Appendix A. Background Information for Barium

Barium is a naturally occurring element. It does not occur in nature as a free metal but is found as the divalent cation in combination with other elements, forming various soluble and insoluble compounds (ATSDR 2007; EPA 2005). It is found in small amounts in drinking water and food and is thought to not be an essential nutrient for humans (ATSDR 2007; EPA 2005; WHO 2008). However, there are some plants, such as legumes, forage plants, Brazil nuts, and mushrooms that accumulate barium. Some Brazil nuts have notably high concentrations of barium (3,000–4,000 ppm) (ATSDR 2007). Barium compounds are used commercially in various industries, and insoluble barium sulfate is used as a contrast agent for x-ray examination of the stomach and intestines (ACGIH 2001; ATSDR 2007; WHO 2008).

A.1 Toxicokinetics

Gastrointestinal absorption of barium is compound-dependent, with very little absorption of insoluble barium sulfate and highly variable (1–80%) absorption of acid-soluble compounds (ATSDR 2007; EPA 2005). Evidence from animal studies suggests that absorption may be increased in children and infants compared with adults (ATSDR 2007; EPA 2005). The extent of absorption of barium in humans following inhalation exposure is unknown (ATSDR 2007; EPA 2005). In laboratory animals, 50–75% of inhaled barium chloride or barium sulfate was estimated to be absorbed from the respiratory tract within 24 hours; barium chloride (soluble) is more rapidly absorbed than barium sulfate (insoluble) (ACGIH 2001; ATSDR 2007). If barium is injected directly into the trachea, clearance times are much longer (up to weeks) than clearance times following inhalation, suggesting that barium compounds are efficiently absorbed (via lung-to-blood transfer) and cleared (via mucociliary transport) in the upper airways, including nasal passages (ATSDR 2007; EPA 2005). There is very little information regarding dermal absorption of barium (ATSDR 2007; EPA 2005); however, based on chemical properties (e.g., high polarity), most barium compounds are not expected to be readily absorbed through the skin (ATSDR 2007).

Following absorption, barium is distributed by the blood throughout the body with rapid disposition of excess barium in bone and teeth (ATSDR 2007; EPA 2005). Approximately 90% of total barium body burden is in the skeleton (ATSDR 2007; EPA 2005). A small percent of barium in the body is also found in various soft tissues (ATSDR 2007; EPA 2005). Barium does not undergo metabolism in the body; however, it may form transport complexes or be incorporated into tissues (ATSDR 2007). Barium is eliminated primarily through feces, with minor excretion in the urine (ATSDR 2007; EPA 2005).

A.2 Health Effects

Potentially fatal hypokalemia (decreased potassium blood levels), resulting in ventricular tachycardia, hyper- and/or hypotension, muscle weakness, and paralysis, has been reported in individuals following accidental or intentional ingestion (at undetermined doses) of water-soluble barium compounds such as barium carbonate or chloride; oral LD_{50} values in rodents range from about 130 to 279 mg/kg (ATSDR 2007). Mild cardiovascular effects (altered blood pressure), kidney damage, gastrointestinal effects (vomiting, abdominal cramps, diarrhea), nervous system effects (altered blood pressure, numbness in the face, muscle weakness), and difficulties in breathing have been reported in individuals ingesting lower (nonfatal) doses (ACGIH 2001; ATSDR 2007; EPA 2005). These effects have not been associated with oral exposure to insoluble barium (e.g., barium sulfate) (ACGIH 2014; ATSDR 2007).

Based on cases of acute poisoning, the primary human health concerns following chronic exposure to elevated soluble barium compounds in drinking water are cardiovascular effects, particularly hypertension (WHO 2008). However, in an epidemiological study, there were no differences in blood pressure or the prevalence of cardiovascular disease between a population drinking water containing a mean barium concentration of 7.3 mg/L compared with a population drinking water containing a mean barium concentration of 0.1 mg/L (WHO 2008). Assuming a body weight of 70 kg and water consumption of 2 L/day, estimated daily intakes in the low- and high-dose groups are 0.003 and 0.21 mg/kg/day, respectively. Similarly, in a controlled exposure study, no cardiovascular effects were observed in volunteers given drinking water containing barium at a concentration of 5 ppm for 4 weeks followed by 10 ppm for an additional 4 weeks, compared with self-control values measured for 2 weeks prior to the exposure period (EPA 2005). The EPA calculated daily intake levels of 0.11 mg/kg/day for the 5 ppm exposure period and 0.21 mg/kg/day for the 10 ppm exposure period (EPA 2005).

Evidence from animal studies indicates that the kidney is the most sensitive target organ of soluble barium compounds following oral exposure in animals fed normal diets, with increased kidney weights and nephropathy following repeat exposure to ≥115 mg/kg/day (ATSDR 2007; EPA 2005; NTP 1994; WHO 2008). Other adverse effects noted in animals repeatedly exposed to higher doses of soluble barium compounds (≥160 mg/kg/day) include decreased immune organ weights, decreased body weight, and increased mortality (ATSDR 2007; EPA 2005; NTP 1994). Additionally, neurological effects were reported in male rats (decreased spontaneous motor activity) and mice (decreased forelimb grip) exposed to barium chloride at 200 and 495 mg/kg/day, respectively, for 90 days (ATSDR 2007; EPA 2005; NTP

1994). Cardiovascular effects were not observed in these animal studies; however, cardiovascular effects (increased blood pressure, decreased cardiac contractility) were observed in animals chronically fed low mineral diets containing barium chloride at doses as low as 0.8 mg/kg/day, suggesting that individuals with dietary deficiencies may be more sensitive to hypokalemic effects of barium exposure (ATSDR 2007; EPA 2005). Data are inadequate to determine if soluble barium compounds are a reproductive or developmental hazard following oral exposure (ATSDR 2007; EPA 2005). Due to low absorption, no adverse health effects have been associated with oral exposure to insoluble barium sulfate in animals (ACGIH 2014; ATSDR 2007).

Very limited data are available regarding health effects following inhalation exposure to barium. Hypokalemia, electrocardiogram abnormalities, muscle weakness and paralysis, and gastrointestinal effects have also been reported in case reports of individuals exposed to very high concentrations of airborne barium (ATSDR 2007). The evidence for lung damage in humans or animals following inhalation exposure to barium compounds is equivocal due to lack of sufficient data (ATSDR 2007); however, baritosis (benign pneumoconiosis) has been described in workers following occupational exposure to barite or barium sulfate (ACGIH 2014; EPA 2005). Additionally, adverse effects (dyspnea, hypoxemia, allergy, fibrosis) and sometimes death have been reported in cases of accidental aspiration of barium sulfate contrast during medical procedures (ACGIH 2014). The potential effects of inhaled barium on the kidney have not been adequately evaluated (ATSDR 2007).

A.3 Mechanisms of Action

Adverse health effects associated with exposure to high levels of barium (cardiovascular and kidney effects, muscle weakness, and paralysis) are attributed to alterations in potassium homeostasis resulting in hypokalemia (ACGIH 2001; Ahlawat and Sachdev 1999; ATSDR 2007). Acute barium poisoning leads to a shift of potassium from the extracellular space to the intracellular space, resulting in altered resting membrane potential and perturbation of various homeostatic mechanisms and physiological functions (e.g., non-excitable muscle cells leading to weakness and paralysis) (ACGIH 2001; Ahlawat and Sachdev 1999; ATSDR 2007). Elevated intracellular potassium levels have been primarily attributed to competitive antagonism of potassium efflux channels by barium (Ahlawat and Sachdev 1999; ATSDR 2007). However, it is possible that barium may also activate the sodium-potassium ATPase pump, which would further elevate intracellular potassium levels (Ahlawat and Sachdev 1999).

A.4 Health Guidelines

For oral exposure, established toxicological values based on kidney toxicity in animal studies include ATSDR intermediate- and chronic-duration MRLs of 0.2 mg/kg/day (ATSDR 2007) and an EPA RfD value of 0.2 mg/kg/day (EPA 2005); see Table H-1 in Appendix H. Several agencies have established drinking water guidelines. To protect against potential cardiovascular effects (hypertension), the World Health Organization (WHO) established a drinking water quality guideline level of 0.7 mg/L (WHO 2008). The EPA established that exposure to barium in drinking water at 0.7 mg/L for 1 or 10 days is not expected to cause any adverse effects in children (EPA 2012). A lifetime health advisory for adults was not established by the EPA; however, a drinking water equivalent level (DWEL) of 7 mg/L (a DWEL is a lifetime exposure level, assuming 100% exposure from that medium, at which adverse, noncarcinogenic health effects would not be expected to occur) (EPA 2012). The Food and Drug Administration (FDA) established that the barium concentration in bottled drinking water should not exceed 2 mg/L (FDA 2015).

Several health guidelines have been established by various agencies to protect against adverse effects from inhalation exposure to barium, including skin, eye, and upper respiratory irritation, gastroenteritis, muscle spasm, cardiovascular effects, and hypokalemia. To protect chronically exposed workers, the ACGIH established a Threshold Limit Value (TLV) (8-hour time-weighted average [TWA]) of 0.5 mg/m^3 for barium and soluble compounds and 5 mg/m^3 for insoluble barium sulfate (ACGIH 2001, 2014); the Occupational Safety and Health Administration (OSHA) established a permissible exposure limit (PEL) (8-hour TWA) of 0.5 mg/m^3 for barium and soluble compounds, 15 mg/m^3 for barium sulfate (total), and 5 mg/m^3 for barium sulfate (respirable fractions) (OSHA 2014, 2015a, 2015b); and the National Institute for Occupational Safety and Health (NIOSH) established a recommended exposure limit (REL) (10-hour TWA) of 0.5 mg/m^3 for barium and soluble compounds, 10 mg/m^3 for barium sulfate (total), and 5 mg/m^3 for barium sulfate (respirable fractions) (NIOSH 2015a, 2015b). ATSDR (2007) and EPA (2005) have not derived inhalation toxicity values.

EPA (2005) and ACGIH (2001) determined that barium and soluble compounds are not classifiable as to human carcinogenicity (Class D and Class A4, respectively). IARC (2015) and NTP (2014) have not assessed barium for carcinogenicity.

A.5 Derivation of Target-organ Toxicity Dose(s)

Following oral exposure, the kidney is the most sensitive target organ following barium exposure, with adverse effects in animals observed following repeat exposure to ≥115 mg/kg/day (ATSDR 2007; EPA 2005). Therefore, kidney effects were used as the basis to derive oral toxicity values. Additional effects observed at ≥160 mg/kg/day include neurobehavioral effects, decreased immune organ weights, decreased body weights, and increased mortality (ATSDR 2007).

Due to neurotoxicity observed following exposure to barium, an oral TTD for barium-induced neurological effects was derived. Adverse neurological effects were observed in male rats and mice in a 13-week drinking water study conducted by the NTP (1994). Rats were identified as the more sensitive species, with a NOAEL of 110 mg/kg/day and a LOAEL of 200 mg/kg/day based on increased locomotor activity (NTP 1994). Using the same uncertainty factor of 300 applied to the oral MRL based on kidney effects (10 for human variability, 10 for animal to human extrapolation, and 3 for database uncertainty; see Table H-1 in Appendix H), a TTD of 0.4 mg/kg/day was derived for neurological effects following oral exposure to barium. This TTD is twice the intermediate MRL of 0.2 mg/kg/day based on adverse kidney effects in the same study.

There is some evidence that high oral intake of barium can cause cardiovascular effects in humans, including hypertension (ATSDR 2007; EPA 2005; WHO 2008). The LOAEL for these effects has not been established; however, no adverse cardiovascular effects were observed in humans exposed to barium doses up to 0.21 mg/kg/day in drinking water in either an epidemiological study or a controlled exposure study (EPA 2005; WHO 2008). Because the oral toxicity value for sodium (another high concentration component of hydraulic fracturing waste fluid) is based on elevated blood pressure, a TTD of 0.21 mg/kg/day was established for hypertensive effects of barium based on this NOAEL (using an uncertainty factor of 1). This TTD is equivalent to the intermediate MRL of 0.2 mg/kg/day based on adverse kidney effects in rats.

Gastrointestinal effects (vomiting, abdominal cramps, diarrhea) have been reported for individuals ingesting nonfatal doses of barium (ACGIH 2001; ATSDR 2007; EPA 2005), but dose-response data are inadequate to derive an intermediate- or chronic-duration oral TTD for barium-induced gastrointestinal effects.

A.6 References

ACGIH. 2001. Barium and soluble compounds. Documentation of the threshold limit values and biological exposure indices. Cincinnati, OH: American Conference of Governmental Industrial Hygienists.

ACGIH. 2014. Barium sulfate. Documentation of the threshold limit values and biological exposure indices. Cincinnati, OH: American Conference of Governmental Industrial Hygienists.

Ahlawat SK, Sachdev A. 1999. Hypokalaemic paralysis. Postgrad Med J 75(882):193-197.

ATSDR. 2007. Toxicological profile for barium and barium compounds. Agency for Toxic Substances Disease Registry. http://www.atsdr.cdc.gov/ToxProfiles/tp24.pdf. September 3, 2015.

EPA. 2005. Toxicological review of barium and compounds. Washington, DC: U.S. Environmental Protection Agency. http://www.epa.gov/iris/toxreviews/0010tr.pdf. September 3, 2015.

EPA. 2012. 2012 Edition of the drinking water standards and health advisories. Washington, DC: U.S. Environmental Protection Agency. EPA822S12001. http://water.epa.gov/action/advisories/drinking/upload/dwstandards2012.pdf. September 3, 2015.

FDA. 2015. Beverages. Bottled water. Code of Federal Regulations 21 CFR 165.110. http://www.gpo.gov/fdsys/pkg/CFR-2015-title21-vol2/pdf/CFR-2015-title21-vol2-part165.pdf. September 4, 2015.

IARC. 2015. Agents classified by the IARC Monographs, Volumes 1-113. International Agency for Research on Cancer. http://monographs.iarc.fr/ENG/Classification/List_of_Classifications_Vol1-113.pdf. September 3, 2015.

NIOSH. 2015a. Barium chloride (as Ba). NIOSH Pocket guide to chemical hazards. National Institute for Occupational Safety and Health. http://www.cdc.gov/niosh/npg/npgd0045.html. September 3, 2015.

NIOSH. 2015b. Barium sulfate. NIOSH Pocket guide to chemical hazards. National Institute for Occupational Safety and Health. http://www.cdc.gov/niosh/npg/npgd0047.html. September 3, 2015.

NTP. 1994. Toxicology and carcinogenesis studies of barium chloride dihydrate (CAS No. 10326-27-9) in F344/N rats and B6C3F1 mice (drinking water studies). Research Triangle Park, NC: National Toxicology Program. TR432. http://ntp.niehs.nih.gov/ntp/htdocs/lt_rpts/tr432.pdf. September 3, 2015.

NTP. 2014. Report on carcinogens. Thirteenth edition. National Toxicology Program. http://ntp.niehs.nih.gov/pubhealth/roc/roc13/index.html. September 3, 2015.

OSHA. 2014. Subpart D - Occupational health and environmental controls. Gasses, vapors, fumes, dusts, and mists. Occupational Safety and Health Administration. Code of Federal Regulations 29 CFR 1926.55. http://www.gpo.gov/fdsys/pkg/CFR-2014-title29-vol8/pdf/CFR-2014-title29-vol8-sec1926-55.pdf. September 4, 2015.

OSHA. 2015a. Subpart Z - Toxic and hazardous substances. 1910.1000. Air contaminants. Occupational Safety and Health Administration. Code of Federal Regulations 29 CFR 1910.1000. http://www.gpo.gov/fdsys/pkg/CFR-2014-title29-vol6/pdf/CFR-2014-title29-vol6-sec1910-1000.pdf. September 4, 2015.

OSHA. 2015b. Subpart Z - Toxic and hazardous substances. 1915.1000. Air contaminants. Occupational Safety and Health Administration. Code of Federal Regulations 29 CFR 1915.1000. http://www.gpo.gov/fdsys/pkg/CFR-2015-title29-vol7/pdf/CFR-2015-title29-vol7-sec1915-1000.pdf. September 4, 2015.

WHO. 2008. Guidelines for drinking-water quality. Third edition incorporating the first and second addenda. Volume 1 recommendations. Geneva, Switzerland: World Health Organization. http://www.who.int/water_sanitation_health/dwq/fulltext.pdf. September 3, 2015.

Appendix B. Background Information for Calcium

Calcium is an essential nutrient that is important in making and maintaining teeth and bone and in many other physiological functions including cell signaling, coagulation of blood, neural transmission, and muscle contraction (EFSA 2012, 2015a; NAS 1997, 2011; SCF 2003). About 99% of calcium in the human body exists in teeth and bone, mainly as insoluble calcium hydroxyapatite; the remaining calcium exists in divalent cationic form in serum and intra- and inter-cellular fluids (EFSA 2012, 2015a; NAS 1997, 2011; SCF 2003). Calcium ingested by adults in the United States, Canada, and Europe is thought to come primarily from food sources and dietary supplements ingested as calcium carbonate or calcium citrate, but calcium in mineral-rich drinking water can make substantial contributions to total calcium intakes (EFSA 2012, 2015a; NAS 2011; WHO 2009).

B.1 Toxicokinetics

Calcium ingested in food and water is absorbed into intestinal cells by an active, saturable transport system and a passive, non-saturable system (EFSA 2012, 2015a; NAS 1997, 2011; SCF 2003). Active transport into cells involves binding to calcitriol (the hydroxylated form of vitamin D), binding to an intestinal vitamin D receptor, transport through calcium-selective transmembrane channels, intracellular movement via a calcium-binding protein, and basolateral extrusion by a calcium pump (Christakos 2012; EFSA 2015a; NAS 2011; Perez et al. 2008). Passive transport occurs through tight junctions within intercellular spaces in epithelial layers of the intestine, and accounts for lower proportions of total absorbed calcium (8–23%) than active transport in adults, especially at low and moderate calcium intake levels (Bronner 2003; McCormick 2002). The relative importance of passive transport absorption in adults increases under conditions of high calcium intake (EFSA 2015a; NAS 2011). In newborn infants, passive absorption is relatively more important than in adults, until full development of the gastrointestinal system occurs (NAS 2011). Although most absorbed calcium ends up as structural hydroxyapatite in teeth and bone, calcium in bone can be released as cationic calcium via bone resorption and serves as a calcium reservoir in the maintenance of circulating levels of calcium (EFSA 2012, 2015a; NAS 1997, 2011; SCF 1993).

Circulating levels of divalent calcium are controlled between about 8.5 and 10.5 mg/dL by a homeostatic system involving calcitriol, PTH, and calcitonin (EFSA 2015a; NAS 2011). Decreases in serum calcium levels signal the secretion of PTH from parathyroid glands via the CaSR. PTH stimulates the kidney to produce calcitriol, activates bone resorption, and stimulates calcium reabsorption in the kidney (EFSA

2015a; NAS 2011; Perez et al. 2008). With increases in serum calcium, feedback mechanisms suppress the secretion of PTH by parathyroid glands, and stimulate thyroid secretion of calcitonin, which suppresses bone resorption (EFSA 2015a; NAS 2011). Elevated serum levels of calcitriol also suppress PTH secretion (NAS 2011).

Calcium excretion from the body in urine is controlled by homeostatic reabsorption processes in the kidney (NAS 2011). In the proximal tubule, high calcium levels in the glomerular filtrate suppress active reabsorption via a CaSR, whereas low calcium levels activate the CaSR and stimulate active reabsorption (NAS 2011). In the renal distal convoluted and connecting tubules, active calcium reabsorption is regulated by calcitriol, PTH, and calcitonin and consists of three steps: entry across the apical plasma membrane via calcium channels, cytosolic diffusion of calcium bound to calbindin, and extrusion across the basolateral membrane via a sodium-calcium exchanger and a calcium-ATPase (Hoenderop et al. 2000).

Unabsorbed calcium in the intestines is excreted in feces, along with calcium in sloughed mucosal cells and intestinal secretions including bile (EFSA 2015a; NAS 2011). The extent of calcium excretion in sweat from the skin increases with increases in ambient temperature and physical activity (EFSA 2015a; NAS 2011).

B.2 Health Effects

Persistent hypercalcemia, a condition defined by EFSA (2012) as serum calcium concentrations >11 mg/dL and by NAS (2011) as concentrations ≥10.5 mg/dL, has been associated in case reports with several symptoms including fatigue, muscular weakness, anorexia, nausea, vomiting, constipation, tachycardic arrhythmia, and weight loss (EFSA 2015a; NAS 2011). Chronic hypercalcemia has been associated with nephrolithiasis (stone formation in the kidney), soft tissue calcifications (i.e., nephrocalcinosis and vascular calcification), and impaired kidney concentration ability (EFSA 2015a; NAS 2011). Hypercalcemia in human subjects has been infrequently linked to excessive intakes of calcium or vitamin D (primarily from dietary supplements), but has been more frequently linked to the presence of malignant tumors or hyperthyroidism (EFSA 2012; Moe 2008). Renal insufficiency, which has been estimated to occur in 30% of North Americans over the age of 60 (NAS 2011), is thought to make individuals more susceptible to the possible adverse effects of excess calcium intake.

Studies have been conducted to examine possible associations between excessive calcium intakes and a number of potentially adverse human health conditions including calcium-alkali syndrome (a condition, formerly named milk-alkali syndrome, characterized by metabolic alkalosis and hypercalcemia with varying degrees of hydration, renal failure, nephrocalcinosis, or nephrolithiasis), kidney stone formation, vascular calcification, cardiovascular disease, and prostate cancer (EFSA 2012; NAS 2011).

Calcium-Alkali Syndrome. NAS (2011) analysis of evidence for associations with calcium-alkali syndrome comes from modern (post-2000) case reports of this condition, mostly in patients with impaired renal function, who repeatedly took calcium carbonate at intake levels ranging from >1,000 to 44,000 mg Ca/day (NAS 2011). NAS (2011) concluded that the data suggest that calcium intakes ≥3,000 mg/day would lead to hypercalcemia and associated symptoms in patients with renal insufficiency. An earlier meta-analysis found no evidence for milk-alkali syndrome in studies of various groups of people (children; pregnant, premenopausal, and perimenopausal women; and elderly subjects) who took lower levels of calcium supplementation (500–2,000 mg/day) for 12 weeks to 4 years (SCF 2003).

Soft Tissue Calcification. NAS (2011) and EFSA (2012) found no data linking the use of calcium supplements with nephrocalcification in human subjects but cited a report of high calcium intake during preadolescence inducing nephrocalcinosis in female rats (Peterson et al. 1996). Evidence for calcium-induced vascular calcification has been reported in several cases of subjects with high calcium intakes and renal insufficiency (Asmus et al. 2005; Block et al. 2005; Goodman et al. 2000; Raggi et al. 2005).

Cardiovascular Disease Mortality and Events. Consistent evidence for associations between increased calcium intakes from dietary supplementation and increased risk of cardiovascular disease mortality or events (such as myocardial infarction and stroke) was not found in meta-analyses of increased cardiovascular mortality or events in 11 randomized control trials in subjects ≥40 years of age (Bolland et al. 2010) or in a meta-analysis of myocardial infarction, stroke, or a composite cardiovascular endpoint (consisting of myocardial infarction, stroke, or sudden death) in four randomized control trials (Wang et al. 2010). Some studies found associations for increased risks for some endpoints and others did not. Bolland et al. (2011) updated their 2010 meta-analysis by including three studies comparing calcium and vitamin D supplements against placebo treatment. The NAS (2011) review of the available data concluded that study limitations, such as a lack of recording of the total calcium intakes, makes it "difficult to conclude that calcium intakes per se in the range of 1,000 to 1,200 mg/day can be associated with cardiovascular events." The EFSA (2012) Panel concluded from review of the available data that

long-term calcium intakes from diet and supplements up 2,500–3,000 mg Ca/day are not associated with an increased risk of cardiovascular disease in adults.

WHO (2009) reported that a number of geographical epidemiology studies have found inverse associations (i.e., protection) between cardiovascular disease mortality and drinking water levels of hardness, calcium, or magnesium, whereas other studies found no association. In a meta-analysis of six case-control studies of cardiovascular disease mortality associations with drinking water levels of calcium or magnesium, only one study found a statistically significant inverse association with drinking water calcium levels, but four of the six studies found significant inverse associations with magnesium levels (Catling et al. 2005; WHO 2009). It is expected that calcium intakes from drinking water in these studies would be well below intakes from calcium dietary supplements and diet in the trials described in the previous paragraph.

Nephrolithiasis (Kidney Stones). NAS (2011) concluded that the best human evidence for calcium-induction of kidney stones comes from a large, well-designed cohort study of bone fracture frequency, bone density, and kidney stone frequency in 36,000 postmenopausal women (ages 50–79 years) who received a placebo or a supplement of 1,000 mg Ca/day and 400 IU vitamin D/day for up to 5 years (Jackson et al. 2006). The total average calcium intake for the treated group was about 2,100 mg Ca/day. The reported hazard ratio for kidney stones in the treated group was 1.17 (95% confidence interval [CI] 1.02–1.34), compared with the placebo group (449 cases in treated group versus 381 cases in the placebo group). NAS (2011) noted that earlier, smaller trials of calcium supplementation in older subjects reported no evidence for a link with nephrolithiasis (Borghi et al. 2002; Levine et al. 1994; Riggs et al. 1998; Williams et al. 2001). NAS (2011) also noted that a series of studies of younger women (Curhan et al. 1997, 2004) or men between 40 and 75 years old (Curhan et al. 1993) did not find consistent evidence for an association with kidney stone formation and provided limited evidence that taking calcium supplements with food may suppress the formation of kidney stones.

In an independent review of the available data on possible associations between high calcium intakes and kidney effects, including kidney stones, EFSA (2012) concluded that: (1) calcium intakes up to about 2,400 mg Ca/day have not been associated with an increased risk of chronic hypercalciuria or impaired kidney function, and (2) calcium intakes up to 3,000 mg Ca/day have not been associated with an increased risk of nephrolithiasis in the general adult population. The EFSA (2012) Panel noted that the evidence for an association between calcium dietary supplementation and kidney stones presented by Jackson et al. (2006) was not found in a subsequent analysis of the same study; Wallace et al. (2011)

found that when only subjects with stone outcomes during the time of treatment ("during active adherence") were included in the analysis, the increased risk estimate was of a similar magnitude (as in the earlier analysis, but was not statistically significant (hazard ratio 1.21; 95% CI 0.98–1.34). The EFSA (2012) Panel concluded that the Jackson et al. (2006) study "did not provide evidence for an increased risk of kidney stones which could be attributed to high calcium intakes."

Other recent reviews present evidence that kidney stone disease in humans has multiple risk factors including sex, age, race, family history, several dietary factors other than calcium (e.g., higher dietary intakes of animal protein, sugar, or oxalates may increase risk and higher potassium or phytates may decrease risk), systemic disorders (e.g., obesity and diabetes increase risk), and fluid intake (e.g., low fluid intake increases risk) (Curhan 2007; Prochaska et al. 2016). The multiplicity of risk factors reflects the inconsistency among epidemiology studies finding positive associations between calcium intake and kidney stones. A number of prospective epidemiology studies have now consistently found an inverse relationship between dietary calcium and kidney stone risk, but intake of calcium supplements has been found to present a 20% added risk for kidney stones in older women, but not in younger women (Prochaska et al. 2016).

Prostate Cancer. In a meta-analysis of 12 cohort studies of possible associations between calcium intake and risk for prostate cancer in men with mean ages ranging from 53 to 67 years, DHHS (2009) reported that seven studies found no statistically significant associations between calcium intakes and prostate cancer risk, whereas five studies found higher prostate cancer risk in high-calcium groups (total intakes from 921 to >2,000 mg Ca/day), compared with low-calcium intake groups.

The NAS (2011) review of this meta-analysis and additional evidence from case-control studies and a randomized control trial concluded that the available evidence was "not sufficiently robust to serve as an indicator for a UL."

The EFSA (2012) review of the available data concluded that "long-term calcium intakes from diets and supplements above 2,000 mg Ca/day are not associated with an increased risk of prostate cancer." The EFSA (2012) conclusion was accompanied by notes that: (1) associations were found in 1/12 case-control studies and 5/12 prospective cohort studies; (2) these studies did not control for factors other than calcium, which may have been responsible for the associations; and (3) a single randomized control trial found no association between calcium supplemental at doses of 1,200 mg Ca/day (total calcium intake of about 2,000 mg Ca/day) and increased risk of prostate cancer.

B.3 Mechanisms of Action

The mechanisms by which high-level calcium intake may produce kidney effects, such as kidney stones (nephrolithiasis) or nephrocalcinosis, are poorly understood. Nephrocalcinosis has not been clearly associated with high intakes of calcium dietary supplements in humans, presumably due to the efficacy of complex homeostatic mechanisms for calcium but was associated with high calcium intakes by preadolescent rats (NAS 2011; Peterson et al. 1996). Symptoms associated with nephrocalcinosis are similar to those associated with renal dysfunction, such as painful and frequent urination, nausea, vomiting, or swelling. Although nephrocalcinosis has been proposed to be linked to the formation of kidney stones (in humans, the principal component of kidney stones is calcium oxalate), Vervaet et al. (2009) have proposed that nephrocalcinosis and calcium nephrolithiasis are independent pathologies associated with multiple factors including compromised or surpassed crystal clearance mechanisms and crystal adherence differences between normal renal epithelial cells and dedifferentiated or regenerating renal epithelial cells. Reflecting this putative mechanistic complexity, some human studies have reported statistically significant associations between high calcium intakes and increased risk for kidney stones (e.g., Jackson et al. 2006) and others have not found significant positive associations (Borghi et al. 2002; Levine et al. 1994; Riggs et al. 1998; Williams et al. 2001). Other studies of humans (Curhan et al. 1993, 1997, 2004), rodents (Mourad et al. 2006), and cats and dogs (Lekcharoensuk et al. 2001a, 2001b; Lulich et al. 2016) have observed inverse relationships between calcium dietary intakes and kidney stone risk (less risk with higher dietary calcium) and provided evidence that other components of the diet can influence the apparent association between high calcium intakes and formation of kidney stones (e.g., increased intakes of oxalates and sugar increase risk and increased intakes of potassium and phytates may decrease risk). A postulated mechanistic explanation for the apparent discrepancy between dietary calcium and supplemental calcium is that dietary calcium may inhibit oxalate absorption in the gut more effectively than supplementary calcium not taken with meals (Prochaska et al. 2016). Overall, the mechanism of kidney stone formation in humans is not clearly understood, but multiple risk factors other than calcium intake from supplements are widely recognized, including low fluid intake, increased urinary calcium and oxalate excretion, increased dietary sugar, high body mass index, and type II diabetes (Curhan 2007; EFSA 2012; NAS 2011; Prochaska et al. 2016).

Limited evidence is available for increased risk of cardiovascular events in older women on calcium supplementation (Bolland et al. 2008, 2010, 2011), but the reported increased risks were small and not seen in other studies (see EFSA 2012; NAS 2011). Calcification of vascular tissues has been reported

with high calcium intake (Asmus et al. 2005; Block et al. 2005; Goodman et al. 2000; Raggi et al. 2005); however, the reports are based on individuals with compromised kidney function. The mechanisms whereby high calcium intakes may produce adverse effects on the cardiovascular system are poorly understood and studied.

B.4 Health Guidelines

The Institute of Medicine of the NAS has determined age-group specific dietary recommendations for calcium (NAS 2011). The preparation of the NAS (2011) report was a collaborative effort with support from both the U.S. and Canadian governments. Recommended adequate intakes (AIs) for infants 0–6 months and 6–12 months were 200 and 260 mg Ca/day, respectively (NAS 2011). The value for 0–6-month-old infants was based on the presumption that calcium requirements for infants are met by human breast milk, reference intake values for infants fed breast milk (780 mL milk/day), estimates of mean breast milk calcium concentrations (259 mg Ca/L milk), and estimates that 60% of ingested calcium is absorbed (NAS 2011). The higher AI value for infants 6–12 months of age was based on estimates of intake from food (140 mg Ca/day) and breast milk (120 mg Ca/day). Recommended dietary allowances (RDAs) for calcium were established for children 1–3 years old (700 mg Ca/day) and 4–8 years old (1,000 mg Ca/day), based on studies that established average calcium accretion via bone measures and average calcium retention via calcium balance studies (NAS 2011). RDAs for children 9–13 years old (1,300 mg Ca/day) and 14–18 years old (1,300 mg Ca/day) were based on a study of bone accretion in children and adolescents (Vatanparast et al. 2010). RDAs for adults 19–30 years old (1,000 mg Ca/day) and 31–50 years old (1,000 mg Ca/day) were based on an analysis of calcium balance data indicating intakes associated with neutral calcium balance (Hunt and Johnson 2007). RDAs for men 51–70 years old (1,000 mg Ca/day) and women 51–70 years old (1,200 mg Ca/day) were based on a presumption that postmenopausal women need more calcium during this life phase than men (NAS 2011). The RDA for adults >70 years old (1,200 mg Ca/day) was based on a presumption that older men and women experience greater loss of bone and bone mass density than at younger stages of life and limited evidence that dietary calcium supplemental may reduce bone fracture risk.

NAS (2011) also established UL values for calcium in various life stages. ULs for children 0–6 months old (1,000 mg/day) and 6–12 months old (1,500 mg Ca/day) were based on a randomized trial of the effects of calcium-supplemented formula in infants from 3 to 9 months of age on calcium excretion, which reported that 1,750 mg Ca/day was without effect on calcium excretion in these infants (Sargent et al. 1999). For 0–6-month-old infants, the NOAEL for calcium excretion was divided by an uncertainty

factor of 2 to account for body weight differences between newborns and infants in the principal study and rounded up to 1,000 mg Ca/day for the UL (NAS 2011). For 6–12-month-old infants, the NOAEL point of departure (POD) was decreased to 1,500 mg Ca/day for the UL to account for the "paucity of data." The ULs for children 1–3 years old (2,500 mg Ca/day) and 4–8 years old (2,500 mg Ca/day) were the same as those established for adults, because no appropriate data were available for these age groups. The ULs for children 9–13 years old and adolescents 14–18 years old of 3,000 mg Ca/day were based on the adult UL value (2,500 mg Ca/day) adjusted upwardly by 500 units to account for increased metabolic demands associated with bone accretion during the 9–18-years-of-age period (NAS 2011). The ULs for adults 19–30 and 31–50 years old were each 2,500 mg Ca/day; they were based on a reported LOAEL of 2,000 mg Ca/day for increased kidney stone risk in postmenopausal women (Jackson et al. 2006), which was adjusted up to 2,500 mg/day, based on the presumption that younger adults tolerate high calcium intakes better than older adults with decreased kidney function (NAS 2011). The UL for adults >50 years old of 2,000 mg/day was the LOAEL for increased kidney stone risk in postmenopausal women (Jackson et al. 2006). The ULs established for nonpregnant and nonlactating women of the same age groups were established for pregnant and lactating women (i.e., 14–18 years old [3,000 mg Ca/day]; 19–30 years old [2,500 mg Ca/day]; 31–50 years old [2,500 mg Ca/day]), based on evidence that calcium requirements for women are not significantly changed by pregnancy and lactation (NAS 2011).

EFSA (2015a) established several age-group-specific dietary recommendations for calcium. An AI for infants aged 7–11 months of 280 mg Ca/day was derived by estimating the amount of calcium absorbed by breastfed infants (120 mg Ca/day) and extrapolating up using isometric scaling. For children aged 1–3, 4–10, and 11–17 years, Population Reference Intakes (PRIs) of 450, 800, and 1,150 mg Ca/day were derived based on estimates of calcium intakes sufficient for calcium accretion in bone and replacement of calcium loss from the body. The PRIs were 1,000 mg/day for young adults (18–24 years old) and 950 mg Ca/day for adults ≥25 years old. These values were based on estimates of calcium intakes equaling fecal and urinary losses from calcium balance studies.

EFSA (2012) established a UL for oral intake of calcium by adults of 2,500 mg Ca/day. This value was based on analysis of numerous studies (published before 2003 and between 2003 and 2011) in which adults (including pregnant and lactating women) with prolonged total intakes of up to 2,500 mg Ca/day from diet and supplements were reported to be without adverse effects (i.e., 2,500 mg Ca/day for adults is a NOAEL). In the assessment of evidence for calcium-induced adverse effects from long-term calcium intakes, the EFSA (2012) Panel concluded that the evidence for dose-response relationships for calcium-alkali syndrome, nephrolithiasis, cardiovascular disease, or prostate cancer was insufficient for UL

development. EFSA (2012) also concluded that available data for infants, children, or adolescents were insufficient for UL derivation. Although the EFSA evaluation of the available evidence differed from that of the NAS (2011), the EFSA (2012) UL for adults, 2,500 mg Ca/day, is numerically the same as the NAS (2011) UL for adults 19–50 years of age.

ATSDR (2018) and EPA (IRIS 2019) have not derived noncancer toxicity values for calcium. The EPA (IRIS 2019), IARC (2018), and NTP (2016) have not assessed calcium for carcinogenicity.

Because ATSDR MRLs and EPA RfDs for calcium have not been developed, the NAS (2011) ULs for calcium (based on increased risk for kidney stones in postmenopausal women taking dietary calcium supplements) are recommended to be the basis for provisional orals MRLs in ATSDR public health assessments of water-soluble forms of calcium. Thus, the NAS (2011) UL for adults 19–50 years old, 2,500 mg/day, is divided by a reference body weight of 70 kg to derive a surrogate oral MRL for soluble forms of calcium of 36 mg Ca/kg/day. For use in screening level assessments, it is thought that this oral MRL would be suitable for intermediate- and chronic-duration exposure scenarios. Other lifestage-specific oral MRLs could be derived similarly from age-group-specific NAS (2011) ULs, using appropriate reference body weights.

B.5 Derivation of Target-organ Toxicity Dose(s)

Following oral exposure, the most sensitive adverse effect associated with excess calcium intake is kidney stone formation, as determined by NAS (2011). A single value provisional oral MRL of 36 mg/kg/day for intermediate- and chronic-duration exposure of adults was based on the adult UL of 2,500 mg Ca/day, which was based on a LOAEL of 2,000 mg Ca/day for increased kidney stone risk in postmenopausal women (Jackson et al. 2006). The EFSA (2012) derived an adult UL of the same value (2,500 mg Ca/day), but interpreted available data somewhat differently than the NAS (2011); their interpretation was that repeated intakes of 2,500 mg Ca/day was a NOAEL for potential adverse effects from calcium including kidney stones, calcium-alkali syndrome, vascular calcification, cardiovascular disease, and prostate cancer.

Limited evidence exists for associations between prolonged high calcium intakes and calcium-alkali syndrome, vascular calcification, cardiovascular disease and prostate cancer. However, available exposure-response data associating repeated high calcium intakes with these other health endpoints were judged by NAS (2011) to be inadequate for UL derivation, and thus for TTD derivation. The NAS (2011)

evaluation of the inadequacy of the available exposure-response data for these adverse health effects associated with high calcium intakes is consistent with the overall EFSA (2012) evaluation.

B.6 References

Asmus H, Braun J, Krause R, et al. 2005. Two year comparison of sevelamer and calcium carbonate effects on cardiovascular calcification and bone density. Nephrol Dial Transplant 20:1653-1661. http://doi.org/10.1093/ndt/gfh894.

ATSDR. 2018. Framework for assessing health impacts of multiple chemicals and other stressors (update). Atlanta, GA: Agency for Toxic Substances and Disease Registry. https://www.atsdr.cdc.gov/interactionprofiles/ip-ga/ipga.pdf. December 22, 2020.

Block GA, Spiegel DM, Ehrlich J, et al. 2005. Effects of sevelamer and calcium on coronary artery calcification in patients new to hemodialysis. Kidney Int 68:1815-1824.

Bolland MJ, Grey AB, Avenell A, et al. 2011. Calcium supplements with or without vitamin D and risk of cardiovascular events: Reanalysis of the Women's Health Initiative limited access dataset with meta-analysis. Br Med J 342:2040.

Bolland MJ, Barber PA, Doughty RN, et al. 2008. Vascular events in healthy older women receiving calcium supplementation: Randomised controlled trial. British Medical Journal 336(7638):262-266. http://doi.org/10.1136/bmj.39440.525752.BE.

Bolland MJ, Bacon CJ, Horne AM, et al. 2010. Vitamin D insufficiency and health outcomes over 5 y in older women. Am J Clin Nutr 91:82-89.

Borghi L, Schianchi T, Meschi T, et al. 2002. Comparison of two diets for the prevention of recurrent stones in idiopathic hypercalciuria. N Engl J Med 346(2):77-84.

Bronner F. 2003. Mechanisms of intestinal calcium absorption. J Cell Biochem 88(2):387-393.

Catling L, Abubakar I, Lake I, et al. 2005. Review of evidence for relationship between incidence of cardiovascular disease and hard water. London, England: University of East Anglia and Drinking Water Inspectorate. http://www.fwr.org/waterq/dwi0844.htm. December 22, 2020.

Christakos S. 2012. Mechanism of action of 1,25-dihydroxyvitamin D3 on intestinal calcium absorption. Rev Endocr Metab Disord 13:39-44.

Curhan GC. 2007. Epidemiology of stone disease. Urol Clin North Am 34(3):287-293. http://doi.org/10.1016/j.ucl.2007.04.003.

Curhan GC, Willett WC, Rimm EB, et al. 1993. A prospective study of dietary calcium and other nutrients and the risk of symptomatic kidney stones. N Engl J Med 328(12):833-838.

Curhan GC, Willett WC, Knight EL, et al. 2004. Dietary factors and the risk of incident kidney stones in younger women. Arch Intern Med 164:885-891.

Curhan GC, Willett WC, Speizer FE, et al. 1997. Comparison of dietary calcium with supplemental calcium and other nutrients as factors affecting the risk for kidney stones in women. Ann Intern Med 126(7):497-504.

DHHS. 2009. Vitamin D and calcium: A systematic review of health outcomes. Rockville, MD: U.S. Department of Health and Human Services. Evidence Report/Technology Assessment: Number 183. https://www.ncbi.nlm.nih.gov/books/NBK32603. December 22, 2020.

EFSA. 2012. Scientific opinion on the tolerable upper intake level of calcium. European Food Safety Authority. EFSA J 10(7):2814. www.efsa.europa.eu/efsajournal. November 13, 2018.

EFSA. 2015a. Scientific opinion on dietary reference values for calcium. European Food Safety Authority. EFSA J 13(5):4101.

Goodman WG, Goldin J, Kuizon BD, et al. 2000. Coronary-artery calcification in young adults with end-stage renal disease who are undergoing dialysis. N Engl J Med 342:1478-1483.

Hoenderop JGJ, Muller D, Suzuki M, et al. 2000. Epithelial calcium channel: Gate-keeper of active calcium reabsorption. Curr Opin Nephrol Hypertens 9:335-340.
Hunt CD, Johnson LK. 2007. Calcium requirements: New estimations for men and women by cross-sectional statistical analyses of calcium balance data from metabolic studies. Am J Clin Nutr 86:1054-1063.
IARC. 2018. Agents classified by the IARC monographs, volumes 1-123. International Agency for Research on Cancer. https://monographs.iarc.fr/wp-content/uploads/2018/09/ClassificationsCASOrder.pdf. February 22, 2019.
IRIS. 2019. IRIS advanced search. International Risk Information System. U.S. Environmental Protection Agency. https://cfpub.epa.gov/ncea/iris/search/index.cfm. February 22, 2019.
Jackson RD, LaCroix AZ, Gass M, et al. 2006. Calcium plus vitamin D supplementation and the risk of fractures. N Engl J Med 354(7):669-683.
Lekcharoensuk C, Osborne CA, Lulich JP. 2001a. Epidemiologic study of risk factors for lower urinary tract diseases in cats. J Am Vet Med Assoc 218(9):1429-1435. http://doi.org/10.2460/javma.2001.218.1429.
Lekcharoensuk C, Osborne CA, Lulich JP, et al. 2001b. Association between dietary factors and calcium oxalate and magnesium ammonium phosphate urolithiasis in cats. J Am Vet Med Assoc 219(9):1228-1237. http://doi.org/10.2460/javma.2001.219.1228.
Levine BS, Rodman JS, Wienerman S, et al. 1994. Effect of calcium citrate supplementation on urinary calcium oxalate saturation in female stone formers: Implications for prevention of osteoporosis. Am J Clin Nutr 60:592-596.
Lulich JP, Berent AC, Adams LG, et al. 2016. ACVIM Small animal consensus recommendations on the treatment and prevention of uroliths in dogs and cats. J Vet Int Med 30(5):1564-1574. http://doi.org/10.1111/jvim.14559.
McCormick CC. 2002. Passive diffusion does not play a major role in the absorption of dietary calcium in normal adults. J Nutr 132:3428-3420.
Moe SM. 2008. Disorders involving calcium, phosphorus, and magnesium. Prim Care 35:215-237.
Mourad B, Fadwa N, Mounir T, et al. 2006. Influence of hypercalcic and/or hyperoxalic diet on calcium oxalate renal stone formation in rats. Scand J Urol Nephrol 40:187-191.
NAS. 1997. Dietary reference intakes for calcium, phosphorus, magnesium, vitamin D, and fluoride. Washington, DC: National Academy of Sciences.
NAS. 2011. Dietary reference intakes for calcium and vitamin D. National Academies of Sciences.
NTP. 2016. Substances listed in the fourteenth report on carcinogens. National Toxicology Program. https://ntp.niehs.nih.gov/ntp/roc/content/listed_substances_508.pdf. February 22, 2019.
Perez AP, Picotto G, Carpentieri AR, et al. 2008. Minireview on regulation of intestinal calcium absorption. Emphasis on molecular mechanisms of transcellular pathway. Digestion 77:22-34.
Peterson CA, Baker DH, Erdman JWJ. 1996. Diet-induced nephrocalcinosis in female rats is irreversible and is induced primarily before the completion of adolescence. J Nutr 126:259-265.
Prochaska ML, Taylor EN, Curhan GC. 2016. Insights into nephrolithiasis from the Nurses' Health Studies. Am J Public Health 106(9):1638-1643. http://doi.org/10.2105/ajph.2016.303319.
Raggi P, James G, Burke SK, et al. 2005. Decrease in thoracic vertebral bone attenuation with calcium-based phosphate binders in hemodialysis. J Bone Miner Res 20(5):764-772.
Riggs BL, O'Fallon WM, Muhs J, et al. 1998. Long-term effects of calcium supplementation on serum parathyroid hormone level, bone turnover, and bone loss in elderly women. J Bone Miner Res 13:168-174.
Sargent JD, Dalton MA, O'Connor GT, et al. 1999. Randomized trial of calcium glycerophosphate-supplemented infant formula to prevent lead absorption. Am J Clin Nutr 69:1224-1230.
SCF. 1993. Reports of the Scientific Committee for Food (Thirty-first series). Nutrient and energy intakes for the European Community. Luxembourg: Scientific Committee on Food. European Commission.

SCF. 2003. Opinion of the Scientific Committee on Food on the tolerable upper intake level of calcium. Brussels, Belgium: Scientific Committee on Food. European Commission.

Vatanparast H, Bailey DA, Baxter-Jones ADG, et al. 2010. Calcium requirements for bone growth in Canadian boys and girls during adolescence. British Journal of Nutrition 103:575-580.

Vervaet BA, Verhulst A, D'Haese PC, et al. 2009. Nephrocalcinosis: New insights into mechanisms and consequences. Nephrol Dial Transplant 24:2030-2035. http://doi.org/10.1093.ndt/gfp115.

Wallace RB, Wactawski-Wende J, O'Sullivan MJ, et al. 2011. Urinary tract stone occurrence in the Women's Health Initiative (WHI) randomized clinical trial of calcium and vitamin D supplements. Am J Clin Nutr 94:270-277.

Wang L, Manson JE, Song Y, et al. 2010. Systematic review: Vitamin D and calcium supplementation in prevention of cardiovascular events. Ann Intern Med 152(5):315-323. http://doi.org/10.7326/0003-4819-152-5-201003020-00010.

WHO. 2009. Calcium and magnesium in drinking-water. Public health significance. Geneva, Switzerland: World Health Organization. https://www.who.int/water_sanitation_health/publications/publication_9789241563550/en/. December 22, 2020.

Williams CP, Child DF, Hudson PR, et al. 2001. Why oral calcium supplements may reduce renal stone disease: Report of a clinical pilot study. J Clin Pathol 54:54-62.

Appendix C. Background Information for Iron

Iron is a naturally occurring element and an essential nutrient (NAS 2001a; NIH 2016). Iron can exist in oxidation states ranging from -2 to +6, but it is most commonly found in ferrous (+2), ferric (+3), and ferryl (+4) states in biological systems (NAS 2001a). Four major classes of iron-containing proteins are found in the mammalian system: iron-containing heme proteins (hemoglobin, myoglobin, cytochromes), iron-sulfur enzymes (flavoproteins, heme-flavoproteins), proteins for iron storage and transport (transferrin, lactoferrin, ferritin, hemosiderin), and other iron-containing or activating enzymes (sulfur, non-heme enzymes) (NAS 2001a). Approximately 60% of the total body burden of iron is bound to hemoglobin in circulating red blood cells, which are essential for the transport of oxygen to tissues throughout the body; another 25% is stored as a readily mobilizable iron source (Kew 2014; NAS 2001a; NIH 2016). The remaining 15% is found as a component of myoglobin in skeletal muscle and a variety of enzymes necessary for oxidative metabolism in cells throughout the body (NAS 2001a; NIH 2016). In addition to being essential for oxygen transport and oxidative metabolism, iron is essential for a variety of other vital functions, including growth and development, normal cellular function, and synthesis of some hormones and connective tissues (Gammella et al. 2015; NIH 2016). The main adverse effect of iron deficiency is anemia; however, adverse effects observed at low iron levels insufficient to cause anemia include impaired physical work performance, developmental delay, cognitive impairment, and adverse pregnancy outcomes such as low birth weight and preterm delivery (NAS 2001a; NIH 2016). Associations between iron deficiency, anemia, and deficits in cognitive and motor skill development in infants have been reported (NIH 2016), and some portion of the neurodevelopmental effects may be attributable to increased tissue concentrations of the neurotoxicant, manganese, under iron-deficient conditions (Park et al. 2013).

C.1 Toxicokinetics

The absorption of iron following oral exposure is highly regulated in the upper small intestine (NAS 2001a). There are two primary absorption systems. The first mediates the uptake of small amounts of heme iron from hemoglobin and myoglobin from ingested meat while the second mediates the uptake of larger amounts of non-heme iron (primarily iron salts) from ingested plant and dairy products (NAS 2001a). Heme iron is highly bioavailable, and readily absorbed, while non-heme iron absorption depends on the solubilization and reduction of predominantly ferric food iron into ferrous form by compounds in the duodenum, such as ascorbic acid or ferrireductase (NAS 2001a; NIH 2016). Vitamin C (ascorbic acid) and certain types of meat such as meat, poultry and seafood can enhance absorption of non-heme

iron, while some non-animal foods, including cereals and legumes, can decrease absorption of non-heme iron due to polyphenol content (NIH 2016). Based on biological need, bioavailable iron is absorbed in the duodenum via mucosal cells formed in the crypts of Lieberkuhn (NAS 2001a). These cells have a high turnover rate (48–72 hours), and the amount of iron in the plasma during early development of the cells determines how much iron they will uptake during their short period of functionality (NAS 2001a). The main iron transporter in these cells is thought to be the DMT-1 protein. The expression of this protein is tightly regulated by modifications of mRNA by iron response proteins and the iron response elements (NAS 2001a).

Once iron is absorbed from the gastrointestinal tract, it binds the transport protein, transferrin, in blood and is distributed throughout the body (NAS 2001a). It can move between cells via reversible binding to transferrin. Both absorption and distribution of iron throughout the body are tightly regulated; the circulating peptide hormone hepcidin and its receptor ferroportin play key roles in these processes (Gammella et al. 2015; Kew 2014; NIH 2016). Additionally, DMT-1 has been implicated in endosomal transport (NAS 2001a). Once in the cell, iron can be incorporated into functional compounds (e.g., hemoglobin), stored as ferritin or hemosiderin, or used to regulate future cellular iron metabolism by modifying the activity of iron response proteins (NAS 2001a). All cells in the body are capable of storing iron; however, the main iron storage sites in humans are cells in the liver, spleen, and bone marrow (NAS 2001a). The iron levels in the body are highly conserved, with very little iron lost to elimination except during heavy bleeding (including menstruation) and pregnancy (NAS 2001a; NIH 2016). Due to age-related loss of processes to excrete excess iron, the general level of stored iron can increase with age, particularly in postmenopausal women (Gammella et al. 2015; Kew 2014; NIH 2016). Small, basal losses of 0.90–1.02 mg/day are observed in of men and non-menstruating women (NAS 2001a). The majority of absorbed iron is eliminated in the feces, with minimal losses via the urine and skin (NAS 2001a). In menstruating women, daily loss of iron via menses is approximately 0.6–0.7 mg/day (NAS 2001a).

C.2 Health Effects

Effects in Humans. The most sensitive effect of acute iron overdose is gastrointestinal upset (vomiting, diarrhea) with acute ingestion of doses ≥20 mg/kg (NAS 2001a; NIH 2016). At higher doses (~60 mg/kg), potentially fatal toxicity can occur due to iron overload, with involvement of the cardiovascular system, central nervous system, kidney, liver, and hematological systems (NAS 2001a; NIH 2016). Daily ingestion of iron supplements has been associated with constipation and other

gastrointestinal effects, such as nausea, vomiting, and diarrhea (Health Canada 2016; NAS 2001a). Moderate-to-severe gastrointestinal effects have been reported with repeated exposure to supplemental doses of 50–60 mg/day (0.71–0.86 mg/kg/day assuming 70-kg body weight) (NAS 2001a).

Iron overload, associated with elevated body iron stores (>5 g), can lead to various health effects due to buildup of iron in tissues (CDC 2003; Farina et al. 2013; Gammella et al. 2015; Gujja et al. 2010; Kew 2014; NIH 2016). The main adverse effects associated with chronic iron overload include liver cirrhosis and cardiomyopathy; other associated effects include neurodegeneration, impotence and infertility, premature menopause, metabolic syndrome, diabetes, and arthritis (CDC 2003; Farina et al. 2013; Gammella et al. 2015; Gujja et al. 2010; Kew 2014; Khamseekaew et al. 2016; NIH 2016). More generalized symptoms of fatigue, weakness, and abdominal pain may also occur, and excess iron deposition in the skin may cause a bronze skin discoloration (CDC 2003). Primary iron overload is found in individuals with primary hereditary hemochromatosis, mostly commonly caused by a mutation in the hemochromatosis (HFE gene) leading to decreased secretion of hepcidin, while secondary iron overload can be observed in individuals with transfusion-dependent diseases (e.g., hemoglobinopathies, aplastic anemia, sideroblastic anemia, myelodysplasia) or individuals with genetic alterations (e.g., DMT-1 polymorphism) or diseases (e.g., chronic liver disease) that alter absorption or transport of iron in the body (CDC 2003; Gammella et al. 2015; Gujja et al. 2010; Kew 2014; Khamseekaew et al. 2016; NAS 2001a; NIH 2016). Evidence is limited for iron overload symptoms in some populations with unusually high dietary intakes in food or drinking water. There is some evidence of secondary iron overload (and/or diseases associated with iron overload) with high dietary intake, such as excess iron intake in a region of South Africa/Zimbabwe (in the form of a traditional beer with an average iron content of 80 mg/L) or elevated iron content in drinking water in parts of Taiwan (average iron content of 1.04 mg/L); however, the potential contribution of genetic and environmental factors in these regions is unclear (Gujja et al. 2010; Kew 2014; NAS 2001a).

Potential associations between measures of iron load (e.g., serum ferritin concentration, serum transferrin saturation, serum iron concentrations, total iron-binding capacity) and chronic heart diseases such as myocardial infarction and carotid vascular disease have been reported in a few studies evaluating the general population (i.e., not individuals susceptible to primary or secondary overload); however, several other population studies did not find an association between measures of iron load and chronic heart disease (NAS 2001a). A systematic review of 12 prospective epidemiological studies did not support a strong association between iron status and heart disease in the general population (NAS 2001a).

Neurodegenerative disease such as Parkinson's disease have been associated with increased levels of iron and other metals in brain regions (Chen et al. 2019; Dusek et al. 2015a, 2015b; Huat et al. 2019), but the etiology leading to brain accumulation of iron is unclear (Berg et al. 2001; Double et al. 2000; Gerlach et al. 2003).

Hepatic iron overload has been clearly associated with hepatocellular carcinoma (CDC 2003; Kew 2014; NAS 2001a; NIH 2016). Evidence for an association between high iron levels and cancer in the general population, however, is inconclusive (NAS 2001a). There is limited evidence that elevated iron levels may be associated with colon cancer (NAS 2001a).

Effects in Laboratory Animals. Standard toxicology studies of laboratory animals repeatedly exposed to supplemental ferric citrate at moderate concentrations in drinking water or diet found no exposure-related histological changes in tissues of B6C3F1 mice provided drinking water doses of up to 53 mg Fe/kg/day for 13 weeks or 39 mg Fe/kg/day for 96 weeks (Inai et al. 1994) and evidence of gastroenteritis or hemosiderosis in F344 rats fed 500 mg Fe/kg/day, but not 105 mg Fe/kg/day, in the diet for 13 weeks (Toyoda et al. 2014).

Statistically significant increased incidences of mild to severe inflammation with eosinophilic infiltration in the colon and cecum, colonic mucosal hyperplasia, and hemosiderosis of the spleen were found in F344 rats fed a diet containing 4% (w/w) supplemental ferric citrate (~500 mg Fe/kg/day) for 13 weeks, compared with control incidences (10 males and 10 females per group) (Toyoda et al. 2014). These histological changes were accompanied with decreased body weight gain during exposure (about 13 and 6% decreased for males and females). No histological changes were found in a comprehensive set of examined tissues in the 0.25 or 1.0% groups (~25 or ~105 mg Fe/kg/day). The results indicate that 105 and 500 mg Fe/kg/day were the NOAEL and LOAEL values, respectively, in this 13-week dietary study for decreased body weight gain and histological changes reflecting gastroenteritis and hemosiderosis.

In groups of B6C3F1 mice (10 males, 10 females) provided drinking water containing 0, 0.06, 0.12, 0.25, 0.5, or 1% ferric citrate (average doses of 0, ~26, ~53, ~108, ~215, or ~432 mg Fe/kg/day) for up to 13 weeks, mice in the highest three dose groups had severely decreased body weight gain (<10% of control values within any 1-week period of exposure) and 5/10 males and 7/10 females in the highest dose group died before the end of the study (Inai et al. 1994). Histological examination of a comprehensive set of tissues from the three highest dose groups showed liver cell atrophy and atrophy of lymphoid tissue in

the spleen or thymus, which were interpreted by the study authors to be due to starvation atrophy. No statistically significant effects on survival, body weight gain, or histological changes in a comprehensive examination of tissues were found in the two lowest dose groups, compared with controls. Although tissue levels of iron were not measured in this study, the study authors speculated that iron overload conditions did not occur, because serum levels of ferritin were not statistically significantly different among the control and exposed groups. Thus, the results from this 13-week drinking water study identified 53 mg Fe/kg/day as a NOAEL for body weight and tissue histological changes, 108 mg Fe/kg/day as a serious adverse effect level for severe body weight gain decreases (~90% decrease compared with control), and 432 mg Fe/kg/day as a frank effect level (FEL) for mortality. The results from this study guided the study authors' selection of dose levels for a subsequent 96-week drinking water exposure study of B6C3F1 mice.

Histological examination of a comprehensive set of tissues found no statistically significantly increased incidence of non-neoplastic or neoplastic lesions in a study of B6C3F1 mice (50 males and 50 females per group) provided drinking water containing 0, 0.06, or 0.12% ferric citrate (0, ~18, or ~39 and 0, ~12, or ~28 mg Fe/kg/day for males and females, respectively) for 96 weeks (Inai et al. 1994). Tissues were collected from surviving mice 4 weeks after the exposure period ended and prepared for histological examinations. No exposure-related effects on body weight were found in either gender, but male mice had decreased survival, compared with controls, starting at 78 weeks of exposure: 40 versus 54% at 78 weeks and 30 versus 48% at 100 weeks. The study identified 39 mg Fe/kg day as a chronic-duration NOAEL for non-neoplastic and neoplastic lesions in a comprehensive set of tissues from mice provided supplemental ferric citrate in drinking water for 96 weeks. This dose level was associated with shortened lifespan, reflected by 30% survival at 100 weeks, compared with 48% survival in control mice.

Several studies have found deficits in performance on neurobehavioral tests in adult laboratory animals following intermediate-duration oral exposure to iron compounds (Huang et al. 2019; Sobotka et al. 1996; Wang et al. 2019).

In male weanling Wistar rats exposed to supplemental carbonyl iron in the diet at concentrations of 0 (n=11), 350 (n=10), 3,500 ppm (n=10), or 20,000 ppm (n=17) for 12 weeks, decreased performance in several neurobehavioral tests and increased brain iron concentrations were only found in the highest dose group (Sobotka et al. 1996). The control diet in this study was reported to contain 35 ppm iron (presumably not carbonyl iron). Daily intakes of iron were estimated based on TWAs of reported body weights (0.266, 0.264, 0.230, and 0.101 kg for control through high-dose groups) and an equation for

food intake based on body weight (EPA 1988): 2, 19, 203, and 1,600 mg Fe/kg/day. The high-dose group was clearly iron overloaded as reflected in 16% and ~30-fold increases in respective brain and liver iron contents, compared with control values. Brain iron contents in the other exposed groups were not significantly elevated, but mean liver iron content in the mid-dose group was ~9-fold higher than the control mean. Average body weight in the high-dose group was ~62% lower than the control average. No marked changes in body weight were found in the lower dose groups. Behavioral testing found statistically significant decreases in spontaneous motor activity, reflex startle tests, and a test of conditioned avoidance response in the high-dose group, but no significant changes in the other exposed groups, compared with control values. The results are consistent with designating 1,602 mg Fe/kg/day as an intermediate-duration LOAEL for deficits in tests of motor activity, startle reflex, and conditioned avoidance response and serious deficits in body weight in young adult rats and 205 mg Fe/kg/day as the highest NOAEL.

In adult male C57BL/6 mice given daily gavage doses of ferric citrate in physiological saline of 0, 83.3, or 333.3 mg/kg/day (0, 19, or 76 mg Fe/kg/day) for 16 weeks starting at 9 months of age, statistically significant deficits were found in the high-dose group, especially after 12 and 16 weeks of exposure, in several motor skills test (open-field, accelerated rotarod, pole, and traction tests), and one test of cognitive skill (Y-maze test) (Huang et al. 2019). Iron concentration in diet was not provided, so total iron intake for the groups in this study could not be estimated. Behavioral deficits in the high-dose group were accompanied with statistically significant increases in iron content of the heart, liver, spleen, liver, and certain regions of the brain (substantia nigra, caudate putamen, olfactory bulb, and thalamus, but not cortex, cerebellum, or hypothalmus). High-dose group tissue iron contents were more highly increased in the affected brain areas (e.g., ~5-, 1.75- and 2-fold increases in the caudate putamen, substantia nigra, and olfactory bulb, respectively) than in the liver and spleen (~17 and ~14% increases, respectively). Histological brain changes were restricted to the high-dose group and included nerve cell swelling in the substantia nigra, white matter edema, vasodilation in the caudate putamen, decreased number of neurons in the substantia nigra and caudate putamen, and increased markers for apoptosis (terminal dUTP nick end labeling [TUNEL] and cleaved caspase-3 staining) in the substantia nigra and caudate putamen. The neuronal loss in the substantia nigra was suspected to be due primarily to dopaminergic neurons based on decreased tyrosine hydroxylase immunostaining, decreased levels of tyrosine hydroxylase mRNA, and decreased levels of dopamine and its metabolite, dihydroxyphenyl acetic acid. The results are consistent with designating 76 and 19 mg Fe/kg/day as respective intermediate-duration oral LOAEL and NOAEL for deficits in tests of motor skills and cognition and degenerative histological changes and marked iron

accumulation in the substantia nigra and caudate putamen regions of the brain in middle-aged mice receiving daily gavage doses of ferric citrate for 16 weeks.

Statistically significant performance deficits in three neurobehavioral tests of cognition were reported in exposed adult male C57BL/6 mice provided drinking water containing 10 mg Fe/L as iron(III) chloride hexahydrate for 6 months starting at 3 months of age (Wang et al. 2019). Control and exposed groups (n=15 per group) were provided a standard diet (iron content of the diet was not reported) and drinking water with 0 or 10 mg supplemental Fe/kg/day for 6 months before the administration of three tests of cognition: novel object recognition, step-down passive avoidance, and Morris water maze tests. After testing, proteomic analysis of hippocampal proteins was conducted. The study report did not state whether or not water intake or body weight data were collected. The estimated daily dose in the exposed mice was estimated at 2.4 mg Fe/kg/day, based on EPA (1988) reference values for body weight for B6C3F1 mice in chronic studies (0.0373 kg) and water intake of 0.0088 L/day. Exploration time in the novel object recognition task in iron-treated mice was shorter than in control mice. The step-down passive avoidance test showed decreased latency and increased number of mistakes in iron-treated mice. The Morris water maze test showed increased escape latency and decreased probe time, number of crossing movements, percentage of time spent in the target quadrant, and percentage of distance traveled in the target quadrant in iron-supplemented mice, compared with controls. Proteomic analysis showed 66 differentially expressed hippocampal proteins in iron-treated mice (30 increased and 36 decreased, compared with controls). Based on bioinformatics analysis, the study authors suggested that iron-induced dysregulation of synaptic, mitochondrial, and cytoskeleton proteins may be involved in the development of the observed neurobehavioral deficits. The results identified a free-standing intermediate-duration LOAEL of 2.4 mg Fe/kg/day for neurobehavioral deficits associated with changes in hippocampal protein expression in middle-aged male C57BL mice provided iron (III) chloride in drinking water for 6 months.

Performance in neurobehavioral tests was examined in studies of male Wistar rats (Schroder et al. 2001) and NMRI and C57BL6 mice (Fredriksson et al. 1999, 2000) involving acute oral gavage exposure to iron succinate (Ferromyn®S) on PNDs 10–12 (a critical period of brain development before the establishment of the blood/brain barrier) and subsequent testing when the animals were 3–4 months old. The results from these studies suggest that gavage doses as low as 7.5 mg Fe/kg/day as iron succinate administered on PNDs 10–12 can produce neurobehavioral deficits in open-field and radial arm maze learning tests administered later in life, but comparison of the results to results from other studies is limited due to the lack of reporting of the iron content of the diet administered to the rats and mice in these studies.

In groups of male Wistar rats (n=12 rats per group) given doses of 0, 2.5, 7.5, 15.0, or 30.0 mg Fe/kg as iron succinate on PNDs 10–12, statistically significant decreased ambulatory activities, but no effects on rearing activities, were measured in open-field testing of the 30.0-mg/kg rats, but not in the lower dose group, compared with controls (Schroder et al. 2001). Rats in this study were fed a "standardized pellet food" for which iron content was not reported; thus, total iron intakes of the rats in this study cannot be estimated. Statistically significant deficits in the radial arm maze learning test were measured in all exposed groups compared with controls, but the magnitude of the deficits did not increase with increasing dose (Schroder et al. 2001). In inhibitory avoidance conditioning and retention testing of rats (n=13–19 per group) given gavage doses of 0, 2.5, 7.5, or 22.5 mg Fe/kg/day on PNDs 10–12, statistically significant deficits in retention of inhibitory avoidance conditioning were measured in the 7.5 and 22.5 mg/kg groups compared with controls, but not in the 2.5 mg/kg group. Mean body weights at testing were not statistically significantly different among groups in these experiments. Iron contents in dissected sections of the substantia nigra of rats sacrificed within 2 weeks of testing were statistically significantly increased in the 7.5- through 30-mg/kg groups, compared with controls. Reported mean values were (for the control through 30-mg/kg groups): 31.36, 36.38, 45.63, 50.75, and 60.13 µg/g. The overall results are consistent with designating 2.5 and 7.5 mg Fe/kg/day as the NOAEL and LOAEL values, respectively, for statistically significant deficits in two out of three neurobehavioral tests associated with increased iron content of the substantia nigra, 3 months after administering gavage doses of iron succinate to male Wistar rats on PNDs 10–12.

In other studies conducted by the same group using the same exposure and testing protocols, 7.5 mg Fe/kg/day as iron succinate administered on PNDs 10–12 was shown to induce statistically significant deficits in neurobehavioral tests (open-field and radial arm maze learning tests) administered at 3–4 months of age in NMR1 male mice (n=10 per group), compared with control mice (Fredriksson et al. 2000). Deficits in open-field testing included locomotor and rearing activities. In the radial arm maze learning test, deficits were for increased latency to finding the last pellet and number of errors. Exposure during PNDs 3–5 or 19–21 did not induce neurobehavioral deficits to the same degree as PND 10–12 exposure. Brain tissue collected after testing had statistically significant increased iron content in basal ganglia, but not frontal cortex, for mice exposed on PNDs 10–12 (53.1 µg/g exposed versus 35.9 µg/g control basal ganglia). This study identified 7.5 mg Fe/kg/day as a free-standing LOAEL for neurobehavioral deficits associated with increased iron content of basal ganglia, 3 months after dose administration to NMRI mice on PNDs 10–12. In a similar study with NMRI male mice fed 0, 3.7, or 37.5 mg Fe/kg/day as iron succinate on PNDs 10–12, statistically significant deficits in the open-field and

radial arm maze learning tests were associated with significantly increased iron content of basal ganglia in the 37.5 mg Fe/kg/day group, but changes observed in the 3.7 mg Fe/kg/day group mostly were not statistically significant (Fredriksson et al. 1999).

C.3 Mechanisms of Action

Systemic toxicity following iron overload can occur when excess levels of free iron are chelated by cellular compounds such as citrate or adenyosyl diphosphate, which can readily participate in redox reactions forming highly toxic free radicals or initiating lipid peroxidation (Gammella et al. 2015; Gujja et al. 2010; Kew 2014; NAS 2001a). It is proposed that the heart is a main target of iron-overload toxicity because all the cell types in the heart, including endothelial cells, are susceptible to damage by reactive oxygen species due to high oxygen demand but low levels of antioxidant enzymes (Gammella et al. 2015). Because iron can enter cardiomyocytes through L-type calcium channels (key mediators of calcium regulation in the heart), it has been proposed that iron overload can also lead to impaired calcium homeostasis in the heart, potentially altering cardiac excitation-contraction coupling (Khamseekaew et al. 2016). Oxidative stress induced by iron overload can also lead to imbalances in calcium homeostasis (Khamseekaew et al. 2016).

Iron-mediated oxidative stress is also proposed to be involved in: development of hepatocellular carcinoma associated with iron overload, due to oxidative-stress-induced deoxyribonucleic acid (DNA) damage (Kew 2014); changes in the immune system reducing immune surveillance of malignant cells (Kew 2014); and development of possible central nervous system toxicity from brain accumulation of iron and other metals (Chen et al. 2019; Gerlach et al. 2003; Huat et al. 2019).

C.4 Health Guidelines

The Institute of Medicine of the NAS has recommended several dietary iron reference intake values for various life stages, including an AI of 0.27 mg/day for infants 0–6 months old (data inadequate to calculate a RDA), and RDAs of 11 mg/day for infants 7–12 months old, 7–10 mg/day for children 1–8 years of age, 8–11 mg/day for boys 9–18 years of age, 8–15 mg/day for girls 9–18 years of age, 8 mg/day for men ≥19 years of age, 18 mg/day for women 19–50 years of age, 27 mg/day for pregnant women, 9–10 mg/day for lactating women, and 8 mg/day for women ≥51 years of age (NAS 2001a). Health Canada (2016) adopted NAS AI recommendations.

Based on gastrointestinal effects, the Institute of Medicine of the NAS has set a UL of 45 mg/day (0.6 mg/kg/day assuming 70-kg body weight) for iron intake in adults ≥19 years of age (NAS 2001a); see Table H-1 in Appendix H. This UL is considered to be protective for pregnant and lactating women; however, it may not be protective for individuals with iron-overloading disorders (NAS 2001a). Based on a lack of adverse effects in iron-supplemented infants, a UL of 40 mg/day was set for iron intake in infants 0–12 months (5.4 mg/kg/day assuming 7.4-kg body weight for a 6-month-old infant [EPA 2011] and young children 1–3 years of age (2.8 mg/kg/day assuming 13.8-kg body weight for a 2-year-old child [EPA 2011]) (NAS 2001a). Data for other age groups were inadequate to derive ULs; therefore, the infant UL of 40 mg/day is applied to children 4–13 years of age (1.2 mg/kg/day assuming 31.8-kg body weight for children (EPA 2011) and the adult UL of 45 mg/day is applied to adolescents 14–18 years of age (0.79 mg/kg/day assuming 56.8-kg body weight for adolescents [EPA 2011]) (NAS 2001a). Health Canada (2016) adopted NAS upper limit recommendations. The EPA has a secondary drinking water advisory of 0.3 mg/L for iron based on aesthetic effects of iron content in water (e.g., taste, odor, color) (EPA 2012). WHO has not proposed a drinking water quality guideline level for iron, as adverse taste and appearance are anticipated to occur at levels lower than those posing health risks (WHO 2008).

ATSDR (2015) and EPA (IRIS 2019) have not derived noncancer toxicity values for iron. The EPA (IRIS 2019), IARC (2015), and NTP (2014) have not assessed iron for carcinogenicity.

The NAS (2001a) ULs for iron are recommended as intermediate- or chronic-duration surrogate MRLs for use in public health assessments, in the absence of ATSDR MRLs or an EPA RfD for oral exposure to iron compounds.

C.5 Derivation of Target-organ Toxicity Dose(s)

The only effects clearly associated with elevated levels of iron following chronic iron ingestion in healthy individuals are gastrointestinal effects (NAS 2001a). Other systemic health effects, particularly cardiomyopathy, have been associated with iron overload in individuals with hemochromatosis and hemoglobinopathies (due to transfusion therapy). Gastrointestinal effects are the basis for the NAS (2001a) ULs, recommended as intermediate- and chronic-duration surrogate MRLs for iron (0.6, 2.8, or 5.4 mg Fe/kg/day for adults, young children, or infants).

Standard toxicology studies identified NOAELs for histological tissue changes of 53 and 39 mg Fe/kg/day for mice provided ferric citrate in drinking water for 13 or 96 weeks, respectively (Inai et al.

1994) and 105 mg Fe/kg/day for F344 rats fed ferric citrate in the diet for 13 weeks (Toyoda et al. 2014). In the rat study, significantly increased incidences of inflammation and eosinophilic changes in the colon and hemosiderosis in the spleen occurred in the 500 mg Fe/kg/day group (Toyoda et al. 2014). If the rat NOAEL of 105 mg Fe/kg/day for the lack of gastrointestinal histological changes was used as the basis of an ATSDR intermediate-duration MRL, a value of 1.05 mg Fe/kg/day would be calculated using a standard uncertainty factor of 100 (10 for interspecies extrapolation and 10 for human variability). This value is above the NAS UL for adults and below the NAS ULs for young children and infants, and the NAS ULs are recommended because they are based on human data for gastrointestinal discomfort.

Possible data on which to base neurotoxicity TTDs for repeated oral exposure to iron comes from studies of neurobehavior in laboratory animals repeatedly exposed by the oral route to supplemental iron. These studies have identified:

1. 1,600 mg Fe/kg/day as an intermediate-duration LOAEL for deficits in tests of motor activity, startle reflex, conditioned avoidance response, and frank effects on body weight and 203 mg Fe/kg/day as the highest NOAEL for young adult Wistar rats fed supplemental carbonyl iron in the diet for 12 weeks (from 3 to 15 weeks of age) (Sobotka et al. 1996);
2. 76 and 19 mg Fe/kg/day as intermediate-duration oral LOAEL and NOAEL for deficits in tests of motor skills and cognition and degenerative histological changes and marked iron accumulation in the substantia nigra and caudate putamen regions of the brain in middle-aged C57BL/6 mice receiving daily gavage doses of ferric citrate for 16 weeks (from 9 to 13 months of age) (Huang et al. 2019); and
3. 2.4 mg Fe/kg/day as a free-standing intermediate-duration LOAEL for neurobehavioral deficits associated with changes in hippocampal protein expression in adult male C57BL/6 mice provided iron(III) chloride hexahydrate in drinking water for 6 months (from 3 to 9 months of age) (Wang et al. 2019).

The lowest neurotoxic LOAEL, 2.4 mg Fe/kg/day, identified in the study of C57BL/6 mice exposed to iron chloride in drinking water for 6 months (Wang et al. 2019) is above the current NAS (2001a) UL for adults (0.6 mg Fe/kg/day), but below the NOAELs identified in this data set: 19 mg Fe/kg/day in middle-aged adult C57BL/6 mice given daily gavage doses of ferric citrate for 16 weeks (Huang et al. 2019) and 203 mg Fe/kg/day in Wistar rats fed carbonyl iron in the diet for 12 weeks (Sobotka et al. 1996). The basis of the apparent differences in dose-response relationships across the animal studies is not clear; possible explanatory factors include differences in administered test substances (carbonyl iron, ferric

citrate, iron(III) chloride), mode of oral exposure (diet, gavage, drinking water), duration of exposure (12 weeks, 16 weeks, 6 months), genetic strain or species (Wistar rat, C57BL/6 mouse), age of animals (young adult or middle-aged adult), and lack of knowledge of the total iron intake in mice in the studies reported by Huang et al. (2019) and Wang et al. (2019).

The results provide consistent evidence that repeated oral exposure of adult rats and mice to supplemental doses of iron compounds can impair performance in neurobehavior tests, but consideration of dose-response data across the studies and derivation of a TTD for neurological effects is limited by the lack of information on the iron content of the basal diet in the Huang et al. (2019) and Wang et al. (2019) studies. The iron levels in a commercial diet can vary greatly, with some diets containing >200 mg Fe/kg diet (approximately 35 mg Fe/kg body weight/day). In the absence of information on the dietary iron content in these studies, total iron intakes cannot be estimated, thus precluding derivation of a TTD.

C.6 References

ATSDR. 2015. Toxic substances portal: Toxicological profiles. Agency for Toxic Substances and Disease Registry. http://www.atsdr.cdc.gov/toxprofiles/index.asp. September 3, 2015.

Berg D, Gerlach M, Youdim MBH, et al. 2001. Brain iron pathways and their relevance to Parkinson's disease. J Neurochem 79:225-236.

CDC. 2003. Hemochromatosis. An iron overload disease. Centers for Disease Control and Prevention. http://www.cdc.gov/ncbddd/hemochromatosis/pdf/IronOverload_508.pdf. September 4, 2015.

Chen P, Totten M, Zhang Z, et al. 2019. Iron and manganese-related CNS toxicity: mechanisms, diagnosis and treatment. Expert Rev Neurother 19(3):243-260. http://doi.org/10.1080/14737175.2019.1581608.

Double KL, Gerlach M, Youdim MBH, et al. 2000. Impaired iron homeostasis in Parkinson's disease. J Neural Transm [Suppl] 60:21-42.

Dusek P, Litwin T, Czlonkowska A. 2015a. Wilson disease and other neurodegenerations with metal accumulations. Neurol Clin 33(1):175-204. http://doi.org/10.1016/j.ncl.2014.09.006.

Dusek P, Roos PM, Litwin T, et al. 2015b. The neurotoxicity of iron, copper and manganese in Parkinson's and Wilson's diseases. J Trace Elem Med Biol 31:193-203. http://doi.org/10.1016/j.jtemb.2014.05.007.

EPA. 1988. Recommendations for and documentation of biological values for use in risk assessment. Washington, DC: U.S. Environmental Protection Agency. EPA600687008. https://cfpub.epa.gov/ncea/risk/recordisplay.cfm?deid=34855. December 22, 2020.

EPA. 2011. Exposure factors handbook. Washington, DC: U.S. Environmental Protection Agency. EPA600R090052F. http://www.epa.gov/ncea/efh/pdfs/efh-complete.pdf. September 4, 2015.

EPA. 2012. 2012 Edition of the drinking water standards and health advisories. Washington, DC: U.S. Environmental Protection Agency. EPA822S12001. http://water.epa.gov/action/advisories/drinking/upload/dwstandards2012.pdf. September 3, 2015.

Farina M, Avila DS, da Rocha JB, et al. 2013. Metals, oxidative stress and neurodegeneration: A focus on iron, manganese and mercury. Neurochem Int 62(5):575-594. http://doi.org/10.1016/j.neuint.2012.12.006.

Fredriksson A, Schröder N, Eriksson P, et al. 1999. Neonatal iron exposure induces neurobehavioural dysfunctions in adult mice. Toxicol Appl Pharmacol 159(1):25-30. http://doi.org/10.1006/taap.1999.8711.

Fredriksson A, Schröder N, Eriksson P, et al. 2000. Maze learning and motor activity deficits in adult mice induced by iron exposure during a critical postnatal period. Dev Brain Res 119(1):65-74. http://doi.org/10.1016/s0165-3806(99)00160-1.

Gammella E, Recalcati S, Rybinska I, et al. 2015. Iron-induced damage in cardiomyopathy: Oxidative-dependent and independent mechanisms. Oxid Med Cell Longev 2015:230182. http://doi.org/10.1155/2015/230182.

Gerlach M, Double KL, Ben-Shachar D, et al. 2003. Neuromelanin and its interaction with iron as a potential risk factor for dopaminergic neurodegeneration underlying Parkinson's disease. Neurotox Res 5(1-2):35-44. http://doi.org/10.1007/BF03033371.

Gujja P, Rosing DR, Tripodi DJ, et al. 2010. Iron overload cardiomyopathy. J Am Coll Cardiol 56(13):1001-1012.

Health Canada. 2016. Drug and health products. Iron. Ottawa, Ontario: Health Canada. http://webprod.hc-sc.gc.ca/nhpid-bdipsn/atReq.do?atid=iron_fer. September 7, 2016.

Huang C, Ma W, Luo Q, et al. 2019. Iron overload resulting from the chronic oral administration of ferric citrate induces parkinsonism phenotypes in middle-aged mice. Aging 11(21):9846-9861. http://doi.org/10.18632/aging.102433.

Huat TJ, Camats-Perna J, Newcombe EA, et al. 2019. Metal toxicity links to Alzheimer's disease and neuroinflammation. J Mol Biol 431(9):1843-1868. http://doi.org/10.1016/j.jmb.2019.01.018.

IARC. 2015. Agents classified by the IARC Monographs, Volumes 1-113. International Agency for Research on Cancer. http://monographs.iarc.fr/ENG/Classification/List_of_Classifications_Vol1-113.pdf. September 3, 2015.

Inai K, Fujihara M, Yonehara S, et al. 1994. Tumorigenicity study of ferric citrate administered orally to mice. Food Chem Toxicol 32(6):493-498. http://doi.org/10.1016/0278-6915(94)90104-x.

IRIS. 2019. IRIS advanced search. International Risk Information System. U.S. Environmental Protection Agency. https://cfpub.epa.gov/ncea/iris/search/index.cfm. February 22, 2019.

Kew MC. 2014. Hepatic iron overload and hepatocellular carcinoma. Cancer Lett 3:31-40.

Khamseekaew J, Kumfu S, Chattipakorn SC, et al. 2016. Effects of iron overload on cardiac calcium regulation: Translational insights into mechanisms and management of a global epidemic. Can J Cardiol 32:1009-1016.

NAS. 2001a. Iron. In: Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. National Academy of Sciences, http://www.nap.edu/catalog/10026/dietary-reference-intakes-for-vitamin-a-vitamin-k-arsenic-boron-chromium-copper-iodine-iron-manganese-molybdenum-nickel-silicon-vanadium-and-zinc. September 8, 2015.

NIH. 2016. Iron. Dietary supplement fact sheet. National Institutes of Health. https://ods.od.nih.gov/factsheets/Iron-HealthProfessional/. August 24, 2016.

NTP. 2014. Report on carcinogens. Thirteenth edition. National Toxicology Program. http://ntp.niehs.nih.gov/pubhealth/roc/roc13/index.html. September 3, 2015.

Park S, Sim CS, Lee H, et al. 2013. Blood manganese concentration is elevated in infants with iron deficiency. Biol Trace Elem Res 155(2):184-189. http://doi.org/10.1007/s12011-013-9782-9.

Schroder N, Fredriksson A, Vianna MR, et al. 2001. Memory deficits in adult rats following postnatal iron administration. Behav Brain Res 124(1):77-85. http://doi.org/10.1016/s0166-4328(01)00236-4.

Sobotka TJ, Whittaker P, Sobotka JM, et al. 1996. Neurobehavioral dysfunctions associated with dietary iron overload. Physiol Behav 59(2):213-219. http://doi.org/10.1016/0031-9384(95)02030-6.

Toyoda T, Cho YM, Mizuta Y, et al. 2014. A 13-week subchronic toxicity study of ferric citrate in F344 rats. Food Chem Toxicol 74:68-75. http://doi.org/10.1016/j.fct.2014.09.005.

Wang X, Zhang J, Zhou L, et al. 2019. Long-term iron exposure causes widespread molecular alterations associated with memory impairment in mice. Food Chem Toxicol 130:242-252. http://doi.org/10.1016/j.fct.2019.05.038.

WHO. 2008. Guidelines for drinking-water quality. Third edition incorporating the first and second addenda. Volume 1 recommendations. Geneva, Switzerland: World Health Organization. http://www.who.int/water_sanitation_health/dwq/fulltext.pdf. September 3, 2015.

Appendix D. Background Information for Magnesium

Magnesium is an essential nutrient that has many physiological functions that are dependent on two important properties: the ability to chelate with important intracellular anionic sites in biological molecules (e.g., ATP) and the ability to compete with calcium for binding sites on membranes and proteins (Swaminathan 2003). Magnesium is needed for the function of many enzymatic systems including those in synthesis of nucleic acids and proteins, intermediary metabolism of carbohydrates and lipids and other cellular energy generation systems (EFSA 2006, 2015b; NAS 1997; Swaminathan 2003). Magnesium's importance to enzyme functions includes binding to ATP in ATP-requiring enzymes, enzyme activation through active site binding and conformational changes, and aggregation of multiple enzyme complexes (Swaminathan 2003). Magnesium is also important to membrane transport systems and skeletal and cardiac muscle functions (EFSA 2006, 2015b; NAS 1997; Swaminathan 2003). Magnesium competes with calcium membrane binding sites and regulates low intracellular calcium concentrations by stimulating the trapping of free calcium ions within the sarcoplasmic reticulum (Swaminathan 2003). In sympathetic and neuromuscular nerve junctions, magnesium is thought to competitively inhibit the entry of calcium into pre-synaptic nerve terminals and prevent the release of neurotransmitters (Swaminathan 2003). Magnesium also plays a role in the structure of bone, proteins, polyribosomes, nucleic acids, and mitochondria (Swaminathan 2003).

Magnesium occurs as a divalent cation in aqueous solutions. In cells, it exists as the free cation or is bound to biological molecules (EFSA 2006, 2015b). Ingestion from foods rich in magnesium (e.g., nuts, whole grains, fish, green leafy vegetables, legumes, coffee, and cocoa beverages) is thought to be the principal source of magnesium in adults, but significant contributions to intake can be made from water, especially from mineral-enriched or "hard" water (EFSA 2006, 2015b).

D.1 Toxicokinetics

Magnesium ingested in food and water is absorbed into intestinal cells by two paths, saturable transcellular transport, which predominates at low or normal magnesium intakes (<20 mmol/day), and passive diffusion, also known as the paracellular pathway, which predominates when magnesium intake is further elevated (de Baaij et al. 2015; EFSA 2006, 2015b; Houillier 2014; NAS 1997). Percentage absorption of ingested magnesium has been reported to range widely from about 65% when magnesium intakes are low to about 10% when intakes are high (Houillier 2014). This wide range of values likely reflects varying conditions across studies, such as ingestion from food or water, whole-body magnesium

status, and amount of magnesium ingested; because of homeostatic processes, percentage absorption is expected to decrease with increasing oral intakes of magnesium (EFSA 2006, 2015b; Houillier 2014; NAS 1997; Swaminathan 2003). Saturable transport across intestinal cells has been proposed to occur primarily in the colon and involve transient receptor potential melastatin subtype 6 and 7 (TRPM6, TRPM7) channels for luminal uptake, CNNM4 channels for basolateral extrusion and driving forces from sodium gradients (de Baaij et al. 2015; Houillier 2014). The paracellular pathway has been proposed to proceed through tight junctions between cells in the small and large intestine whose permeability is influenced by multimolecular complexes containing claudins (de Baaij et al. 2015; Houillier 2014). Which claudins are involved in magnesium permeability and how they are regulated is under investigation (de Baaij et al. 2015; Houillier 2014).

Following absorption, magnesium in blood is distributed to bone and soft tissues to nearly equal proportions of total body magnesium (EFSA 2015b). Serum magnesium exists as the free cation (~54%), magnesium bound to proteins (~33%), and magnesium complexed to anions (~13%), but serum magnesium represents only a small fraction of total body magnesium (~0.3%) (EFSA 2015b). In normal adults, total serum magnesium concentration displays a narrow range from about 0.70 to 1.1 mmol/L that is under homeostatic control from gastrointestinal absorption and kidney reabsorption processes (Houillier 2014; Swaminathan 2003). The normal kidney is thought to be efficient in protecting against hypomagnesemia without the need for bone (or muscle tissues) to release a significant amount of magnesium, although release from soft tissues and bones can be important under low magnesium intakes (de Baaij et al. 2015; Houillier 2014). About 60% of total body magnesium exists in bones, either strongly bound to hydroxyapatite or more loosely bound to the surface of bone mineral crystals (Musso 2009; Swaminathan 2003). Mitochondria in muscle tissue are also important accumulation sites, representing about 25% of total body magnesium (EFSA 2015b). Typical intracellular concentrations of magnesium are in the range of 10–30 mM, but most intracellular magnesium is complexed to ribosomes, polynucleotides and ATP, and free intracellular magnesium ions are expected to be in the 0.5–1.2 mM range (de Baaij et al. 2015). Intracellular magnesium concentrations are tightly regulated and molecular mechanisms are under ongoing investigations. Numerous transport proteins that are thought to be involved in magnesium homeostasis include transient receptor potential M6 and M7 ion channel kinases (TRPM6, TPPM7), Na+/K+ ATPases, Solute Carrier family 41 member A1 (SLC41A1), and Cystathionine-β synthase (CBS)-pair domain divalent metal cation transport mediators (CNNMs: e.g., CNNM2) (Arjona and de Baaij 2018; Arjona et al. 2019; de Baaij et al. 2015; Funato et al. 2018; Garcia-Castano et al. 2020; Flatman 1991; Kolisek et al. 2019; Mittermeier et al. 2019; Romani 2011; Schlingmann et al. 2007, 2018; Watanabe et al. 2005; Workinger et al. 2018). A recent review by

Kolisek et al. (2019) proposed that TRPM6/7 is the principal way that magnesium enters mammalian cells and is reabsorbed in renal tubular cells and that SLC41A1 is an important magnesium efflux pathway out of cells but recognized the possible contributions of several other magnesium transporters and homeostatic factors and the limited understanding of how all of the identified components work together to maintain cellular and organismal magnesium homeostasis. Reflecting the emerging understanding of the complexity of magnesium homeostasis, other recent studies using conditional mouse knock-out models provided evidence that TRPM7 in the intestine is essential for maintaining magnesium, calcium, and zinc homeostasis, whereas the function of this transporter in kidney was expendable, presumably due to the presence and function of other transport systems regulating renal reabsorption of magnesium and other divalent cations (Mittermeier et al. 2019).

Magnesium is excreted from the body primarily in urine, and to lesser extents in feces, sweat, and breast milk (EFSA 2006, 2015b). Magnesium homeostasis and maintenance of serum concentrations are thought to involve reabsorption of magnesium in glomerular filtrate by the thick ascending loop of Henle in the kidney, with lesser fine-tuning reabsorption occurring in the distal convoluted tubule (Dai et al. 2001; de Baaij et al. 2015; Musso 2009). Molecular components of the reabsorption process are under intensive investigation (e.g., de Baaij et al. 2015; Kolisek et al. 2019).

Magnesium in feces is comprised of magnesium not absorbed by the intestine, absorbed magnesium excreted in bile and pancreatic secretions, and sloughed intestinal cells (Lakshmanan et al. 1984; Swaminathan 2003). Other less important elimination routes for absorbed magnesium are sweat and breast milk (EFSA 2015b).

Because magnesium has been identified as a calcium antagonist in isolated membrane transport systems, isolated neurotransmission systems, and certain enzymatic reactions, it has been proposed that calcium and magnesium may inhibit each other's absorption in the gastrointestinal tract (EFSA 2006). However, Spencer et al. (1994) showed that increased magnesium intake of 826 mg Mg/day (about 250 mg Mg/day in the diet plus 576 mg Mg/day as MgO tablets) did not affect intestinal calcium absorption, determined with tracer doses of ^{47}Ca at intakes of 241 or 812 mg Ca/day in adult males. The absence of a competitive inhibition could be due to locational differences in absorption sites for these metallic cations, as well to the complexity and independence of homeostatic mechanisms regulating whole-body status of these essential elements.

D.2 Health Effects

Effects in Humans. Magnesium ingested in foods has not been associated with any adverse health effects, but depression of the nervous system, sometimes leading to death, has been reported many times in cases following the ingestion of single or a few large doses of magnesium sulfate (Epsom salts) (EFSA 2006; Huey et al. 1995; Stevens and Wolff 1950; Swaminathan 2003). Reports of accidental lethal poisoning with magnesium sulfate include the death of a woman <2 hours after ingesting a dose of about 120 g of Epsom salts in a tumbler of hot water (EFSA 2006; Stevens and Wolff 1950; Swaminathan 2003). An estimate of the magnesium dose ingested in this case is about 11,833 mg magnesium/kg body weight (using the atomic weight of magnesium, 24.305 mg/mmol; the molecular weight of $MgSO_4$ $7H_2O$, 246.47 mg/mmol, and an assumed body weight of 60 kg). These acute effects are thought to be due to excess magnesium preventing the release of neurotransmitters from pre-synaptic sympathetic and neuromuscular nerve junctions, an effect that has been shown to be counteracted by excess calcium (EFSA 2006; Huey et al. 1995; Stevens and Wolff 1950; Swaminathan 2003). The acute effect of magnesium on sympathetic and neuromuscular nerve junctions is thought to occur only when serum magnesium concentrations attain levels beyond the normal range, about 0.7–1.1 mmol/L (EFSA 2006; Huey et al. 1995; Swaminathan 2003). Huey et al. (1995) reported the following associations between serum magnesium concentrations and clinical symptoms: skin flushing, nausea, hypotension, and vomiting at 1.5–4.5 mmol/L; electrocardiogram changes, decreased tendon reflexes, bradycardia, and drowsiness at 2–5 mmol/L; respiratory depression, absent deep tendon reflexes, and voluntary muscle paralysis at >5 mmol/L; and cardiac arrest at >7–7.5 mmol/L.

NAS (1997) reviewed several reports of diarrhea induction or gastrointestinal distress by repeated oral doses of nonfood magnesium compounds (Bashir et al. 1993; Fine et al. 1991; Marken et al. 1989; Ricci et al. 1991). Effective doses ranged from about 168 to 2,320 mg Mg/day as magnesium hydroxide (Fine et al. 1991), 360 mg Mg/day as magnesium chloride in 6 of 21 male and female patients aged 51–71 years (Bashir et al. 1993), and 476 mg Mg/day as magnesium oxide in 18 of 50 male and female patients aged 31–50 years (Marken et al. 1989). NAS (1997) noted the existence of several other reports of the lack of diarrheal induction or gastrointestinal distress in subjects given similar or more elevated daily doses of nonfood magnesium compounds (Nadler et al. 1992; Nagy et al. 1988; Spencer et al. 1994; Stendig-Lindberg et al. 1993). From these studies, NAS (1997) selected diarrhea as the most sensitive adverse effect from excess magnesium intake from nonfood sources and identified a LOAEL of 360 mg Mg/day, based on the results of Bashir et al. (1993), to be the basis for deriving an UL for nonfood magnesium intakes.

In a more recent review, EFSA (2006) identified 20 reports of examinations of mild diarrhea in subjects ingesting daily oral magnesium supplements with doses ranging from 180 to 1,095 mg Mg/day for durations ranging from 1 to 72 weeks. All of these studies only reported magnesium doses in the supplements; they did not report magnesium intakes from food or beverages. Four studies with daily doses ranging from 180 to 250 mg Mg/day reported no mild diarrhea in 130 subjects (Classen et al. 1986, as cited in EFSA 2006), 112 subjects (Schimatschek et al. 1997, as cited in EFSA 2006), 181 subjects (Schimatschek et al. 2001, as cited in EFSA 2006), or 31 subjects (Stendig-Lindberg et al. 1993). The first three studies administered magnesium aspartate hydrochloride daily for 3 weeks, and the last study administered magnesium hydroxide daily for 72 weeks. At least one case of mild diarrhea occurred in four of six studies that administered daily doses of 360–365 mg Mg/day for 4 to 26 weeks to groups with 17–278 subjects (Cappuccio et al. 1985; Fehlinger et al. 1988; Gullestad et al. 1992; Plum-Wirell et al. 1994; Spätling and Spätling 1988; Rasmussen et al. 1989, as cited in EFSA 2006). Daily doses ranged from 384 to 1,095 mg Mg/day in the remaining 10 studies, of which 8 studies reported at least one subject with mild diarrhea (Bashir et al. 1993; Daly and Harris 1990; Marken et al. 1989; Muehlbauer et al. 1991, as cited in EFSA 2006; Nadler et al. 1992; Ricci et al. 1991; Ruddel et al. 1990; Spätling et al. 1998; Spencer et al. 1994; Widman et al. 1993). Based on its review of results from these 20 studies of adult men and women (some of which included pregnant and lactating women), EFSA (2006) concluded that 250 mg Mg/day was a NOAEL for laxative effects from nonfood readily dissociable magnesium salts or magnesium oxide. This NOAEL was used as the basis for a recommended UL for magnesium that does not include magnesium present in foods or beverages (EFSA 2006).

Low magnesium levels have been proposed to be involved in the development of high blood pressure, cardiovascular dysfunctions (e.g., cardiac arrhythmias, stroke, coronary heart disease), type 2 diabetes mellitus, and bone disorders (see EFSA 2015b; NAS 1997; WHO 2009 for reviews). In the most recent authoritative review for setting dietary requirements, EFSA (2015b) concluded that the available data on magnesium intake and hypertension or cardiovascular disease outcomes were inadequate for setting dietary reference values for magnesium. EFSA (2015b) also concluded that available data on magnesium intake and type 2 diabetes mellitus cannot be used for setting dietary reference values for magnesium. This conclusion was accompanied by notes that there was: (1) evidence (from meta-analyses) for an inverse association between magnesium intakes and risk for type 2 diabetes; (2) inconsistent evidence from dietary supplementation studies with type 1 or 2 diabetes or insulin-resistant overweight individuals; and (3) insufficient evidence for a dose-response relationship between magnesium intake and risk for type 2 diabetes. With respect to bone disorders, EFSA (2015b) concluded that the essential role of magnesium

and bone structure and physiology is well established, but quantitative data for this relationship were inadequate for setting dietary reference values. The most recent recommended dietary reference values for magnesium by NAS (1997) and EFSA (2015b) are based on balance studies of healthy individuals or intakes in certain populations without evidence of magnesium deficiency, rather than on exposure-response relationships for magnesium intakes and amelioration of physiological disorders from magnesium deficiency (see Section D.4).

Effects in Laboratory Animals. In studies of mice and rats repeatedly exposed to supplementary magnesium chloride via the oral route, signs of kidney toxicity have been observed only at very high dose levels (>1,000 mg Mg/kg/day) (Kurata et al. 1989; Takizawa et al. 2000; Tanaka et al. 1994). Single oral doses of magnesium oxide nanoparticles produced histological changes in the liver (necrosis and degenerative changes) and the kidney (focal tubular damage and swelling of the glomerulus) in female Wistar rats at a dose of 1,206.9 mg Mg/kg, but no tissue damage at doses ≤180.9 mg Mg/kg (Mangalampalli et al. 2017).

In a 13-week study of B6C3F1 mice (10 males, 10 females) fed supplementary magnesium chloride ($MgCl_2$-$6H_2O$) in the diet at concentrations of 0, 0.3, 0.6, 1.25, 2.5 or 5%, no statistically significant exposure-related effects were found on clinical signs or hematological and blood biochemistry endpoints (Tanaka et al. 1994). From reported food intake data, calculated doses were: 0, 72.9, 145.9, 321.6, 646.8, and 1,362.8 mg Mg/kg/day males; and 0, 91.0, 186.8, 385.3, 804.9, and 1,634.7 mg Mg/kg/day females. Terminal body weight decreases >10%, compared with controls, were only found in the 5% group (~15% males; ~10% females). Comprehensive histopathological examinations found increased incidences of histologically detected lesions only in kidneys of male mice in the 5% group (vacuolation of kidney tubules). Incidences for kidney tubular vacuolation in the male groups were (listed from control to the 5% groups): 2/10 (1 slight, 1 moderate); 0/10; 0/10; 1/10 (slight); 2/10 (both slight); and 10/10 (4 slight, 5 moderate, 1 severe). The results are consistent with designating 5% (1,362 mg Mg/kg/day) as the LOAEL and 2.5% (647 mg Mg/kg/day) as the NOAEL for histological changes in the kidney in B6C3F1 mice fed supplements magnesium chloride for 13 weeks.

In a 104-week study of B6C3F1 mice (50 males, 50 females) fed supplementary magnesium chloride ($MgCl_2$-$6H_2O$) in the diet at concentrations of 0, 0.5, or 2%, no statistically significant exposure-related effects were found on clinical signs or survival; hematological and blood biochemistry endpoints; or in a comprehensive histological examination of tissues for non-neoplastic and neoplastic changes (Kurata et al. 1989). Based on reported food intake values, calculated daily doses were: 0, 68.1, and 335.9 mg

Mg/kg/day for males and 0, 87.3, and 469.8 mg Mg/kg/day for females. Females in the 2% group had decreased mean body weight (~25%), compared with controls, from about 8 weeks of exposure to the end of the study. As an example of the reported incidences for noncancer histological lesions, incidences for chronic nephropathy in the control through high-dose groups were: 2/50, 3/50, and 3/50 males; and 2/49, 0/50, and 0/50 females. No neoplastic changes were found in examined kidneys, and neoplastic lesions in other tissues in exposed groups were not found at statistically significant elevated incidences, compared with controls (Kurata et al. 1989). The highest dose in this study, 470 mg Mg/kg/day, is a NOAEL for non-neoplastic and neoplastic tissue changes in B6C3F1 mice fed supplementary magnesium in the diet for 104 weeks.

In a 90-day study of F344 rats (10 males, 10 females) fed $MgCl_2$-$6H_2O$ in the diet at supplementary concentrations of 0, 0.1, 0.5, or 2.5%, no statistically significant exposure-related effects were found on survival, organ weights, hematology, biochemistry, or in histopathological examinations of a comprehensive set of tissues (Takizawa et al. 2000). The only effects observed in the 2.5% group were transient soft stool, increased water consumption, and a slight reduction in body weight gain.

In female Wistar rats given single oral doses of 2,000 mg magnesium oxide nanoparticles/kg (1,206.9 mg Mg/kg), histological changes were noted in the liver (necrosis and hepatocyte degenerative changes) and kidney (focal tubular damage and swelling of the glomerulus) (Mangalampalli et al. 2017). These changes were reported as absent in other groups (n=5) dosed with 0, 5, 50, and 300 mg MgO nanoparticles/kg (0, 3.0, 30.1, and 180.9 mg Mg/kg).

Therapeutic Uses of Magnesium. Therapeutic uses of magnesium compounds include: (1) intravenously administered magnesium sulfate to prevent convulsions in women with severe preeclampsia, the mechanism of which is not understood (Belfort et al. 2003; Greene 2003; The Magpie Trial Collaborative Group 2002); (2) 24-hour intravenous magnesium sulfate to patients with suspected acute myocardial infarction, presumably by inhibiting calcium influx into ischemic myocardial cells, and thereby reducing injury, arrhythmias, and post-event mortality (Antman 2002; ISIS 1995; Woods 1991; Woods and Fletcher 1994); (3) oral administration of magnesium sulfate or other easily dissociable magnesium compounds (e.g., magnesium chloride, magnesium oxide) to empty the intestine from an osmotic effect (EFSA 2006, 2015b; NAS 1997; Swaminathan 2003); and (4) oral or intravenous magnesium supplementation has been used to treat hypomagnesemia in patients with mostly rare mutations in genes encoding proteins involved in magnesium homeostasis (see de Baaij et al. 2015 for review). The use of magnesium to counter nephropathy in cancer patients treated with cisplatin is another therapeutic use that

has received research attention (Ashrafi et al. 2012; Oka et al. 2014; Saito et al. 2017). Use of magnesium to empty the intestines is the most widely used of these therapeutic uses; daily oral doses ranging from about 1,200 to 3,000 mg magnesium as magnesium sulfate have been associated with intestinal emptying (Marken et al. 1989).

D.3 Mechanisms of Action

Gastrointestinal effects from magnesium compounds (e.g., mild diarrhea or emptying of the intestines) appear to be the most sensitive effects from oral exposure and are thought to be due to an osmotic effect from both the magnesium cation and its accompanying anion (EFSA 2006; NAS 1997). Mild diarrhea has been associated with doses as low as 360 mg Mg/day, whereas intestinal emptying has been associated with daily doses in the 1,200–3,000 mg Mg/day range (EFSA 2006; Marken et al. 1989; NAS 1997).

The neurological effects from higher acute doses of magnesium (that surpass homeostatic mechanisms and produce serum magnesium levels higher than the normal range of 0.7–1.1 mmol/L) are thought to be due to excess magnesium inhibiting calcium entry and preventing the release of neurotransmitters from pre-synaptic sympathetic and neuromuscular nerve junctions (EFSA 2006; Huey et al. 1995; Stevens and Wolff 1950; Swaminathan 2003). Similarly, the therapeutic use of intravenous magnesium to counteract adverse effects (cytological injury, arrhythmias, and mortality) from myocardial infarction have been proposed to be due to inhibition of calcium influx into ischemic myocardial cells (Antman 2002; ISIS 1995; Woods 1991; Woods and Fletcher 1994).

D.4 Health Guidelines

The Institute of Medicine of the NAS has determined age-group specific dietary recommendations for magnesium (NAS 1997). RDAs for men aged 19–30 years and women aged 19–30 years were 400 and 310 mg Mg/day, respectively (NAS 1997). The RDAs for men aged 31–70 years and women aged 31–70 years were 420 and 320 mg Mg/day, respectively. These RDA values were based on magnesium balance studies in healthy individuals (NAS 1997). Based on the assumption that weight gain during pregnancy increases magnesium requirements, the RDAs for pregnant women ages 19–30 years and ages 31–50 years were 350 mg Mg/day and 360 mg/day, respectively (NAS 1997).

NAS (1997) established UL values for magnesium from nonfood sources. The UL of 350 mg Mg/day from supplementary magnesium was based on a LOAEL of 360 mg Mg/day for mild diarrhea in studies of dietary magnesium supplementation and an uncertainty factor "very close to 1.0." NAS (1997) noted that a few studies found mild diarrhea in small percentages of subjects at doses of 360–380 mg Mg/day and that this effect has not been found in other individuals at doses substantially higher than the UL of 350 mg Mg/day.

EFSA (2015b) established several age-group-specific dietary recommendations for magnesium. After reviewing balance studies and prospective observational studies, EFSA (2015b) established AIs of 350 mg Mg/day for adult men ≥18 years of age and 300 mg Mg/day for adult women (including pregnant and lactating women), based on observed intakes in several EU countries. Based on similar reasoning, AIs were established at 80 mg Mg/day for infants aged 7–11 months, 170 mg Mg/day for children aged 1–<3 years, 230 mg Mg/day for children aged 3–<10 years, 300 mg Mg/day for boys aged 10–<18 years, and 250 mg/day for girls aged 10–<18 years (EFSA 2015b).

EFSA (2006) established a UL of 250 mg Mg/day for magnesium not in food and beverages derived from a NOAEL for mild diarrhea of 250 mg Mg/day (from studies by Classen et al. 1986, as cited in EFSA 2006; Schimatschek et al. 1997, 2001, as cited in EFSA 2006; and Stendig-Lindberg et al. 1993 of subjects given oral dietary supplements of magnesium) and an uncertainty factor of 1.0. EFSA (2006) justified the uncertainty factor of 1.0 because: (1) data were available from many human studies involving a large number of subjects from a spectrum of lifestage groups, including adults, pregnant and lactating women, and children; and (2) the NOAEL was based on "a mild, transient laxative effect, without pathological sequelae, which is readily reversible and for which considerable adaptation can develop within days." This UL was meant for adults, including pregnant and lactating women, and children from 4 years on.

ATSDR (2018) and EPA (IRIS 2019) have not derived noncancer toxicity values for magnesium or magnesium compounds. The EPA (IRIS 2019), IARC (2018), and NTP (2016) have not assessed magnesium or magnesium compounds for carcinogenicity.

Because ATSDR MRLs and EPA RfDs for magnesium have not been developed, the NAS (1997) UL for magnesium, 350 mg Mg/day (5 mg Mg/kg/day assuming a body weight of 70 kg), is recommended to be the basis for a provisional surrogate intermediate-duration oral MRL for adults in ATSDR public health assessments of magnesium compounds that are readily dissociated in the human body, including

magnesium chloride, magnesium sulfate, magnesium acetate, magnesium hydroxide, and magnesium oxide.

D.5 Derivation of Target-organ Toxicity Dose(s)

Following oral exposure, the most sensitive adverse effect associated with excess magnesium is gastrointestinal discomfort and diarrhea, as determined by NAS (1997) and EFSA (2006). A provisional oral MRL of 5 mg Mg/kg/day for intermediate- and chronic-duration exposure of adults was based on the NAS (1997) UL for adults, which was based on an estimated NOAEL of 350 mg/day and a LOAEL of 360 mg Mg/day for mild diarrhea in adults and an uncertainty factor slightly larger than 1.0.

Limited evidence also exists for associations between high acute doses of magnesium sulfate and neurological effects. Although associations between ranges of serum levels of magnesium and neurological symptoms of varying severity have been presented for humans (e.g., Huey et al. 1995), quantitative data relating acute oral doses and neurological symptoms in humans are not available. Thus, a TTD for neurological effects from acute oral exposure to magnesium was not derived.

Statistically significant increased incidences of histological changes in the kidney were found in B6C3F1 mice fed 5% magnesium chloride in the diet (1.362 mg Mg/kg/day), but not in mice fed 2.5% in the diet (647 mg Mg/kg/day) (Tanaka et al. 1994). These kidney effects, or histological changes in other tissues, were not found in F344 rats fed up to 5% magnesium chloride in the diet for 13 week (Takizawa et al. 2000) or in B6C3F1 mice fed supplementary magnesium chloride in the diet for 104 weeks at doses as high as 470 mg Mg/kg/day (Kurata et al. 1989). Following ATSDR MRL derivation protocols, a provisional intermediate-duration TTD for kidney effects of 6 mg Mg/kg/day is derived by dividing the NOAEL, 647 mg Mg/kg/day, for kidney effects in the 13-week mouse study by (Tanaka et al. 1994) by an uncertainty factor of 100 (10 for interspecies extrapolation and 10 for human variation in susceptibility). The value for this provisional intermediate-duration oral TTD for kidney effects from magnesium compounds, 6 mg Mg/kg/day, is slightly higher that the provisional surrogate intermediate-duration MRL of 5 mg Mg/kg/day, based on the NAS (1997) UL of 350 mg Mg/day for mild diarrhea in adults.

D.6 References

Antman EM. 2002. Early administration of intravenous magnesium to high-risk patients with acute myocardial infarction in the Magnesium in Coronaries (MAGIC) Trial: A randomised controlled trial. Lancet 360(9341):1189-1196.

Arjona FJ, de Baaij JHF. 2018. CrossTalk opposing view: CNNM proteins are not Na(+) /Mg(2+) exchangers but Mg(2+) transport regulators playing a central role in transepithelial Mg(2+) (re)absorption. J Physiol 596(5):747-750. http://doi.org/10.1113/JP275249.

Arjona FJ, Latta F, Mohammed SG, et al. 2019. SLC41A1 is essential for magnesium homeostasis in vivo. Pflugers Arch 471(6):845-860. http://doi.org/10.1007/s00424-018-2234-9.

Ashrafi F, Haghshenas S, Nematbakhsh M, et al. 2012. The role of magnesium supplementation in cisplatin-induced nephrotoxicity in a rat model: No nephroprotectant effect. Int J Prev Med 3(9):637-643.

ATSDR. 2018. Framework for assessing health impacts of multiple chemicals and other stressors (update). Atlanta, GA: Agency for Toxic Substances and Disease Registry. https://www.atsdr.cdc.gov/interactionprofiles/ip-ga/ipga.pdf. December 22, 2020.

Bashir Y, Sneddon JF, Staunton HA, et al. 1993. Effects of long-term oral magnesium chloride replacement in congestive heart failure secondary to coronary artery disease. Am J Cardiol 72:1156-1162.

Belfort MA, Anthony J, Saade GR, et al. 2003. A comparison of magnesium sulfate and nimodipine for the prevention of eclampsia. N Engl J Med 348:304-311.

Cappuccio FP, Markandu ND, Benynon GW, et al. 1985. Lack of effect of oral magnesium on high blood pressure: A double blind study. Br Med J 291:235-239.

Dai LJ, Ritchie G, Kerstan D, et al. 2001. Magnesium transport in the renal distal convoluted tubule. Physiol Rev 81(1):51-81.

Daly NM, Harris AM. 1990. Magnesium supplementation and blood pressure in borderline hypertensive subjects: A double blind study. Magnesium Bulletin 12(4):149-154.

de Baaij JH, Hoenderop JG, Bindels RJ. 2015. Magnesium in man: implications for health and disease. Physiol Rev 95(1):1-46. http://doi.org/10.1152/physrev.00012.2014.

EFSA. 2006. Tolerable upper intake levels for vitamins and minerals. European Food Safety Authority. https://www.efsa.europa.eu/sites/default/files/efsa_rep/blobserver_assets/ndatolerableuil.pdf. February 26, 2019.

EFSA. 2015b. Scientific opinion on dietary reference values for magnesium. European Food Safety Authority. EFSA J 13(7):4186.

Fehlinger R, Kemnitz C, Stephan A, et al. 1988. Clinical study of the effectiveness of pyrrolidone carboxylic acid magnesium in the treatment of patients with chronic tetanic syndrome. Curr Ther Res 43(1):160-170.

Fine KD, Santa Ana CA, Fordtran JS. 1991. Diagnosis of magnesium-induced diarrhea. N Engl J Med 324:1012-1017.

Flatman PW. 1991. Mechanisms of magnesium transport. Annu Rev Physiol 53:259-271.

Funato Y, Furutani K, Kurachi Y, et al. 2018. CrossTalk proposal: CNNM proteins are Na(+) /Mg(2+) exchangers playing a central role in transepithelial Mg(2+) (re)absorption. J Physiol 596(5):743-746. http://doi.org/10.1113/JP275248.

García-Castaño A, Bandapalli OR, Madariaga L, et al. 2020. Novel variant in the CNNM2 gene associated with dominant hypomagnesemia. PLoS ONE 15(9):e0239965. http://doi.org/10.1371/journal.pone.0239965.

Greene MF. 2003. Magnesium sulfate for preeclampsia. N Engl J Med 384(4):275-276.

Gullestad L, Dolva LO, Birkeland K, et al. 1992. Oral versus intravenous magnesium supplementation in patients with magnesium deficiency. Magnes Trace Elem 10:11-16.

Houillier P. 2014. Mechanisms and regulation of renal magnesium transport. Annu Rev Physiol 76:411-430. http://doi.org/10.1146/annurev-physiol-021113-170336.

Huey CG, Chan KM, Wong ET, et al. 1995. Los Angeles County-University of Southern California Medical Center Clinical Pathology Case Conference: Extreme hypermagnesemia in a neonate. Clin Chem 41(4):615-618.

IARC. 2018. Agents classified by the IARC monographs, volumes 1-123. International Agency for Research on Cancer. https://monographs.iarc.fr/wp-content/uploads/2018/09/ClassificationsCASOrder.pdf. February 22, 2019.

IRIS. 2019. IRIS advanced search. International Risk Information System. U.S. Environmental Protection Agency. https://cfpub.epa.gov/ncea/iris/search/index.cfm. February 22, 2019.

ISIS. 1995. ISIS-4: A randomised factorial trial assessing early oral captopril, oral mononitrate, and intravenous magnesium sulphate in 58,050 patients with suspected acute myocardial infarction. Lancet 345:669-685.

Kolisek M, Sponder G, Pilchova I, et al. 2019. Magnesium extravaganza: A critical compendium of current research into cellular Mg(2+) transporters other than TRPM6/7. Rev Physiol Biochem Pharmacol 176:65-105. http://doi.org/10.1007/112_2018_15.

Kurata Y, Tamano S, Shibata MA, et al. 1989. Lack of carcinogenicity of magnesium chloride in a long-term feeding study in B6C3F1 mice. Food Chem Toxicol 27(9):559-563. http://doi.org/10.1016/0278-6915(89)90014-8.

Lakshmanan FL, Rao RB, Kim WW, et al. 1984. Magnesium intakes, balances, and blood levels of adults consuming self-selected diets. Am J Clin Nutr 40:1380-1389.

Mangalampalli B, Dumala N, Grover P. 2017. Acute oral toxicity study of magnesium oxide nanoparticles and microparticles in female albino Wistar rats. Regul Toxicol Pharmacol 90:170-184. http://doi.org/10.1016/j.yrtph.2017.09.005.

Marken PA, Weart CW, Stier Carson D, et al. 1989. Effects of magnesium oxide on the lipid profile of healthy volunteers. Atherosclerosis 77:37-42.

Mittermeier L, Demirkhanyan L, Stadlbauer B, et al. 2019. TRPM7 is the central gatekeeper of intestinal mineral absorption essential for postnatal survival. Proc Natl Acad Sci 116(10):4706-4715. http://doi.org/10.1073/pnas.1810633116.

Musso D. 2009. Magnesium metabolism in health and disease. Int Urol Nephrol 41:357-362.

Nadler JL, Malayan S, Lluong H, et al. 1992. Intracellular free magnesium deficiency plays a key role in increased platelet reactivity in Type II diabetes mellitus. Diabetes Care 15(7):835-841.

Nagy L, Tarnok F, Past T, et al. 1988. Human tolerability and pharmacodynamic study of tisacid tablet in duodenal ulcer patients. A prospective, randomized, self-controlled clinicopharmacological study. Acta Med Hung 45(2):231-247.

NAS. 1997. Dietary reference intakes for calcium, phosphorus, magnesium, vitamin D, and fluoride. Washington, DC: National Academy of Sciences.

NTP. 2016. Substances listed in the fourteenth report on carcinogens. National Toxicology Program. https://ntp.niehs.nih.gov/ntp/roc/content/listed_substances_508.pdf. February 22, 2019.

Oka T, Kimura T, Suzumura T, et al. 2014. Magnesium supplementation and high volume hydration reduce the renal toxicity caused by cisplatin-based chemotherapy in patients with lung cancer: a toxicity study. BMC Pharmacol Toxicol 15:70. http://doi.org/10.1186/2050-6511-15-70.

Plum-Wirell M, Stegmayr BG, Wester PO. 1994. Nutritional magnesium supplementation does not change blood pressure nor serum or muscle potassium and magnesium in untreated hypertension. A double-blind crossover study. Magnes Res 7(3/4):277-283.

Ricci JM, Hariharan S, Helfgott A, et al. 1991. Oral tocolysis with magnesium chloride: A randomized controlled prospective clinical trial. Am J Obstet Gynecol 165:603-610.

Romani AMP. 2011. Cellular magnesium homeostasis. Arch Biochem Biophys 512(1):1-23. http://doi.org/10.1016/j.abb.2011.05.010.

Ruddel H, Werner C, Ising H. 1990. Impact of magnesium supplementation on performance data in young swimmers. Magnes Res 3:103-107.

Saito Y, Okamoto K, Kobayashi M, et al. 2017. Magnesium co-administration decreases cisplatin-induced nephrotoxicity in the multiple cisplatin administration. Life Sci 189:18-22. http://doi.org/10.1016/j.lfs.2017.08.028.

Schlingmann KP, Waldegger S, Konrad M, et al. 2007. TRPM6 and TRPM7- gatekeepers of human magnesium metabolism. Biochim Biophys Acta 1772:813-821.

Schlingmann KP, Bandulik S, Mammen C, et al. 2018. Germline de novo mutations in ATP1A1 cause renal hypomagnesemia, refractory seizures, and intellectual disability. Am J Hum Genet 103(5):808-816. http://doi.org/10.1016/j.ajhg.2018.10.004.

Spatling L, Bubeck J, Schulz U, et al. 1998. Magnesium supplementation in der stillzeit? Ergebnisse-einer klinischen und experimentellen studie. Geburtsh U Frauenheilk 58:561-565.

Spencer H, Fuller H, Norris C, et al. 1994. Effect of magnesium on the intestinal absorption of calcium in man. J Am Coll Nutr 13(5):485-492.

Stendig-Lindberg G, Tepper R, Leichter I. 1993. Trabecular bone density in a two year controlled trial of peroral magnesium in osteoporosis. Magnes Res 6(2):155-163.

Stevens AR, Wolff HG. 1950. Magnesium intoxication. Absorption from the intact gastrointestinal tract. Arch Neurol Psychiatry 63:749-759.

Swaminathan R. 2003. Magnesium metabolism and its disorders. Clin Biochem Rev 24:47-66.

Takizawa T, Yasuhara K, Mitsumori K, et al. 2000. [A 90-day repeated dose oral toxicity study of magnesium chloride in F344 rats]. Kokuritsu Iyakuhin Shokuhin Eisei Kenkyusho Hokoku 118:63-70.

Tanaka H, Hagiwara A, Kurata Y, et al. 1994. Thirteen-week oral toxicity study of magnesium chloride in B6C3F1 mice. Toxicol Lett 73:25-32.

The Magpie Trial Collaborative Group. 2002. Do women with pre-eclampsia, and their babies, benefit from magnesium sulfate? The Magpie Trial: A randomised placebo-controlled trial. Lancet 359:1877-1890.

Watanabe M, Konishi M, Ohkido I, et al. 2005. Enhanced sodium-dependent extrusion of magnesium in mutant cells established from a mouse renal tubular cell line. Am J Physiol Renal Physiol 289(4):F742-748. http://doi.org/10.1152/ajprenal.00091.2005.

WHO. 2009. Calcium and magnesium in drinking-water. Public health significance. Geneva, Switzerland: World Health Organization. https://www.who.int/water_sanitation_health/publications/publication_9789241563550/en/. December 22, 2020.

Widman L, Wester PO, Stegmayr BK, et al. 1993. The dose-dependent reduction in blood pressure through administration of magnesium. A double blind placebo controlled cross-over study. Am J Hypertens 6(1):41-45.

Woods KL. 1991. Possible pharmacological actions of magnesium in acute myocardial infarction. Br J Clin Pharmacol 32:3-10.

Woods KL, Fletcher S. 1994. Long-term outcome after intravenous magnesium sulphate in suspected acute myocardial infarction: The second Leicester Intravenous Magnesium Intervention Trial (LIMIT-2). Lancet 343:816-819.

Workinger JL, Doyle RP, Bortz J. 2018. Challenges in the diagnosis of magnesium status. Nutrients 10:1202.

Appendix E. Background Information for Manganese

Manganese is a naturally occurring element (ATSDR 2012; Health Canada 2010; NAS 2001b). It does not occur in nature as a free metal, rather it is found mainly as oxides, sulphides, carbonates, and silicates in over 100 minerals (ATSDR 2012; Health Canada 2010). In aqueous solutions, manganese can exist as divalent or trivalent cations. The most common naturally occurring form is pyrolusite (manganese dioxide) (ATSDR 2012). Manganese is an essential nutrient, required for normal amino acid, lipid, protein, and carbohydrate metabolism, the urea cycle, and the formation of healthy cartilage and bone (ATSDR 2012; Health Canada 2010; NAS 2001b). Several enzyme systems also rely on manganese for catalytic or regulatory functions (ATSDR 2012; Health Canada 2010). Additionally, manganese is also important for wound healing (ATSDR 2012). Manganese demonstrates a "U-shaped" dose-response curve, indicating that low oral intake can lead to essential nutrient deficiency and adverse effects on bone, reproduction, and brain function, while high oral intake can lead to toxicity (ATSDR 2012; Chung et al. 2015; Health Canada 2010).

E.1 Toxicokinetics

Dietary absorption of manganese from the gastrointestinal tract is very small, approximately 1–5% of the administered dose (ATSDR 2012; Health Canada 2010; NAS 2001b). Manganese is transported across cellular membranes either via simple diffusion or high-affinity, low-capacity, active-transport mechanisms, thought to be mediated by DMT-1 (ATSDR 2012; Health Canada 2010). However, more recent studies with mice with intestinal DMT-1 deficiency showed no defects in manganese absorption or tissue levels, indicating that other transport system can operate in the gastrointestinal tract (Shawki et al. 2015). Various factors influence gastrointestinal absorption, including iron levels (shared membrane transport with other divalent cations, especially iron), trace minerals, phytate, ascorbic acid, and solubility of manganese compounds (ATSDR 2012; Health Canada 2010; IRIS 2002a). The absorption and hepatobiliary excretion of manganese is tightly regulated by complex homeostatic mechanisms (ATSDR 2012; IRIS 2002a). Under conditions of excess manganese load in the body, adaptive changes include reduced gastrointestinal absorption of manganese and increased biliary and pancreatic excretion of manganese (Health Canada 2010). Manganese homeostatic mechanisms at the cellular level include multiple transporters mediating cellular influx and efflux (Chen et al. 2015). One hypothesis is that the principal transport systems for cellular influx are DMT-1 for divalent manganese and the transferrin/TfR complex for trivalent manganese (Chen et al. 2015). Trivalent manganese binds to transferrin in blood, and following binding of Mn-transferrin to the transmembrane protein, TfR, endocytosis occurs (Chen et

al. 2015). Other transporters proposed to be involved in cellular influx of manganese include zinc transporters (ZIP8 and ZIP14), dopamine transporters, calcium channels, choline transporters, and citrate transporters (Chen et al. 2015). Evidence that ZIP8 and ZIP14 are the most important transporters for cellular influx of manganese comes from studies of diseases of manganese deficiency and excess, respectively, and studies of animal models of these diseases (Anagianni and Tuschl 2019). Manganese efflux transporters identified in various mammalian experimental systems include: SLC30A10, ferroportin, and secretory pathway Ca^{+2}-ATPase 1 (SPCA1) (Chen et al. 2015). There is evidence from human and animal studies that absorption and retention of ingested manganese is elevated in neonates, likely due to the immaturity of the hepatobiliary excretion system (ATSDR 2012; Health Canada 2010).

Following inhalation exposure, particle size determines the deposition in the respiratory tract, with deposition of fine particles (<2.5 μM) in the pulmonary region and deposition of coarse particles (>2.5 μM) in the tracheobronchial and extrathoracic regions (IRIS 2002a). Fine particles are readily absorbed into the blood and cleared through the lymphatic system, while coarse particles are either transported to the gastrointestinal system via mucociliary clearance mechanisms or directly transported into olfactory or trigeminal presynaptic nerve endings in the nasal mucosa with subsequent delivery to the brain, bypassing the liver and first-pass clearance (ATSDR 2012; Health Canada 2010; IRIS 2002a). The rate of lung clearance is faster for more water-soluble forms of manganese (e.g., manganese sulfate or manganese chloride) than for forms with lower solubility (e.g., manganese oxides or manganese phosphate) (Health Canada 2010).

Following absorption, manganese is transported by blood throughout the body. In the plasma, >80% of manganese is bound to β-globulin and albumin as the divalent ion; the remainder is trivalent manganese bound to transferrin with a small amount existing as free ion (ATSDR 2012; Health Canada 2010). No unique mammalian transporters for manganese have been identified (Health Canada 2010). In the cell, manganese concentrates in mitochondria, and is expected to form a complex with ATP (Health Canada 2010). The tissues with the highest manganese concentrations include the liver, pancreas, and kidney; with inhalation exposure, manganese has also been shown to preferentially accumulate in specific brain regions of laboratory animals due to bypass of "first-pass elimination" by the liver, including the substantia nigra, caudate nucleus, putamen, and globus pallidus (ATSDR 2012; Health Canada 2010; IRIS 2002a). Manganese in the blood can directly enter the brain across the choroid plexus; proposed transport mechanisms include facilitated diffusion, active transport, transferrin/TfR-mediated endocytosis, and DMT-1 transporters (ATSDR 2012; Health Canada 2010; NAS 2001b). In contrast, elimination from the brain is thought to be via passive diffusion, which would allow for manganese accumulation in the

brain under high exposure conditions (Health Canada 2010). While in the body, manganese may undergo changes in oxidation state; these oxidation state changes allow manganese to mimic other essential metals, such as Fe^{3+} (Mn^{3+}) and Ca^{2+} (Mn^{2+}) (ATSDR 2012; Health Canada 2010). The potential transfer of manganese across the placenta is low; however, evidence of developmental effects in laboratory animals at high, but non-maternally toxic doses, indicate that some transfer may occur (Health Canada 2010). Placental transfer is also likely via transferrin/TfR and/or DMT-1 transporters (Health Canada 2010).

The principal route of elimination for manganese is through the feces via hepatobiliary excretion; urinary excretion is generally low (ATSDR 2012; Health Canada 2010; NAS 2001b). Whole-body elimination of ingested manganese occurs in two phases: an initial rapid elimination of unabsorbed manganese in the gastrointestinal tract (half-life of <2 days) followed by a slower elimination of absorbed manganese (half-life of 10–30 days) (Health Canada 2010).

E.2 Health Effects

The nervous system is the main target of manganese toxicity in humans (ATSDR 2012; Health Canada 2010; IRIS 2002a; NAS 2001b). Permanent neurological damage (manganism) is well established in workers chronically exposed to manganese at air concentrations >1 mg/m^3 (ATSDR 2012; Health Canada 2010; IRIS 2002a). Manganism is characterized by a slow and clumsy gait, speech disturbances, a masklike face, and tremors that progress to dystonia and hyperreflexia; psychological disturbances also occur in some patients with manganism (ATSDR 2012; Health Canada 2010; IRIS 2002a). Occupational exposure to lower concentrations (0.07–0.97 mg/m^3) has been associated with subclinical neurological effects, including impaired coordination, decreased postural stability, and impaired cognition (ATSDR 2012; Health Canada 2010; IRIS 2002a). There is limited evidence from human studies that oral exposure to excess manganese in drinking water at levels as low as 0.06–0.08 mg/kg/day may also lead to neurological impairments, particularly in children (such as poor school performance, impaired cognitive function, abnormal performance on neurobehavioral tests, and increased oppositional behavior and hyperactivity) (ATSDR 2012; NAS 2001b). However, these values are lower than the RDAs for manganese (up to 10 mg/kg or 0.14 mg/kg/day), which have generally been considered adequate for essential functions and without adverse effects (IRIS 2002a). Too little and too much manganese can have adverse impacts on neurodevelopment, as demonstrated in a study of mental and psychomotor development in infants: lower developmental scores were associated with both low and high maternal blood manganese concentrations (Chung et al. 2015). Iron deficiency has also been associated with

elevated levels of manganese in body fluids and adverse impacts on neurodevelopment (ATSDR 2012; Health Canada 2010; Kim et al. 2012; Park et al. 2013). Numerous animal studies have identified acute and repeated exposure levels for oral and inhalation routes resulting in neurological impairments and/or damage, included impaired neurodevelopment at inhalation concentrations of manganese as low as 0.009 mg/m^3 and oral doses as low as 4.4 mg/kg/day (ATSDR 2012; Health Canada 2010; NAS 2001b)

The male reproductive system has also been identified as a potential target of manganese toxicity, potentially via disturbance of the neuroendocrine axis (ATSDR 2012; Health Canada 2010; IRIS 2002a). Complaints of decreased libido and impotence have been reported in chronically exposed workers at air levels similar to those causing neurobehavioral deficits (ATSDR 2012; Health Canada 2010; IRIS 2002a). A single occupational study reported impaired fertility in workers exposed to manganese at concentrations of 0.97 mg/m^3, and abnormal sperm have been reported in workers chronically exposed to >2 mg/m^3 (ATSDR 2012). Several animal studies have shown damage to male reproductive organs, decreased sperm motility and counts, and impaired development of the male reproductive tract following oral exposure to manganese at doses as low as 4.8 mg/kg/day (ATSDR 2012; Health Canada 2010).

Respiratory system effects, predominantly inflammatory responses in the lung, have been observed in both humans and animals following inhalation exposure to manganese (ATSDR 2012; Health Canada 2010). Pulmonary inflammation has been associated with increased incidence of cough and bronchitis, mild-to-moderate lung injury, and minor decreases in lung function in workers chronically exposed to ≥0.97 mg/m^3 (ATSDR 2012). Evidence from animal studies indicates that that chronic inhalation of manganese can increase susceptibility to pulmonary infections at high exposure levels (>40 mg/m^3) (ATSDR 2012; Health Canada 2010). However, observed lung effects have been observed following exposure to a variety of inhalable particulates, and are not unique to manganese-containing dusts (ATSDR 2012).

Other organ systems and tissues are not considered primary targets of manganese toxicity (ATSDR 2012; Health Canada 2010).

E.3 Mechanisms of Action

Manganese is a cellular toxicant that can lead to numerous alterations in transport systems, enzyme activities, and receptor functions; due to the complexity of its cellular actions, the mechanisms by which manganese causes neurotoxicity have not been fully elucidated (ATSDR 2012). One proposed

mechanism is that excess manganese leads to alterations in the dopaminergic system, potentially via damage to dopaminergic neurons, damage to dopamine receptors, and/or alterations in dopamine production/release (ATSDR 2012; IRIS 2002a). Similarities between Parkinsonism and manganism support this mechanism of action; however, patients with manganism generally do not respond well to the classic treatment for Parkinson's disease (levo-dopa), suggesting that damage to the dopaminergic system is more wide-spread than damage to dopamine-secreting cells in the substantia nigra observed with Parkinson's disease (ATSDR 2012). Other proposed mechanisms include free radical formation, oxidative stress, alterations in mitochondrial energy metabolism, disruption of cellular calcium and iron homeostasis, impaired astrocyte function resulting in excess extracellular glutamate, and apoptosis and/or necrosis of neurons (ATSDR 2012; Health Canada 2010; IRIS 2002a). These processes are likely interrelated, culminating in cytotoxicity and selective neurodegeneration (Health Canada 2010). Accumulation of manganese in the hypothalamus is a proposed mechanism underlying male reproductive findings (IRIS 2002a).

E.4 Health Guidelines

Several health guidelines have been established by various agencies to protect against neurological damage from inhalation exposure to manganese. To protect chronically exposed workers from neurological effects, ACGIH established a TLV (8-hour TWA) of 0.02 mg/m^3 (ACGIH 2013), OSHA established a PEL (8-hour TWA) of 5 mg/m^3 (OSHA 2014, 2015a, 2015b), and NIOSH established a REL (10-hour TWA) of 1 mg/m^3 (NIOSH 2015c). Chronic toxicological values include an EPA IRIS RfC of $5x10^{-5}$ mg/m^3 (IRIS 2002a) and an ATSDR chronic inhalation MRL of 0.0003 mg/m^3 (ATSDR 2012) based on neurotoxicity; see Table H-1 in Appendix H. Health Canada has also derived an inhalation RfC of $5x10^{-5}$ mg/m^3 to protect against neurotoxicity (Health Canada 2010).

For oral exposure, the Institute of Medicine of the NAS recommended AI values for manganese of 2.3 and 1.8 mg/day for adult men and women, respectively (0.03 mg/kg/day assuming 70-kg body weight) (NAS 2001b). Based on a lack of adverse effects associated with manganese intake levels in Western diets, the Institute of Medicine of the NAS set a UL of 11 mg/day (0.16 mg/kg/day assuming 70-kg body weight) for manganese intake (NAS 2001b). Similarly, the EPA derived an RfD value of 0.14 mg/kg/day based on a lack of adverse effects at RDAs (IRIS 2002a) (see Table H-1 in Appendix H). Although the ATSDR (2012) did not derive a MRL for repeated oral exposure to manganese, the NAS UL (NAS 2001b) and EPA RfD (IRIS 2002a) guidance values are similar and represent reasonable

estimates of acceptable oral intakes of manganese. For ATSDR public health assessments of chronic oral exposure to manganese, the EPA RfD is recommended.

Several agencies have established drinking water guidelines. The EPA established that lifetime exposure to manganese in drinking water at concentrations of 0.3 mg/L is not expected to cause any adverse effects, and exposure to 1 mg/L for 1 or 10 days is not expected to cause any adverse effects in children (EPA 2012). WHO established a drinking water quality guideline level of 0.4 mg/L (WHO 2008), and the FDA has established that the manganese concentration in bottled drinking water should not exceed 0.05 mg/L (FDA 2015).

The EPA determined that manganese was not classifiable as to human carcinogenicity (Class D) (IRIS 2002a). IARC (2015), NTP (2014), and ACGIH (2013) have not assessed manganese for carcinogenicity.

E.5 Derivation of Target-organ Toxicity Dose(s)

The neurological system is the most sensitive target organ following inhalation exposure to manganese in both animals and humans; neurological effects were the basis for the inhalation MRL of 0.0004 mg/m^3 and RfC of 0.00005 mg/m^3 (ATSDR 2012; IRIS 2002a). In chronically exposed workers, neurological effects have been reported at air concentrations as low as 0.07 mg/m^3, while other target organ effects (impaired lung function and decreased fertility) were reported at concentrations ≥0.97 mg/m^3 (ATSDR 2012). TTDs were not derived for these effects from manganese, because they are principally associated with inhalation exposure and this profile focuses on repeated oral exposure scenarios.

Evidence from limited human data and extensive animal data indicates that the neurological system is also the most sensitive target organ following oral exposure to manganese; a lack of neurological effects at RDAs was the basis of the oral RfD of 0.14 mg/kg/day (ATSDR 2012; IRIS 2002a). In laboratory animals, the lowest effective oral doses for neurological effects (altered neurobehavior) and male reproductive effects (decreased sperm motility and counts) are very similar, at 4.4 and 4.8 mg/kg/day, respectively (ATSDR 2012). As male reproductive effects have not been associated with other metals evaluated in this interaction profile, a TTD for adverse reproductive effects from manganese was not derived.

E.6 References

ACGIH. 2013. Manganese, elemental and inorganic compounds. Documentation of the threshold limit values and biological exposure indices. Cincinnati, OH: American Conference of Governmental Industrial Hygienists.

Anagianni S, Tuschl K. 2019. Genetic disorders of manganese metabolism. Curr Neurol Neurosci Rep 19(6):33. http://doi.org/10.1007/s11910-019-0942-y.

ATSDR. 2012. Toxicological profile for manganese. Agency for Toxic Substances Disease Registry. http://www.atsdr.cdc.gov/ToxProfiles/tp151.pdf. September 3, 2015.

Chen P, Chakraborty S, Mukhopadhyay S, et al. 2015. Manganese homeostasis in the nervous system. J Neurochem 134(4):601-610.

Chung SE, Cheong HK, Ha EH, et al. 2015. Maternal blood manganese and early neurodevelopment: The Mothers and Children's Environmental Health (MOCEH) Study. Environ Health Perspect 123(7):717-722.

EPA. 2012. 2012 Edition of the drinking water standards and health advisories. Washington, DC: U.S. Environmental Protection Agency. EPA822S12001. http://water.epa.gov/action/advisories/drinking/upload/dwstandards2012.pdf. September 3, 2015.

FDA. 2015. Beverages. Bottled water. Code of Federal Regulations 21 CFR 165.110. http://www.gpo.gov/fdsys/pkg/CFR-2015-title21-vol2/pdf/CFR-2015-title21-vol2-part165.pdf. September 4, 2015.

Health Canada. 2010. Human health risk assessment for inhaled manganese-document summary. Health Canada. HC pub: 100122. http://healthycanadians.gc.ca/publications/healthy-living-vie-saine/manganese/index-eng.php. September 2, 2015.

IARC. 2015. Agents classified by the IARC Monographs, Volumes 1-113. International Agency for Research on Cancer. http://monographs.iarc.fr/ENG/Classification/List_of_Classifications_Vol1-113.pdf. September 3, 2015.

IRIS. 2002a. Manganese (CASRN 7439-96-5). IRIS summaries. Washington, DC: Integrated Risk Information System. U.S. Environmental Protection Agency. http://www.epa.gov/iris/subst/0373.htm. September 4, 2015.

Kim J, Li Y, Buckett PD, et al. 2012. Iron-responsive olfactory uptake of manganese improves motor function deficits associated with iron deficiency. PLoS ONE 7(3):e33533. http://doi.org/10.1371/journal.pone.0033533.

NAS. 2001b. Manganese. In: Dietary reference intakes for Vitamin A, Vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. National Academy of Sciences, http://www.nap.edu/catalog/10026/dietary-reference-intakes-for-vitamin-a-vitamin-k-arsenic-boron-chromium-copper-iodine-iron-manganese-molybdenum-nickel-silicon-vanadium-and-zinc. September 8, 2015.

NIOSH. 2015c. Manganese compounds and fume (as Mn). NIOSH Pocket guide to chemical hazards. National Institute for Occupational Safety and Health. http://www.cdc.gov/niosh/npg/npgd0379.html. September 8, 2015.

NTP. 2014. Report on carcinogens. Thirteenth edition. National Toxicology Program. http://ntp.niehs.nih.gov/pubhealth/roc/roc13/index.html. September 3, 2015.

OSHA. 2014. Subpart D - Occupational health and environmental controls. Gasses, vapors, fumes, dusts, and mists. Occupational Safety and Health Administration. Code of Federal Regulations 29 CFR 1926.55. http://www.gpo.gov/fdsys/pkg/CFR-2014-title29-vol8/pdf/CFR-2014-title29-vol8-sec1926-55.pdf. September 4, 2015.

OSHA. 2015a. Subpart Z - Toxic and hazardous substances. 1910.1000. Air contaminants. Occupational Safety and Health Administration. Code of Federal Regulations 29 CFR 1910.1000. http://www.gpo.gov/fdsys/pkg/CFR-2014-title29-vol6/pdf/CFR-2014-title29-vol6-sec1910-1000.pdf. September 4, 2015.

OSHA. 2015b. Subpart Z - Toxic and hazardous substances. 1915.1000. Air contaminants. Occupational Safety and Health Administration. Code of Federal Regulations 29 CFR 1915.1000. http://www.gpo.gov/fdsys/pkg/CFR-2015-title29-vol7/pdf/CFR-2015-title29-vol7-sec1915-1000.pdf. September 4, 2015.

Park S, Sim CS, Lee H, et al. 2013. Blood manganese concentration is elevated in infants with iron deficiency. Biol Trace Elem Res 155(2):184-189. http://doi.org/10.1007/s12011-013-9782-9.

Shawki A, Anthony SR, Nose Y, et al. 2015. Intestinal DMT1 is critical for iron absorption in the mouse but is not required for the absorption of copper or manganese. Am J Physiol Gastrointest Liver Physiol 309(8):G635-647. http://doi.org/10.1152/ajpgi.00160.2015.

WHO. 2008. Guidelines for drinking-water quality. Third edition incorporating the first and second addenda. Volume 1 recommendations. Geneva, Switzerland: World Health Organization. http://www.who.int/water_sanitation_health/dwq/fulltext.pdf. September 3, 2015.

Appendix F. Background Information for Sodium

Sodium is an essential nutrient (Health Canada 2012; NAS 2005). It is required for various homeostatic and physiological functions, including osmotic regulation (salt-water balance), establishment of membrane potential, and regulation of active transport (NAS 2005). The principal form of dietary sodium is sodium chloride (NaCl; >90% of dietary intake), but sodium is also consumed as sodium bicarbonate and as sodium in a variety of forms present in processed foods (e.g., monosodium glutamate, sodium benzoate, etc.) (NAS 2005).

F.1 Toxicokinetics

Following ingestion, approximately 98% of sodium can be absorbed in the small intestine via active transport mechanisms, primarily via nutrient-coupled Na^+ absorption (e.g., Na^+/glucose cotransporters) and NaCl absorption mediated via Na^+/H^+ and Cl^-/HCO_3 exchangers (Kato and Romero 2011; NAS 2005). Once absorbed, sodium is widely distributed throughout the body, about 10% in cells and 90% in extracellular fluids (Pohl et al. 2013). Sodium is not metabolized to other valence states. Excess sodium is primarily excreted in urine, but it can also be excreted through sweat (NAS 2005). Fecal excretion of sodium is minimal (NAS 2005). The urinary excretion of sodium is tightly regulated through various homeostatic mechanisms in the kidney in order to maintain proper osmotic balance. Urinary loss of sodium can be increased with increased levels of dietary potassium, presumably through inhibition of sodium reabsorption in the distal tubule of the kidney (NAS 2005).

F.2 Health Effects

The most sensitive adverse effect associated with excess sodium intake is elevated blood pressure, and evidence from population-based studies in various populations throughout the world indicate a significant positive association between dietary salt intake and elevated blood pressure (Choi et al. 2015; de Wardener and MacGregor 2002; Galletti and Strazzullo 2016; NAS 2005; Subasinghe et al. 2016). Additionally, several intervention studies and two meta-analysese have shown that moderate reductions in sodium intake are associated with decreased blood pressure in both hypertensive and normotensive individuals (Aburto et al. 2012; Galletti and Strazzullo 2016; He et al. 2013; NAS 2005). For example, the pooled analysis by He et al. (2013) indicates that a decrease in dietary salt intake of 4–6 g/day is associated with a 5.39 mm Hg decrease in systolic blood pressure and 2.82 mm Hg decrease in diastolic blood pressure in hypertensive individuals. In normotensive individuals, the associated decreases are

2.42 and 1.00 mm Hg for systolic and diastolic blood pressure, respectively (He et al. 2013). Available data indicate that blood pressure increases progressively with increased sodium intake in nonlinear manner, with greater increases in blood pressure per unit increase of sodium at intake levels up to 2.3 g/day compared with increases in blood pressure per unit increase of sodium at intake levels >2.3 g/day (NAS 2005). This suggests a plateauing of sodium-induced hypertension at dose levels above the UL of 2.3 g/day. Individuals with certain conditions may be more sensitive to the hypertensive effects of sodium, including individuals with pre-existing conditions, such as hypertension, diabetes, and chronic kidney disease; increased sensitivity has also been observed in older individuals and African Americans (NAS 2005). Additionally, certain individuals (termed "salt-sensitive") are more susceptible to changes in blood pressure associated with sodium intake levels, while those without salt sensitivity ("salt-resistant") maintain normal blood pressure despite high sodium intake (Choi et al. 2015; Galletti and Strazzullo 2016; NAS 2005). Human evidence is supported by findings in animal studies, which indicate a correlation between increased sodium intake and elevated blood pressure (Choi et al. 2015).

In several epidemiological studies and meta-analyses, elevated sodium intake has been associated with increased risk of stroke, cardiovascular disease, and left ventricular hypertrophy; increased renal damage has also been associated with elevated sodium intake in patients with preexisting renal disease (Aburto et al. 2012; Choi et al. 2015; de Wardener and MacGregor 2002; Galletti and Strazzullo 2016; Health Canada 2012; NAS 2005; Strazzullo et al. 2009). The observed increases in risk may directly result from the known hypertensive effects of sodium, as high blood pressure is a known risk factor for cardiovascular and renal disease (Health Canada 2012; NAS 2005). However, some studies report an increased risk of cardiovascular effects (stroke, cardiovascular disease, left ventricular hypertrophy) independent of hypertensive effects, suggesting additional mechanisms of sodium-induced cardiovascular effects (de Wardener and MacGregor 2002; Galletti and Strazzullo 2016; Strazzullo et al. 2009). For example, in a meta-analysis by Strazzullo et al. (2009), the pooled relative risk (95% CI) per 5 g increase in daily salt intake was 1.23 (1.06, 1.43) for stroke and 1.17 (1.02, 1.32) for cardiovascular disease. However, when the pooled analysis was corrected for baseline blood pressure, the relative risk for stroke remained significant (1.22; 95% CI 1.02, 1.45) and the relative risk for cardiovascular disease was borderline significant (1.25; 95% CI 0.99, 1.57) (Strazzullo et al. 2009). Human evidence is supported by findings in animal studies, which indicate a strong association between increased sodium intake and adverse cardiovascular effects, often independent of blood pressure effects (de Wardener and MacGregor 2002; NAS 2005).

Limited evidence suggests that excess sodium intake may lead to decreased bone density (osteoporosis) and increased kidney stones due to increased urinary calcium excretion associated with elevated sodium levels (de Wardener and MacGregor 2002; NAS 2005). In several human studies, urinary calcium excretion was significantly elevated in individuals with dietary sodium intake levels ≥3.2 g/day compared with individuals with intake levels ≤2.3 g/day (NAS 2005). Limited evidence also indicates that a high salt intake may worsen the severity of asthma and bronchial responsiveness to agents (e.g., histamines) at intake levels ≥4.6 g/day; data do not indicate that elevated salt intake will alter airway responsiveness in healthy individuals (de Wardener and MacGregor 2002; NAS 2005).

Several epidemiological studies have reported associations between high sodium intake and/or high sodium levels in urine and risk of gastric cancer; animal studies have reported similar findings when high sodium intake is accompanied by exposure to various known carcinogens (de Wardener and MacGregor 2002; NAS 2005). Sodium itself is not considered to be carcinogenic; rather, it is thought that the irritative effects of high sodium intake, and subsequent destruction of the mucosal barrier of the stomach, may allow carcinogens greater access to the epithelial layer (NAS 2005).

F.3 Mechanisms of Action

Hypertension associated with elevated sodium salt intake has historically been attributed to increased renal retention of sodium, which leads to increased water retention, elevated plasma volume, and increased blood pressure (Choi et al. 2015). While disturbance in sodium and water homeostasis is still considered to contribute to elevated blood pressure in some individuals with high salt intake, the role of sodium in the maintenance of normal blood pressure may be more complicated (Blaustein et al. 2012; Choi et al. 2015). Current evidence indicates that sodium accumulation outside the kidney may also contribute to hypertension, particularly non-osmotic accumulation of sodium in vascular endothelium (e.g., sodium accumulation without water retention) (Choi et al. 2015). In animal studies, excess sodium intake leads to damage of the soft layer of the endothelium (endothelial glycocalyx layer), which is a negative charged biopolymer known to preferentially bind sodium (Choi et al. 2015). This damage could decrease the sodium-buffering capacity of the endothelial glycocalyx layer, allowing for increased sodium entry into endothelial cells, with the end result of increased vascular tone (Choi et al. 2015). Endothelial damage may contribute to the observed stiffening of arterial walls, decreased reactivity of smaller vessels, and left ventricular hypertrophy observed in humans and animal models associated with high sodium intake (de Wardener and MacGregor 2002). In animal models, these vascular changes have been associated with generation of reactive oxygen species (de Wardener and MacGregor 2002). Additionally,

animal models have indicated that left ventricular hypertrophy induced by elevated sodium intake is associated with increased myocardial angiotensin-converting enzyme, TGF-β, and endothelin gene expression, increased non-collagenous protein and total collagen content, and increased intramyocardial interstitial fibrosis in the left ventricle, intramyocardial arteries, and arterioles (de Wardener and MacGregor 2002). Blaustein et al. (2012) reviewed evidence for a complex molecular pathogenesis of salt-dependent hypertension involving the key role of sodium-induced secretion of endogenous ouabain (a cardiotonic steroid that is a natural ligand and inhibitor for α-2-sodium pumps) by the hypothalamus in the brain and the adrenals. In this paradigm, elevated endogenous ouabain induces acute augmentation of calcium signaling associated with cardiotonic and vasotonic effects, as well as mediates slow pathways in the brain and the periphery that lead to sustained sympathetic nerve activity and changes in expression and/or phosphorylation of calcium and sodium transport proteins including the sodium calcium exchanger (NCX1) and TRPC proteins. The net result is sustained enhancement of vasoconstriction and blood pressure elevation.

Both genetic and acquired traits have been proposed to underlie the difference in susceptibility to salt-induced changes in blood pressure. Abnormalities in the renin-angiotensin-aldosterone system, the sympathetic nervous system, the renal transmembrane sodium transport system, the kallikrein-kinin system, the nitric oxide system, eicosanoids, natriuretic peptides, insulin, leptin, and the vascular endothelium and various endothelial factors have all been considered as potential contributors to salt sensitivity (Choi et al. 2015; Galletti and Strazzullo 2015; Nishimoto and Fujita 2015). In particular, mutations in genes involved in renal transport of sodium have been suggested to underlie salt-sensitivity, including those involved in the β2-adrenergic stimulant-glucocorticoid receptor-with-no-lysine kinase 4-Na^+Cl^- cotransporter pathway in the distal convoluted tubule and the Ras-related C3 botulinum toxin substrate (Rac)1-mineralocorticoid receptor pathway in the distal convoluted tubule, connecting tubules, and collecting ducts (Choi et al. 2015; Nishimoto and Fujita 2015). Specific genetic polymorphisms have also been implicated as risk factors for development of hypertension, including those affecting the α-adducin molecule, the glucagon receptor, the serum and glucocorticoid-regulated kinase SGK1, the G-protein β-3 subunit, and the renal isoenzyme of 11β-hydroxysteroid dehydrogenase (Galletti and Strazzullo 2016). Abdominal adiposity and metabolic syndrome, which are attributed to both genetic and lifestyle factors, have also been associated with increased proximal tubular sodium reabsorption (Galletti and Strazzullo 2016).

Elevated sodium intake has also been associated with a significant increase in platelet aggregation in humans (de Wardener and MacGregor 2002). Increased platelet aggregation may contribute to the

increased stroke risk associated with elevated sodium intake levels, independent of (or in addition to) sodium-induced elevations in blood pressure.

F.4 Health Guidelines

The Institute of Medicine of the NAS has recommended AIs of 0.11 g/day for infants 0–6 months old 0.370 g/day for infants 7–12 months old, 0.800 g/day for children 1–3 years old, 1.00 g/day for children 4–8 years old, 1.2 g/day for children 9–13 years old, and 1.5 g/day for teenagers 14–18 years old (NAS 2019). The recommended AI for adults is 1.5 g/day (21 mg/kg/day assuming 70-kg body weight); higher levels are recommended for individuals exposed to high temperatures and/or increased physical activity to accommodate for loss of sodium in sweat (NAS 2019). AI values for sodium recommended by Health Canada (2012) are comparable to NAS values: 1.5 g/day for teens and adults (9–50 years), 1.3 g/day for 51–70 years of age, and 1.2 g/day for >70 years of age. Health Canada (2012) recommendations for infants and children are: 0.12 g/day for infants 0–6 months of age, 0.37 g/day for infants 7–12 months of age, 1 g/day for children 1–3 years of age, and 1.2 g/day for children 4–7 years of age.

The Institute of Medicine of the NAS has determined that there is insufficient evidence of sodium toxicity risk within the apparently health population to establish a sodium tolerable upper limit (NAS 2019). The EPA has a drinking water advisory of 20 mg/L for individuals restricted to a dietary intake of 500 mg sodium/day (EPA 2012). Due to lack of a clear relationship between sodium levels in drinking water and risk of hypertension, WHO did not establish a drinking water quality guideline level for sodium (WHO 2008).

ATSDR (2015) and EPA (IRIS 2019) have not derived noncancer toxicity values for sodium. The EPA (IRIS 2019), IARC (2015), and NTP (2014) have not assessed sodium for carcinogenicity.

F.5 Derivation of Target-organ Toxicity Dose(s)

Following oral exposure, the most sensitive adverse effect associated with excess sodium intake appears to be hypertension; chronic hypertension has been associated with increased risk for stroke, cardiovascular disease, left ventricular hypertrophy, and kidney damage. There is also limited evidence of adverse skeletal effects (osteoporosis) and exacerbation of asthma symptoms in individuals with excess sodium intake. However, available data are inadequate to derive TTDs for these endpoints.

F.6 References

Aburto NJ, Ziolkovska A, Hooper L, et al. 2012. Effect of lower sodium intake on health: Systematic review and meta-analyses. Br Med J 346:f1326. http://doi.org/10.1136/bmj.f1326.

ATSDR. 2015. Toxic substances portal: Toxicological profiles. Agency for Toxic Substances and Disease Registry. http://www.atsdr.cdc.gov/toxprofiles/index.asp. September 3, 2015.

Blaustein MP, Leenen FHH, Chen L, et al. 2012. How NaCl raises blood pressure: A new paradigm for the pathogenesis of salt-dependent hypertension. Am J Physiol Heart Circ Physiol 302:H1031-H1049.

Choi HY, Park HC, Ha SK. 2015. Salt sensitivity and hypertension: A paradigm shift from kidney malfunction to vascular endothelial dysfunction. Electrolyte Blood Press 13(1):7-16. http://doi.org/10.5049/ebp.2015.13.1.7.

de Wardener HE, MacGregor GA. 2002. Harmful effects of dietary salt in addition to hypertension. J Hum Hypertens 16:213-223.

EPA. 2012. 2012 Edition of the drinking water standards and health advisories. Washington, DC: U.S. Environmental Protection Agency. EPA822S12001. http://water.epa.gov/action/advisories/drinking/upload/dwstandards2012.pdf. September 3, 2015.

Galletti F, Strazzullo P. 2016. The blood pressure salt sensitivity paradigm: Pathophysiologically sound yet of no practical matter. Nephrol Dial Transplant 31:1-6.

He FJ, Li J, MacGregor GA. 2013. Effect of longer term modest salt reduction on blood pressure: Cochrane systematic review and meta-analysis of randomised trials. Br Med J 346:f1325. http://doi.org/10.1136/bmj.f1325.

Health Canada. 2012. Food and nutrition. Sodium in Canada. Health Canada. http://www.hc-sc.gc.ca/fn-an/nutrition/sodium/index-eng.php. August 23, 2016.

IARC. 2015. Agents classified by the IARC Monographs, Volumes 1-113. International Agency for Research on Cancer. http://monographs.iarc.fr/ENG/Classification/List_of_Classifications_Vol1-113.pdf. September 3, 2015.

IRIS. 2019. IRIS advanced search. International Risk Information System. U.S. Environmental Protection Agency. https://cfpub.epa.gov/ncea/iris/search/index.cfm. February 22, 2019.

Kato A, Romero MF. 2011. Regulation of electroneutral NaCl absorption by the small intestine. Annu Rev Physiol 73:261-281. http://doi.org/10.1146/annurev-physiol-012110-142244.

NAS. 2005. Sodium and chloride. In: Dietary reference intakes for water, potassium, sodium chloride and sulfate National Academy of Sciences, http://www.nap.edu/catalog/10925/dietary-reference-intakes-for-water-potassium-sodium-chloride-and-sulfate. September 8, 2015.

NAS. 2019. Dietary reference intakes for sodium and potassium. National Academies of Sciences. https://www.ncbi.nlm.nih.gov/books/NBK538102/pdf/Bookshelf_NBK538102.pdf. January 11, 2022.

Nishimoto M, Fujita T. 2015. Renal mechanisms of salt-sensitive hypertension: Contribution of two steroid receptor-associated pathways. Am J Physiol Renal Physiol 308:F377-F387.

NTP. 2014. Report on carcinogens. Thirteenth edition. National Toxicology Program. http://ntp.niehs.nih.gov/pubhealth/roc/roc13/index.html. September 3, 2015.

Pohl HR, Wheeler JS, Murray HE. 2013. Sodium and potassium in health and disease. Met Ions Life Sci 13:29-47. http://doi.org/10.1007/978-94-007-7500-8_2.

Strazzullo P, D'Elia L, Ngianga-Bakwin K, et al. 2009. Salt intake, stroke, and cardiovascular disease: Meta-analysis of prospective studies. Br Med J 339:b4567. http://doi.org/10.1136/bmh.b4567.

Subasinghe AK, Arabshahi S, Businge D, et al. 2016. Association between salt and hypertension in rural and urban populations of low to middle income countries: a systematic review and meta-analysis of population based studies. Asia Pac J Clin Nutr 25(2):402-413.

WHO. 2008. Guidelines for drinking-water quality. Third edition incorporating the first and second addenda. Volume 1 recommendations. Geneva, Switzerland: World Health Organization. http://www.who.int/water_sanitation_health/dwq/fulltext.pdf. September 3, 2015.

Appendix G. Background Information for Strontium

Strontium is a natural and commonly occurring element that can exist in two oxidation states (0 or +2); however, only the +2 oxidation state is stable under normal environmental conditions (ATSDR 2004b). It does not occur in nature as a free metal but is found in a variety of compounds in mineral form. Natural strontium exists in four stable isotopes (^{84}Sr, ^{86}Sr, ^{87}Sr, ^{88}Sr) with similar chemical characteristics. Radioactive strontium is not a naturally occurring isotope but can be generated in nuclear reactors or during the explosion of nuclear weapons by the nuclear fission of ^{235}U, ^{238}U, or ^{239}P (ATSDR 2004b).

G.1 Toxicokinetics

Strontium is readily absorbed via the inhalation route, poorly absorbed via the oral route, and minimally absorbed via the dermal route (ATSDR 2004b). Following inhalation exposure, particle size determines the deposition in the respiratory tract, with fine particles (<2.5 μM) preferentially depositing in the pulmonary region and coarse particles (>2.5 μM) depositing in the tracheobronchial and extrathoracic regions (ATSDR 2004b). Fine particles can be absorbed after extracellular dissolution or ingested by alveolar macrophages; the relative contributions of these pathways are unknown (ATSDR 2004b). Coarse particles are expected to be transported to the gastrointestinal system via mucociliary clearance mechanisms (ATSDR 2004b). Compounds of greater solubility are more rapidly absorbed and cleared from the lung than compounds with low solubility (ATSDR 2004b). Following oral exposure in humans, approximately 11–35% of the ingested dose (generally administered as $SrCl_2$) is absorbed from the gastrointestinal tract, with similar estimates in infants, children, and adults (ATSDR 2004b; EPA 1990). However, increased absorption has been observed in neonatal rats compared with adult rats, suggesting that strontium absorption may be age-related, with up to 90% absorption in young animals (ATSDR 2004b; EPA 1990). Carbohydrates, particularly lactose, as well as vitamin D, also enhance gastrointestinal absorption of strontium (EPA 1990). Studies in animals show that strontium is absorbed both in the stomach and the small intestine; however, the mechanisms of strontium absorption in the gastrointestinal tract are not clear (ATSDR 2004b). *In vivo* studies in hamsters suggest passive uptake of strontium (based on a serosal:mucosal strontium ratio <1); however, data from *in vivo* rat small intestine slice cultures show a saturable uptake mechanism, suggesting that absorption may be an active process (ATSDR 2004b; EPA 1990). Strontium absorption may occur in concert with calcium absorption, because both metallic ions appear to share common membrane transport mechanisms, and the fractional absorption of a gavage dose of strontium demonstrates a relatively constant ratio to that of calcium (0.75). Proposed mechanisms include transport via a calcitriol-inducible Ca^{2+}-ATPase and/or binding to

calbindin-D, which is a $1,25(OH)_2D_3$-inducible calcium binding protein with a proposed role in calcium absorption (ATSDR 2004b). Therefore, absorption of strontium may be also greater in individuals with higher calcium demands (e.g., children, women who are pregnant or lactating) and/or insufficient calcium intake (ATSDR 2004b; EPA 1990). Absorption through intact skin in humans is low (0.14–0.37%), with higher absorption (up to 57%) through scratched or abraded skin (ATSDR 2004b).

After absorption, strontium is initially distributed from blood into three main compartments: plasma extracellular fluid (bound to plasma proteins, although specific proteins have not been characterized), soft tissue and superficial zone of bone tissue, and bone, with ultimate disposition in bone and teeth (ATSDR 2004b; EPA 1990; IRIS 2002b). Similar to calcium, 99% of total strontium body burden is in the skeleton (ATSDR 2004b; EPA 1990). Strontium can be released from bone during ion exchange and bone remodeling (EPA 1990). The similar distribution patterns between calcium and strontium are due to the binding of strontium to ligands that normally bind calcium, such as hydroxyapatite (the main component of mineralized bone) and various calcium binding and transport proteins involved in the disposition of calcium in cells (Ca^{2+}-ATPases, Na^{+}-Ca^{+}-antiport, and Ca^{2+}channels). Small amounts of maternal strontium can be transferred to the fetus through the placenta and to the neonate via breast milk (ATSDR 2004b). Strontium is not metabolized in the body, but it may bind to cellular macromolecules such as proteins. Based on its similarity to calcium, it is expected to form complexes with various inorganic anions (e.g., carbonate and phosphate) and carboxylic acids (e.g., citrate and lactate) (ATSDR 2004b).

Whole-body elimination of strontium displays biphasic kinetics, with rapid elimination of non-absorbed compound and very slow (i.e., years) elimination of strontium deposited in the skeleton (ATSDR 2004b). The predominant route of elimination is via urinary excretion, with smaller amounts excreted in the feces; the urinary:fecal excretion ratio of strontium is approximately 3:1 (ATSDR 2004b). Based on excretion kinetic studies in volunteers, it appears that strontium undergoes net tubular reabsorption in the kidney. While the molecular mechanisms of reabsorption are not known, it is likely that it utilizes calcium transport mechanisms, such as Ca^{2+}-ATPases, Na^{+}-Ca^{+}-antiport, and/or membrane Ca^{2+} channels (ATSDR 2004b). For fecal excretion, there is evidence of direct secretion of strontium into the small intestines and passive transport into the colon; the contribution of biliary secretion to fecal elimination is unknown (ATSDR 2004b).

G.2 Health Effects

The only adverse health effect associated with excess oral strontium (stable, not radioactive) intake in humans is rickets (skeletal abnormalities) in children with poor diets (vitamin D, calcium, and protein deficiencies), characterized by craniomalacia, rachitic rosary, bulging at the wrist, bony deformities of the leg, and delayed closure of the fontanelles (ATSDR 2004b). Animal studies also identify bones as the most sensitive target of strontium toxicity, with severe effects on bone growth occurring in young animals exposed to very high oral doses of strontium (≥350 mg/kg/day) (ATSDR 2004b; EPA 1990; IRIS 2002b). Skeletal effects are not expected to occur in healthy individuals with adequate diets at environmentally occurring levels of strontium (ATSDR 2004b). The only chemical form of stable strontium that is harmful via inhalation is strontium chromate; however, toxic effects are attributable to chromium rather than strontium (ATSDR 2004b). No adverse health effects have been associated with inhalation or dermal exposure to other forms of stable strontium (ATSDR 2004b). Following intravenous exposure to strontium in dialysis water, adverse skeletal effects (osteomalacia) have also been observed in adults (ATSDR 2004b). In animals, intravenous exposure to large doses of strontium can interfere with several physiological processes, including heart and skeletal muscle contractions and ionic transport across red blood cell membranes and nerve cells (EPA 1990; IRIS 2002b). The relevance of these high intravenous dose findings to oral toxicity is unknown.

G.3 Mechanisms of Action

High levels of strontium can potentially disrupt calcium homeostasis. Excess strontium is deposited into bone, where it interferes with bone mineralization in the developing skeleton by replacing calcium in the hydroxyapatite crystal during bone calcification or displacing calcium from existing calcified matrix (ATSDR 2004b; IRIS 2002b). Children with poor diets, particularly diets low in calcium and vitamin D, are more susceptible to strontium-mediated skeletal effects (ATSDR 2004b).

G.4 Health Guidelines

Toxicological values for oral exposure to strontium include an ATSDR intermediate MRL of 2 mg/kg/day (ATSDR 2004b) and an EPA IRIS RfD of 0.6 mg/kg/day (IRIS 2002b) based on skeletal toxicity; see Table H-1 in Appendix H. The EPA has established that exposure to strontium in drinking water at concentrations of 4 mg/L for life will not cause any adverse effects, and exposure to 25 mg/L for 1 or

10 days will not cause any adverse effects in children (EPA 2012). For ATSDR public health assessments of chronic oral exposure to strontium, the EPA RfD is recommended (see Appendix H).

The EPA determined that strontium was not classifiable as to human carcinogenicity (Class D) (EPA 2012). IARC (2015) and NTP (2014) have not assessed strontium for carcinogenicity.

G.5 Derivation of Target-organ Toxicity Dose(s)

The only adverse effect associated with excess oral strontium (stable) exposure is skeletal toxicity (ATSDR 2004b; EPA 1990; IRIS 2002b), precluding the derivation of TTDs for other adverse health outcomes.

G.6 References

ATSDR. 2004b. Toxicological profile for strontium. Agency for Toxic Substances Disease Registry. http://www.atsdr.cdc.gov/ToxProfiles/tp159.pdf. September 3, 2015.

EPA. 1990. Health and environmental effects document for stable strontium. Cincinnati, OH: U.S. Environmental Protection Agency. ECAOCING111.

EPA. 2012. 2012 Edition of the drinking water standards and health advisories. Washington, DC: U.S. Environmental Protection Agency. EPA822S12001. http://water.epa.gov/action/advisories/drinking/upload/dwstandards2012.pdf. September 3, 2015.

IARC. 2015. Agents classified by the IARC Monographs, Volumes 1-113. International Agency for Research on Cancer. http://monographs.iarc.fr/ENG/Classification/List_of_Classifications_Vol1-113.pdf. September 3, 2015.

IRIS. 2002b. Strontium (CASRN 7440-24-6). IRIS summaries. Washington, DC: Integrated Risk Information System. U.S. Environmental Protection Agency. http://www.epa.gov/iris/subst/0550.htm. September 3, 2015.

NTP. 2014. Report on carcinogens. Thirteenth edition. National Toxicology Program. http://ntp.niehs.nih.gov/pubhealth/roc/roc13/index.html. September 3, 2015.

Appendix H. Noncancer Health Guidance Values for Selected Metals

Table H-1. Critical Effects and PODs for Noncancer Health Guidance Values for Adult Exposure to Selected Metals

Chemical	Critical effect	POD			Toxicity value	Species and exposure	Uncertainty factors[a]	Source
		NOAEL	LOAEL	BMDL				
Chronic inhalation exposure (mg/m^3)								
Manganese	Neurobehavioral impairment	NI	0.15	NI	0.00005 (RfC)	Human, occupational exposure for an average duration of 5.3 years	10 HV, 10 LN, 10 DB/SCC; adjusted for continued exposure	IRIS 2002a
Manganese	Neurobehavioral impairment	NI	0.179	0.142	0.0003 (MRL)	Human, occupational exposure for an average duration of 5.3 years	10 HV, 10 DB; adjusted for continued exposure	ATSDR 2012
Intermediate oral exposure (mg/kg/day)								
Barium	Increased kidney weight	65	115	NI	0.2 (MRL)	Rat, drinking water, 90 days	10 HV,10 AH, 3 DB	ATSDR 2007
Strontium	Skeletal toxicity	140	550	NI	2 (MRL)	Young rat, dietary, 20 days	3 HV, 10 AH, 3 MF (limited endpoints, short duration)	ATSDR 2004b
Chronic oral exposure (mg/kg/day)								
Barium	Nephropathy	75	160	61.13	0.2 (MRL)	Mouse, drinking water, 2 years	10 HV,10 AH, 3 DB	ATSDR 2007
Barium	Nephropathy	75	160	63	0.2 (RfD)	Mouse, drinking water, 2 years	10 HV,10 AH, 3 DB	EPA 2005
Strontium	Skeletal toxicity	190	380	NI	0.6 (RfD)	Young rat, dietary, 20 days	3 HV,10 AH, 10 DB	IRIS 2002b
Calcium	Kidney stones	NI	36	NI	36 (UL)	Human, dietary supplement, intermediate and chronic (based on post-menopausal women)	1	NAS 2011
Magnesium	Gastrointestinal discomfort and diarrhea	5	NI	NI	5 (UL)	Human, dietary supplement, intermediate and chronic	1	NAS 1997; EFSA 2006

Table H-1. Critical Effects and PODs for Noncancer Health Guidance Values for Adult Exposure to Selected Metals

Chemical	Critical effect	POD			Toxicity value	Species and exposure	Uncertainty factors[a]	Source
		NOAEL	LOAEL	BMDL				
Iron	Gastrointestinal effects	NI	70[c]	NI	45 (UL)	Human, dietary, chronic	1.5 LN	NAS 2001a
Manganese	No adverse neurological effects	0.14	NI	NI	0.14 (RfD)	Human, chronic ingestion data (recommended dietary allowances)	1	IRIS 2002a
Manganese	No adverse neurological effects	0.16	NI	NI	0.16 (UL)	Human, dietary, chronic (based on intakes in Western diet)	1	NAS 2001b

[a]Uncertainty factor abbreviations: AH for animal to human extrapolation; DB for database deficiency; HV for human variability; LN for LOAEL to NOAEL extrapolation; SCC for subchronic to chronic extrapolation.
[b]A NOAEL could not be identified because the relationship between blood pressure and sodium intake is a progressive, dose-response relationship without a threshold (NAS 2005).
[c]LOAEL is based on iron content of supplement (60 mg/day) plus the estimated mean dietary intake of iron (11 mg/day). There is supportive evidence for a LOAEL of 50–120 mg/day of supplemental iron from various studies, but these studies either failed to include a placebo group and/or had fewer study subjects than the study identifying a supplemental LOAEL of 60 mg/day (NAS 2001a).

ATSDR = Agency for Toxic Substances and Disease Registry; BMDL = benchmark dose limit; EPA = U.S. Environmental Protection Agency; IRIS = Integrated Risk Information System; LOAEL = lowest-observed-adverse-effect level; MRL = minimal risk level; NAS = National Academy of Sciences; NI = not identified; NOAEL = no-observed-adverse-effect level; POD = point of departure; RfC = reference concentration; RfD = reference dose; UL = tolerable upper intake level

H.1 References

ATSDR. 2004b. Toxicological profile for strontium. Agency for Toxic Substances Disease Registry. http://www.atsdr.cdc.gov/ToxProfiles/tp159.pdf. September 3, 2015.

ATSDR. 2007. Toxicological profile for barium and barium compounds. Agency for Toxic Substances Disease Registry. http://www.atsdr.cdc.gov/ToxProfiles/tp24.pdf. September 3, 2015.

ATSDR. 2012. Toxicological profile for manganese. Agency for Toxic Substances Disease Registry. http://www.atsdr.cdc.gov/ToxProfiles/tp151.pdf. September 3, 2015.

EFSA. 2006. Tolerable upper intake levels for vitamins and minerals. European Food Safety Authority. https://www.efsa.europa.eu/sites/default/files/efsa_rep/blobserver_assets/ndatolerableuil.pdf. February 26, 2019.

EPA. 2005. Toxicological review of barium and compounds. Washington, DC: U.S. Environmental Protection Agency. http://www.epa.gov/iris/toxreviews/0010tr.pdf. September 3, 2015.

IRIS. 2002a. Manganese (CASRN 7439-96-5). IRIS summaries. Washington, DC: Integrated Risk Information System. U.S. Environmental Protection Agency. http://www.epa.gov/iris/subst/0373.htm. September 4, 2015.

IRIS. 2002b. Strontium (CASRN 7440-24-6). IRIS summaries. Washington, DC: Integrated Risk Information System. U.S. Environmental Protection Agency. http://www.epa.gov/iris/subst/0550.htm. September 3, 2015.

NAS. 1997. Dietary reference intakes for calcium, phosphorus, magnesium, vitamin D, and fluoride. Washington, DC: National Academy of Sciences.

NAS. 2001a. Iron. In: Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. National Academy of Sciences, http://www.nap.edu/catalog/10026/dietary-reference-intakes-for-vitamin-a-vitamin-k-arsenic-boron-chromium-copper-iodine-iron-manganese-molybdenum-nickel-silicon-vanadium-and-zinc. September 8, 2015.

NAS. 2001b. Manganese. In: Dietary reference intakes for Vitamin A, Vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. National Academy of Sciences, http://www.nap.edu/catalog/10026/dietary-reference-intakes-for-vitamin-a-vitamin-k-arsenic-boron-chromium-copper-iodine-iron-manganese-molybdenum-nickel-silicon-vanadium-and-zinc. September 8, 2015.

NAS. 2005. Sodium and chloride. In: Dietary reference intakes for water, potassium, sodium chloride and sulfate National Academy of Sciences, http://www.nap.edu/catalog/10925/dietary-reference-intakes-for-water-potassium-sodium-chloride-and-sulfate. September 8, 2015.

NAS. 2011. Dietary reference intakes for calcium and vitamin D. National Academies of Sciences.

Appendix I. Mixtures of Inorganic Components Identified in Waste Water from Unconventional Gas Extraction Activities

Table I-1. Inorganic Components of Waste Water from Unconventional Gas Extraction Activities in Pennsylvania's Marcellus Shale

	Average concentration (range)	Number of samples
Anions (mg/L)		
Cl	57,447 (64–196,000)	154
Br	511 (0.2–1,990)	95
SO_4	71 (0–763)	113
$CaCO_3$	165 (7.5–577)	144
Cations (mg/L)		
Na	24,123 (69–117,000)	157
Ca	7,220 (38–41,000)	159
Ba	2,224 (0.2–13,800)	159
Sr	1,694 (0.6–8,460)	151
Mg	632 (17–2,550)	157
Fe (total)	76 (2.6–321)	141
Radioactivity (pCi/L)		
Ra^{228}	120 (0–1,360)	46
Ra^{226}	623 (2.75–9,280)	46
U^{235}	1 (0–20)	14
U^{238}	42 (0-497)	14
Gross alpha	1,509 (37.7-9,551)	32
Gross beta	43,415 (75.2-597,600)	32

Source: Barbot et al. 2013

Table I-2. Concentrations of Inorganic Ions Identified in Waste Water from Unconventional Gas Extraction Activities in Three U.S. Shale Formations[a]

Components	Range of reported concentrations (mg/L)
Anions	
Cl	8,042–43,578
HCO_3	261–1,281
SO_4	9.1–149
Cations	
Na	5,363–24,445
Ca	256–2,921
Ba	0.8–679
Sr	21–357
Mg	77–263
Fe	26–33
Mn	3.9–44

[a]U.S. shale formations: Fayetteville, Marcellus, and Barnett.

Source: Jackson et al. 2013b

Table I-3. Concentrations of Inorganic Components Identified in Injected Fluid and Flowback Water from Seven Hydraulically Fractured Gas Wells in Pennsylvania

Component	Injected fluid day 0; median concentration (mg/L)	Flowback water day 14; median concentration
Anions		
Cl	82	98,300
Br	<10	872
SO_4	59	<50
$CaCO_3$	126	71
Cations		
Na	80	36,400
Ca	32	11,200
Ba	0.6	1,990
Sr	0.82	2,330
Mg	3.7	875
Fe	0.68	47
Mn	0.074	5.6
Li	0.04	95
Zn	0.08	0.09
Al	0.3	0.5

Source: Reprinted from Haluszczak et al. 2013 with permission from Elsevier.

I.1 References

Barbot E, Vidic NS, Gregory KB, et al. 2013. Spatial and temporal correlation of water quality parameters of produced waters from Devonian-Age shale following hydraulic fracturing. Environ Sci Technol 47(6):2562-2569. http://doi.org/10.1021/es304638h.

Haluszczak LO, Rose AW, Kump LR. 2013. Geochemical evaluation of flowback brine from Marcellus gas wells in Pennsylvania, USA. Appl Geochem 28:55-61. http://doi.org/10.1016/j.apgeochem.2012.10.002.

Jackson RE, Gorody AW, Mayer B, et al. 2013b. Groundwater protection and unconventional gas extraction: The critical need for field-based hydrogeological research. Groundwater 51(4):488-510. http://doi.org/10.1111/gwat.12074.

Appendix J. Database Query Strings for Combinations of Selected Metallic Ions (Barium, Calcium, Iron, Magnesium, Manganese, Sodium, and Strontium)

Information to prepare this profile was obtained via searches of the literature. The search objective was to identify noncancer and cancer toxicity, toxicokinetic, and interaction data from studies of humans and laboratory animals, as well as mechanistic studies using tissue, cell, or *in vitro* systems. An initial search of PubMed, Toxline, and Toxcenter was conducted in May 2015 to identify references with records mentioning two or more of five metals of interest (barium, iron, manganese, sodium, and strontium) using Chemical Abstracts Service Registry Numbers (CASRNs), Unique Ingredient Identifiers (UNIIs), and synonyms. In October 2018, an update, date-limited search of the same databases was conducted for these same five metallic cations, along with a non-date-limited search of PubMed for references with records mentioning calcium or magnesium and at least one other of seven metals of interest (the previously mentioned five metals, plus calcium and magnesium), augmented by a gray literature search in selected institutional websites: FDA, EFSA, INCHEM.org, eChemPortal, WHO, government of Canada, government of Japan, and Australia National Industrial Chemicals Notification and Assessment Scheme (NICNAS). Table J-1 presents the CASRNs and names of the compounds, as well as synonyms used in the search. An inclusive list of various metallic compounds was used since ATSDR does not know the specific cation/anion (ionic) compound(s) present in UOG extraction waste water and/or groundwater near UOG activities (e.g., sodium chloride versus sodium carbonate or barium sulfate versus barium chloride). The ATSDR Toxicological Profiles for barium, manganese, and strontium and the ChemID database were consulted to identify CASRNs and synonyms for each metallic cation and compounds of environmental interest (metal salts with acetate, carbonate, chloride, and sulfate). To narrow the list of synonyms, all pharmaceuticals and trade names were removed unless they were listed in the Medical Subject Heading record for a specific CASRN. The synonym list for sodium chloride was further narrowed by removing synonyms with the word, “salt” (e.g., table salt, sea salt, rock salt).

Table J-1. Substances Searched for Joint Toxic Action Studies in PubMed, Toxline, and Toxcenter

Component	Synonyms	Chemical Abstracts Service Registry Numbers and compounds searched	
Barium	Barite; barium; baritop; e-z-cat; micropaque oral	22541-12-4	Barium cation
		543-80-6	Barium acetate
		513-77-9	Barium carbonate
		10361-37-2	Barium chloride
		7727-43-7	Barium sulfate
Calcium	Calcium; monocalcium carbonate; acetate of lime; anhydrite; anhydrous sulfate of lime; gypsum; karstenite; lime acetate; muriacite; phoslo; slaker rejects; aragonite; calcite; chalk; limestone; marble; vaterite	14127-61-8	Calcium cation
		62-54-4	Calcium acetate
		471-34-1	Calcium carbonate
		10043-52-4	Calcium chloride anhydrous
		10035-04-8	Calcium chloride dihydrate
		7778-18-9	Calcium sulfate
Iron	Iron; ferrous; ferric; lawrencite; polyferric; aktiferrin; biofer; ceferro; conferon; eisendragees-ratiopharm; eisensulfat stada; feospan; fer-gen-sol; fer-in-sol; feratab; fero-gradumet; ferodan; ferogradumet; ferro-gradumet; ferrogamma; ferrograd; ferroinfant; haemoprotect; hämatopan; hemobion; hemofer; kendural; mol-iron; plastufer; slow-Fe; vitaferro kapseln	15438-31-0	Ferrous cation
		20074-52-6	Ferric cation
		563-71-3	Ferrous carbonate
		10290-71-8	Iron carbonate
		7705-08-0	Ferric chloride
		7758-94-3	Ferrous chloride
		12040-57-2	Iron chloride
		16480-60-7	
		23444-30-6	
		2140-52-5	Iron acetate
		1834-30-6	Ferric acetate
		7720-78-7	Ferrous sulfate
		10028-22-5	Ferric sulfate
		10124-49-9	Sulfuric acid, iron salt
		16547-58-3	Sulfuric acid, iron (2+) salt
Magnesium	Magnesium; epsom salt; epsom salts; hydromagnesite; magnesite; nesquehonite	22537-22-0	Magnesium cation
		142-72-3	Magnesium acetate
		546-93-0	Magnesium carbonate
		17968-26-2	Magnesium carbonate (1:1) hydrate
		23389-33-5	Magnesium carbonate (1:1) hydrate
		7786-30-3	Magnesium chloride anhydrous
		7791-18-6; 14989-29-8	Magnesium chloride
		7487-88-9	Magnesium sulfate anhydrous
		10034-99-8; 18939-43-0	Magnesium sulfate

Table J-1. Substances Searched for Joint Toxic Action Studies in PubMed, Toxline, and Toxcenter

Component	Synonyms	Chemical Abstracts Service Registry Numbers and compounds searched	
Manganese	Manganese; manganous chloride; scacchite; manganous carbonate; rhodochrosite; manganous acetate; $MnCl_2$	19768-33-3	Manganese, ion
		7773-01-5	Mn(II) chloride
		7785-87-7	Manganese sulfate
		598-62-9	Mn(II) carbonate
		638-38-0	Manganous acetate
Sodium	Bisodium carbonate; bisodium sulfate; disodium carbonate; disodium monosulfate; disodium sulfate; disodium sulphate; Na sulphate; sodium; trisodium trichloride; saline solution; mangxiao; mirabilitum; natrii sulphas; puxiao; thenardite	17341-25-2	Sodium cation
		127-09-3	Sodium acetate
		6131-90-4	
		497-19-8	Sodium carbonate
		5968-11-6	
		7647-14-5	Sodium chloride
		7757-82-6	Sodium sulfate, dried
		7727-73-3	Sodium sulfate
		15124-09-1	
Strontium	Strontium; strontianite; metastron	22537-39-9	Strontium cation
		543-94-2	Strontium acetate
		1633-05-2	Strontium carbonate
		10476-85-4	Strontium chloride
		7759-02-6	Strontium sulfate

The initial 2015 search resulted in about 17,645 records, after removal of duplicates. An electronic screening of the 17,645 initial records retained records using one or more of the following terms in the title or abstract: additivity, antagonism, inhibition, joint action, masking, potentiation, or synergism. The screening retained 5,494 records of the initial 17,645 records. An additional electronic screening step excluded records that mentioned bacteria or plants; this step excluded an additional 1,730 records, leaving 3,764 records of the initial 17,645 records for manual screening. A senior toxicologist manually screened titles and abstracts of the retained 3,764 records to select potential studies of interest; 110 records were selected for retrieval and full text evaluation. The toxicologist selected interaction studies with whole-body exposure scenarios with mammals but did not exclude interaction studies conducted with isolated cell components, cells, or tissues. To check the accuracy of the electronic screening, a senior toxicologist examined a random sample of 100 of the excluded records. The sample of excluded records was selected using a publicly available random number generator (www.random.org). The toxicologist concluded that the excluded sample of 100 contained no records of potential interest. Additional potentially useful references were identified by authors of this report during full-text evaluations of the retrieved references and pertinent published reviews and government reports on the health effects of the five metallic cations

and by supplemental searches (in August 2015) of PubMed for more recent reports from key investigators.

The updated and expanded search conducted in October 2018 resulted in about 35,564 records. An electronic screening of the initial results retained records containing the terms additivity, antagonism, inhibition, joint action, masking, potentiation, or synergism. It also excluded records that mentioned bacteria or plants terms. An additional electronic screening, applied to the voluminous calcium/ magnesium results, retained records containing either chemical synonyms in the titles, or any of the following terms anywhere in the records: mixture, pollutants, metal, drinking water, ground water, groundwater, waste water, fracking, hydraulic fracturing, or wells. In all, the applied terms excluded about 29,156 records, resulting in a total of 4,604 retained records following duplicate removal. A senior toxicologist manually screened titles and abstracts of the retained records to select potential studies of interest; 268 records were selected for retrieval and full text evaluation. The toxicologist selected interaction studies with whole-body exposure scenarios with mammals but did not exclude interaction studies conducted with isolated cell components, cells, or tissues. To check the accuracy of the electronic screening, a senior toxicologist examined a random sample of 929 of the excluded records. The sample of excluded records was selected using a publicly available random number generator (www.random.org). The toxicologist concluded that the excluded sample contained no relevant records.

An additional 245 potentially useful references were identified by authors of this report during full-text evaluations of the retrieved references and pertinent published reviews and government reports on the health effects and nutritional values of calcium and magnesium, selected from computer literature searches of the gray literature.

In addition, targeted supplemental PubMed searches were conducted in October 2020 for individual binary combinations of the metallic cations, which the previous search protocol had indicated were data poor (barium and calcium; barium and iron; barium and magnesium; barium and manganese; barium and sodium; barium and strontium; iron and sodium; iron and strontium; magnesium and sodium; magnesium and strontium; manganese and sodium; manganese and strontium; and sodium and strontium). These individual PubMed searches linked the elements' names to either "transport proteins," "interactions," or "gastrointestinal absorption." This search was successful in identifying many studies of interactions among the cations and various membrane transport systems in isolated membrane vesicles, cells, or tissues that the previous protocol had missed. From these searches, about 240 additional reports were selected for full text evaluation; a preference for the most recent reviews was used for the supplemental

searches identifying more than 300 reports (e.g., barium and calcium; barium and sodium; iron and sodium; magnesium and sodium; and manganese and sodium).

The query strings used for the literature searches are presented in Table J-2. Keywords used in the additional electronic screenings of the search results prior to manual screening are presented in Table J-3.

Table J-2. Database Query Strings

Database search date	Query string
PubMed	
5/2015	Barium ion or compound, and another chemical of concern: (((22541-12-4[rn] OR 543-80-6[rn] OR 513-77-9[rn] OR 10361-37-2[rn] OR 7727-43-7[rn] OR 6P669D8HQ8[rn] OR 0VK51DA1T2[rn] OR 25BB7EKE2E[rn]) AND ((15438-31-0[rn] OR 20074-52-6[rn] OR 563-71-3[rn] OR 10290-71-8[rn] OR 7705-08-0[rn] OR 7758-94-3[rn] OR 12040-57-2[rn] OR 16480-60-7[rn] OR 23444-30-6[rn] OR 2140-52-5[rn] OR 1834-30-6[rn] OR 7720-78-7[rn] OR 10028-22-5[rn] OR 10124-49-9[rn] OR 16547-58-3[rn] OR U38V3ZVV3V[rn] OR S3Y25PHP1W[rn] OR CZZ8832SI5[rn] OR 39R4TAN1VT[rn] OR 3HWS7HF5XD[rn] OR 3HWS7HF5XD[rn]) OR (19768-33-3[rn] OR 7773-01-5[rn] OR 7785-87-7[rn] OR 598-62-9[rn] OR 638-38-0[rn] OR QQE170PANO[rn] OR 9ZV57512ZM[rn]) OR (17341-25-2[rn] OR 127-09-3[rn] OR 6131-90-4[rn] OR 497-19-8[rn] OR 5968-11-6[rn] OR 7647-14-5[rn] OR 7727-73-3[rn] OR 7757-82-6[rn] OR 15124-09-1[rn] OR 4550K0SC9B[rn] OR 45P3261C7T[rn] OR 45P3261C7T[rn] OR 7647-14-5[rn] OR 0YPR65R21J[rn] OR 0YPR65R21J[rn] OR 0YPR65R21J[rn]) OR (22537-39-9[rn] OR 543-94-2[rn] OR 1633-05-2[rn] OR 10476-85-4[rn] OR 7759-02-6[rn] OR 4SL32YMY7B[rn] OR 41YPU4MMCA[rn] OR EKE8PS9J6Z[rn]))))) OR (((Barite[tw] OR Barium[tw] OR Baritop[tw] OR "E-Z-CAT"[tw] OR "Micropaque Oral"[tw]) NOT medline[sb]) AND (((Iron[tw] OR Ferrous[tw] OR Ferric [tw] OR Lawrencite[tw] OR Polyferric[tw] OR Aktiferrin[tw] OR Biofer[tw] OR Ceferro[tw] OR Conferon[tw] OR "Eisendragees-ratiopharm"[tw] OR "Eisensulfat Stada"[tw] OR Feospan[tw] OR "Fer-Gen-Sol"[tw] OR "Fer-in-Sol"[tw] OR "Feratab"[tw] OR "Fero-Gradumet"[tw] OR "Ferodan"[tw] OR "Ferogradumet"[tw] OR "Ferro-Gradumet"[tw] OR "Ferrogamma"[tw] OR "Ferrograd"[tw] OR "FERROinfant"[tw] OR "Haemoprotect"[tw] OR "Hämatopan"[tw] OR "Hemobion"[tw] OR "Hemofer"[tw] OR "Kendural"[tw] OR "Mol-Iron"[tw] OR "Plastufer"[tw] OR "Slow-Fe"[tw] OR "Vitaferro Kapseln"[tw]) NOT medline[sb]) OR (("Manganese"[tw] OR "Manganous chloride"[tw] OR "Scacchite"[tw] OR "Manganous carbonate"[tw] OR "Rhodochrosite"[tw] OR "Manganous acetate"[tw] OR "MnCl2"[tw]) NOT medline[sb]) OR (("Bisodium carbonate"[tw] OR "Bisodium sulfate"[tw] OR "Disodium carbonate"[tw] OR "Disodium monosulfate"[tw] OR "Disodium sulfate"[tw] OR "Disodium sulphate"[tw] OR "Na sulphate"[tw] OR "Sodium"[tw] OR "Trisodium trichloride"[tw] OR "Saline Solution"[tw] OR "Mangxiao"[tw] OR "mirabilitum"[tw] OR "natrii sulphas"[tw] OR "puxiao"[tw] OR "thenardite"[tw]) NOT medline[sb]) OR ((Strontium[tw] OR Strontianite[tw] OR Metastron[tw]) NOT medline[sb]))))
5/2015	Iron ion or compound, and another chemical of concern other than barium: (((15438-31-0[rn] OR 20074-52-6[rn] OR 563-71-3[rn] OR 10290-71-8[rn] OR 7705-08-0[rn] OR 7758-94-3[rn] OR 12040-57-2[rn] OR 16480-60-7[rn] OR 23444-30-6[rn] OR 2140-52-5[rn] OR 1834-30-6[rn] OR 7720-78-7[rn] OR 10028-22-5[rn] OR 10124-49-9[rn] OR 16547-58-3[rn] OR U38V3ZVV3V[rn] OR S3Y25PHP1W[rn] OR CZZ8832SI5[rn] OR 39R4TAN1VT[rn] OR 3HWS7HF5XD[rn] OR 3HWS7HF5XD[rn]) AND ((19768-33-3[rn] OR 7773-01-5[rn] OR 7785-87-7[rn] OR 598-62-9[rn] OR 638-38-0[rn] OR QQE170PANO[rn]

Table J-2. Database Query Strings

Database search date	Query string
	OR 9ZV57512ZM[rn]) OR (17341-25-2[rn] OR 127-09-3[rn] OR 6131-90-4[rn] OR 497-19-8[rn] OR 5968-11-6[rn] OR 7647-14-5[rn] OR 7727-73-3[rn] OR 7757-82-6[rn] OR 15124-09-1[rn] OR 4550K0SC9B[rn] OR 45P3261C7T[rn] OR 45P3261C7T[rn] OR 7647-14-5[rn] OR 0YPR65R21J[rn] OR 0YPR65R21J[rn] OR 0YPR65R21J[rn]) OR (22537-39-9[rn] OR 543-94-2[rn] OR 1633-05-2[rn] OR 10476-85-4[rn] OR 7759-02-6[rn] OR 4SL32YMY7B[rn] OR 41YPU4MMCA[rn] OR EKE8PS9J6Z[rn]))))) OR (((Iron[tw] OR Ferrous[tw] OR Ferric [tw] OR Lawrencite[tw] OR Polyferric[tw] OR Aktiferrin[tw] OR Biofer[tw] OR Ceferro[tw] OR Conferon[tw] OR "Eisendragees-ratiopharm"[tw] OR "Eisensulfat Stada"[tw] OR Feospan[tw] OR "Fer-Gen-Sol"[tw] OR "Fer-in-Sol"[tw] OR "Feratab"[tw] OR "Fero-Gradumet"[tw] OR "Ferodan"[tw] OR "Ferogradumet"[tw] OR "Ferro-Gradumet"[tw] OR "Ferrogamma"[tw] OR "Ferrograd"[tw] OR "FERROinfant"[tw] OR "Haemoprotect"[tw] OR "Hämatopan"[tw] OR "Hemobion"[tw] OR "Hemofer"[tw] OR "Kendural"[tw] OR "Mol-Iron"[tw] OR "Plastufer"[tw] OR "Slow-Fe"[tw] OR "Vitaferro Kapseln"[tw]) NOT medline[sb]) AND (("Manganese"[tw] OR "Manganous chloride"[tw] OR "Scacchite"[tw] OR "Manganous carbonate"[tw] OR "Rhodochrosite"[tw] OR "Manganous acetate"[tw] OR "MnCl2"[tw]) NOT medline[sb]) OR (("Bisodium carbonate"[tw] OR "Bisodium sulfate"[tw] OR "Disodium carbonate"[tw] OR "Disodium monosulfate"[tw] OR "Disodium sulfate"[tw] OR "Disodium sulphate"[tw] OR "Na sulphate"[tw] OR "Sodium"[tw] OR "Trisodium trichloride"[tw] OR "Saline Solution"[tw] OR "Mangxiao"[tw] OR "mirabilitum"[tw] OR "natrii sulphas"[tw] OR "puxiao"[tw] OR "thenardite"[tw]) NOT medline[sb]) OR ((Strontium[tw] OR Strontianite[tw] OR Metastron[tw]) NOT medline[sb]))
5/2015	Manganese ion or compound, and another chemical of concern other than barium or iron: (((19768-33-3[rn] OR 7773-01-5[rn] OR 7785-87-7[rn] OR 598-62-9[rn] OR 638-38-0[rn] OR QQE170PANO[rn] OR 9ZV57512ZM[rn]) AND ((17341-25-2[rn] OR 127-09-3[rn] OR 6131-90-4[rn] OR 497-19-8[rn] OR 5968-11-6[rn] OR 7647-14-5[rn] OR 7727-73-3[rn] OR 7757-82-6[rn] OR 15124-09-1[rn] OR 4550K0SC9B[rn] OR 45P3261C7T[rn] OR 45P3261C7T[rn] OR 7647-14-5[rn] OR 0YPR65R21J[rn] OR 0YPR65R21J[rn] OR 0YPR65R21J[rn]) OR (22537-39-9[rn] OR 543-94-2[rn] OR 1633-05-2[rn] OR 10476-85-4[rn] OR 7759-02-6[rn] OR 4SL32YMY7B[rn] OR 41YPU4MMCA[rn] OR EKE8PS9J6Z[rn]))))) OR ((("Manganese"[tw] OR "Manganous chloride"[tw] OR "Scacchite"[tw] OR "Manganous carbonate"[tw] OR "Rhodochrosite"[tw] OR "Manganous acetate"[tw] OR "MnCl2"[tw]) NOT medline[sb]) AND ((("Bisodium carbonate"[tw] OR "Bisodium sulfate"[tw] OR "Disodium carbonate"[tw] OR "Disodium monosulfate"[tw] OR "Disodium sulfate"[tw] OR "Disodium sulphate"[tw] OR "Na sulphate"[tw] OR "Sodium"[tw] OR "Trisodium trichloride"[tw] OR "Saline Solution"[tw] OR "Mangxiao"[tw] OR "mirabilitum"[tw] OR "natrii sulphas"[tw] OR "puxiao"[tw] OR "thenardite"[tw]) NOT medline[sb]) OR ((Strontium[tw] OR Strontianite[tw] OR Metastron[tw]) NOT medline[sb])))
5/2015	Sodium ion or compound, and another chemical of concern other than barium, iron or manganese: (((17341-25-2[rn] OR 127-09-3[rn] OR 6131-90-4[rn] OR 497-19-8[rn] OR 5968-11-6[rn] OR 7647-14-5[rn] OR 7727-73-3[rn] OR 7757-82-6[rn] OR 15124-09-1[rn] OR 4550K0SC9B[rn] OR 45P3261C7T[rn] OR 45P3261C7T[rn] OR 7647-14-5[rn] OR 0YPR65R21J[rn] OR 0YPR65R21J[rn] OR 0YPR65R21J[rn]) AND (22537-39-9[rn] OR 543-94-2[rn] OR 1633-05-2[rn] OR 10476-85-4[rn] OR 7759-02-6[rn] OR 4SL32YMY7B[rn] OR 41YPU4MMCA[rn] OR EKE8PS9J6Z[rn]))) OR ((("Bisodium carbonate"[tw] OR "Bisodium sulfate"[tw] OR "Disodium carbonate"[tw] OR "Disodium monosulfate"[tw] OR "Disodium sulfate"[tw] OR "Disodium sulphate"[tw] OR "Na sulphate"[tw] OR "Sodium"[tw] OR "Trisodium trichloride"[tw] OR "Saline Solution"[tw] OR "Mangxiao"[tw] OR "mirabilitum"[tw] OR "natrii sulphas"[tw] OR "puxiao"[tw] OR "thenardite"[tw]) NOT medline[sb]) AND ((Strontium[tw] OR Strontianite[tw] OR Metastron[tw]) NOT medline[sb]))

Table J-2. Database Query Strings

Database search date	Query string
10/2018	Barium ion or compound, and another chemical of concern: ((((((22541-12-4[rn] OR 543-80-6[rn] OR 513-77-9[rn] OR 10361-37-2[rn] OR 7727-43-7[rn] OR 6P669D8HQ8[rn] OR 0VK51DA1T2[rn] OR 25BB7EKE2E[rn]) AND ((15438-31-0[rn] OR 20074-52-6[rn] OR 563-71-3[rn] OR 10290-71-8[rn] OR 7705-08-0[rn] OR 7758-94-3[rn] OR 12040-57-2[rn] OR 16480-60-7[rn] OR 23444-30-6[rn] OR 2140-52-5[rn] OR 1834-30-6[rn] OR 7720-78-7[rn] OR 10028-22-5[rn] OR 10124-49-9[rn] OR 16547-58-3[rn] OR U38V3ZVV3V[rn] OR S3Y25PHP1W[rn] OR CZZ8832SI5[rn] OR 39R4TAN1VT[rn] OR 3HWS7HF5XD[rn] OR 3HWS7HF5XD[rn]) OR (19768-33-3[rn] OR 7773-01-5[rn] OR 7785-87-7[rn] OR 598-62-9[rn] OR 638-38-0[rn] OR QQE170PANO[rn] OR 9ZV57512ZM[rn]) OR (17341-25-2[rn] OR 127-09-3[rn] OR 6131-90-4[rn] OR 497-19-8[rn] OR 5968-11-6[rn] OR 7647-14-5[rn] OR 7727-73-3[rn] OR 7757-82-6[rn] OR 15124-09-1[rn] OR 4550K0SC9B[rn] OR 45P3261C7T[rn] OR 45P3261C7T[rn] OR 7647-14-5[rn] OR 0YPR65R21J[rn] OR 0YPR65R21J[rn] OR 0YPR65R21J[rn]) OR (22537-39-9[rn] OR 543-94-2[rn] OR 1633-05-2[rn] OR 10476-85-4[rn] OR 7759-02-6[rn] OR 4SL32YMY7B[rn] OR 41YPU4MMCA[rn] OR EKE8PS9J6Z[rn])))) OR (((Barite[ot] OR Barium[ot] OR Baritop[ot] OR "E-Z-CAT"[ot] OR "Micropaque Oral"[ot])) AND (((Iron[ot] OR Ferrous[ot] OR Ferric [ot] OR Lawrencite[ot] OR Polyferric[ot] OR Aktiferrin[ot] OR Biofer[ot] OR Ceferro[ot] OR Conferon[ot] OR "Eisendragees-ratiopharm"[ot] OR "Eisensulfat Stada"[ot] OR Feospan[ot] OR "Fer-Gen-Sol"[ot] OR "Fer-in-Sol"[ot] OR "Feratab"[ot] OR "Fero-Gradumet"[ot] OR "Ferodan"[ot] OR "Ferogradumet"[ot] OR "Ferro-Gradumet"[ot] OR "Ferrogamma"[ot] OR "Ferrograd"[ot] OR "FERROinfant"[ot] OR "Haemoprotect"[ot] OR "Hämatopan"[ot] OR "Hemobion"[ot] OR "Hemofer"[ot] OR "Kendural"[ot] OR "Mol-Iron"[ot] OR "Plastufer"[ot] OR "Slow-Fe"[ot] OR "Vitaferro Kapseln"[ot])) OR (("Manganese"[ot] OR "Manganous chloride"[ot] OR "Scacchite"[ot] OR "Manganous carbonate"[ot] OR "Rhodochrosite"[ot] OR "Manganous acetate"[ot] OR "MnCl2"[ot])) OR (("Bisodium carbonate"[ot] OR "Bisodium sulfate"[ot] OR "Disodium carbonate"[ot] OR "Disodium monosulfate"[ot] OR "Disodium sulfate"[ot] OR "Disodium sulphate"[ot] OR "Na sulphate"[ot] OR "Sodium"[ot] OR "Trisodium trichloride"[ot] OR "Saline Solution"[ot] OR "Mangxiao"[ot] OR "mirabilitum"[ot] OR "natrii sulphas"[ot] OR "puxiao"[ot] OR "thenardite"[ot])) OR ((Strontium[ot] OR Strontianite[ot] OR Metastron[ot])))) OR (((Barite[tiab] OR Barium[tiab] OR Baritop[tiab] OR "E-Z-CAT"[tiab] OR "Micropaque Oral"[tiab])) AND (((Iron[tiab] OR Ferrous[tiab] OR Ferric [tiab] OR Lawrencite[tiab] OR Polyferric[tiab] OR Aktiferrin[tiab] OR Biofer[tiab] OR Ceferro[tiab] OR Conferon[tiab] OR "Eisendragees-ratiopharm"[tiab] OR "Eisensulfat Stada"[tiab] OR Feospan[tiab] OR "Fer-Gen-Sol"[tiab] OR "Fer-in-Sol"[tiab] OR "Feratab"[tiab] OR "Fero-Gradumet"[tiab] OR "Ferodan"[tiab] OR "Ferogradumet"[tiab] OR "Ferro-Gradumet"[tiab] OR "Ferrogamma"[tiab] OR "Ferrograd"[tiab] OR "FERROinfant"[tiab] OR "Haemoprotect"[tiab] OR "Hämatopan"[tiab] OR "Hemobion"[tiab] OR "Hemofer"[tiab] OR "Kendural"[tiab] OR "Mol-Iron"[tiab] OR "Plastufer"[tiab] OR "Slow-Fe"[tiab] OR "Vitaferro Kapseln"[tiab])) OR (("Manganese"[tiab] OR "Manganous chloride"[tiab] OR "Scacchite"[tiab] OR "Manganous carbonate"[tiab] OR "Rhodochrosite"[tiab] OR "Manganous acetate"[tiab] OR "MnCl2"[tiab])) OR (("Bisodium carbonate"[tiab] OR "Bisodium sulfate"[tiab] OR "Disodium carbonate"[tiab] OR "Disodium monosulfate"[tiab] OR "Disodium sulfate"[tiab] OR "Disodium sulphate"[tiab] OR "Na sulphate"[tiab] OR "Sodium"[tiab] OR "Trisodium trichloride"[tiab] OR "Saline Solution"[tiab] OR "Mangxiao"[tiab] OR "mirabilitum"[tiab] OR "natrii sulphas"[tiab] OR "puxiao"[tiab] OR "thenardite"[tiab])) OR ((Strontium[tiab] OR Strontianite[tiab] OR Metastron[tiab]))))) AND (2014/05/01:3000[dp] OR 2015/05/01:3000[mhda] OR 2015/05/01:3000[crdat] OR 2015/05/01:3000[edat])) AND (additiv* OR antagonis* OR inhibit* OR mask* OR potentiat* OR synergis* OR "joint action" OR interact* OR combin* OR transport*)) NOT ("aeruginosa"[tw] OR "anguillarum"[tw] OR "anophagefferens"[tw] OR "aureococcus"[tw]

Table J-2. Database Query Strings

Database search date	Query string
	OR "azobacter"[tw] OR "azotobacter"[tw] OR "bacillus"[tw] OR "bacteria"[tw] OR "bacterium"[tw] OR "barkeri"[tw] OR "campylobacter"[tw] OR "chlorella"[tw] OR "crassa"[tw] OR "cryptococcus"[tw] OR "elongatus"[tw] OR "enterococcus"[tw] OR "escherichia"[tw] OR "faecalis"[tw] OR "falciparum"[tw] OR "flexneri"[tw] OR "fungi"[tw] OR "fungus"[tw] OR "fusarium"[tw] OR "helicobacter"[tw] OR "hyphomycetes"[tw] OR "methanosarcina"[tw] OR "microbial"[tw] OR "microcystus"[tw] OR "microorganism"[tw] OR "neurospora"[tw] OR "penicillium"[tw] OR "pestis"[tw] OR "plasmodium"[tw] OR "pseudomonas"[tw] OR "pylori"[tw] OR "saccharomyces"[tw] OR "shewanella"[tw] OR "shigella"[tw] OR "siderophore"[tw] OR "streptococcus"[tw] OR "thermosynechococcus"[tw] OR "trichothecium"[tw] OR "tuberculosis"[tw] OR "vibrio"[tw] OR "vinelandii"[tw] OR "yeast"[tw] OR "yersinia"[tw] OR "salmonella"[tw] OR "typhimurium"[tw] OR "plankton"[tw] OR "arum maculatum"[tw] OR "phaseolus lunatus"[tw] OR "cissus populnea"[tw] OR "potentilla recta"[tw] OR "zingiber officinale"[tw] OR "aframomum danielli"[tw] OR "lantana camara"[tw] OR "solanum torvum"[tw] OR "eugenia uniflora"[tw] OR "tribulus terrestris"[tw] OR "leucaena leucocephala"[tw] OR "dichapetalum madagascasiense"[tw] OR "funtumia elastica"[tw] OR "mallotus oppositifolius"[tw] OR "coli"[tw] OR "legume"[tw] OR "legumes"[tw] OR "plant"[tw] OR "plants"[tw] OR "pea"[tw] OR "peas"[tw])
10/2018	Calcium ion or compound, and another chemical of concern (((((14127-61-8[rn] OR 62-54-4[rn] OR 471-34-1[rn] OR 10043-52-4[rn] OR 10035-04-8[rn] OR 7778-18-9[rn] OR Y882YXF34X[rn] OR H0G9379FGK[rn] OR M4I0D6VV5M[rn] OR WAT0DDB505[rn]) AND ((22537-22-0[rn] OR 142-72-3[rn] OR 546-93-0[rn] OR 17968-26-2[rn] OR 23389-33-5[rn] OR 7786-30-3[rn] OR 7791-18-6[rn] OR 14989-29-8[rn] OR 7487-88-9[rn] OR 10034-99-8[rn] OR 18939-43-0[rn] OR 0E53J927NA[rn] OR 02F3473H9O[rn]) OR (22541-12-4[rn] OR 543-80-6[rn] OR 513-77-9[rn] OR 10361-37-2[rn] OR 7727-43-7[rn] OR 6P669D8HQ8[rn] OR 0VK51DA1T2[rn] OR 25BB7EKE2E[rn]) OR (15438-31-0[rn] OR 20074-52-6[rn] OR 563-71-3[rn] OR 10290-71-8[rn] OR 7705-08-0[rn] OR 7758-94-3[rn] OR 12040-57-2[rn] OR 16480-60-7[rn] OR 23444-30-6[rn] OR 2140-52-5[rn] OR 1834-30-6[rn] OR 7720-78-7[rn] OR 10028-22-5[rn] OR 10124-49-9[rn] OR 16547-58-3[rn] OR U38V3ZVV3V[rn] OR S3Y25PHP1W[rn] OR CZZ8832SI5[rn] OR 39R4TAN1VT[rn] OR 3HWS7HF5XD[rn] OR 3HWS7HF5XD[rn]) OR (19768-33-3[rn] OR 7773-01-5[rn] OR 7785-87-7[rn] OR 598-62-9[rn] OR 638-38-0[rn] OR QQE170PANO[rn] OR 9ZV57512ZM[rn]) OR (17341-25-2[rn] OR 127-09-3[rn] OR 6131-90-4[rn] OR 497-19-8[rn] OR 5968-11-6[rn] OR 7647-14-5[rn] OR 7727-73-3[rn] OR 7757-82-6[rn] OR 15124-09-1[rn] OR 4550K0SC9B[rn] OR 45P3261C7T[rn] OR 45P3261C7T[rn] OR 7647-14-5[rn] OR 0YPR65R21J[rn] OR 0YPR65R21J[rn] OR 0YPR65R21J[rn]) OR (22537-39-9[rn] OR 543-94-2[rn] OR 1633-05-2[rn] OR 10476-85-4[rn] OR 7759-02-6[rn] OR 4SL32YMY7B[rn] OR 41YPU4MMCA[rn] OR EKE8PS9J6Z[rn])))) OR (((Calcium[ot] OR "Monocalcium carbonate"[ot] OR "Acetate of lime"[ot] OR Anhydrite[ot] OR "Anhydrous sulfate of lime"[ot] OR Gypsum[ot] OR Karstenite[ot] OR "Lime acetate"[ot] OR Muriacite[ot] OR Phoslo[ot] OR "Slaker rejects"[ot] OR Aragonite[ot] OR Calcite[ot] OR Chalk[ot] OR Limestone[ot] OR Marble[ot] OR Vaterite[ot])) AND ((Magnesium[ot] OR "Epsom salt"[ot] OR "Epsom salts"[ot] OR Hydromagnesite[ot] OR Magnesite[ot] OR nesquehonite[ot]) OR (Barite[ot] OR Barium[ot] OR Baritop[ot] OR "E-Z-CAT"[ot] OR "Micropaque Oral"[ot]) OR ((Iron[ot] OR Ferrous[ot] OR Ferric [ot] OR Lawrencite[ot] OR Polyferric[ot] OR Aktiferrin[ot] OR Biofer[ot] OR Ceferro[ot] OR Conferon[ot] OR "Eisendragees-ratiopharm"[ot] OR "Eisensulfat Stada"[ot] OR Feospan[ot] OR "Fer-Gen-Sol"[ot] OR "Fer-in-Sol"[ot] OR "Feratab"[ot] OR "Fero-Gradumet"[ot] OR "Ferodan"[ot] OR "Ferogradumet"[ot] OR "Ferro-Gradumet"[ot] OR "Ferrogamma"[ot] OR "Ferrograd"[ot] OR "FERROinfant"[ot] OR "Haemoprotect"[ot] OR "Hämatopan"[ot] OR "Hemobion"[ot] OR "Hemofer"[ot] OR "Kendural"[ot] OR "Mol-Iron"[ot] OR "Plastufer"[ot] OR "Slow-Fe"[ot] OR "Vitaferro

Table J-2. Database Query Strings

Database search date	Query string
	Kapseln"[ot])) OR (("Manganese"[ot] OR "Manganous chloride"[ot] OR "Scacchite"[ot] OR "Manganous carbonate"[ot] OR "Rhodochrosite"[ot] OR "Manganous acetate"[ot] OR "MnCl2"[ot])) OR (("Bisodium carbonate"[ot] OR "Bisodium sulfate"[ot] OR "Disodium carbonate"[ot] OR "Disodium monosulfate"[ot] OR "Disodium sulfate"[ot] OR "Disodium sulphate"[ot] OR "Na sulphate"[ot] OR "Sodium"[ot] OR "Trisodium trichloride"[ot] OR "Saline Solution"[ot] OR "Mangxiao"[ot] OR "mirabilitum"[ot] OR "natrii sulphas"[ot] OR "puxiao"[ot] OR "thenardite"[ot])) OR ((Strontium[ot] OR Strontianite[ot] OR Metastron[ot])))) OR (((Calcium[tiab] OR "Monocalcium carbonate"[tiab] OR "Acetate of lime"[tiab] OR Anhydrite[tiab] OR "Anhydrous sulfate of lime"[tiab] OR Gypsum[tiab] OR Karstenite[tiab] OR "Lime acetate"[tiab] OR Muriacite[tiab] OR Phoslo[tiab] OR "Slaker rejects"[tiab] OR Aragonite[tiab] OR Calcite[tiab] OR Chalk[tiab] OR Limestone[tiab] OR Marble[tiab] OR Vaterite[tiab])) AND ((Magnesium[tiab] OR "Epsom salt"[tiab] OR "Epsom salts"[tiab] OR Hydromagnesite[tiab] OR Magnesite[tiab] OR nesquehonite[tiab]) OR (Barite[tiab] OR Barium[tiab] OR Baritop[tiab] OR "E-Z-CAT"[tiab] OR "Micropaque Oral"[tiab]) OR ((Iron[tiab] OR Ferrous[tiab] OR Ferric [tiab] OR Lawrencite[tiab] OR Polyferric[tiab] OR Aktiferrin[tiab] OR Biofer[tiab] OR Ceferro[tiab] OR Conferon[tiab] OR "Eisendragees-ratiopharm"[tiab] OR "Eisensulfat Stada"[tiab] OR Feospan[tiab] OR "Fer-Gen-Sol"[tiab] OR "Fer-in-Sol"[tiab] OR "Feratab"[tiab] OR "Fero-Gradumet"[tiab] OR "Ferodan"[tiab] OR "Ferogradumet"[tiab] OR "Ferro-Gradumet"[tiab] OR "Ferrogamma"[tiab] OR "Ferrograd"[tiab] OR "FERROinfant"[tiab] OR "Haemoprotect"[tiab] OR "Hämatopan"[tiab] OR "Hemobion"[tiab] OR "Hemofer"[tiab] OR "Kendural"[tiab] OR "Mol-Iron"[tiab] OR "Plastufer"[tiab] OR "Slow-Fe"[tiab] OR "Vitaferro Kapseln"[tiab])) OR (("Manganese"[tiab] OR "Manganous chloride"[tiab] OR "Scacchite"[tiab] OR "Manganous carbonate"[tiab] OR "Rhodochrosite"[tiab] OR "Manganous acetate"[tiab] OR "MnCl2"[tiab])) OR (("Bisodium carbonate"[tiab] OR "Bisodium sulfate"[tiab] OR "Disodium carbonate"[tiab] OR "Disodium monosulfate"[tiab] OR "Disodium sulfate"[tiab] OR "Disodium sulphate"[tiab] OR "Na sulphate"[tiab] OR "Sodium"[tiab] OR "Trisodium trichloride"[tiab] OR "Saline Solution"[tiab] OR "Mangxiao"[tiab] OR "mirabilitum"[tiab] OR "natrii sulphas"[tiab] OR "puxiao"[tiab] OR "thenardite"[tiab])) OR ((Strontium[tiab] OR Strontianite[tiab] OR Metastron[tiab]))))) AND (additiv* OR antagonis* OR inhibit* OR mask* OR potentiat* OR synergis* OR "joint action" OR interact* OR combin* OR transport*)) NOT ("aeruginosa"[tw] OR "anguillarum"[tw] OR "anophagefferens"[tw] OR "aureococcus"[tw] OR "azobacter"[tw] OR "azotobacter"[tw] OR "bacillus"[tw] OR "bacteria"[tw] OR "bacterium"[tw] OR "barkeri"[tw] OR "campylobacter"[tw] OR "chlorella"[tw] OR "crassa"[tw] OR "cryptococcus"[tw] OR "elongatus"[tw] OR "enterococcus"[tw] OR "escherichia"[tw] OR "faecalis"[tw] OR "falciparum"[tw] OR "flexneri"[tw] OR "fungi"[tw] OR "fungus"[tw] OR "fusarium"[tw] OR "helicobacter"[tw] OR "hyphomycetes"[tw] OR "methanosarcina"[tw] OR "microbial"[tw] OR "microcystus"[tw] OR "microorganism"[tw] OR "neurospora"[tw] OR "penicillium"[tw] OR "pestis"[tw] OR "plasmodium"[tw] OR "pseudomonas"[tw] OR "pylori"[tw] OR "saccharomyces"[tw] OR "shewanella"[tw] OR "shigella"[tw] OR "siderophore"[tw] OR "streptococcus"[tw] OR "thermosynechococcus"[tw] OR "trichothecium"[tw] OR "tuberculosis"[tw] OR "vibrio"[tw] OR "vinelandii"[tw] OR "yeast"[tw] OR "yersinia"[tw] OR "salmonella"[tw] OR "typhimurium"[tw] OR "plankton"[tw] OR "arum maculatum"[tw] OR "phaseolus lunatus"[tw] OR "cissus populnea"[tw] OR "potentilla recta"[tw] OR "zingiber officinale"[tw] OR "aframomum danielli"[tw] OR "lantana camara"[tw] OR "solanum torvum"[tw] OR "eugenia uniflora"[tw] OR "tribulus terrestris"[tw] OR "leucaena leucocephala"[tw] OR "dichapetalum madagascasiense"[tw] OR "funtumia elastica"[tw] OR "mallotus oppositifolius"[tw] OR "coli"[tw] OR "legume"[tw] OR "legumes"[tw] OR "plant"[tw] OR "plants"[tw] OR "pea"[tw] OR "peas"[tw])

Table J-2. Database Query Strings

Database search date	Query string
10/2018	Iron ion or compound, and another chemical of concern other than barium: ((((((15438-31-0[rn] OR 20074-52-6[rn] OR 563-71-3[rn] OR 10290-71-8[rn] OR 7705-08-0[rn] OR 7758-94-3[rn] OR 12040-57-2[rn] OR 16480-60-7[rn] OR 23444-30-6[rn] OR 2140-52-5[rn] OR 1834-30-6[rn] OR 7720-78-7[rn] OR 10028-22-5[rn] OR 10124-49-9[rn] OR 16547-58-3[rn] OR U38V3ZVV3V[rn] OR S3Y25PHP1W[rn] OR CZZ8832SI5[rn] OR 39R4TAN1VT[rn] OR 3HWS7HF5XD[rn] OR 3HWS7HF5XD[rn]) AND ((19768-33-3[rn] OR 7773-01-5[rn] OR 7785-87-7[rn] OR 598-62-9[rn] OR 638-38-0[rn] OR QQE170PANO[rn] OR 9ZV57512ZM[rn]) OR (17341-25-2[rn] OR 127-09-3[rn] OR 6131-90-4[rn] OR 497-19-8[rn] OR 5968-11-6[rn] OR 7647-14-5[rn] OR 7727-73-3[rn] OR 7757-82-6[rn] OR 15124-09-1[rn] OR 4550K0SC9B[rn] OR 45P3261C7T[rn] OR 45P3261C7T[rn] OR 7647-14-5[rn] OR 0YPR65R21J[rn] OR 0YPR65R21J[rn] OR 0YPR65R21J[rn]) OR (22537-39-9[rn] OR 543-94-2[rn] OR 1633-05-2[rn] OR 10476-85-4[rn] OR 7759-02-6[rn] OR 4SL32YMY7B[rn] OR 41YPU4MMCA[rn] OR EKE8PS9J6Z[rn]))))) OR (((Iron[ot] OR Ferrous[ot] OR Ferric [ot] OR Lawrencite[ot] OR Polyferric[ot] OR Aktiferrin[ot] OR Biofer[ot] OR Ceferro[ot] OR Conferon[ot] OR "Eisendragees-ratiopharm"[ot] OR "Eisensulfat Stada"[ot] OR Feospan[ot] OR "Fer-Gen-Sol"[ot] OR "Fer-in-Sol"[ot] OR "Feratab"[ot] OR "Fero-Gradumet"[ot] OR "Ferodan"[ot] OR "Ferogradumet"[ot] OR "Ferro-Gradumet"[ot] OR "Ferrogamma"[ot] OR "Ferrograd"[ot] OR "FERROinfant"[ot] OR "Haemoprotect"[ot] OR "Hämatopan"[ot] OR "Hemobion"[ot] OR "Hemofer"[ot] OR "Kendural"[ot] OR "Mol-Iron"[ot] OR "Plastufer"[ot] OR "Slow-Fe"[ot] OR "Vitaferro Kapseln"[ot])) AND ((("Manganese"[ot] OR "Manganous chloride"[ot] OR "Scacchite"[ot] OR "Manganous carbonate"[ot] OR "Rhodochrosite"[ot] OR "Manganous acetate"[ot] OR "MnCl2"[ot])) OR (("Bisodium carbonate"[ot] OR "Bisodium sulfate"[ot] OR "Disodium carbonate"[ot] OR "Disodium monosulfate"[ot] OR "Disodium sulfate"[ot] OR "Disodium sulphate"[ot] OR "Na sulphate"[ot] OR "Sodium"[ot] OR "Trisodium trichloride"[ot] OR "Saline Solution"[ot] OR "Mangxiao"[ot] OR "mirabilitum"[ot] OR "natrii sulphas"[ot] OR "puxiao"[ot] OR "thenardite"[ot])) OR ((Strontium[ot] OR Strontianite[ot] OR Metastron[ot])))) OR (((Iron[tiab] OR Ferrous[tiab] OR Ferric [tiab] OR Lawrencite[tiab] OR Polyferric[tiab] OR Aktiferrin[tiab] OR Biofer[tiab] OR Ceferro[tiab] OR Conferon[tiab] OR "Eisendragees-ratiopharm"[tiab] OR "Eisensulfat Stada"[tiab] OR Feospan[tiab] OR "Fer-Gen-Sol"[tiab] OR "Fer-in-Sol"[tiab] OR "Feratab"[tiab] OR "Fero-Gradumet"[tiab] OR "Ferodan"[tiab] OR "Ferogradumet"[tiab] OR "Ferro-Gradumet"[tiab] OR "Ferrogamma"[tiab] OR "Ferrograd"[tiab] OR "FERROinfant"[tiab] OR "Haemoprotect"[tiab] OR "Hämatopan"[tiab] OR "Hemobion"[tiab] OR "Hemofer"[tiab] OR "Kendural"[tiab] OR "Mol-Iron"[tiab] OR "Plastufer"[tiab] OR "Slow-Fe"[tiab] OR "Vitaferro Kapseln"[tiab])) AND ((("Manganese"[tiab] OR "Manganous chloride"[tiab] OR "Scacchite"[tiab] OR "Manganous carbonate"[tiab] OR "Rhodochrosite"[tiab] OR "Manganous acetate"[tiab] OR "MnCl2"[tiab])) OR (("Bisodium carbonate"[tiab] OR "Bisodium sulfate"[tiab] OR "Disodium carbonate"[tiab] OR "Disodium monosulfate"[tiab] OR "Disodium sulfate"[tiab] OR "Disodium sulphate"[tiab] OR "Na sulphate"[tiab] OR "Sodium"[tiab] OR "Trisodium trichloride"[tiab] OR "Saline Solution"[tiab] OR "Mangxiao"[tiab] OR "mirabilitum"[tiab] OR "natrii sulphas"[tiab] OR "puxiao"[tiab] OR "thenardite"[tiab])) OR ((Strontium[tiab] OR Strontianite[tiab] OR Metastron[tiab]))))) AND (2014/05/01:3000[dp] OR 2015/05/01:3000[mhda] OR 2015/05/01:3000[crdat] OR 2015/05/01:3000[edat])) AND (additiv* OR antagonis* OR inhibit* OR mask* OR potentiat* OR synergis* OR "joint action" OR interact* OR combin* OR transport*)) NOT ("aeruginosa"[tw] OR "anguillarum"[tw] OR "anophagefferens"[tw] OR "aureococcus"[tw] OR "azobacter"[tw] OR "azotobacter"[tw] OR "bacillus"[tw] OR "bacteria"[tw] OR "bacterium"[tw] OR "barkeri"[tw] OR "campylobacter"[tw] OR "chlorella"[tw] OR "crassa"[tw] OR "cryptococcus"[tw] OR "elongatus"[tw] OR "enterococcus"[tw] OR "escherichia"[tw] OR "faecalis"[tw] OR "falciparum"[tw] OR "flexneri"[tw] OR "fungi"[tw] OR "fungus"[tw] OR

Table J-2. Database Query Strings

Database search date	Query string
	"fusarium"[tw] OR "helicobacter"[tw] OR "hyphomycetes"[tw] OR "methanosarcina"[tw] OR "microbial"[tw] OR "microcystus"[tw] OR "microorganism"[tw] OR "neurospora"[tw] OR "penicillium"[tw] OR "pestis"[tw] OR "plasmodium"[tw] OR "pseudomonas"[tw] OR "pylori"[tw] OR "saccharomyces"[tw] OR "shewanella"[tw] OR "shigella"[tw] OR "siderophore"[tw] OR "streptococcus"[tw] OR "thermosynechococcus"[tw] OR "trichothecium"[tw] OR "tuberculosis"[tw] OR "vibrio"[tw] OR "vinelandii"[tw] OR "yeast"[tw] OR "yersinia"[tw] OR "salmonella"[tw] OR "typhimurium"[tw] OR "plankton"[tw] OR "arum maculatum"[tw] OR "phaseolus lunatus"[tw] OR "cissus populnea"[tw] OR "potentilla recta"[tw] OR "zingiber officinale"[tw] OR "aframomum danielli"[tw] OR "lantana camara"[tw] OR "solanum torvum"[tw] OR "eugenia uniflora"[tw] OR "tribulus terrestris"[tw] OR "leucaena leucocephala"[tw] OR "dichapetalum madagascasiense"[tw] OR "funtumia elastica"[tw] OR "mallotus oppositifolius"[tw] OR "coli"[tw] OR "legume"[tw] OR "legumes"[tw] OR "plant"[tw] OR "plants"[tw] OR "pea"[tw] OR "peas"[tw])
10/2018	Magnesium ion or compound, and another chemical of concern other than calcium: (((((22537-22-0[rn] OR 142-72-3[rn] OR 546-93-0[rn] OR 17968-26-2[rn] OR 23389-33-5[rn] OR 7786-30-3[rn] OR 7791-18-6[rn] OR 14989-29-8[rn] OR 7487-88-9[rn] OR 10034-99-8[rn] OR 18939-43-0[rn] OR 0E53J927NA[rn] OR 02F3473H9O[rn]) AND ((22541-12-4[rn] OR 543-80-6[rn] OR 513-77-9[rn] OR 10361-37-2[rn] OR 7727-43-7[rn] OR 6P669D8HQ8[rn] OR 0VK51DA1T2[rn] OR 25BB7EKE2E[rn]) OR (15438-31-0[rn] OR 20074-52-6[rn] OR 563-71-3[rn] OR 10290-71-8[rn] OR 7705-08-0[rn] OR 7758-94-3[rn] OR 12040-57-2[rn] OR 16480-60-7[rn] OR 23444-30-6[rn] OR 2140-52-5[rn] OR 1834-30-6[rn] OR 7720-78-7[rn] OR 10028-22-5[rn] OR 10124-49-9[rn] OR 16547-58-3[rn] OR U38V3ZVV3V[rn] OR S3Y25PHP1W[rn] OR CZZ8832SI5[rn] OR 39R4TAN1VT[rn] OR 3HWS7HF5XD[rn] OR 3HWS7HF5XD[rn]) OR (19768-33-3[rn] OR 7773-01-5[rn] OR 7785-87-7[rn] OR 598-62-9[rn] OR 638-38-0[rn] OR QQE170PANO[rn] OR 9ZV57512ZM[rn]) OR (17341-25-2[rn] OR 127-09-3[rn] OR 6131-90-4[rn] OR 497-19-8[rn] OR 5968-11-6[rn] OR 7647-14-5[rn] OR 7727-73-3[rn] OR 7757-82-6[rn] OR 15124-09-1[rn] OR 4550K0SC9B[rn] OR 45P3261C7T[rn] OR 45P3261C7T[rn] OR 7647-14-5[rn] OR 0YPR65R21J[rn] OR 0YPR65R21J[rn] OR 0YPR65R21J[rn]) OR (22537-39-9[rn] OR 543-94-2[rn] OR 1633-05-2[rn] OR 10476-85-4[rn] OR 7759-02-6[rn] OR 4SL32YMY7B[rn] OR 41YPU4MMCA[rn] OR EKE8PS9J6Z[rn]))))) OR (((Magnesium[ot] OR "Epsom salt"[ot] OR "Epsom salts"[ot] OR Hydromagnesite[ot] OR Magnesite[ot] OR nesquehonite[ot])) AND ((Barite[ot] OR Barium[ot] OR Baritop[ot] OR "E-Z-CAT"[ot] OR "Micropaque Oral"[ot]) OR ((Iron[ot] OR Ferrous[ot] OR Ferric [ot] OR Lawrencite[ot] OR Polyferric[ot] OR Aktiferrin[ot] OR Biofer[ot] OR Ceferro[ot] OR Conferon[ot] OR "Eisendragees-ratiopharm"[ot] OR "Eisensulfat Stada"[ot] OR Feospan[ot] OR "Fer-Gen-Sol"[ot] OR "Fer-in-Sol"[ot] OR "Feratab"[ot] OR "Fero-Gradumet"[ot] OR "Ferodan"[ot] OR "Ferogradumet"[ot] OR "Ferro-Gradumet"[ot] OR "Ferrogamma"[ot] OR "Ferrograd"[ot] OR "FERROinfant"[ot] OR "Haemoprotect"[ot] OR "Hämatopan"[ot] OR "Hemobion"[ot] OR "Hemofer"[ot] OR "Kendural"[ot] OR "Mol-Iron"[ot] OR "Plastufer"[ot] OR "Slow-Fe"[ot] OR "Vitaferro Kapseln"[ot])) OR (("Manganese"[ot] OR "Manganous chloride"[ot] OR "Scacchite"[ot] OR "Manganous carbonate"[ot] OR "Rhodochrosite"[ot] OR "Manganous acetate"[ot] OR "MnCl2"[ot])) OR (("Bisodium carbonate"[ot] OR "Bisodium sulfate"[ot] OR "Disodium carbonate"[ot] OR "Disodium monosulfate"[ot] OR "Disodium sulfate"[ot] OR "Disodium sulphate"[ot] OR "Na sulphate"[ot] OR "Sodium"[ot] OR "Trisodium trichloride"[ot] OR "Saline Solution"[ot] OR "Mangxiao"[ot] OR "mirabilitum"[ot] OR "natrii sulphas"[ot] OR "puxiao"[ot] OR "thenardite"[ot])) OR ((Strontium[ot] OR Strontianite[ot] OR Metastron[ot])))) OR (((Magnesium[tiab] OR "Epsom salt"[tiab] OR "Epsom salts"[tiab] OR Hydromagnesite[tiab] OR Magnesite[tiab] OR nesquehonite[tiab])) AND ((Barite[tiab] OR Barium[tiab] OR Baritop[tiab] OR "E-Z-CAT"[tiab] OR "Micropaque Oral"[tiab]) OR

Table J-2. Database Query Strings

Database search date	Query string
	((Iron[tiab] OR Ferrous[tiab] OR Ferric [tiab] OR Lawrencite[tiab] OR Polyferric[tiab] OR Aktiferrin[tiab] OR Biofer[tiab] OR Ceferro[tiab] OR Conferon[tiab] OR "Eisendragees-ratiopharm"[tiab] OR "Eisensulfat Stada"[tiab] OR Feospan[tiab] OR "Fer-Gen-Sol"[tiab] OR "Fer-in-Sol"[tiab] OR "Feratab"[tiab] OR "Fero-Gradumet"[tiab] OR "Ferodan"[tiab] OR "Ferogradumet"[tiab] OR "Ferro-Gradumet"[tiab] OR "Ferrogamma"[tiab] OR "Ferrograd"[tiab] OR "FERROinfant"[tiab] OR "Haemoprotect"[tiab] OR "Hämatopan"[tiab] OR "Hemobion"[tiab] OR "Hemofer"[tiab] OR "Kendural"[tiab] OR "Mol-Iron"[tiab] OR "Plastufer"[tiab] OR "Slow-Fe"[tiab] OR "Vitaferro Kapseln"[tiab])) OR (("Manganese"[tiab] OR "Manganous chloride"[tiab] OR "Scacchite"[tiab] OR "Manganous carbonate"[tiab] OR "Rhodochrosite"[tiab] OR "Manganous acetate"[tiab] OR "MnCl2"[tiab])) OR (("Bisodium carbonate"[tiab] OR "Bisodium sulfate"[tiab] OR "Disodium carbonate"[tiab] OR "Disodium monosulfate"[tiab] OR "Disodium sulfate"[tiab] OR "Disodium sulphate"[tiab] OR "Na sulphate"[tiab] OR "Sodium"[tiab] OR "Trisodium trichloride"[tiab] OR "Saline Solution"[tiab] OR "Mangxiao"[tiab] OR "mirabilitum"[tiab] OR "natrii sulphas"[tiab] OR "puxiao"[tiab] OR "thenardite"[tiab])) OR ((Strontium[tiab] OR Strontianite[tiab] OR Metastron[tiab]))))) AND (additiv* OR antagonis* OR inhibit* OR mask* OR potentiat* OR synergis* OR "joint action" OR interact* OR combin* OR transport*)) NOT ("aeruginosa"[tw] OR "anguillarum"[tw] OR "anophagefferens"[tw] OR "aureococcus"[tw] OR "azobacter"[tw] OR "azotobacter"[tw] OR "bacillus"[tw] OR "bacteria"[tw] OR "bacterium"[tw] OR "barkeri"[tw] OR "campylobacter"[tw] OR "chlorella"[tw] OR "crassa"[tw] OR "cryptococcus"[tw] OR "elongatus"[tw] OR "enterococcus"[tw] OR "escherichia"[tw] OR "faecalis"[tw] OR "falciparum"[tw] OR "flexneri"[tw] OR "fungi"[tw] OR "fungus"[tw] OR "fusarium"[tw] OR "helicobacter"[tw] OR "hyphomycetes"[tw] OR "methanosarcina"[tw] OR "microbial"[tw] OR "microcystus"[tw] OR "microorganism"[tw] OR "neurospora"[tw] OR "penicillium"[tw] OR "pestis"[tw] OR "plasmodium"[tw] OR "pseudomonas"[tw] OR "pylori"[tw] OR "saccharomyces"[tw] OR "shewanella"[tw] OR "shigella"[tw] OR "siderophore"[tw] OR "streptococcus"[tw] OR "thermosynechococcus"[tw] OR "trichothecium"[tw] OR "tuberculosis"[tw] OR "vibrio"[tw] OR "vinelandii"[tw] OR "yeast"[tw] OR "yersinia"[tw] OR "salmonella"[tw] OR "typhimurium"[tw] OR "plankton"[tw] OR "arum maculatum"[tw] OR "phaseolus lunatus"[tw] OR "cissus populnea"[tw] OR "potentilla recta"[tw] OR "zingiber officinale"[tw] OR "aframomum danielli"[tw] OR "lantana camara"[tw] OR "solanum torvum"[tw] OR "eugenia uniflora"[tw] OR "tribulus terrestris"[tw] OR "leucaena leucocephala"[tw] OR "dichapetalum madagascasiense"[tw] OR "funtumia elastica"[tw] OR "mallotus oppositifolius"[tw] OR "coli"[tw] OR "legume"[tw] OR "legumes"[tw] OR "plant"[tw] OR "plants"[tw] OR "pea"[tw] OR "peas"[tw])
10/2018	Manganese ion or compound, and another chemical of concern other than barium or iron: ((((((19768-33-3[rn] OR 7773-01-5[rn] OR 7785-87-7[rn] OR 598-62-9[rn] OR 638-38-0[rn] OR QQE170PANO[rn] OR 9ZV57512ZM[rn]) AND ((17341-25-2[rn] OR 127-09-3[rn] OR 6131-90-4[rn] OR 497-19-8[rn] OR 5968-11-6[rn] OR 7647-14-5[rn] OR 7727-73-3[rn] OR 7757-82-6[rn] OR 15124-09-1[rn] OR 4550K0SC9B[rn] OR 45P3261C7T[rn] OR 45P3261C7T[rn] OR 7647-14-5[rn] OR 0YPR65R21J[rn] OR 0YPR65R21J[rn] OR 0YPR65R21J[rn]) OR (22537-39-9[rn] OR 543-94-2[rn] OR 1633-05-2[rn] OR 10476-85-4[rn] OR 7759-02-6[rn] OR 4SL32YMY7B[rn] OR 41YPU4MMCA[rn] OR EKE8PS9J6Z[rn])))) OR ((("Manganese"[ot] OR "Manganous chloride"[ot] OR "Scacchite"[ot] OR "Manganous carbonate"[ot] OR "Rhodochrosite"[ot] OR "Manganous acetate"[ot] OR "MnCl2"[ot])) AND ((("Bisodium carbonate"[ot] OR "Bisodium sulfate"[ot] OR "Disodium carbonate"[ot] OR "Disodium monosulfate"[ot] OR "Disodium sulfate"[ot] OR "Disodium sulphate"[ot] OR "Na sulphate"[ot] OR "Sodium"[ot] OR "Trisodium trichloride"[ot] OR "Saline Solution"[ot] OR "Mangxiao"[ot] OR "mirabilitum"[ot] OR "natrii sulphas"[ot] OR "puxiao"[ot] OR "thenardite"[ot])) OR ((Strontium[ot] OR Strontianite[ot]

Table J-2. Database Query Strings

Database search date	Query string
	OR Metastron[ot])))) OR ((("Manganese"[tiab] OR "Manganous chloride"[tiab] OR "Scacchite"[tiab] OR "Manganous carbonate"[tiab] OR "Rhodochrosite"[tiab] OR "Manganous acetate"[tiab] OR "MnCl2"[tiab])) AND ((("Bisodium carbonate"[tiab] OR "Bisodium sulfate"[tiab] OR "Disodium carbonate"[tiab] OR "Disodium monosulfate"[tiab] OR "Disodium sulfate"[tiab] OR "Disodium sulphate"[tiab] OR "Na sulphate"[tiab] OR "Sodium"[tiab] OR "Trisodium trichloride"[tiab] OR "Saline Solution"[tiab] OR "Mangxiao"[tiab] OR "mirabilitum"[tiab] OR "natrii sulphas"[tiab] OR "puxiao"[tiab] OR "thenardite"[tiab])) OR ((Strontium[tiab] OR Strontianite[tiab] OR Metastron[tiab]))))) AND (2014/05/01:3000[dp] OR 2015/05/01:3000[mhda] OR 2015/05/01:3000[crdat] OR 2015/05/01:3000[edat])) AND (additiv* OR antagonis* OR inhibit* OR mask* OR potentiat* OR synergis* OR "joint action" OR interact* OR combin* OR transport*)) NOT ("aeruginosa"[tw] OR "anguillarum"[tw] OR "anophagefferens"[tw] OR "aureococcus"[tw] OR "azobacter"[tw] OR "azotobacter"[tw] OR "bacillus"[tw] OR "bacteria"[tw] OR "bacterium"[tw] OR "barkeri"[tw] OR "campylobacter"[tw] OR "chlorella"[tw] OR "crassa"[tw] OR "cryptococcus"[tw] OR "elongatus"[tw] OR "enterococcus"[tw] OR "escherichia"[tw] OR "faecalis"[tw] OR "falciparum"[tw] OR "flexneri"[tw] OR "fungi"[tw] OR "fungus"[tw] OR "fusarium"[tw] OR "helicobacter"[tw] OR "hyphomycetes"[tw] OR "methanosarcina"[tw] OR "microbial"[tw] OR "microcystus"[tw] OR "microorganism"[tw] OR "neurospora"[tw] OR "penicillium"[tw] OR "pestis"[tw] OR "plasmodium"[tw] OR "pseudomonas"[tw] OR "pylori"[tw] OR "saccharomyces"[tw] OR "shewanella"[tw] OR "shigella"[tw] OR "siderophore"[tw] OR "streptococcus"[tw] OR "thermosynechococcus"[tw] OR "trichothecium"[tw] OR "tuberculosis"[tw] OR "vibrio"[tw] OR "vinelandii"[tw] OR "yeast"[tw] OR "yersinia"[tw] OR "salmonella"[tw] OR "typhimurium"[tw] OR "plankton"[tw] OR "arum maculatum"[tw] OR "phaseolus lunatus"[tw] OR "cissus populnea"[tw] OR "potentilla recta"[tw] OR "zingiber officinale"[tw] OR "aframomum danielli"[tw] OR "lantana camara"[tw] OR "solanum torvum"[tw] OR "eugenia uniflora"[tw] OR "tribulus terrestris"[tw] OR "leucaena leucocephala"[tw] OR "dichapetalum madagascasiense"[tw] OR "funtumia elastica"[tw] OR "mallotus oppositifolius"[tw] OR "coli"[tw] OR "legume"[tw] OR "legumes"[tw] OR "plant"[tw] OR "plants"[tw] OR "pea"[tw] OR "peas"[tw])
10/2018	Sodium ion or compound, and another chemical of concern other than barium, iron or manganese: ((((((17341-25-2[rn] OR 127-09-3[rn] OR 6131-90-4[rn] OR 497-19-8[rn] OR 5968-11-6[rn] OR 7647-14-5[rn] OR 7727-73-3[rn] OR 7757-82-6[rn] OR 15124-09-1[rn] OR 4550K0SC9B[rn] OR 45P3261C7T[rn] OR 45P3261C7T[rn] OR 7647-14-5[rn] OR 0YPR65R21J[rn] OR 0YPR65R21J[rn] OR 0YPR65R21J[rn]) AND (22537-39-9[rn] OR 543-94-2[rn] OR 1633-05-2[rn] OR 10476-85-4[rn] OR 7759-02-6[rn] OR 4SL32YMY7B[rn] OR 41YPU4MMCA[rn] OR EKE8PS9J6Z[rn]))) OR ((("Bisodium carbonate"[ot] OR "Bisodium sulfate"[ot] OR "Disodium carbonate"[ot] OR "Disodium monosulfate"[ot] OR "Disodium sulfate"[ot] OR "Disodium sulphate"[ot] OR "Na sulphate"[ot] OR "Sodium"[ot] OR "Trisodium trichloride"[ot] OR "Saline Solution"[ot] OR "Mangxiao"[ot] OR "mirabilitum"[ot] OR "natrii sulphas"[ot] OR "puxiao"[ot] OR "thenardite"[ot])) AND ((Strontium[ot] OR Strontianite[ot] OR Metastron[ot]))) OR ((("Bisodium carbonate"[tiab] OR "Bisodium sulfate"[tiab] OR "Disodium carbonate"[tiab] OR "Disodium monosulfate"[tiab] OR "Disodium sulfate"[tiab] OR "Disodium sulphate"[tiab] OR "Na sulphate"[tiab] OR "Sodium"[tiab] OR "Trisodium trichloride"[tiab] OR "Saline Solution"[tiab] OR "Mangxiao"[tiab] OR "mirabilitum"[tiab] OR "natrii sulphas"[tiab] OR "puxiao"[tiab] OR "thenardite"[tiab])) AND ((Strontium[tiab] OR Strontianite[tiab] OR Metastron[tiab])))) AND (2014/05/01:3000[dp] OR 2015/05/01:3000[mhda] OR 2015/05/01:3000[crdat] OR 2015/05/01:3000[edat])) AND (additiv* OR antagonis* OR inhibit* OR mask* OR potentiat* OR synergis* OR "joint action" OR interact* OR combin* OR transport*)) NOT

Table J-2. Database Query Strings

Database search date	Query string
	("aeruginosa"[tw] OR "anguillarum"[tw] OR "anophagefferens"[tw] OR "aureococcus"[tw] OR "azobacter"[tw] OR "azotobacter"[tw] OR "bacillus"[tw] OR "bacteria"[tw] OR "bacterium"[tw] OR "barkeri"[tw] OR "campylobacter"[tw] OR "chlorella"[tw] OR "crassa"[tw] OR "cryptococcus"[tw] OR "elongatus"[tw] OR "enterococcus"[tw] OR "escherichia"[tw] OR "faecalis"[tw] OR "falciparum"[tw] OR "flexneri"[tw] OR "fungi"[tw] OR "fungus"[tw] OR "fusarium"[tw] OR "helicobacter"[tw] OR "hyphomycetes"[tw] OR "methanosarcina"[tw] OR "microbial"[tw] OR "microcystus"[tw] OR "microorganism"[tw] OR "neurospora"[tw] OR "penicillium"[tw] OR "pestis"[tw] OR "plasmodium"[tw] OR "pseudomonas"[tw] OR "pylori"[tw] OR "saccharomyces"[tw] OR "shewanella"[tw] OR "shigella"[tw] OR "siderophore"[tw] OR "streptococcus"[tw] OR "thermosynechococcus"[tw] OR "trichothecium"[tw] OR "tuberculosis"[tw] OR "vibrio"[tw] OR "vinelandii"[tw] OR "yeast"[tw] OR "yersinia"[tw] OR "salmonella"[tw] OR "typhimurium"[tw] OR "plankton"[tw] OR "arum maculatum"[tw] OR "phaseolus lunatus"[tw] OR "cissus populnea"[tw] OR "potentilla recta"[tw] OR "zingiber officinale"[tw] OR "aframomum danielli"[tw] OR "lantana camara"[tw] OR "solanum torvum"[tw] OR "eugenia uniflora"[tw] OR "tribulus terrestris"[tw] OR "leucaena leucocephala"[tw] OR "dichapetalum madagascasiense"[tw] OR "funtumia elastica"[tw] OR "mallotus oppositifolius"[tw] OR "coli"[tw] OR "legume"[tw] OR "legumes"[tw] OR "plant"[tw] OR "plants"[tw] OR "pea"[tw] OR "peas"[tw])
Toxline	
5/2015	Barium ion or compound, and another chemical of concern: (22541-12-4[rn] OR 543-80-6[rn] OR 513-77-9[rn] OR 10361-37-2[rn] OR 7727-43-7[rn] OR Barite OR Barium OR Baritop OR "E-Z-CAT" OR "Micropaque Oral") AND ((15438-31-0[rn] OR 20074-52-6[rn] OR 563-71-3[rn] OR 10290-71-8[rn] OR 7705-08-0[rn] OR 7758-94-3[rn] OR 12040-57-2[rn] OR 16480-60-7[rn] OR 23444-30-6[rn] OR 2140-52-5[rn] OR 1834-30-6[rn] OR 7720-78-7[rn] OR 10028-22-5[rn] OR 10124-49-9[rn] OR 16547-58-3[rn] OR Iron OR Ferrous OR Ferric OR Lawrencite OR Polyferric OR Aktiferrin OR Biofer OR Ceferro OR Conferon OR "Eisendragees-ratiopharm" OR "Eisensulfat Stada" OR Feospan OR "Fer-Gen-Sol" OR "Fer-in-Sol" OR "Feratab" OR "Fero-Gradumet" OR "Ferodan" OR "Ferogradumet" OR "Ferro-Gradumet" OR "Ferrogamma" OR "Ferrograd" OR "FERROinfant" OR "Haemoprotect" OR "Hämatopan" OR "Hemobion" OR "Hemofer" OR "Kendural" OR "Mol-Iron" OR "Plastufer" OR "Slow-Fe" OR "Vitaferro Kapseln") OR (19768-33-3[rn] OR 7773-01-5[rn] OR 7785-87-7[rn] OR 598-62-9[rn] OR 638-38-0[rn] OR "Manganese" OR "Manganous chloride" OR "Scacchite" OR "Manganous carbonate" OR "Rhodochrosite" OR "Manganous acetate" OR "MnCl2") OR (17341-25-2[rn] OR 127-09-3[rn] OR 6131-90-4[rn] OR 497-19-8[rn] OR 5968-11-6[rn] OR 7647-14-5[rn] OR 7727-73-3[rn] OR 7757-82-6[rn] OR 15124-09-1[rn] OR "Bisodium carbonate" OR "Bisodium sulfate" OR "Disodium carbonate" OR "Disodium monosulfate" OR "Disodium sulfate" OR "Disodium sulphate" OR "Na sulphate" OR "Sodium" OR "Trisodium trichloride" OR "Saline Solution" OR "Mangxiao" OR "mirabilitum" OR "natrii sulphas" OR "puxiao" OR "thenardite") OR (22537-39-9[rn] OR 543-94-2[rn] OR 1633-05-2[rn] OR 10476-85-4[rn] OR 7759-02-6[rn] OR Strontium OR Strontianite OR Metastron))
5/2015	Iron ion or compound, and another chemical of concern other than barium: (15438-31-0[rn] OR 20074-52-6[rn] OR 563-71-3[rn] OR 10290-71-8[rn] OR 7705-08-0[rn] OR 7758-94-3[rn] OR 12040-57-2[rn] OR 16480-60-7[rn] OR 23444-30-6[rn] OR 2140-52-5[rn] OR 1834-30-6[rn] OR 7720-78-7[rn] OR 10028-22-5[rn] OR 10124-49-9[rn] OR 16547-58-3[rn] OR Iron OR Ferrous OR Ferric OR Lawrencite OR Polyferric OR Aktiferrin OR Biofer OR Ceferro OR Conferon OR "Eisendragees-ratiopharm" OR "Eisensulfat Stada" OR Feospan OR "Fer-Gen-Sol" OR "Fer-in-Sol" OR "Feratab" OR "Fero-Gradumet" OR "Ferodan" OR "Ferogradumet" OR "Ferro-Gradumet" OR "Ferrogamma" OR "Ferrograd" OR "FERROinfant" OR "Haemoprotect" OR "Hämatopan" OR "Hemobion" OR

Table J-2. Database Query Strings

Database search date	Query string
	"Hemofer" OR "Kendural" OR "Mol-Iron" OR "Plastufer" OR "Slow-Fe" OR "Vitaferro Kapseln") AND ((19768-33-3[rn] OR 7773-01-5[rn] OR 7785-87-7[rn] OR 598-62-9[rn] OR 638-38-0[rn] OR "Manganese" OR "Manganous chloride" OR "Scacchite" OR "Manganous carbonate" OR "Rhodochrosite" OR "Manganous acetate" OR "MnCl2") OR (17341-25-2[rn] OR 127-09-3[rn] OR 6131-90-4[rn] OR 497-19-8[rn] OR 5968-11-6[rn] OR 7647-14-5[rn] OR 7727-73-3[rn] OR 7757-82-6[rn] OR 15124-09-1[rn] OR "Bisodium carbonate" OR "Bisodium sulfate" OR "Disodium carbonate" OR "Disodium monosulfate" OR "Disodium sulfate" OR "Disodium sulphate" OR "Na sulphate" OR "Sodium" OR "Trisodium trichloride" OR "Saline Solution" OR "Mangxiao" OR "mirabilitum" OR "natrii sulphas" OR "puxiao" OR "thenardite") OR (22537-39-9[rn] OR 543-94-2[rn] OR 1633-05-2[rn] OR 10476-85-4[rn] OR 7759-02-6[rn] OR Strontium OR Strontianite OR Metastron))
5/2015	Manganese ion or compound, and another chemical of concern or than barium or iron: (19768-33-3[rn] OR 7773-01-5[rn] OR 7785-87-7[rn] OR 598-62-9[rn] OR 638-38-0[rn] OR "Manganese" OR "Manganous chloride" OR "Scacchite" OR "Manganous carbonate" OR "Rhodochrosite" OR "Manganous acetate" OR "MnCl2") AND ((17341-25-2[rn] OR 127-09-3[rn] OR 6131-90-4[rn] OR 497-19-8[rn] OR 5968-11-6[rn] OR 7647-14-5[rn] OR 7727-73-3[rn] OR 7757-82-6[rn] OR 15124-09-1[rn] OR "Bisodium carbonate" OR "Bisodium sulfate" OR "Disodium carbonate" OR "Disodium monosulfate" OR "Disodium sulfate" OR "Disodium sulphate" OR "Na sulphate" OR "Sodium" OR "Trisodium trichloride" OR "Saline Solution" OR "Mangxiao" OR "mirabilitum" OR "natrii sulphas" OR "puxiao" OR "thenardite") OR (22537-39-9[rn] OR 543-94-2[rn] OR 1633-05-2[rn] OR 10476-85-4[rn] OR 7759-02-6[rn] OR Strontium OR Strontianite OR Metastron))
5/2015	Sodium ion or compound, and another chemical of concern other than barium, iron or manganese: (17341-25-2[rn] OR 127-09-3[rn] OR 6131-90-4[rn] OR 497-19-8[rn] OR 5968-11-6[rn] OR 7647-14-5[rn] OR 7727-73-3[rn] OR 7757-82-6[rn] OR 15124-09-1[rn] OR "Bisodium carbonate" OR "Bisodium sulfate" OR "Disodium carbonate" OR "Disodium monosulfate" OR "Disodium sulfate" OR "Disodium sulphate" OR "Na sulphate" OR "Sodium" OR "Trisodium trichloride" OR "Saline Solution" OR "Mangxiao" OR "mirabilitum" OR "natrii sulphas" OR "puxiao" OR "thenardite") AND (22537-39-9[rn] OR 543-94-2[rn] OR 1633-05-2[rn] OR 10476-85-4[rn] OR 7759-02-6[rn] OR Strontium OR Strontianite OR Metastron)
10/2018	Barium ion or compound, and another chemical of concern: (22541-12-4[rn] OR 543-80-6[rn] OR 513-77-9[rn] OR 10361-37-2[rn] OR 7727-43-7[rn] OR Barite OR Barium OR Baritop OR "E-Z-CAT" OR "Micropaque Oral") AND ((15438-31-0[rn] OR 20074-52-6[rn] OR 563-71-3[rn] OR 10290-71-8[rn] OR 7705-08-0[rn] OR 7758-94-3[rn] OR 12040-57-2[rn] OR 16480-60-7[rn] OR 23444-30-6[rn] OR 2140-52-5[rn] OR 1834-30-6[rn] OR 7720-78-7[rn] OR 10028-22-5[rn] OR 10124-49-9[rn] OR 16547-58-3[rn] OR Iron OR Ferrous OR Ferric OR Lawrencite OR Polyferric OR Aktiferrin OR Biofer OR Ceferro OR Conferon OR "Eisendragees-ratiopharm" OR "Eisensulfat Stada" OR Feospan OR "Fer-Gen-Sol" OR "Fer-in-Sol" OR "Feratab" OR "Fero-Gradumet" OR "Ferodan" OR "Ferogradumet" OR "Ferro-Gradumet" OR "Ferrogamma" OR "Ferrograd" OR "FERROinfant" OR "Haemoprotect" OR "Hämatopan" OR "Hemobion" OR "Hemofer" OR "Kendural" OR "Mol-Iron" OR "Plastufer" OR "Slow-Fe" OR "Vitaferro Kapseln") OR (19768-33-3[rn] OR 7773-01-5[rn] OR 7785-87-7[rn] OR 598-62-9[rn] OR 638-38-0[rn] OR "Manganese" OR "Manganous chloride" OR "Scacchite" OR "Manganous carbonate" OR "Rhodochrosite" OR "Manganous acetate" OR "MnCl2") OR (17341-25-2[rn] OR 127-09-3[rn] OR 6131-90-4[rn] OR 497-19-8[rn] OR 5968-11-6[rn] OR 7647-14-5[rn] OR 7727-73-3[rn] OR 7757-82-6[rn] OR 15124-09-1[rn] OR "Bisodium carbonate" OR "Bisodium sulfate" OR "Disodium carbonate" OR "Disodium monosulfate" OR "Disodium sulfate" OR "Disodium sulphate" OR "Na sulphate" OR "Sodium" OR "Trisodium trichloride" OR "Saline

Table J-2. Database Query Strings

Database search date	Query string
	Solution" OR "Mangxiao" OR "mirabilitum" OR "natrii sulphas" OR "puxiao" OR "thenardite") OR (22537-39-9[rn] OR 543-94-2[rn] OR 1633-05-2[rn] OR 10476-85-4[rn] OR 7759-02-6[rn] OR Strontium OR Strontianite OR Metastron)) AND 2014:2018 [yr] AND (ANEUPL [org] OR BIOSIS [org] OR CIS [org] OR DART [org] OR EMIC [org] OR EPIDEM [org] OR FEDRIP [org] OR HEEP [org] OR HMTC [org] OR IPA [org] OR RISKLINE [org] OR MTGABS [org] OR NIOSH [org] OR NTIS [org] OR PESTAB [org] OR PPBIB [org]) AND NOT PubMed [org] AND NOT pubdart [org]
10/2018	Iron ion or compound, and another chemical of concern other than barium: (15438-31-0[rn] OR 20074-52-6[rn] OR 563-71-3[rn] OR 10290-71-8[rn] OR 7705-08-0[rn] OR 7758-94-3[rn] OR 12040-57-2[rn] OR 16480-60-7[rn] OR 23444-30-6[rn] OR 2140-52-5[rn] OR 1834-30-6[rn] OR 7720-78-7[rn] OR 10028-22-5[rn] OR 10124-49-9[rn] OR 16547-58-3[rn] OR Iron OR Ferrous OR Ferric OR Lawrencite OR Polyferric OR Aktiferrin OR Biofer OR Ceferro OR Conferon OR "Eisendragees-ratiopharm" OR "Eisensulfat Stada" OR Feospan OR "Fer-Gen-Sol" OR "Fer-in-Sol" OR "Feratab" OR "Fero-Gradumet" OR "Ferodan" OR "Ferogradumet" OR "Ferro-Gradumet" OR "Ferrogamma" OR "Ferrograd" OR "FERROinfant" OR "Haemoprotect" OR "Hämatopan" OR "Hemobion" OR "Hemofer" OR "Kendural" OR "Mol-Iron" OR "Plastufer" OR "Slow-Fe" OR "Vitaferro Kapseln") AND ((19768-33-3[rn] OR 7773-01-5[rn] OR 7785-87-7[rn] OR 598-62-9[rn] OR 638-38-0[rn] OR "Manganese" OR "Manganous chloride" OR "Scacchite" OR "Manganous carbonate" OR "Rhodochrosite" OR "Manganous acetate" OR "MnCl2") OR (17341-25-2[rn] OR 127-09-3[rn] OR 6131-90-4[rn] OR 497-19-8[rn] OR 5968-11-6[rn] OR 7647-14-5[rn] OR 7727-73-3[rn] OR 7757-82-6[rn] OR 15124-09-1[rn] OR "Bisodium carbonate" OR "Bisodium sulfate" OR "Disodium carbonate" OR "Disodium monosulfate" OR "Disodium sulfate" OR "Disodium sulphate" OR "Na sulphate" OR "Sodium" OR "Trisodium trichloride" OR "Saline Solution" OR "Mangxiao" OR "mirabilitum" OR "natrii sulphas" OR "puxiao" OR "thenardite") OR (22537-39-9[rn] OR 543-94-2[rn] OR 1633-05-2[rn] OR 10476-85-4[rn] OR 7759-02-6[rn] OR Strontium OR Strontianite OR Metastron)) AND 2014:2018 [yr] AND (ANEUPL [org] OR BIOSIS [org] OR CIS [org] OR DART [org] OR EMIC [org] OR EPIDEM [org] OR FEDRIP [org] OR HEEP [org] OR HMTC [org] OR IPA [org] OR RISKLINE [org] OR MTGABS [org] OR NIOSH [org] OR NTIS [org] OR PESTAB [org] OR PPBIB [org]) AND NOT PubMed [org] AND NOT pubdart [org]
10/2018	Manganese ion or compound, and another chemical of concern other than barium or iron: (19768-33-3[rn] OR 7773-01-5[rn] OR 7785-87-7[rn] OR 598-62-9[rn] OR 638-38-0[rn] OR "Manganese" OR "Manganous chloride" OR "Scacchite" OR "Manganous carbonate" OR "Rhodochrosite" OR "Manganous acetate" OR "MnCl2") AND ((17341-25-2[rn] OR 127-09-3[rn] OR 6131-90-4[rn] OR 497-19-8[rn] OR 5968-11-6[rn] OR 7647-14-5[rn] OR 7727-73-3[rn] OR 7757-82-6[rn] OR 15124-09-1[rn] OR "Bisodium carbonate" OR "Bisodium sulfate" OR "Disodium carbonate" OR "Disodium monosulfate" OR "Disodium sulfate" OR "Disodium sulphate" OR "Na sulphate" OR "Sodium" OR "Trisodium trichloride" OR "Saline Solution" OR "Mangxiao" OR "mirabilitum" OR "natrii sulphas" OR "puxiao" OR "thenardite") OR (22537-39-9[rn] OR 543-94-2[rn] OR 1633-05-2[rn] OR 10476-85-4[rn] OR 7759-02-6[rn] OR Strontium OR Strontianite OR Metastron)) AND 2014:2018 [yr] AND (ANEUPL [org] OR BIOSIS [org] OR CIS [org] OR DART [org] OR EMIC [org] OR EPIDEM [org] OR FEDRIP [org] OR HEEP [org] OR HMTC [org] OR IPA [org] OR RISKLINE [org] OR MTGABS [org] OR NIOSH [org] OR NTIS [org] OR PESTAB [org] OR PPBIB [org]) AND NOT PubMed [org] AND NOT pubdart [org]
10/2018	Sodium ion or compound, and another chemical of concern other than barium, iron or manganese: (17341-25-2[rn] OR 127-09-3[rn] OR 6131-90-4[rn] OR 497-19-8[rn] OR 5968-11-6[rn] OR 7647-14-5[rn] OR 7727-73-3[rn] OR 7757-82-6[rn] OR 15124-09-1[rn] OR "Bisodium

Table J-2. Database Query Strings

Database search date	Query string
	carbonate" OR "Bisodium sulfate" OR "Disodium carbonate" OR "Disodium monosulfate" OR "Disodium sulfate" OR "Disodium sulphate" OR "Na sulphate" OR "Sodium" OR "Trisodium trichloride" OR "Saline Solution" OR "Mangxiao" OR "mirabilitum" OR "natrii sulphas" OR "puxiao" OR "thenardite") AND (22537-39-9[rn] OR 543-94-2[rn] OR 1633-05-2[rn] OR 10476-85-4[rn] OR 7759-02-6[rn] OR Strontium OR Strontianite OR Metastron) AND 2014:2018 [yr] AND (ANEUPL [org] OR BIOSIS [org] OR CIS [org] OR DART [org] OR EMIC [org] OR EPIDEM [org] OR FEDRIP [org] OR HEEP [org] OR HMTC [org] OR IPA [org] OR RISKLINE [org] OR MTGABS [org] OR NIOSH [org] OR NTIS [org] OR PESTAB [org] OR PPBIB [org]) AND NOT PubMed [org] AND NOT pubdart [org]
Toxcenter	
5/2015	FILE 'TOXCENTER' ENTERED AT 14:26:28 ON 12 MAY 2015 L1 7872 SEA (22541-12-4 OR 543-80-6 OR 513-77-9 OR 10361-37-2 OR 7727-43-7) L2 35993 SEA (15438-31-0 OR 20074-52-6 OR 563-71-3 OR 10290-71-8 OR 7705-08-0 OR 7758-94-3 OR 12040-57-2 OR 16480-60-7 OR 23444-30-6 OR 2140-52-5 OR 1834-30-6 OR 7720-78-7 OR 10028-22-5 OR 10124-49-9 OR 16547-58-3) L3 7242 SEA (19768-33-3 OR 7773-01-5 OR 7785-87-7 OR 598-62-9 OR 638-38-0) L4 90904 SEA (17341-25-2 OR 127-09-3 OR 6131-90-4 OR 497-19-8 OR 5968-11-6 OR 7647-14-5 OR 7727-73-3 OR 7757-82-6 OR 15124-09-1) L5 2024 SEA (22537-39-9 OR 543-94-2 OR 1633-05-2 OR 10476-85-4 OR 7759-02-6) L6 1875 SEA L1 AND (L2 OR L3 OR L4 OR L5) L7 5735 SEA L2 AND (L3 OR L4 OR L5) L8 1534 SEA L3 AND (L4 OR L5) L9 478 SEA L4 AND L5 L10 478 SEA L4 AND L5 L11 8162 SEA L6 OR L7 OR L8 OR L9 L12 8157 SEA L11 NOT TSCATS/FS L13 2905 SEA L12 NOT PATENT/DT ACT TOXQUERY/Q --------- L14 QUE (CHRONIC OR IMMUNOTOX? OR NEUROTOX? OR TOXICOKIN? OR BIOMARKER? OR NEUROLOG?) L15 QUE (PHARMACOKIN? OR SUBCHRONIC OR PBPK OR EPIDEMIOLOGY/ST,CT, IT) L16 QUE (ACUTE OR SUBACUTE OR LD50# OR LD(W)50 OR LC50# OR LC(W)50) L17 QUE (TOXICITY OR ADVERSE OR POISONING)/ST,CT,IT L18 QUE (INHAL? OR PULMON? OR NASAL? OR LUNG? OR RESPIR?) L19 QUE ((OCCUPATION? OR WORKPLACE? OR WORKER?) AND EXPOS?) L20 QUE (ORAL OR ORALLY OR INGEST? OR GAVAGE? OR DIET OR DIETS OR DIETARY OR DRINKING(W)WATER?) L21 QUE (MAXIMUM AND CONCENTRATION? AND (ALLOWABLE OR PERMISSIBLE)) L22 QUE (ABORT? OR ABNORMALIT? OR EMBRYO? OR CLEFT? OR FETUS?) L23 QUE (FOETUS? OR FETAL? OR FOETAL? OR FERTIL? OR MALFORM? OR OVUM?) L24 QUE (OVA OR OVARY OR PLACENTA? OR PREGNAN? OR PRENATAL?) L25 QUE (PERINATAL? OR POSTNATAL? OR REPRODUC? OR STERIL? OR TERATOGEN?) L26 QUE (SPERM OR SPERMAC? OR SPERMAG? OR SPERMATI? OR SPERMAS? OR SPERMATOB? OR SPERMATOC? OR SPERMATOG?)

Table J-2. Database Query Strings

Database search date	Query string
	L27 QUE (SPERMATOI? OR SPERMATOL? OR SPERMATOR? OR SPERMATOX? OR SPERMATOZ? OR SPERMATU? OR SPERMI? OR SPERMO?)
	L28 QUE (NEONAT? OR NEWBORN? OR DEVELOPMENT OR DEVELOPMENTAL?)
	L29 QUE (ENDOCRIN? AND DISRUPT?)
	L30 QUE (ZYGOTE? OR CHILD OR CHILDREN OR ADOLESCEN? OR INFANT?)
	L31 QUE (WEAN? OR OFFSPRING OR AGE(W)FACTOR?)
	L32 QUE (DERMAL? OR DERMIS OR SKIN OR EPIDERM? OR CUTANEOUS?)
	L33 QUE (CARCINOG? OR COCARCINOG? OR CANCER? OR PRECANCER? OR NEOPLAS?)
	L34 QUE (TUMOR? OR TUMOUR? OR ONCOGEN? OR LYMPHOMA? OR CARCINOM?)
	L35 QUE (GENETOX? OR GENOTOX? OR MUTAGEN? OR GENETIC(W)TOXIC?)
	L36 QUE (NEPHROTOX? OR HEPATOTOX?)
	L37 QUE (ENDOCRIN? OR ESTROGEN? OR ANDROGEN? OR HORMON?)
	L38 QUE (OCCUPATION? OR WORKER? OR WORKPLACE? OR EPIDEM?)
	L39 QUE L14 OR L15 OR L16 OR L17 OR L18 OR L19 OR L20 OR L21 OR L22 OR L23 OR L24 OR L25 OR L26 OR L27 OR L28 OR L29 OR L30 OR L31 OR L32 OR L33 OR L34 OR L35 OR L36 OR L37 OR L38
	L40 QUE (RAT OR RATS OR MOUSE OR MICE OR GUINEA(W)PIG? OR MURIDAE OR DOG OR DOGS OR RABBIT? OR HAMSTER? OR PIG OR PIGS OR SWINE OR PORCINE OR MONKEY? OR MACAQUE?)
	L41 QUE (MARMOSET? OR FERRET? OR GERBIL? OR RODENT? OR LAGOMORPHA OR BABOON? OR CANINE OR CAT OR CATS OR FELINE OR MURINE)
	L42 QUE L39 OR L40 OR L41
	L43 QUE (NONHUMAN MAMMALS)/ORGN
	L44 QUE L42 OR L43
	L45 QUE (HUMAN OR HUMANS OR HOMINIDAE OR MAMMALS OR MAMMAL? OR PRIMATES OR PRIMATE?)
	L46 QUE L44 OR L45

	L47 764 SEA L13 AND L46
	L48 31 SEA L47 AND MEDLINE/FS
	L49 81 SEA L47 AND BIOSIS/FS
	L50 589 SEA L47 AND CAPLUS/FS
	L51 63 SEA L47 NOT (MEDLINE/FS OR BIOSIS/FS OR CAPLUS/FS)
	L52 753 DUP REM L48 L49 L51 L50 (11 DUPLICATES REMOVED)
	L*** DEL 31 S L47 AND MEDLINE/FS
	L*** DEL 31 S L47 AND MEDLINE/FS
	L53 31 SEA L52
	L*** DEL 81 S L47 AND BIOSIS/FS
	L*** DEL 81 S L47 AND BIOSIS/FS
	L54 81 SEA L52
	L*** DEL 589 S L47 AND CAPLUS/FS
	L*** DEL 589 S L47 AND CAPLUS/FS
	L55 578 SEA L52
	L*** DEL 63 S L47 NOT (MEDLINE/FS OR BIOSIS/FS OR CAPLUS/FS)
	L*** DEL 63 S L47 NOT (MEDLINE/FS OR BIOSIS/FS OR CAPLUS/FS)
	L56 63 SEA L52
	L57 722 SEA (L53 OR L54 OR L55 OR L56) NOT MEDLINE/FS
10/2018	FILE 'TOXCENTER' ENTERED AT 09:38:46 ON 12 OCT 2018
	L1 10225 SEA FILE=TOXCENTER (22541-12-4 OR 543-80-6 OR 513-77-9 OR 10361-37-2 OR 7727-43-7)
	L2 50967 SEA FILE=TOXCENTER (15438-31-0 OR 20074-52-6 OR 563-71-3 OR 10290-71-8 OR 7705-08-0 OR 7758-94-3 OR 12040-57-2 OR 16480-60-7 OR 23444-30-6 OR 2140-52-5 OR 1834-30-6 OR 7720-78-7 OR 10028-22-5 OR 10124-49-9 OR 16547-58-3)
	L3 10389 SEA FILE=TOXCENTER (19768-33-3 OR 7773-01-5 OR 7785-87-7 OR

Table J-2. Database Query Strings

Database search date	Query string
	598-62-9 OR 638-38-0)
	L4 120965 SEA FILE=TOXCENTER (17341-25-2 OR 127-09-3 OR 6131-90-4 OR 497-19-8 OR 5968-11-6 OR 7647-14-5 OR 7727-73-3 OR 7757-82-6 OR 15124-09-1)
	L5 2721 SEA FILE=TOXCENTER (22537-39-9 OR 543-94-2 OR 1633-05-2 OR 10476-85-4 OR 7759-02-6)
	L6 2532 SEA FILE=TOXCENTER L1 AND (L2 OR L3 OR L4 OR L5)
	L7 8646 SEA FILE=TOXCENTER L2 AND (L3 OR L4 OR L5)
	L8 2170 SEA FILE=TOXCENTER L3 AND (L4 OR L5)
	L9 643 SEA FILE=TOXCENTER L4 AND L5
	L10 12013 SEA FILE=TOXCENTER L6 OR L7 OR L8 OR L9
	L11 12008 SEA FILE=TOXCENTER L10 NOT TSCATS/FS
	L12 3983 SEA FILE=TOXCENTER L11 NOT PATENT/DT
	ACT TOXQUERY/Q

	L13 QUE (CHRONIC OR IMMUNOTOX? OR NEUROTOX? OR TOXICOKIN? OR BIOMARKER? OR NEUROLOG?)
	L14 QUE (PHARMACOKIN? OR SUBCHRONIC OR PBPK OR EPIDEMIOLOGY/ST,CT, IT)
	L15 QUE (ACUTE OR SUBACUTE OR LD50# OR LD(W)50 OR LC50# OR LC(W)50)
	L16 QUE (TOXICITY OR ADVERSE OR POISONING)/ST,CT,IT
	L17 QUE (INHAL? OR PULMON? OR NASAL? OR LUNG? OR RESPIR?)
	L18 QUE ((OCCUPATION? OR WORKPLACE? OR WORKER?) AND EXPOS?)
	L19 QUE (ORAL OR ORALLY OR INGEST? OR GAVAGE? OR DIET OR DIETS OR DIETARY OR DRINKING(W)WATER?)
	L20 QUE (MAXIMUM AND CONCENTRATION? AND (ALLOWABLE OR PERMISSIBLE))
	L21 QUE (ABORT? OR ABNORMALIT? OR EMBRYO? OR CLEFT? OR FETUS?)
	L22 QUE (FOETUS? OR FETAL? OR FOETAL? OR FERTIL? OR MALFORM? OR OVUM?)
	L23 QUE (OVA OR OVARY OR PLACENTA? OR PREGNAN? OR PRENATAL?)
	L24 QUE (PERINATAL? OR POSTNATAL? OR REPRODUC? OR STERIL? OR TERATOGEN?)
	L25 QUE (SPERM OR SPERMAC? OR SPERMAG? OR SPERMATI? OR SPERMAS? OR SPERMATOB? OR SPERMATOC? OR SPERMATOG?)
	L26 QUE (SPERMATOI? OR SPERMATOL? OR SPERMATOR? OR SPERMATOX? OR SPERMATOZ? OR SPERMATU? OR SPERMI? OR SPERMO?)
	L27 QUE (NEONAT? OR NEWBORN? OR DEVELOPMENT OR DEVELOPMENTAL?)
	L28 QUE (ENDOCRIN? AND DISRUPT?)
	L29 QUE (ZYGOTE? OR CHILD OR CHILDREN OR ADOLESCEN? OR INFANT?)
	L30 QUE (WEAN? OR OFFSPRING OR AGE(W)FACTOR?)
	L31 QUE (DERMAL? OR DERMIS OR SKIN OR EPIDERM? OR CUTANEOUS?)
	L32 QUE (CARCINOG? OR COCARCINOG? OR CANCER? OR PRECANCER? OR NEOPLAS?)
	L33 QUE (TUMOR? OR TUMOUR? OR ONCOGEN? OR LYMPHOMA? OR CARCINOM?)
	L34 QUE (GENETOX? OR GENOTOX? OR MUTAGEN? OR GENETIC(W)TOXIC?)
	L35 QUE (NEPHROTOX? OR HEPATOTOX?)
	L36 QUE (ENDOCRIN? OR ESTROGEN? OR ANDROGEN? OR HORMON?)
	L37 QUE (OCCUPATION? OR WORKER? OR WORKPLACE? OR EPIDEM?)
	L38 QUE L13 OR L14 OR L15 OR L16 OR L17 OR L18 OR L19 OR L20 OR L21 OR L22 OR L23 OR L24 OR L25 OR L26 OR L27 OR L28 OR L29 OR L30 OR L31 OR L32 OR L33 OR L34 OR L35 OR L36 OR L37
	L39 QUE (RAT OR RATS OR MOUSE OR MICE OR GUINEA(W)PIG? OR MURIDAE OR DOG OR DOGS OR RABBIT? OR HAMSTER? OR PIG OR PIGS OR SWINE OR PORCINE OR MONKEY? OR MACAQUE?)

Table J-2. Database Query Strings

Database search date	Query string
	L40 QUE (MARMOSET? OR FERRET? OR GERBIL? OR RODENT? OR LAGOMORPHA OR BABOON? OR CANINE OR CAT OR CATS OR FELINE OR MURINE)
	L41 QUE L38 OR L39 OR L40
	L42 QUE (HUMAN OR HUMANS OR HOMINIDAE OR MAMMALS OR MAMMAL? OR PRIMATES OR PRIMATE?)
	L43 QUE L41 OR L42

	L44 986 SEA FILE=TOXCENTER L12 AND L43
	L45 43 SEA FILE=TOXCENTER L44 AND MEDLINE/FS
	L46 90 SEA FILE=TOXCENTER L44 AND BIOSIS/FS
	L47 790 SEA FILE=TOXCENTER L44 AND CAPLUS/FS
	L48 63 SEA FILE=TOXCENTER L44 NOT (L45 OR L46 OR L47)
	L49 973 DUP REM L45 L46 L48 L47 (13 DUPLICATES REMOVED) ANSWERS '1-973' FROM FILE TOXCENTER
	L*** DEL 43 S L44 AND MEDLINE/FS
	L*** DEL 43 S L44 AND MEDLINE/FS
	L50 43 SEA FILE=TOXCENTER L49
	L*** DEL 90 S L44 AND BIOSIS/FS
	L*** DEL 90 S L44 AND BIOSIS/FS
	L51 89 SEA FILE=TOXCENTER L49
	L*** DEL 790 S L44 AND CAPLUS/FS
	L*** DEL 790 S L44 AND CAPLUS/FS
	L52 778 SEA FILE=TOXCENTER L49
	L*** DEL 63 S L44 NOT (L45 OR L46 OR L47)
	L*** DEL 63 S L44 NOT (L45 OR L46 OR L47)
	L53 63 SEA FILE=TOXCENTER L49
	L54 930 SEA FILE=TOXCENTER (L50 OR L51 OR L52 OR L53) NOT MEDLINE/FS
	L55 173 SEA FILE=TOXCENTER L54 AND ED>=20150501
	L56 177 SEA FILE=TOXCENTER L54 AND PY>2014
	L57 188 SEA FILE=TOXCENTER L55 OR L56
	D SCAN L57
	ACT CAMG/A

	L58 (80329)SEA FILE=TOXCENTER (14127-61-8 OR 62-54-4 OR 471-34-1 OR 10043-52-4 OR 10035-04-8 OR 7778-18-9)
	L59 (38690)SEA FILE=TOXCENTER (22537-22-0 OR 142-72-3 OR 546-93-0 OR 17968-26-2 OR 23389-33-5 OR 7786-30-3 OR 7791-18-6 OR 14989-29-8 OR 7487-88-9 OR 10034-99-8 OR 18939-43-0)
	L60 (10225)SEA FILE=TOXCENTER (22541-12-4 OR 543-80-6 OR 513-77-9 OR 10361-37-2 OR 7727-43-7)
	L61 (50967)SEA FILE=TOXCENTER (15438-31-0 OR 20074-52-6 OR 563-71-3 OR 10290-71-8 OR 7705-08-0 OR 7758-94-3 OR 12040-57-2 OR 16480-60-7 OR 23444-30-6 OR 2140-52-5 OR 1834-30-6 OR 7720-78-7 OR 10028-22-5 OR 10124-49-9 OR 16547-58-3)
	L62 (10389)SEA FILE=TOXCENTER (19768-33-3 OR 7773-01-5 OR 7785-87-7 OR 598-62-9 OR 638-38-0)
	L63 (120965)SEA FILE=TOXCENTER (17341-25-2 OR 127-09-3 OR 6131-90-4 OR 497-19-8 OR 5968-11-6 OR 7647-14-5 OR 7727-73-3 OR 7757-82-6 OR 15124-09-1)
	L64 (2721)SEA FILE=TOXCENTER (22537-39-9 OR 543-94-2 OR 1633-05-2 OR 10476-85-4 OR 7759-02-6)
	L65 (28714)SEA FILE=TOXCENTER L58 AND (L59 OR L60 OR L61 OR L62 OR L63 OR L64)
	L66 (18653)SEA FILE=TOXCENTER L59 AND (L60 OR L61 OR L62 OR L63 OR L64)
	L67 (36702)SEA FILE=TOXCENTER L65 OR L66
	L68 (13945)SEA FILE=TOXCENTER L67 NOT (TSCATS/FS OR PATENT/DT)

Table J-2. Database Query Strings

Database search date	Query string	
	L69	QUE (CHRONIC OR IMMUNOTOX? OR NEUROTOX? OR TOXICOKIN? OR BIOMARKER? OR NEUROLOG?)
	L70	QUE (PHARMACOKIN? OR SUBCHRONIC OR PBPK OR EPIDEMIOLOGY/ST,CT, IT)
	L71	QUE (ACUTE OR SUBACUTE OR LD50# OR LD(W)50 OR LC50# OR LC(W)50)
	L72	QUE (TOXICITY OR ADVERSE OR POISONING)/ST,CT,IT
	L73	QUE (INHAL? OR PULMON? OR NASAL? OR LUNG? OR RESPIR?)
	L74	QUE ((OCCUPATION? OR WORKPLACE? OR WORKER?) AND EXPOS?)
	L75	QUE (ORAL OR ORALLY OR INGEST? OR GAVAGE? OR DIET OR DIETS OR DIETARY OR DRINKING(W)WATER?)
	L76	QUE (MAXIMUM AND CONCENTRATION? AND (ALLOWABLE OR PERMISSIBLE))
	L77	QUE (ABORT? OR ABNORMALIT? OR EMBRYO? OR CLEFT? OR FETUS?)
	L78	QUE (FOETUS? OR FETAL? OR FOETAL? OR FERTIL? OR MALFORM? OR OVUM?)
	L79	QUE (OVA OR OVARY OR PLACENTA? OR PREGNAN? OR PRENATAL?)
	L80	QUE (PERINATAL? OR POSTNATAL? OR REPRODUC? OR STERIL? OR TERATOGEN?)
	L81	QUE (SPERM OR SPERMAC? OR SPERMAG? OR SPERMATI? OR SPERMAS? OR SPERMATOB? OR SPERMATOC? OR SPERMATOG?)
	L82	QUE (SPERMATOI? OR SPERMATOL? OR SPERMATOR? OR SPERMATOX? OR SPERMATOZ? OR SPERMATU? OR SPERMI? OR SPERMO?)
	L83	QUE (NEONAT? OR NEWBORN? OR DEVELOPMENT OR DEVELOPMENTAL?)
	L84	QUE (ENDOCRIN? AND DISRUPT?)
	L85	QUE (ZYGOTE? OR CHILD OR CHILDREN OR ADOLESCEN? OR INFANT?)
	L86	QUE (WEAN? OR OFFSPRING OR AGE(W)FACTOR?)
	L87	QUE (DERMAL? OR DERMIS OR SKIN OR EPIDERM? OR CUTANEOUS?)
	L88	QUE (CARCINOG? OR COCARCINOG? OR CANCER? OR PRECANCER? OR NEOPLAS?)
	L89	QUE (TUMOR? OR TUMOUR? OR ONCOGEN? OR LYMPHOMA? OR CARCINOM?)
	L90	QUE (GENETOX? OR GENOTOX? OR MUTAGEN? OR GENETIC(W)TOXIC?)
	L91	QUE (NEPHROTOX? OR HEPATOTOX?)
	L92	QUE (ENDOCRIN? OR ESTROGEN? OR ANDROGEN? OR HORMON?)
	L93	QUE (OCCUPATION? OR WORKER? OR WORKPLACE? OR EPIDEM?)
	L94	QUE L69 OR L70 OR L71 OR L72 OR L73 OR L74 OR L75 OR L76 OR L77 OR L78 OR L79 OR L80 OR L81 OR L82 OR L83 OR L84 OR L85 OR L86 OR L87 OR L88 OR L89 OR L90 OR L91 OR L92 OR L93
	L95	QUE (RAT OR RATS OR MOUSE OR MICE OR GUINEA(W)PIG? OR MURIDAE OR DOG OR DOGS OR RABBIT? OR HAMSTER? OR PIG OR PIGS OR SWINE OR PORCINE OR MONKEY? OR MACAQUE?)
	L96	QUE (MARMOSET? OR FERRET? OR GERBIL? OR RODENT? OR LAGOMORPHA OR BABOON? OR CANINE OR CAT OR CATS OR FELINE OR MURINE)
	L97	QUE L94 OR L95 OR L96
	L98	QUE (HUMAN OR HUMANS OR HOMINIDAE OR MAMMALS OR MAMMAL? OR PRIMATES OR PRIMATE?)
	L99	QUE L97 OR L98
	L100(4611)	SEA FILE=TOXCENTER L68 AND L99
	L101(423)	SEA FILE=TOXCENTER L100 AND MEDLINE/FS
	L102(1165)	SEA FILE=TOXCENTER L100 AND BIOSIS/FS
	L103(2879)	SEA FILE=TOXCENTER L100 AND CAPLUS/FS
	L104(144)	SEA FILE=TOXCENTER L100 NOT (L101 OR L102 OR L103)
	L105(4474)	DUP REM L101 L102 L104 L103 (137 DUPLICATES REMOVED)
	L106(422)	SEA FILE=TOXCENTER L105
	L107(1141)	SEA FILE=TOXCENTER L105
	L108(2770)	SEA FILE=TOXCENTER L105

Table J-2. Database Query Strings

Database search date	Query string
	L109(141)SEA FILE=TOXCENTER L105 L110 4052 SEA FILE=TOXCENTER (L106 OR L107 OR L108 OR L109) NOT MEDLINE/FS --------- L112 3584 SEA FILE=TOXCENTER L110 NOT L44 L113 468 SEA FILE=TOXCENTER L110 AND L44 D SCAN L113 D SCAN L112

Table J-3. Additional Electronic Screening Keywords

Search date	Keywords applied in Endnote
5/2015	Inclusion keywords: additivity, antagonism, inhibition, joint action, masking, potentiation, or synergism
10/2018	Inclusion keywords: additivity, antagonism, inhibition, joint action, masking, potentiation, synergism, combined, or transport Exclusion keywords: aeruginosa, anguillarum, anophagefferens, aureococcus, azobacter, azotobacter, bacillus, bacteria, bacterium, barkeri, campylobacter, chlorella, crassa, cryptococcus, elongatus, enterococcus, escherichia, faecalis, falciparum, flexneri, fungi, fungus, fusarium, helicobacter, hyphomycetes, methanosarcina, microbial, microcystus, microorganism, neurospora, penicillium, pestis, plasmodium, pseudomonas, pylori, saccharomyces, shewanella, shigella, siderophore, streptococcus, thermosynechococcus, trichothecium, tuberculosis, vibrio, vinelandii, yeast, yersinia, salmonella, typhimurium, plankton, arum maculatum, phaseolus lunatus, cissus populnea, potentilla recta, zingiber officinale, aframomum danielli, lantana camara, solanum torvum, eugenia uniflora, tribulus terrestris, leucaena leucocephala, dichapetalum madagascasiense, funtumia elastica, mallotus oppositifolius, coli, legume, legumes, plant, plants, pea, or peas Applied only to calcium and magnesium PubMed search results— Inclusion keywords, appearing in titles: mixture, pollutants, metal, drinking water, ground water, groundwater, waste water, wastewater, wells, hydraulic fracturing, fracking; or synonym or abbreviation for calcium or magnesium ion or compound