroid stimulating hormone levels reflect urinary iodine concentrations? *Therapeutic Drug Monitoring*, 27:178–185.

Soukup-Hein RJ et al. (2007). A general, positive ion mode ESI-MS approach for the analysis of singly charged inorganic and organic anions using a dicationic reagent. *Analytical Chemistry*, 79:7346–7352.

Srinivasan A, Viraraghavan T (2009). Perchlorate: health effects and technologies for its removal from water resources. *International Journal of Environmental Research and Public Health*, 6:1418–1442.

Steinmaus C, Miller MD, 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*, 115:1333–1338.

Stoker TE et al. (2006). Evaluation of ammonium perchlorate in the Endocrine Disruptor Screening and Testing Program's male pubertal protocol: ability to detect effects on thyroid endpoints. *Toxicology*, 10:58–65.

Strawson J, Dourson ML, Zhao QJ (2005). The NAS perchlorate review: is the RfD acceptable? *Environmental Health Perspectives*, 113:A729–A730.

Strawson J, Zhao Q, Dourson M (2004). Reference dose for perchlorate based on thyroid hormone change in pregnant women as the critical effect. *Regulatory Toxicology and Pharmacology*, 39:44–65.

Susala S et al. (1999). Perchlorate identification in fertilizers. *Environmental Science & Technology*, 33:3469–3472.

Tan K et al. (2004). Accumulation of perchlorate in aquatic and terrestrial plants at a field scale. *Journal of Environmental Quality*, 33:1638–1646.

Téllez Téllez R et al. (2005). Long-term environmental exposure to perchlorate through drinking water and thyroid function during pregnancy and the neonatal period. *Thyroid*, 15:963–975.

Theodorakis C et al. (2006). Perchlorate in fish from a contaminated site in east-central Texas. *Environmental Pollution*, 139:59–69.

Thorne PG (2004). *Field screening method for perchlorate in water and soil*. Hanover, NH, United States Cold Regions Research and Engineering Laboratory (ERDC/CRREL TR-04-8; http://www.crrel.usace.army.mil/techpub/CRREL_Reports/reports/TR04-8.pdf).

Thuett KA et al. (2002a). In utero and lactational exposure to ammonium perchlorate in drinking water: effects on developing deer mice at postnatal day 21. *Journal of Toxicology and Environmental Health. Part A*, 65:1061–1076.

Thuett KA et al. (2002b). Effects of in utero and lactational ammonium perchlorate exposure on thyroid gland histology and thyroid and sex hormones in developing deer mice

(*Peromyscus maniculatus*) through postnatal day 21. *Journal of Toxicology and Environmental Health. Part A*, 65:2119–2130.

Ting D et al. (2006). Development of a health-protective drinking-water level for perchlorate. *Environmental Health Perspectives*, 114:881–886.

Tonacchera M et al. (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*, 14:1012–1019.

Toro Guillen M (1991). [Reversibility of proliferative thyroid lesions.] *Dissertation Abstracts International C*, 54:1186 (in Spanish).

Tran N et al. (2008). Thyroid-stimulating hormone increases active transport of perchlorate into thyroid cells. *American Journal of Physiology, Endocrinology and Metabolism*, 294:E802–E806.

USEPA (1998). *Perchlorate environmental contamination: toxicological review and risk characterization based on emerging information (1998 external review draft).* Washington, DC, United States Environmental Protection Agency, Office of Research and Development, National Center for Environmental Assessment (http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=23292).

USEPA (1999a). *Interim assessment guidance for perchlorate*. Washington, DC, United States Environmental Protection Agency, Office of Research and Development, National Center for Environmental Assessment (http://www.epa.gov/fedfac/pdf/interim_guid_perchlorate_6-99.pdf).

USEPA (1999b). *Method 314.0. Determination of perchlorate in drinking water using ion chromatography. Revision 1.0*. Washington, DC, United States Environmental Protection Agency (http://www.epa.gov/safewater/methods/pdfs/methods/met314.pdf).

USEPA (2002). *Perchlorate environmental contamination: toxicological review and risk characterization (2002 external review draft).* Washington, DC, United States Environmental Protection Agency, Office of Research and Development, National Center for Environmental Assessment (NCEA-1-0503; http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=24002).

USEPA (2005a). *Perchlorate and perchlorate salts*. Washington, DC, United States Environmental Protection Agency, Integrated Risk Information System (http://www.epa.gov/iris/subst/1007.htm).

USEPA (2005b). *EPA sets reference dose for perchlorate*. Washington, DC, United States Environmental Protection Agency (News release, 18 February 2005; http://yosemite.epa.gov/opa/admpress.nsf/d0cf6618525a9efb85257359003fb69d/c1a57d2077c4bfda85256fac005b8b32!OpenDocument).

USEPA (2005c). *Method 314.1. Determination of perchlorate in drinking water using inline column concentration/matrix elimination ion chromatography with suppressed conductivity detection. Revision 1.0*. Washington, DC, United States Environmental Protection Agency (http://www.epa.gov/safewater/methods/pdfs/methods/method_314_1.pdf).

USEPA (2005d). *Method 331.0. Determination of perchlorate in drinking water by liquid chromatography electrospray ionization mass spectrometry. Revision 1.0*. Washington, DC, United States Environmental Protection Agency (http://www.epa.gov/safewater/methods/pdfs/methods/met331_0.pdf).

USEPA (2006). *Memorandum: assessment guidance for perchlorate*. Washington, DC, United States Environmental Protection Agency, Office of Solid Waste and Emergency Response (http://www.epa.gov/fedfac/pdf/perchlorate_guidance.pdf).

USEPA (2008a). *Interim drinking water health advisory for perchlorate*. Washington, DC, United States Environmental Protection Agency, Office of Science and Technology, Office of Water (EPA 822-R-08-025; http://www.epa.gov/safewater/contaminants/unregulated/pdfs/healthadvisory_perchlorate_interim.pdf).

USEPA (2008b). *Method 314.2. Determination of perchlorate in drinking water using two-dimensional ion chromatography with suppressed conductivity detection. Version 1.0.* Washington, DC, United States Environmental Protection Agency (http://www.epa.gov/safewater/methods/pdfs/methods/methods_314-2.pdf).

USEPA (2009). *EPA seeks advice on perchlorate in drinking water—Agency issues interim health advisory (01/08/2009).* Washington, DC, United States Environmental Protection Agency (News release, 8 January 2009; http://yosemite.epa.gov/opa/admpress.nsf/3881d73f4d4aaa0b85257359003f5348/467d05245cbb049d8525753800644b1e!OpenDocument).

USFDA (2005). *Rapid determination of perchlorate anion in foods by ion chromatography–tandem mass spectrometry. Revision 2: April 12, 2005.* Silver Spring, MD, United States Department of Health and Human Services, Food and Drug Administration (http://www.fda.gov/Food/FoodSafety/FoodContaminantsAdulteration/ChemicalContaminants/Perchlorate/ucm077793.htm).

USFDA (2007a). *Preliminary estimation of perchlorate dietary exposure based on FDA 2004/2005 exploratory data.* Silver Spring, MD, United States Department of Health and Human Services, Food and Drug Administration, Center for Food Safety and Applied Nutrition (http://www.fda.gov/Food/FoodSafety/FoodContaminantsAdulteration/ChemicalContaminants/Perchlorate/ucm077653.htm, accessed 5 February 2010).

USFDA (2007b). *2004–2005 exploratory survey data on perchlorate in food.* Silver Spring, MD, United States Department of Health and Human Services, Food and Drug Administration, Center for Food Safety and Applied Nutrition (http://www.fda.gov/Food/FoodSafety/FoodContaminantsAdulteration/ChemicalContaminants/Perchlorate/ucm077685.htm, accessed 16 May 2010).

USFDA (2009). *Survey data on perchlorate in food—2005/2006 Total Diet Study results.* Silver Spring, MD, United States Department of Health and Human Services, Food and Drug Administration (http://www.fda.gov/Food/FoodSafety/FoodContaminantsAdulteration/ChemicalContaminants/Perchlorate/ucm077615.htm).

van Wijk N et al. (2008). Perinatal and chronic hypothyroidism impair behavioural development in male and female rats. *Experimental Physiology*, 93:1199–1209.

Wang Z et al. (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*, 57:9250–9255.

WHO (1999). *Progress towards the elimination of iodine deficiency disorders (IDD).* Prepared by the World Health Organization, United Nations Children's Fund and International Council for Control of Iodine Deficiency Disorders. Geneva, World Health Organization (Unpublished document WHO/NHD/99.4.2).

WHO (2001). *WHO, UNICEF and ICCIDD assessment of the iodine deficiency disorders and monitoring their elimination. A guide for programme managers*. Geneva, World Health Organization (WHO/NHD/01.1).

Wolff J (1998). *Perchlorate and the thyroid gland*. *Pharmacological Reviews*, 50:89–105.

York RG et al. (2001a). Two-generation reproduction study of ammonium perchlorate in drinking water in rats evaluates thyroid toxicity. *International Journal of Toxicology*, 20:183–197.

York RG et al. (2001b). Oral (drinking water) developmental toxicity study of ammonium perchlorate in New Zealand White rabbits. *International Journal of Toxicology*, 20: 199–205.

York RG et al. (2003). Oral (drinking water) developmental toxicity study of ammonium perchlorate in Sprague-Dawley rats. *International Journal of Toxicology*, 22:453–464.

York RG et al. (2004). A rat neurodevelopmental evaluation of offspring, including evaluation of adult and neonatal thyroid, from mothers treated with ammonium perchlorate in drinking water. *International Journal of Toxicology*, 23:191–214.

York RG et al. (2005a). Refining the effects observed in a developmental neurobehavioral study of ammonium perchlorate administered orally in drinking water to rats. I. Thyroid and reproductive effects. *International Journal of Toxicology*, 24:403–418.

York RG et al. (2005b). Refining the effects observed in a developmental neurobehavioral study of ammonium perchlorate administered orally in drinking water to rats. II. Behavioral and neurodevelopment effects. *International Journal of Toxicology*, 24:451–467.

Yu L et al. (2006). Challenges in determining perchlorate in biological tissues and fluids: implications for characterizing perchlorate exposure. *Analytica Chimica Acta*, 567:66–72.

Zeiger E (1998a). Salmonella *mutagenicity testing of ammonium perchlorate* [memorandum with attachment to A. Jarabek]. Research Triangle Park, NC, United States Department of Health and Human Services, National Institute of Environmental Health Sciences.

Zeiger E (1998b). *Ammonium perchlorate MN test results* [memorandum with attachment to A. Jarabek]. Research Triangle Park, NC, United States Department of Health and Human Services, National Institute of Environmental Health Sciences.

Zhang P et al. (2007a). Determination of trace level perchlorate in sludge samples by ion chromatography–electrospray tandem mass spectrometry. *Chinese Journal of Analytical Chemistry*, 35:131–134.

Zhang P et al. (2007b). Analysis of perchlorate, bromate and iodide in milk using ion chromatography–electrospray tandem mass spectrometry. *Journal of Instrumental Analysis*, 26:690–693.

Zhou Y (2007). Perchlorate contamination in water. *Engineering and Construction*, 21: 355–356.

Zoeller RT (2006). Collision of basic and applied approaches to risk assessment of thyroid toxicants. *Annals of the New York Academy of Sciences*, 1076:168–190.

Zoeller RT, Rice DC (2004). Critical effect of perchlorate on neonates is iodide uptake inhibition. *Regulatory Toxicology and Pharmacology*, 40:376–377.