2. Joint Toxic Action Data for the Mixture of Concern and Binary Mixtures of Components

2.1 Mixture of Concern

No data were located regarding health or toxicokinetic endpoints in humans or animals exposed to mixtures containing all seven metallic cations of concern. However, Yao et al. (2015) reported that samples of UOG waste fluids were cytotoxic to cultured human BEAS-2B cells and at high concentrations (at 1,000 times higher than levels in drinking water) could transform them into carcinogenic cells that induced tumors in mice after subcutaneous injection. Yao et al. (2015) proposed that at least a portion of the observed biological activity could be attributable to metallic cations within this complex mixture.

No PBPK/PD models were found for mixtures containing all seven metallic cations of concern or any mixture with two or more of the subject cations.

No studies were located examining toxicokinetic or toxicological endpoints in humans or laboratory animals exposed to mixtures containing more than two of the subject metallic cations and comparing the responses to responses from sole exposures to the individual cations. As a result, available interaction data for binary mixtures of the components were evaluated in Section 2.2.

The limitation of this binary approach is recognized due to evidence that overlap in homeostatic mechanisms can occur for more than two metals. For example, in rat pups exposed to high dietary manganese via maternal milk on postnatal days (PNDs) 4–21, brain concentrations of chromium, manganese, and zinc were increased, and brain iron concentration was decreased (Garcia et al. 2007; see Section 2.2.13 for more details). Another example comes from a study of rats given eight daily oral doses of single metallic cations (arsenic, lead, or manganese) and a mixture of all three metallic cations, which showed that kidney, brain, and liver concentrations of lead in the metal-mixture-treated group were significantly increased by 5.4-, 2.5-, and 1.6-fold, respectively, compared to the lead-alone-treated group (Andrade et al. 2014).

2.2 Binary Mixtures of Components

2.2.1 Barium and Calcium

Barium interactions with isolated calcium transport processes have been studied for a long time. A small sample of reported barium interactions with isolated calcium transport proteins include barium (or strontium) substitution for calcium in sodium/calcium exchange channels driving excitation-contraction coupling in the frog heart (Potreau et al. 1987); barium substitution for calcium in bovine cardiac sodium/calcium exchange channels expressed in transfected Chinese hamster ovary cells (Condrescu et al. 1997); barium inhibition of calcium release from bovine heart mitochondria via sodium/calcium exchange (Lukacs and Fonyo 1986); barium inhibition of calcium entry and subsequent neurotransmitter release from isolated frog neuromuscular junctions (Silinsky 2000); barium stimulation of calcium entry into isolated bovine adrenomedullary chromaffin cells via voltage-gated calcium channels and subsequent stimulation of neuropeptide synthesis (Waschek and Eiden 1988); and similar permeation rates of barium and calcium through $Ca_v3.1$ T-type calcium channels expressed in cultured HEK293 cells, similar inhibition by barium and calcium of sodium permeation through the channels, and differential effects of barium and calcium on channel gating (Khan et al. 2008). The physiological and toxicologic relevance of observations of interactions between barium and calcium at binding sites in transport proteins to environmentally relevant oral exposures to both cations, however, is unclear given the complexity of whole-body and cellular homeostatic systems for calcium.

Studies examining toxicokinetic endpoints (such as accumulation in toxicity target tissues), nutritional balance, or toxicological endpoints following repeated oral exposure of humans or laboratory animals to concomitant excess levels of barium and calcium were performed to understand how repeated oral exposure to excess levels of both barium and calcium may influence each other's toxicity. There is evidence that repeated excess exposure to either of these metallic ions alone results in adverse effects in the kidney, but it is unlikely that they occur via the same mode of action (see Appendices A and B). Kidney effects from barium are thought to involve perturbation of potassium homeostatic process in the kidney, whereas calcium-associated kidney effects are associated with precipitation of calcium oxalate crystals. The kidney is the site of the most sensitive adverse effects from repeated oral exposure to barium (nephropathy, Appendix A) and calcium (kidney stones, Appendix B). High exposures to barium (above the lowest levels associated with kidney effects) have been associated with cardiovascular and neurological effects that form the basis of TTDs for barium (Appendix A), but available data are inadequate to derive TTDs for less sensitive effects from excess calcium (Appendix A). In conclusion,

available interaction data are inadequate, however, to determine whether or not co-exposure to excess barium and excess calcium may produce adverse kidney effects in a dose-additive, greater-than-dose-additive, or less-than-dose-additive manner or whether or not co-exposure to both cations may influence barium's cardiovascular or neurological effects.

2.2.2 Barium and Iron

As with other divalent cations included in this profile, interactions with isolated elements of calcium homeostasis (e.g., calcium channels and calcium-binding regulatory proteins) in isolated cells have been extensively studied with barium (see Section 2.2.1) and iron (Section 2.2.7), but the relevance of findings from this type of research to the possible joint action of these metallic cations on toxicity endpoints following concomitant repeated oral exposure to barium and iron is unclear.

No studies were located that examined the effects of concomitant oral exposure of animals or humans to excess barium and excess iron on toxicokinetic endpoints (e.g., gastrointestinal absorption or distribution to expected sites of toxicity) or expected toxicity endpoints (e.g., kidney effects). In deriving health guidance values to protect against the development of adverse effects from repeated oral exposure, the critical effects of exposure to barium are kidney effects, whereas the critical effect of repeated exposure to excess iron is gastrointestinal irritation (see Appendices A and C). TTDs were derived for cardiovascular and neurological effects from barium that occur at higher doses than those associated with kidney effects. Although there are potential toxicity targets associated with excessive iron accumulation in tissues (effects on kidneys, liver, and cardiovascular and nervous system) that overlap with various targets of excess barium exposure, available data are inadequate to derive a TTD for these effects (see Appendix C).

Summary. Available evidence for interactions between barium and iron is inadequate to determine whether or not concomitant oral exposure will influence each other's toxicity.

2.2.3 Barium and Magnesium

Barium and magnesium belong to Group IIA of the periodic table of elements. These elements have the same number of valence electrons and form stable divalent cations. The Stokes radius of Ba^{2+} is approximately 6% smaller than Ca^{2+} and Sr^{2+} and the Stokes radius of Mg^{2+} is approximately 12% larger than Ca^{2+} and Sr^{2+} (Kadhim and Gamaj 2020). Barium and magnesium have been examined for interactions with components of calcium homeostasis: calcium membrane transport processes for barium

(see Section 2.2.1) and several sites of interaction with magnesium including TRPV5-mediated calcium reabsorption in the renal distal tubule, calcium-sensing receptor (CaSR), and parathyroid hormone (PTH) secretion from the parathyroid (see Section 2.2.8). Current understanding, however, is inadequate to explain how these potential coupling sites might work together and influence toxic responses under conditions of high oral intakes of these cations with or without concomitant high oral intakes of calcium.

Under isolated experimental conditions, barium and other divalent cations have been shown to be permeable through ion channels thought to be important for magnesium homeostasis, TRPM6 and TRPM7; however, it is unclear whether or not competitive inhibition of magnesium membrane transport by barium (e.g., in the intestine) occurs under physiological conditions (Bouron et al. 2015). Studies examining toxicokinetic (e.g., gastrointestinal absorption or accumulation in toxicity target tissues), nutritional balance, or toxicological endpoints following repeated oral exposure of humans or laboratory animals to concomitant excess levels of barium and calcium were not located, and would be more useful for understanding how repeated oral exposure to excess levels of both barium and calcium may influence each other's toxicity.

In cases of acute oral poisoning with high doses of soluble barium compounds (such as barium nitrate), the resultant severe hypokalemia has been successfully counteracted with massive potassium supplementation and, in at least one case, with magnesium sulfate, presumably to precipitate non-absorbed barium ions to insoluble barium sulfate (Payen et al. 2011). The therapeutic use of massive potassium supplementation to counteract acute barium poisoning is consistent with evidence that barium's acute toxic effects involve inhibition of key potassium homeostatic mechanisms, but magnesium does not appear to share this property with barium.

In deriving health guidance values to protect against the development of adverse effects from repeated oral exposure, the critical effects for exposure to barium are kidney effects (nephropathy, Appendix A), whereas the critical effect for exposure to magnesium is mild diarrhea (a gastrointestinal effect, Appendix D). There is no clear evidence in humans or laboratory animals that repeated excess exposure to either of these metallic ions alone results in a common adverse outcome via a common mode of action. But the kidney represents a potential common toxicity target when magnesium levels of exposure are sufficiently elevated (see Appendix D for description of a TTD for kidney effects from magnesium). TTDs were derived for cardiovascular and neurological effects from barium exposure levels higher than those associated with kidney effects but repeated oral exposure to magnesium has not been associated with these toxicity targets (see Appendices A and D).

Summary. Studies conducted in subcellular systems, isolated cells, and tissues have found evidence for various interactions between barium and magnesium on membrane transport of ions, neurotransmitters, and hormones. However, available interaction data are inadequate to determine whether or not repeated concomitant oral exposure to excess barium and magnesium may influence the adverse effects of barium on the cardiovascular and nervous systems or the effects of magnesium on the gastrointestinal tract, or whether their joint actions on the kidney may be additive, less-than-additive, or more-than-additive.

2.2.4 Barium and Manganese

Barium and manganese and other divalent cations can interact in complex ways with isolated membrane transport systems including: (1) components of mammalian calcium homeostatic systems including several types of calcium channels (see Sections 2.2.1 and 2.2.9); (2) sodium pumps (Cukierman and Krueger 1990; Gatto et al. 2007); (3) TRP channels (Bouron et al. 2015); (4) calcium-activated BK potassium channels involved in the regulation of neurotransmitter release and neuronal excitability (e.g., Lee and Cui 2010; McLarnon and Sawyer 1993; Zhou et al. 2012); and (5) calcium-activated SK potassium channels expressed in neurons, smooth muscle, neuroendocrine cells, and hematopoietic cells (e.g., Cao and Houamed 1999). Also, barium, manganese, and nickel, like calcium, have been shown to activate purified nitric oxide synthase, a Ca^{+2}/calmodulin binding enzyme that mediates production of nitric oxide free radicals, which are thought to be involved with vasodilator tone, hypertension, and neuronal function (Weaver et al. 2004). The physiological and toxicological relevance of the findings from this type of research is mostly unclear, especially with regard to making reliable predictions of how repeated combined oral exposure to any pair of metallic cations that are the subject of this profile may influence their individual toxicity.

No studies were located that examined toxicokinetic, nutritional balance, or toxicological endpoints following repeated oral exposure of humans or laboratory animals to concomitant excess levels of barium and manganese; these types of studies would be more useful for understanding how repeated oral exposure to excess levels of both barium and manganese may influence each other's toxicity. In deriving health guidance values to protect against the development of adverse effects from repeated oral exposure, the critical effects from exposure to manganese are neurological, whereas the critical effects from exposure to barium are kidney effects (see Appendices A and E). However, neurological effects have been reported with higher barium exposures and kidney effects at lower exposures, indicating a common toxicity target of barium and manganese, albeit by separate modes of action (see Appendices A and E).

Because dose-response data for barium-induced neurological effects are available, a TTD was derived for neurological effects following repeat oral exposure to barium (see Appendix A).

Summary. Studies conducted in subcellular systems, isolated cells, and tissues have found evidence for various interactions between barium and manganese on membrane transport of ions and neurotransmitters and on nitric acid synthetase. However, there is inadequate evidence to make conclusions on whether or not repeated oral exposure to excess barium and manganese may influence each other's toxicity or whether their possible joint actions on the nervous system are additive, less-than-additive, or greater-than-additive.

2.2.5 Barium and Sodium

Barium interactions with isolated cellular sodium/calcium exchangers (a plasma membrane reversible ion transport protein; it typically exchanges three sodium ions for one calcium ion) and sodium channels or pumps have been studied for many years, but the toxicological and physiological relevance of these types of observations are not clear due to the complexity of sodium homeostatic systems at cellular and organismal levels. Examples of isolated systems in which barium interactions with sodium/calcium exchange have been reported include dog sarcolemmal membrane vesicles (Trosper and Philipson 1983), Chinese hamster ovary cells transfected with a bovine sodium/calcium exchanger (Condrescu et al. 1997), isolated rat heart mitochondria (Lukacs and Fonyo1986), and frog atrial fibers (Potreau et al. 1987). Examples of reports of barium interactions with other isolated sodium transport systems include barium interactions with binding sites in the isolated Na, K-ATPase sodium pump from rat kidneys (Gatto et al. 2007); inhibition of the sodium/potassium pump activity in rat peritoneal mast cells by barium and other divalent cations (Knudsen 1995); voltage-dependent blockage by barium and other divalent cations (cadmium, calcium, cobalt, magnesium, manganese, nickel, and zinc) of sodium ion currents in single canine cardiac Purkinje cells (Hanck and Sheets 1992; Sheets and Hanck 1992); and effects by barium and other divalent cations on gating of, and permeability through, an isolated sodium channel from rat brain membranes (Cukierman and Krueger 1990).

In *in vivo* studies involving micro-perfusion of barium through loops of Henle in kidneys of anesthetized rats, sodium reabsorption was inhibited, presumably due to barium blockage of potassium channels and subsequent disruption of the operation of a $Na^+/K^+/Cl^-$ co-transporter (Huang et al. 2000; Walter et al. 2001; Wang et al. 1995). These *in vivo* findings illustrate coupling of a physiological component of

sodium, barium, and potassium homeostasis, but do not provide clear evidence on how oral co-exposure to barium and sodium may influence sodium homeostasis or influence each other's toxic effects.

No studies were located that examined toxicokinetic, nutritional balance, or toxicological endpoints following repeated oral exposure of humans or laboratory animals to concomitant excess levels of barium and sodium; these types of studies would be more useful for understanding how repeated oral exposure to excess levels of both may influence each other's toxicity. In deriving health guidance values to protect against the development of adverse effects from repeated oral exposure, the critical effect for exposure to sodium is hypertension, whereas the critical effects of exposure to barium are kidney effects (see Appendices A and F). However, increased blood pressure is also an endpoint of concern with excess barium exposure, indicating a potential common adverse outcome (cardiovascular effects) of barium and sodium co-exposure, likely via different modes of action (see Appendices A and F). Due to the availability of adequate data, TTDs were derived for cardiovascular and neurological effects following repeated oral exposure to barium at doses higher than those associated with kidney effects (see Appendix A). TTDs for other less sensitive effects from exposure to these metallic cations were not developed due to inadequate data (see Appendix A and F).

Summary. Studies conducted in subcellular systems, isolated cells, and tissues have found evidence for various interactions between barium and sodium on membrane transport. However, available interaction data are inadequate to assess whether the joint action of barium and sodium on blood pressure or other cardiovascular endpoints is dose-additive, greater-than-dose-additive, or less-than-dose-additive and whether or not repeated concomitant oral exposure to barium and sodium would affect barium's kidney or neurological effects (Appendix F).

2.2.6 Barium and Strontium

Barium, calcium, and strontium belong to Group (IIA) of the periodic table of elements. These elements have the same number of valence electrons and form stable divalent cations. The Stokes radius of Ba^{2+} is approximately 6% smaller than Ca^{2+} and Sr^{2+} (Kadhim and Gamaj 2020). These characteristics influence their interactions with binding sites on proteins, and extensive research has focused on their interactions with isolated membrane transport systems including: (1) components of mammalian calcium homeostatic systems including several types of calcium channels (see Sections 2.2.1 and 2.2.11); (2) sodium pumps (Cukierman and Krueger 1990; Gatto et al. 2007); (3) TRP channels (Bouron et al. 2015); (4) calcium-activated BK potassium channels involved in the regulation of neurotransmitter release and neuronal

excitability (e.g., Lee and Cui 2010; McLarnon and Sawyer 1993; Zhou et al. 2012); and (5) calcium-activated SK potassium expressed in neurons, smooth muscle, neuroendocrine cells, and hematopoietic cells (e.g., Cao and Houamed 1999). Findings from these studies have been important to understanding how the systems work in isolation, but the whole-body physiological and toxicological relevance of the findings from this type of research is mostly unclear, especially with respect to making reliable predictions of how combined oral exposure of humans or laboratory animals to any pair of metallic cations that are the subject of this profile may influence each other's toxicity.

Studies examining potential interactions between barium and strontium in whole-body mammals are few. Daily supplemental gavage administration of 33 mg Ba/kg/day plus 21.3 mg Sr/kg/day to young or old rats did not change concentrations of barium, calcium, or strontium in the tibia, compared with tibia concentrations in rats provided supplemental doses of barium or strontium alone (Panahifar et al. 2018). In an early study, gastrointestinal uptake of radiolabeled barium, calcium, radium, and strontium was measured in rats after single gavage administrations of the separate cations, but co-exposure was not part of the study (Taylor et al. 1962).

In deriving health guidance values to protect against the development of adverse effects from repeated oral exposure, the critical effects for exposure to strontium are skeletal effects, whereas the critical effects of repeated exposure to excess barium are kidney effects (see Appendices A and G). There is no clear evidence in humans or laboratory animals that repeated excess exposure to either of these metallic ions alone results in a common adverse outcome via a common mode of action or adverse effects in a common target organ or tissue (see Appendices A and G). No TTDs for other effects from strontium were derived due to inadequate data, but oral TTDs for barium-induced neurological and cardiovascular effects were derived (see Appendices A and G).

Summary. Studies conducted in subcellular systems, isolated cells, and tissues have found evidence for various interactions between barium and strontium on membrane transport. However, the available evidence for interactions between barium and strontium provides limited evidence from one study (Panahifar et al. 2018) that co-exposure to barium and strontium may not affect strontium distribution to the bone and thus have no influence on possible skeletal effects from excess strontium, and inadequate evidence to determine if co-exposure to strontium may influence the potential for barium to induce kidney, neurological, or cardiovascular effects.

2.2.7 Calcium and Iron

Potential interactions between calcium and iron have been investigated in biological systems at several levels of organization.

In short-term *in vivo* studies, oral intake of calcium in various media when consumed with iron has been shown to inhibit the acute gastric absorption of iron in volunteers (Cook et al. 1991; Hallberg et al. 1991, 1993; Gleerup et al. 1993, 1995) and animals (Barton et al. 1983; Wienk et al. 1996). Hallberg et al. (1991) postulated that calcium noncompetitively inhibits membrane proteins that transport non-heme iron into intestinal cells. In longer-term *in vivo* studies, however, calcium supplementation does not appear to interfere with long-term iron status or absorption of iron in humans (Dalton et al. 1997; Gaitan et al. 2011; Grinder-Pedersen et al. 2004; Hoppe and Hulthén 2012; Ilich-Ernst et al. 1998; Kalkwarf and Harrast 1998; Minihane and Fairweather-Tait 1998; Molgaard et al. 2005; Reddy and Cook 1997; Rios-Castillo et al. 2014; Yan et al. 1996) or animals (Wauben and Atkinson 1999), leading to the postulate that long-term calcium supplementation induces as yet unspecified homeostatic adaptive responses that counter the initial calcium inhibition of iron into gastrointestinal cells (Bendich 2001; Lonnerdal 2010; Minihane and Fairweather-Tait 1998).

Mechanistic studies with isolated cells indicate that the underlying short-term responses of iron homeostatic components to calcium may be more complicated than just calcium noncompetitively inhibiting the influx of iron into intestinal cells. An alternative hypothesis proposes that calcium acts to decrease the efflux of iron from enterocytes into the bloodstream (Gaitan et al. 2012; Hallberg et al. 1993). One study provided evidence that calcium is a noncompetitive inhibitor, but not a transported substrate, of divalent metal transporter 1 (DMT-1; a protein required for the influx of non-heme iron) (Shawki and Mackenzie 2010) and another study provided evidence that calcium decreased DMT-1 expression and localization in the apical membrane, inhibited iron-induced ferritin levels, and did not induce changes in ferroportin levels (Thompson et al. 2010). Other studies reported that short-term exposure to calcium (1–4 hours) had no effect on or increased iron influx or retention and decreased iron efflux (Gaitan et al. 2012; Lonnerdal 2010), and decreased levels of ferroportin in basolateral membranes (Lonnerdal 2010). Ferroportin is the only known cellular exporter of iron and evidence suggests that its activity is calcium-dependent, presumably through calcium binding that changes the conformation of ferroportin (Deshpande et al. 2018). Current mechanistic understanding is inadequate to provide evidence-based explanations for the short-term apparent inhibition of iron uptake by calcium and the lack of an effect of long-term calcium supplementation on iron absorption or iron status.

Iron-deficiency in rats has been shown to influence components of calcium homeostasis. After 40 days of nutritional iron deficiency, gastrointestinal absorption of calcium and urinary calcium excretions were increased, without a notable change in overall calcium balance (Campos et al. 1998). Iron-deficient rats also had increased absorption of phosphorus and magnesium, decreased balance of phosphorus and magnesium, and increased serum levels of PTH (Campos et al. 1998).

Excess iron status from hereditary hemochromatosis (i.e., mutations leading to increased intestinal absorption of iron) or repeated blood transfusions in thalassemic patients (leading to excessive red blood cell break down and release of heme-bound iron) has been associated with iron accumulation in the liver and heart, as well as "iron-overload" cardiomyopathy (see Gujja et al. 2010; Lopin et al. 2012 for review). Iron overload cardiomyopathy can present as a wide array of cardiac symptoms, but the most common forms are atrial and ventricular tachyarrhythmias with myocardial damage (Gujja et al. 2010). Under conditions of excess iron (when transferrin binding sites are saturated), the entry of excess non-transferrin bound iron into cardiomyocytes has been associated with changes in cellular structure (Iancu et al. 1987), gene expression (Parkes et al. 2000), intracellular calcium handling (Kim et al. 1995; Sripetchwandee et al. 2014; Wongjaikam et al. 2017), and properties of ion channels (Kuryshev et al. 1999). Several types of transport proteins have been proposed to be involved in transport of excess iron into cardiomyocytes, including L-type calcium channels (Oudit et al. 2003, 2006; Tsushima et al. 1999) and T-type calcium channels (Kumfu et al. 2011; Lopin et al. 2012), but currently, the mechanism for entry of excess iron into heart tissue is not clearly understood (Chen et al. 2014; Kumfu et al. 2013; Mackenzie et al. 2010). Although evidence has been presented that calcium channel blockers can increase iron excretion under iron overload conditions via DMT-1 (Ludwiczek et al. 2007) and randomized control trials of a calcium channel blocker have been proposed for patients with severe myocardial iron deposition (Shakoor et al. 2014), others have presented evidence that the mechanism by which calcium channel blockers increase iron excretion in iron-overload conditions does not involve DMT-1 and remains unclear (Mackenzie et al. 2010). Other investigators have examined other possible therapeutic approaches to iron overload disruption of calcium homeostasis and cardiac function, including the use of iron chelators and antioxidants (Wongjaikam et al. 2017).

Iron overload status also has been associated with disruption of calcium homeostasis in cultured neuronal cells (Lee et al. 2016; Nakamichi et al. 2002; Wang et al. 2017) and the brains of laboratory animals (Bostanci and Bagirici 2013; Wang et al. 2017). Iron overload status in mouse HT-22 hippocampal-derived neurons was associated with mitochondrial fragmentation, increased apoptotic cell death,

increased intracellular calcium concentrations, and activation of calcineurin and calcium signaling pathways (Lee et al. 2016). Iron-chelation or treatment with inhibitors of calcium signaling pathways countered the mitochondrial fragmentation and cell death responses (Lee et al. 2016). Ferrous iron, at concentrations ranging from 10 to 200 µM, inhibited increased intracellular calcium concentrations induced by N-methyl-D-aspartate in immature rat cortical neurons (Nakamichi et al. 2002). Treatment with a blocker of L-type calcium channels (nicardipine) countered the loss of neurons in the brains of iron-overloaded mice (achieved by intracerebroventricular injection of iron) (Bostanci and Bagirici 2013). Another L-type calcium channel blocker, isradipine, countered the degeneration of dopamine neurons and associated increased iron concentration in the substantia nigra of mice given intraperitoneal injections of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (Wang et al. 2017). These observations are consistent with evidence that, although calcium and iron are essential for normal development and function of the brain and are under tight cellular homeostatic control, their presence in excess can cause neuronal damage via calcium and iron mechanistic connections (see Hidalgo and Nunez 2007 for further discussion).

Summary. Although oral administration of calcium has been demonstrated to inhibit the gastrointestinal absorption of iron in short-term studies of humans and animals, long-term dietary supplementation with calcium does not appear to interfere with long-term iron status, presumably due to adaptive responses in as yet uncharacterized homeostatic mechanisms. Coupling between calcium and iron homeostatic processes has been revealed in studies of iron-deficient laboratory animals, studies of cardiomyopathy in humans with hereditary hemochromatosis, and studies of neuronal damage in cultured neuronal cells and iron-overloaded laboratory animals. However, available interaction data are inadequate to conclude whether or not concomitant oral exposure to excess calcium and excess iron will modify the potential for iron to induce gastrointestinal disturbances (the critical effect for the iron UL, Appendix C) or other high-dose iron effects (Appendix C) or calcium to induce kidney stones (the critical effect for the calcium UL, Appendix B).

2.2.8 Calcium and Magnesium

Calcium and magnesium were historically proposed to inhibit each other's absorption in the gastrointestinal tract due to the identification of magnesium as a calcium antagonist in isolated membrane transport systems, isolated neurotransmission systems and certain enzymatic reactions (EFSA 2006), but results from human studies conducted in the 1990s with radiotracer ^{47}Ca under physiological conditions reported no evidence of a competitive interaction (EFSA 2006). For example, 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 evidence for locational differences in absorption sites for these metallic cations along the gastrointestinal tract and different members of the transient receptor potential (TRP) ion channel protein family being involved in calcium (TRPV6) and magnesium (TRPM6) transcellular transport from the intestinal lumen (see Lameris et al. 2015).

Balance studies in humans have not found consistent evidence for negative magnesium balance with dietary calcium supplementation. Although an analysis of early research conducted before 1965 reported that calcium intakes >800 mg Ca/day may reduce magnesium balance (Seelig 1964) and a study of adult men indicated decreased magnesium absorption in the ileum of men consuming 1,900 mg Ca/day for 4 weeks, compared with men consuming 200 mg Ca/day (Norman et al. 1981), others reported no change in magnesium utilization/balance with dietary calcium supplementation (Andon et al. 1996; Greger et al. 1981; Yan et al. 1996). For example, magnesium balance was not significantly changed in adolescent girls eating a controlled diet containing 667 mg Ca/day and 176 mg Mg/day supplemented with 1,000 mg Ca/day for 14 days, compared with girls eating the same diet plus placebo (Andon et al. 1996).

Coupling between calcium and magnesium homeostatic mechanisms has been suspected for some time and is the focus of ongoing investigations, but mechanistic understanding is inadequate to explain the possible toxicological and public health consequences of repeated oral exposure to excess calcium and excess magnesium. As discussed in Appendix B, a complex, multiple-organ homeostatic process for calcium has been identified involving the intestine, kidneys, and bone that transport calcium into and out of extracellular fluid with various types of calcium selective ion channels, sensors of calcium concentrations in blood and cells (e.g., the CaSR), and regulatory hormones such as vitamin D, calcitonin, and PTH. Regulation of reabsorption in the kidney has been thought to be the key homeostatic mechanism for magnesium, but magnesium homeostasis has been less intensively investigated than calcium homeostasis (Appendix D). However, emerging evidence indicates that calcium and magnesium homeostasis may be coupled through magnesium inhibition of TRPV5-mediated calcium reabsorption in the renal distal tubule (Bonny et al. 2008), magnesium inhibition of calcium binding to the K^+-independent Na+/Ca+2 exchanger (NCX1) (Levitsky and Takahashi 2013), and through magnesium interactions with the CaSR, PTH secretion from the parathyroid, and vitamin D (Ferre et al. 2012; Hoorn and Zietse 2013; Quinn et al. 2013; Ritchie et al. 2001; Rodriguez-Ortiz et al. 2014; Rosanoff et al. 2016). Details of how these various sites of potential interactions between calcium and magnesium homeostatic

mechanisms may work together under conditions of excess oral exposure to calcium and magnesium are unclear.

Despite evidence that magnesium may be an effective inhibitor of calcium oxalate stone formation *in vitro*, results from up to seven human clinical trials of magnesium to prevent kidney stone recurrences were inconsistent (Riley et al. 2013; Schwartz et al. 2001). This inconsistency may be reflective of the inconsistent evidence linking high calcium intakes with kidney stone formation (see Appendix B and following discussion) or reflective of the relative effectiveness of citrate as an inhibitor of calcium oxalate stones *in vitro* and an inhibitor of recurrence of kidney stones in clinical trials (Allie and Rogers 2003). As discussed in Appendix B, evidence linking kidney stone formation to calcium dietary supplementation is not consistent across studies and U.S. (NAS 2011) and European (EFSA 2012) agencies differ in their evaluation of the critical effect on which to base a UL for calcium. The NAS (2011) concluded that the best human evidence for calcium induction of kidney stones comes from a study of postmenopausal women with total average intakes of about 2,100 mg Ca/day (Jackson et al. 2006) and based the adult calcium UL on the apparent lowest-observed-adverse-effect level (LOAEL) identified in this study. NAS (2011) recognized the inconsistency of the evidence for an association between high calcium dietary intakes or calcium dietary supplementation and kidney stone formation, citing studies indicating that high dietary calcium intakes may suppress the formation of kidney stones, and others indicating that older women taking calcium supplements may have increased risk for kidney stones (Curhan 2007; Curhan et al. 1993, 1997, 2004). In contrast, EFSA (2012) concluded that available evidence was inadequate to support associations between high calcium intakes and adverse effects of any kind, including formation of kidney stones, and based their calcium UL on a no-observed-adverse-effect level (NOAEL) of 2,500 mg Ca/day from numerous studies reporting no adverse effects with prolonged intakes of calcium from diet and supplements at this level (see Appendix B).

Summary. Emerging evidence indicates that coupling of homeostatic processes for calcium and magnesium exist at several sites (e.g., TRPV5-mediated calcium reabsorption in the renal distal tubule, CaSR, PTH secretion from the parathyroid), but current understanding is inadequate to explain how these potential coupling sites might work together under conditions of high oral intakes of both metallic cations, as is the case in the mixture of concern in UOG extraction wastewater. Evidence from human studies provide inconsistent evidence that calcium dietary supplementation decreases magnesium balance, and clinical trials provide inconsistent evidence that magnesium supplementation is an effective therapy against kidney stone formation in humans.

In deriving health guidance values to protect against the development of adverse effects from repeated oral exposure, the critical effect for calcium is increased risk for kidney stones, whereas the critical effect of repeated exposure to excess magnesium is gastrointestinal irritation (see Appendices B and D). There is no clear evidence in humans or laboratory animals that repeated excess exposure to either of these metallic ions alone results in a common adverse outcome via a common mode of action. But the kidney is a common toxicity target for both (see Appendix D for derivation of a TTD for kidney effects from magnesium). In conclusion, although there is ample evidence that calcium and magnesium homeostatic elements are coupled, studies of calcium dietary supplementation found inconsistent evidence for effects on magnesium balance, and studies of magnesium dietary supplementation found inconsistent evidence for protection against kidney stone formation. Available data are inadequate to determine whether the possible joint actions of calcium and magnesium on the kidney may be additive, less-than-additive, or more-than-additive.

2.2.9 Calcium and Manganese

Competitive inhibitions between calcium and manganese for intestinal absorption mechanisms have been suspected for a long time, but inconsistent evidence for this possible interaction has been reported in studies of animals and humans. Observations in support of short-term mutual competition include decreased absorption of manganese in chickens fed high dietary intakes of calcium or calcium and phosphorus (Smith and Kabaija 1986; Wilgus and Patton 1939); decreased manganese retention from food in mice fed calcium-enriched food (or magnesium-enriched food) (Van Barneveld and Van den Hamer 1984); bone decalcification in rats and negative calcium balance in cows fed high dietary levels of manganese (Chornock et al. 1942; Reid 1947); and decreased absorption of ^{54}Mn in perfused rat intestinal sections in the presence of high concentrations of calcium (0.1 or 0.01 mM manganese in the presence of 0 or 1 mM calcium) (Lutz et al. 1993). The latter observation of calcium inhibition of manganese absorption was observed in perfused sections of proximal jejunum and colon, but a calcium stimulation of manganese absorption was observed in sections of the distal jejunum (Lutz et al. 1993). In 12-hour fasted human subjects, peak plasma manganese concentrations were markedly decreased when an oral dose of 40 mg manganese was accompanied by 800 mg of calcium as either calcium carbonate or 545 mL of 2% milk (Freeland-Graves and Lin 1991). In longer-term human nutritional balance studies, however, small, negative manganese balances after dietary calcium supplementation have been reported in some studies (McDermott and Kies 1987), but not in others (Price and Bunce 1972; Spencer et al. 1979).

Evidence for short-term interactions between calcium and manganese have been reported in a number of isolated biological systems. For example, short-term exposure of primary cultured astrocytes to micromolar concentrations of manganese inhibited ATP-induced calcium signaling by blocking calcium entry through the TRP channel, TRPC3 (Streifel et al. 2013; Tjalkens et al. 2006). Manganese blocked voltage-gated calcium channels in isolated smooth muscle cells (Bolton et al. 1988). Studies with isolated mitochondria indicated that manganese uptake can be accelerated by the presence of calcium and calcium uptake can be inhibited by manganese (Chance and Mela 1966; Gavin et al. 1999; Vinogradow and Scarpa 1973), processes that are thought to be regulated by an ion-selective, mitochondrial calcium-activated calcium channel uniporter complex (Kamer et al. 2018; Kirichok et al. 2004). Studies with human HEK 239T cells genetically modified to be deficient in the pore-forming subunit of the uniporter complex (MCU) suggest that manganese transport into mitochondria by this calcium channel may play a role in manganese cytotoxicity (Kamer et al. 2018). Other studies have shown that manganese can be transported across membranes by a number of calcium channels including voltage-gated calcium channels (Fukuda and Kawa 1977) and the inositol 1,4,5-trisphosphate receptor (IP3R) channel (Striggow and Ehrlich 1996). Calcium and manganese interactions have also been demonstrated in isolated tissues, such as dissected rabbit atria (Sabatini-Smith and Holland 1969) and dissected rat uterine smooth muscle (Sakai and Uchida 1986).

Summary. Although there is evidence for complex interactions between short-term exposures to calcium and manganese at subcellular, cellular, tissue, and whole-body levels of organization (including reports that diets high in calcium can inhibit absorption or retention of manganese), consistent evidence for marked negative manganese balance in the presence of calcium dietary supplements has not been provided by human nutritional balance studies. The lack of strong evidence for interactions between calcium and manganese in the human balance studies may be due to the complexity, adaptability, and efficiency of homeostatic mechanisms for each of these essential elements under the applied experimental conditions. There is no clear evidence in humans or laboratory animals that repeated excess exposure to either of these metallic ions alone results in a common adverse outcome via a common mode of action or adverse effects in a common target organ, tissue, or system (Appendices B and F). In deriving health guidance values to protect against the development of adverse effects from repeated oral exposure, the critical effects from manganese exposure are neurological, whereas the critical effect of repeated exposure to excess calcium is increased risk for kidney stones (see Appendices B and F). TTDs for other less sensitive effects from these metallic cations were not developed due to inadequate data (see Appendix B and F). No studies were located that examined neurological or kidney endpoints after repeated co-exposure to these metallic cations at elevated dose levels.

In conclusion, although there is evidence that diets high in calcium can inhibit absorption or retention of manganese in animals, marked negative balance of manganese was not consistently observed in humans consuming calcium dietary supplements. Overall, there is limited evidence that calcium dietary supplementation may have no influence on manganese balance and hence manganese neurotoxic effects, and inadequate data to assess the potential for manganese co-exposure to modify calcium's potential to increase risk for kidney stones.

2.2.10 Calcium and Sodium

Ongoing investigations have collected evidence for coupling between homeostatic mechanisms for calcium and sodium at molecular, cellular, tissue, and whole-organism levels of organization. Sodium/calcium exchange across cellular membranes is mediated by a family of transport proteins (e.g., NCX1, NCX2, NCX3) that, in complex cooperation with other ion channels and calcium or sodium pumps, play roles in calcium and sodium homeostasis in many types of cells including various types of muscle cells (e.g., heart, vascular, and skeletal muscle cells), nervous system cells, intestinal cells, and blood cells (Blaustein and Lederer 1999; DiPolo and Beaugé 2006; Michel et al. 2015; Philipson and Nicoll 2000). NCXs in the plasma membrane of most cells exchange three sodium ions for one calcium ion and operate to extrude calcium or mediate calcium entry depending on transmembrane ion gradients and overall membrane electrochemical potential (Blaustein and Lederer 1999; Michel et al. 2015; Philipson and Nicoll 2000).

In vascular smooth muscle cells (VSMCs) under basal physiological conditions, NCX1 is thought to mediate calcium entry and play important roles in regulation of blood pressure and development of hypertension (Zhang 2013). This idea is based on observations of correlations between levels of expression of NCX1 in VSMCs and arterial contraction and blood pressure. Transgenic mice with NCX1 deficiency in VSMCs had decreased myogenic tone, vasoconstriction, and blood pressure, and transgenic mice with NCX overexpression in VSMCs had high blood pressure, greater sensitivity to salt (NaCl), and upregulation of cation-selective receptor-operated channels (e.g., TRPC6 protein) involved in regulating the sub-plasma membrane sodium gradient (Zhang 2013). These observations are consistent with a proposed complex, multi-organ, molecular pathogenesis of salt-dependent hypertension involving 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 adrenal glands, acute augmentation of calcium signaling associated with cardiotonic and vasotonic effects, and changes in

expression and/or phosphorylation of calcium and sodium transport proteins including NCX1 and TRPC proteins (Blaustein et al. 2012).

Inverse associations between dietary calcium (and other minerals, such as magnesium and potassium) and blood pressure reported in observational epidemiology studies (see Appel et al. 2006; Cappuccio et al. 1995 for reviews) prompted numerous clinical trials of calcium supplementation to counteract hypertension or the development of hypertension, a multifactor condition strongly associated with age and sodium salt intake (see Appendix F). Meta-analyses of clinical trials indicated small reductions of systolic and/or diastolic blood pressures with calcium supplementation (Allender et al. 1996; Bucher et al. 1996; Griffith et al. 1999; van Mierlo et al. 2006) and several studies of small numbers of subjects indicated an attenuating effect of calcium supplementation on increased blood pressure from high salt intake (e.g., Rich et al. 1991; Saito et al. 1989; Zemel et al. 1986). In addition, studies of animal models of hypertension have provided evidence that high calcium dietary intake can counteract development of hypertension (e.g., Ayachi 1979; Doris 1985; Evans et al. 1990; Ladipo et al. 2006; Makynen et al. 1995; McCarron 1985; McCarron et al. 1981; Pörsti et al. 1992; Resnick et al. 1986; Scrogin et al. 1991; Wuorela et al. 1992). However, an American Heart Association committee concluded that the available evidence from human studies was insufficient to recommend supplemental calcium as a means to lower blood pressure (Appel et al. 2006).

Summary. As reviewed above, there is evidence of coupling between homeostatic mechanisms for calcium and sodium, and there is limited evidence that supplemental oral exposure to calcium may counteract the development of hypertension, a condition associated with multiple factors, including exposure to excess sodium chloride. No other studies were located that examined toxicokinetic, nutritional balance, or toxicological endpoints following repeated oral exposure of humans or laboratory animals to concomitant excess levels of calcium and sodium; these types of studies would be useful for understanding how repeated oral exposure to excess levels of both may influence each other's toxicity. There is no clear evidence in humans or laboratory animals that repeated excess exposure to either of these metallic ions alone results in a common adverse outcome via a common mode of action or adverse effects in a common target organ or tissue (see Appendices B and F). In deriving health guidance values to protect against the development of adverse effects from repeated oral exposure, the critical effect for exposure to sodium is hypertension, whereas the critical effect for exposure to calcium is kidney stone development (see Appendices B and F). TTDs for other less sensitive effects from these metallic cations were not developed due to inadequate data (see Appendices B and F). In conclusion, the available evidence for interactions between calcium and sodium provides limited evidence that repeated oral

exposure to supplemental dietary calcium may counteract sodium salt-associated hypertension and inadequate evidence that excess sodium may modify the potential for calcium to produce kidney stones.

2.2.11 Calcium and Strontium

Calcium and strontium belong to Group (IIA) of the periodic table of elements. These elements have the same number of valence electrons and form stable divalent cations that have very similar Stokes radii (Kadhim and Gamaj 2020). These characteristics influence their interactions with binding sites on proteins and potential interactions between them have been investigated in a number of biological systems.

Many studies have examined the ability of strontium (and other divalent cations) to permeate through and modulate activity of isolated calcium transport systems (e.g., Bouron et al. 2015; Nelson 1986; Potreau et al. 1987; Tsien et al. 1987). In many systems, strontium is permeable (and could potentially compete with calcium) through calcium channels and can compete with calcium at allosteric binding sites. However, due to the complexity of calcium homeostasis at cellular, tissue, and whole-organism levels of organization, the significance of these types of findings to the possible joint action of calcium and strontium on toxicity targets after combined oral exposure of animals or humans is unclear.

Diets relatively high in strontium and low in calcium have been shown to disrupt calcium homeostasis in animals as evidenced by depressed intestinal absorption of calcium, depressed plasma calcium concentrations, and development of bone lesions (Bartley and Reber 1961; Corradino et al. 1971a, 1971b). In chickens, strontium inhibition of intestinal absorption of calcium involved strontium inhibition of the synthesis of calcitriol (1,25-dihydroxycholecalciferol) in the kidney, and strontium inhibition of calcium intestinal absorption was not evident in chickens fed a diet containing normal levels of calcium (Omdahl and DeLuca 1972). In basolateral membrane vesicles isolated from rat renal cortex, calcium was shown to be preferentially absorbed over strontium, demonstrating the discrimination of reabsorption processes in the renal proximal tubules between calcium and strontium; in the presence of 0.1 μM calcium, strontium significantly inhibited calcium uptake rates only when the molar ratio (Sr:Ca) was ≥16; in the presence of a high calcium concentration (1 μM), strontium concentrations up to 20 μM did not inhibit calcium uptake rates (Sugihira et al. 1992). The available evidence suggests that strontium can inhibit membrane transport of calcium only when external strontium concentrations are much higher than calcium concentrations.

Strontium ranelate, a molecule composed of an organic moiety and two strontium ions, has been shown to counteract postmenopausal osteoporosis in clinical trials, resulting in lower risks for bone fractures (Meunier et al. 2004; Reginster et al. 2005; Roux et al. 2006). Mechanistic studies indicate that strontium inhibits bone resorption and stimulates bone formation via interactions with the CaSR (Brown 2003; Hurtel-Lemaire et al. 2009). Strontium stimulation of new bone formation has also been observed in ovariectomized goats with calcium-sufficient diets that were supplemented with strontium phosphate (Li et al. 2009). Studies with isolated osteoblasts and osteoclasts indicated that strontium salts stimulate bone formation and decreases bone resorption, and *in vivo* studies showed that strontium ranelate prevented bone loss and maintained indices of bone formation at high levels (e.g., osteoblast surface, bone formation, and alkaline phosphatase activity) in ovariectomized rats (see Hurtel-Lemaire et al. 2009). While calcium and strontium each were shown to promote apoptosis of mature osteoblasts (thought to be a key step in regulating bone resorption) via the CaSR, the two cations are thought to act through different subsequent cell signaling pathways in a manner in which strontium adds to calcium-induced apoptosis of mature osteoclasts, and vice versa (Hurtel-Lemaire et al. 2009). Strontium stimulation of the AMP-protein kinase and mammalian target of rapamycin (AMPK/mTOR) signaling pathway has been linked to strontium stimulation of autophagy and differentiation in MC3T3 osteoblastic cells (Cheng et al. 2019). Whether or not the joint action of these cations on osteoclast apoptosis or differentiation is dose-additive, greater-than-dose-additive, or less-than-dose-additive has not been determined.

Calcium and strontium interactions with the CaSR also have been examined in a cell line (rat medullary thyroid carcinoma 6–23 cells) that is thought to be a good model for thyroid parafollicular C-cells, which secrete calcitonin in response to CaSR activation, and are key contributors to calcium homeostasis (Thomsen et al. 2012). Activation of the CaSR in parathyroid cells inhibits PTH secretion (a calcium-increasing agent) and stimulates secretion of calcitonin (a calcium-decreasing agent). In this cell model, strontium was more potent than calcium in stimulating calcitonin secretion and produced a different pattern of cell signaling pathways than calcium (Thomsen et al. 2012). Although these results highlight the CaSR as a likely molecular site of interaction between calcium and strontium, they do not address the possible toxicological consequences from exposure scenarios with both calcium and strontium present at elevated levels in drinking water.

Oscillations in calcium concentrations in the cytoplasm of fertilized mammalian oocytes (i.e., “calcium oscillations”) have been associated with different embryonic developmental stages and are thought to be activated by a protein factor from sperm that acts through inositol trisphosphate receptors (InsP3) (see Swann and Lai 1997 for review). Like the sperm factor, high concentrations of strontium (20 mM) have

been shown to activate isolated mouse oocytes and induce calcium oscillations through the action of InsP3 receptors (Zhang et al. 2005). This interaction between calcium and strontium oscillations in mammalian oocytes has been proposed to be useful to dissect the effects of calcium oscillations on cytoplasmic and nuclear developmental events in oocytes (Zhang et al. 2005), but its relevance to the possible toxicological significance of concomitant exposure to environmentally relevant excessive oral intakes of calcium and strontium is unclear.

Summary. Many studies of calcium transport processes and calcium-binding regulatory proteins in isolated systems or cells have shown that strontium can replace and compete with calcium, but the direct relevance of this type of research to questions about how combined oral exposure to excess levels of calcium and strontium may influence toxic responses is unknown, because of the complexity of calcium homeostasis. In addition, available evidence suggests that strontium can inhibit membrane transport of calcium only when external strontium concentrations are much higher than calcium concentrations (e.g., Bartley and Reber 1961; Corradino et al. 1971a, 1971b; Omdahl and DeLuca 1972; Sugihira et al. 1992).

Other studies indicate that the adverse skeletal effects of excess strontium in calcium-deficient animals can be counteracted by adequate dietary intakes of calcium (Omdahl and DeLuca 1972) and that added intake of strontium can also have beneficial skeletal effects. Strontium supplementation of normal calcium diets stimulated bone formation in ovariectomized (and osteoporotic) animals and decreased risk of bone fractures in osteoporotic women, presumably through strontium inhibition of bone resorption and stimulation of bone formation (Brown 2003; Hurtel-Lemaire et al. 2009; Meunier et al. 2004; Reginster et al. 2005; Roux et al. 2006).

In deriving health guidance values to protect against the development of adverse effects from repeated oral exposure, the critical effects for exposure to strontium are adverse skeletal effects, whereas the critical effect for exposure to calcium is increased risk for kidney effects (see Appendices B and G). TTDs for other less sensitive effects from these metallic cations were not developed due to inadequate data (see Appendices B and G). In conclusion, the available evidence for interactions between calcium and strontium is inadequate to conclude whether or not concomitant oral exposure to excess strontium will modify the potential for calcium to induce kidney stones, but there is some evidence from dietary studies in animals that excess calcium may protect against strontium-induced adverse skeletal effects and that excess strontium may stimulate bone formation in osteoporotic animals and humans.

2.2.12 Iron and Magnesium

Coupling between iron and magnesium homeostatic mechanisms has been demonstrated in studies of laboratory animals and isolated tissues and cells.

In short-term studies with isolated tissues and cells, iron uptake has been shown to be decreased by the presence of relatively higher concentrations of magnesium ions in incubating solutions. In isolated mouse intestinal mucosa fragments, the presence of 2, 5, or 10 mM magnesium ion in the incubating solution (containing 90 µM iron (III)) decreased iron uptake rates by about 25–30%, compared with medium containing no magnesium (Raja et al. 1987). The presence of 0.5 or 1 mM calcium had no significant effect on iron uptake rates (Raja et al. 1987). Iron transport from incubating solutions containing 20 µM iron (II) into rabbit erythroid cells was shown to be inhibited by the presence of exterior magnesium, with an IC_{50}=90µM, and stimulated by increased cytoplasmic levels of magnesium (Stonell et al. 1996). Stonell et al. (1996) proposed that iron uptake into erythroid cells may be mediated by a Na^{+}/Mg^{+2} antiport process.

The significance of this short-term inhibition of cellular uptake of iron by higher concentrations of magnesium to the long-term effects of repeated concomitant oral exposure is unclear, given the emerging understanding of the complex adaptability of homeostatic mechanisms for these essential metallic cations. It is conceivable that with long-term repeated oral co-exposure at levels below the respective tolerable upper intake levels, coordinated homeostatic mechanisms may adapt to maintain appropriate concentrations of both cations at the cellular and whole-body levels of organizations. Studies examining toxicokinetic endpoints (such as accumulation in toxicity target tissues), nutritional balance, or toxicological endpoints following repeated oral exposure of humans or laboratory animals to concomitant excess levels of iron and magnesium were not located and would be more useful for understanding how repeated oral exposure to excess levels of both cations may influence each other's toxicity.

Dietary magnesium deficiency in laboratory animals has been associated with hemolytic anemia (Piomelli et al. 1973; Sanchez-Morito et al. 2000), increased iron levels in certain tissues, especially liver and spleen (Gunther et al. 1995; Ishizaki et al. 2011; Kimura and Itokawa 1989; Piomelli et al. 1973; Sanchez-Morito et al. 2000; Schumann et al. 1997), increased intestinal iron absorption (Sanchez-Morito et al. 1999), and increased liver levels of oxidative stress indicators (Ishizaki et al. 2011). Reciprocally, perfused jejunum-ileum sections from rats fed an iron-deficient diet for 40 days had higher magnesium absorption rates than sections from control rats fed an iron-sufficient diet (Gomez-Ayala et al. 1997).

Although the underlying mechanisms for this apparent interaction are not understood, Sanchez-Morito et al. (2000) proposed the possible involvement of (1) increased fragility of erythrocytes due to magnesium deficiency and subsequent release of iron into blood and accumulation in liver and spleen; and (2) increased intestinal iron absorption in response to altered iron status. Ishizaki et al. (2011) proposed that iron accumulation in the liver from magnesium deficiency may involve dysregulation of gene expression of hepcidin, which negatively regulates cellular iron absorption.

Summary. Coupling between iron and magnesium homeostasis has been demonstrated in studies of laboratory animals and isolated cells and tissues, including the effects of magnesium-deficiency on iron status and homeostasis, iron-deficiency on magnesium absorption by perfused jejunum-ileum sections, and apparent inhibition of magnesium on short-term cellular uptake of iron, but the relevance of these findings to how concomitant oral exposure may influence their toxic actions is unclear. Studies examining toxicokinetic endpoints, nutritional balance, or toxicological endpoints following repeated oral exposure of humans or laboratory animals to concomitant excess levels of iron and magnesium were not located, and would be more useful for understanding how repeated oral exposure to excess levels of both iron and magnesium may influence each other's toxicity. There is some evidence that repeated excess exposure to these metallic ions alone results in adverse effects in a common tissue. In deriving health guidance values to protect against the development of adverse effects from repeated oral exposure, the gastrointestinal tract has been identified as the site of the critical adverse effect for exposure to excess magnesium (i.e., mild diarrhea) (Appendix D) and excess iron (gastrointestinal irritation) (Appendix C), but the mechanisms for these effects may differ. Diarrhea from magnesium is thought to be due to an osmotic effect (Appendix D), whereas gastrointestinal irritation from iron is thought to be due to reactive oxygen species from redox cycling of iron (Appendix C). Available data were inadequate for deriving TTDs for less-sensitive iron overload effects (Appendix C) but were adequate to derive a kidney effects TTD for magnesium (Appendix D). In conclusion, available data are inadequate to conclude whether or not repeated concomitant oral exposure to excess iron and excess magnesium may produce gastrointestinal effects in a dose-additive, greater-than-dose-additive, or less-than-dose-additive manner, whether co-exposure to magnesium may influence neurotoxic effect or other effects associated iron overload conditions, or whether co-exposure to iron may influence kidney effects of magnesium.

2.2.13 Iron and Manganese

There is clear evidence for competitive toxicokinetic interactions between iron and manganese at the level of binding to transport proteins (e.g., the transmembrane DMT-1, which binds to Fe^{+2}, Mn^{+2}, and other

divalent metallic cations, and transferrin (Tf), a blood transport protein that binds to Fe^{+3} and Mn^{+3}) (reviewed by Aschner et al. 2005; ATSDR 2012; Erikson et al. 2005a, 2005b; Ehrnstorfer et al. 2017; Fitsanakis et al. 2010, 2011; Gunter et al. 2013; Health Canada 2010; Vincent and Love 2012).

Studies in both animals and humans indicate that the gastrointestinal absorption of manganese is inversely related to dietary iron intake; therefore, relatively high levels of dietary iron intake can decrease gastrointestinal absorption of manganese and relatively low levels of dietary iron intake can increase gastrointestinal uptake of manganese (Davis et al. 1992a, 1992b; Diez-Ewald et al. 1968; Meltzer et al. 2010; Mena et al. 1969; Thomson et al. 1971; Zhang et al. 2016). Conversely, relatively high levels of manganese intake can lead to decreased gastrointestinal absorption of iron (Diez-Ewald et al. 1968; Li et al. 2006; Rossander-Hulten et al. 1991; Thomson et al. 1971). Animal studies also indicate that low-iron status or intake can lead to increased absorption of manganese in the nose and lungs or reduced clearance to the blood, following intratracheal or intranasal instillation; conversely, high iron intake can lead to decreased absorption of manganese in the lungs following intratracheal instillation (Heilig et al. 2005, 2006; Kim et al. 2012; Seo et al. 2013; Thompson et al. 2006, 2007). One study reported that intratracheal instillation of 2 mg MnO_2/kg/day + 2 mg Fe_3O_4/kg/day to rats for 4 weeks did not produce the decreased body weight gain and altered brain electrical activities observed in rats exposed to 2 mg MnO_2/kg/day alone, indicating a protective effect of iron under these conditions (Mate et al. 2017). In rats exposed to iron and manganese, manganese levels in blood and brain tissues were not different from levels in rats exposed to manganese alone, but manganese levels in lungs were 40% lower in rats exposed to iron and manganese, compared with rats exposed to iron alone (Mate et al. 2017).

Iron deficiency in rats also has been associated with increased manganese accumulation in the brain that is thought to involve increased binding of manganese to DMT-1 and transferrin due to decreased competition from iron, as well as upregulation of these transport proteins under iron-deficient conditions (Anderson et al. 2007; Aschner and Aschner 1990; Chua and Morgan 1996; Erikson and Aschner 2002; Erikson et al. 2002, 2004, 2005a, 2005b; Fitsanakis et al. 2011; Garcia et al. 2006, 2007).

Exposing rats to an iron-deficient and high-manganese diet (3.5 mg Fe/g and 100 mg Mn/kg) was reported to increase brain levels of oxygen stress indicators and alter performance results in a water maze behavioral test, compared with rats fed a control diet with 35 mg Fe/kg and 10 mg Mn/kg (Fitsanakis et al. 2009). Paradoxically, iron dietary supplementation also has been reported to increase manganese accumulation in the brains of rats (Chua and Morgan 1996; Fitsanakis et al. 2011), suggesting that the iron:manganese dietary ratio is important. Fitsanakis et al. (2011) concluded that both iron deficiency and

iron supplementation can lead to increased manganese accumulation in the adult brain, and that iron supplementation may not necessarily counter manganese brain accumulation. Fitsanakis et al. (2011) presented a hypothetical explanation based on observations that high dietary iron levels can lead to degradation of ferroportin, a transport protein that is hypothesized to decrease the plasma iron levels allowing for increased manganese binding to transferrin and subsequent increased manganese transport to, and accumulation in, the brain. Tests of this mechanistic hypothesis have not been conducted. Nevertheless, a case-control study of iron-deficient (n=31) and control (n=36) infants found that iron-deficient infants had higher mean blood manganese concentrations than controls [2.555 versus 1.499 μg/L) and that iron therapy of 19 iron-deficient infants significantly decreased mean blood manganese concentrations, compared with pre-therapy values (2.971 versus 2.045 μg/L) (Park et al. 2013).

Other studies indicated that oral exposure of rat dams between embryonic day 15 through PND 28 to excess manganese (10 mg Mn/kg by gavage every other day) or iron deficiency (diet containing 10% of normal diet iron) caused some changes in multiple behavioral tests in PND 29 offspring, but offspring exposed to both excess iron and manganese deficiency through their mothers did not show distinct or marked differences in behavioral tests from either condition alone (Amos-Kroohs et al. 2015, 2016, 2017). The available data on iron deficiency and excess manganese indicate that interactions between iron and manganese are complex and not well understood in relation to their accumulation in the brain, a well-known toxicity target of excess manganese.

The complexity of interactions between iron and manganese is reinforced by observations that repeated exposure of adult rats, with normal iron nutritional status, to gavage or intraperitoneal doses of excess manganese decreased levels of iron in serum, while increasing iron levels in the cerebrospinal fluid (Li et al. 2005, 2006; Wang et al. 2008a; Zheng et al. 1999). The manganese-induced change in iron distribution between blood and cerebrospinal fluid was associated with increased levels of transferrin receptor (TfR) messenger ribonucleic acid (mRNA) and concomitant decreased levels of ferritin mRNA (ferritin is a cytosolic iron-storing protein that protects against iron deficiency and iron overload) in the choroid plexus and striatum (Li et al. 2005, 2006). Li et al. (2006) hypothesized that manganese-induced increased expression of TfR and decreased expression of ferritin in rat brain regions may facilitate iron influx into the brain and contribute to manganese-induced neurotoxicity. Supporting evidence that excess manganese can disrupt iron homeostasis leading to increased iron in the brain comes from studies with intact rat choroid plexus (Wang et al. 2008b), rat choroidal epithelial cells (Wang et al. 2008a), and cultured neuronal cells (Zheng and Zhao 2001). Other *in vitro* and *in vivo* studies have provided evidence

that excess manganese can shift the Fe^{+2}/Fe^{+3} ratio in brain tissue towards the redox active Fe^{+2} (Fernsebner et al. 2014; Kwik-Uribe et al. 2003; Neth et al. 2015) and that this manganese-induced shift involves manganese blockage of the translation of iron homeostatic proteins, amyloid precursor protein, and heavy-chain Ferritin (Venkataramani et al. 2018).

The influence of excess manganese on iron homeostasis may be dose-, route-, age-, or media-dependent, as suggested by the observation that exposure of nutritionally iron-sufficient rats to drinking water with 10 mg Mn/mL had no influence on levels of iron in tissues from three brain regions (globus pallidus, striatum, and inferior colliculus) or three regions of the cochlea, compared with controls without added manganese in drinking water (Mullin et al. 2015). Differences between animal species or stage of development also may influence potential interactions between iron and manganese. Gavage exposure of neonatal mice to excess manganese (11 or 25 mg Mn/kg/day) from PND 1 through 28 produced dose-dependent decreased motor activity on PND 19 that was associated with dose-dependent increased manganese levels in all tissues examined: olfactory bulb, striatum, frontal cortex, liver, and femur (Foster et al. 2017, 2018). Under these experimental conditions, however, excess manganese intake decreased iron levels in the liver, but produced no significant changes in iron levels in the femur or the various brain regions examined (Foster et al. 2017, 2018). In studies of developing rats exposed to a diet high in manganese via maternal milk during lactation (PNDs 4–21), exposed pups showed an increase in brain manganese, chromium, and zinc and a decrease in brain iron, accompanied with enhanced protein expression of DMT-1 and TfR in the brain and an increase in gamma-aminobutyric acid (GABA) and the ratio of GABA to glutamate, indicating enhanced inhibitory transmission in the brain (Garcia et al. 2007). These latter results illustrate complex coupling of iron and manganese homeostatic mechanisms and suggest that high intake of manganese can influence not only brain concentrations of iron, but concentrations of other metals as well.

Results from studies with mice in which the gene (SLC11A2) encoding DMT-1 was inactivated in the intestine further illustrate the complexity of physiological interactions between iron and manganese (Shawki et al. 2015). These genetically modified mice (intestinal knockout mice, $DMT1^{int/int}$) showed a severe anemia, depleted blood iron, depleted tissue stores of iron, and depressed mRNA expression of liver hepcidin, all of which could be countered with intraperitoneal iron injection. In contrast, the $DMT1^{int/int}$ mice showed no marked differences in tissue concentrations of copper, manganese, or zinc, compared with wild-type mice, thereby indicating that intestinal absorption of manganese (and these other divalent metallic cations) may proceed via transport mechanisms other than DMT-1. This explanation was reinforced with studies showing that acute gastric doses of ^{64}Cu or ^{54}Mn were absorbed to a similar

extent in DMT1$^{int/int}$ and DMT$^{+/+}$ mice, but ^{59}Fe absorption was essentially abolished in DMT1$^{int/int}$ mice (Shawki et al. 2015).

The SLC39A14 protein (also known as ZIP14) can facilitate membrane transport of manganese and other metals, including iron. The protein has been proposed to be important for transporting excess manganese from the blood to the liver, where excess manganese can be eliminated in the bile, but its possible importance to whole-body iron and manganese homeostasis is not well understood. One study of mice deficient in the SLC39A14 gene reported abnormally low manganese levels in liver and markedly elevated manganese levels in blood and most other organs, including the brain (Jenkitkasemwong et al. 2018), whereas another study reported that global knockout of the SLC39A14 gene in mice produced (compared with wild-type mice) no change in liver manganese levels, markedly increased manganese levels in other tissues including brain, and no significant changes in iron levels in any examined tissue (Xin et al. 2017). The elevated manganese levels in the global knock-out mice were associated with motor deficits (Jenkitkasemwong et al. 2018; Xin et al. 2017). When the expression of the SLC39A14 gene was only inactivated in hepatocytes, levels of manganese were markedly decreased in the liver, but not in extrahepatic tissues including the brain, and no motor deficits were observed (Xin et al. 2017). The data indicate that the SLC39A14 protein is involved in regulating manganese transport from the blood to the liver. It is not currently understood how global deficiency in this protein leads to excess brain manganese levels and motor deficits, but inactivation only in hepatocytes does not.

The nervous system may be a common toxicity target of excess iron and excess manganese, as evidenced by observations of: (1) neurological dysfunction in human subjects with genetic disorders or neurodegenerative diseases such as Parkinson's and Alzheimer's disease and accumulation of iron, manganese, and other metals in brain tissue (Chen et al. 2019; Dusek et al. 2015a, 2015b; Huat et al. 2019); (2) neurological dysfunction and accumulation of manganese in brains of human subjects with high occupational exposures to manganese (e.g., manganese miners, see ATSDR 2012); and (3) common proposed toxic actions related to generation of reactive oxygen species (see Appendices C and E).

Although brain accumulation of iron has been observed in patients with neurodegenerative diseases, the etiology of the iron accumulation in the brain is unclear (Berg et al. 2001; Double et al. 2000; Gerlach et al. 2003). Nevertheless, the substantia nigra, a region of iron accumulation in the brain, is the same brain region associated with manganese accumulation in patients with manganism (see ATSDR 2012 and Appendix E). In addition, elevated levels of oxidative stress indicators and altered behavioral endpoints have been observed in rodents fed an iron-deficient and high-manganese diet (Fitsanakis et al. 2009).

However, studies designed to examine how concomitant elevated exposures to both cations may act jointly on neurological endpoints were not located, with the exception of the earlier mentioned study by Mate et al. (2017), reporting that concomitant intratracheal exposure of rats to iron and manganese counteracted the effect of manganese alone on electrophysiological measurements in cortical regions of the brain.

Summary. Toxicokinetic evidence from human and animal studies indicates that iron deficiency can result in increased gastrointestinal absorption of manganese and increased brain accumulation of manganese, potentially resulting in increased susceptibility to manganese-induced neurotoxicity (Anderson et al. 2007; Aschner and Aschner 1990; Chua and Morgan 1996; Davis et al. 1992a, 1992b; Diez-Ewald et al. 1968; Erikson and Aschner 2002; Erikson et al. 2002, 2004, 2005a, 2005b; Fitsanakis et al. 2009, 2011; Garcia et al. 2006, 2007; Meltzer et al. 2010; Mena et al. 1969; Thomson et al. 1971). Complementing the toxicokinetic evidence, rats fed an iron-deficient and high-manganese diet had increased brain levels of oxygen stress indicators and altered results in a water maze behavioral test, compared with rats fed a control diet with normal levels of iron and manganese (Fitsanakis et al. 2009). Studies in animals show that excess dietary iron supplementation can also increase brain accumulation of manganese (Chua and Morgan 1996; Fitsanakis et al. 2011), although iron-deficient infants given iron supplementation showed significant decreases in their mean blood manganese concentrations (Park et al. 2013) and exposure of rat offspring to excess manganese and iron deficiency during gestation and early postnatal periods did not show distinct or marked differences in behavioral tests from either condition alone (Amos-Kroohs et al. 2015, 2016, 2017). Additionally, evidence from human and animal studies suggests that exposure to excess manganese could lead to iron deficiency due to decreased intestinal absorption of dietary iron (Diez-Ewald et al. 1968; Li et al. 2006; Rossander-Hulten et al. 1991; Thomson et al. 1971), but other studies have found that repeated exposure of adult rats to excess manganese, under normal iron nutritional status, produced decreased levels of iron in serum, while increasing iron levels in the cerebrospinal fluid or brain (Li et al. 2005, 2006; Wang et al. 2008a; Zheng et al. 1999). Other studies found that excess manganese shifted the Fe^{+2}/Fe^{+3} ratio in brain tissue towards the redox active Fe^{+2}, which has been associated with manganese blockage of the translation of genes for neuroprotective, iron-homeostatic proteins (Fernsebner et al. 2014; Kwik-Uribe et al. 2003; Neth et al. 2015; Venkataramani et al. 2018). Still other animal studies reported conditions in which excess manganese did not produce elevated iron levels in the brain (Foster et al. 2017, 2018; Garcia et al. 2007; Mullin et al. 2015).

The available data indicate that although homeostatic mechanisms for iron and manganese may share some components, each mechanism is complex and incompletely understood. In addition, homeostatic

mechanisms for these two metallic cations may overlap with other metals (see results from Garcia et al. 2007 above). Emerging concepts from genetic modification studies indicate that: (1) DMT-1 is essential for iron intestinal absorption, but other transporters may be involved in the absorption of manganese and other divalent cations (Shawki et al. 2015), and (2) the SLC39A14 protein, a protein that has been reported to transport iron and manganese across cellular membranes, plays a role in regulating manganese uptake into the liver and an uncharacterized role in regulating manganese levels in the brain (Jenkitkasemwong et al. 2018; Xin et al. 2017).

Thus, there is: (1) a wealth of evidence that interactions between iron and manganese at molecular, cellular, and physiological levels of organization are complex and may involve coupling of homeostatic mechanisms; (2) some evidence that iron deficiency in rats may enhance the neurotoxicity of manganese; and (3) some evidence that accumulations of both iron and manganese in brain tissue are associated with various neurological dysfunctions. A single study reported that electrophysiological brain measurements after intratracheal exposure of rats to iron and manganese was less than those in rats exposed only to manganese, suggesting a possible protective effect. However, no studies were located that examined brain levels of both cations or neurological endpoints after repeated oral exposure of laboratory animals (or humans) to excess iron and excess manganese, as may be the case following exposure to groundwater contaminated with UOG extraction wastewater.

In deriving health guidance values to protect against the development of adverse effects from repeated oral exposure, the most sensitive adverse effects (i.e., critical effect) for exposure to manganese are neurological, whereas the critical effects of exposure to excess iron are gastrointestinal discomfort and irritation. Iron, manganese, and other metals may jointly act to produce central nervous system toxicity, but available evidence is inadequate to determine whether possible joint action on neurotoxic endpoints following oral exposure to high levels of iron and manganese may be additive, less-than-additive, or more-than-additive. Available dose-response data were inadequate to derive an oral neurological TTD for iron (see Appendix C). A TTD for male reproductive effects from oral exposure to manganese was considered but would have been essentially the same as the recommended health guidance value based on neurological effects (see Appendix E). In conclusion, the available evidence provides limited evidence that concomitant oral exposure to excess iron and excess manganese may increase risk for neurological effects and inadequate evidence to determine whether or not co-exposure will modify the potential for iron to induce gastrointestinal discomfort and irritation.

2.2.14 Iron and Sodium

Interactions between iron and sodium with membrane transport processes in isolated cells have been examined in many studies. Findings from a few representative reports follows. In isolated pyramidal neurons from rats, Fe^{+2} altered currents through K^+ and Na^+ channels (Ge et al. 2001). Iron was required for the induction of the functional epithelial Na^+ channels by oxygen in primary rat fetal lung cells (Rafii et al. 2000). In cultured dog kidney cells (MDCK cells), low external potassium induced an increase in $Na^+/K+$ -ATPase activity that was dependent on the presence of serum or transferrin in the external media and was accompanied by increased uptake of radiolabeled iron (Yin et al. 2003). This transferrin-dependent response of Na^+/K^+-ATPase activity was inhibited by deferoxamine (an iron chelator) and superoxide dismutase (which catalyzes the dismutation of oxygen radicals) suggesting that the low potassium effect was linked to increased iron transport and reactive oxygen species activity (Zhou et al. 2003). In human primary bronchial epithelial cells, iron accumulation: (1) did not occur in the absence of external sodium: (2) was diminished by inhibitors of various sodium transport channels; and (3) was accompanied by increased potassium efflux and phosphate influx (Turi et al. 2008). These results are consistent with the idea that iron uptake into cells is dependent on maintenance of a Na^+/K^+ gradient across cells, but their relevance to whole-body homeostasis of iron and sodium or to the toxicological significance of concurrent oral exposure to excess iron and excess sodium is unclear.

The beneficial effects of decreased iron intake on hypertension, renal tubule morphological changes, and changes in renal mineralocorticoid signaling pathways have been examined in rat models of chronic kidney disease (Naito et al. 2012, 2013a). Iron-restriction beneficial effects also have been examined in rat models of sodium-salt hypertensive nephropathy (Naito et al. 2013b) and sodium-salt hypertensive cardiovascular disease (Naito et al. 2011). Taken together, the findings from these studies (see following three paragraphs for more details) illustrate complex interactions between elements of iron and sodium homeostasis and suggest the involvement of iron in the development of hypertensive chronic kidney disease and sodium-salt-induced hypertension and effects on the kidney and cardiovascular system.

In Sprague-Dawley rats with 5/6 of nephrons surgically removed (a model of human chronic kidney disease), hypertension and renal structural damage spontaneously developed starting at 8 weeks and continued until 16 weeks after surgery at the end of the study, compared with control rats without surgery (Naito et al. 2012, 2013a). The development of hypertension and renal damage was counteracted by administration of an iron-restricted diet between weeks 1 and 16 (Naito et al. 2012, 2013a). The normal diet contained cornstarch 33%, casein 22%, cellulose 5%, sucrose 30%, corn oil 5%, mineral mixture 4%,

and vitamin mix 1%. The mineral mixture was a mixture of minerals containing 0.623% ferric citrate; the ferric citrate was omitted from the iron-restricted diet. Later administration of the iron-restricted diet between 8 and 16 weeks after surgery did not prevent hypertension and renal damage but did counteract their further development (Naito et al. 2013a). Iron restriction for the full 16 weeks also counteracted other responses to the kidney surgery including increased renal mineralocorticoid receptor signaling (important in renal regulation of sodium and fluids), increased urinary iron and protein excretion, increased renal content of iron and an oxidative stress indicator (8-hydroxy-2'-deoxyguanosine [8OHdG]), and increased aortic expression of iron transporter, transferrin receptor 1 (TfR1). Iron restriction did not seem to counteract the increased expression of TfR1 and DMT-1 in renal tubules produced by the surgery (Naito et al. 2012, 2013a). Surgically modified rats with the full-term iron restriction showed increased urinary sodium and decreased urinary potassium excretion, compared with surgically modified and control rats fed the control diet. The authors concluded that the beneficial effects of dietary iron restriction against renal damage in this model appear to be mediated through mineralocorticoid receptor signaling.

In spontaneously hypertensive, stroke prone (SHRSP) rats, the pronounced effects of a high-salt diet for 4 weeks on survival and body weight, systolic blood pressure, kidney morphology and gene expression profiles, and kidney iron accumulation (compared with SHRSP fed a control diet) were significantly lessened or absent in SHRSP fed a high-salt and iron-restricted diet for 4 weeks (Naito et al. 2013b). The control diet in these experiments was the same as those reported in the previous paragraph (Naito et al. 2013a). High-salt diets contained 8% NaCl (versus 0.3% in control), and iron-restricted diets omitted ferric citrate from the mineral mixture in the control diet. High-salt treatment induced decreased mean body weight at the end of treatment, and decreased survival during a 4-week post-treatment observation period (~40 versus 100% in control). These effects were not seen in the group fed the high-salt, iron-restricted diet. At the end of the study period, high-salt treatment also produced (compared with values for the control diet group) increased: (1) systolic blood pressure (~25%); (2) renal iron content (~500%); (3) urinary iron (~90%) and 8OHdG (~90%) excretion; (4) scores for renal glomerulosclerosis and tubular dilation and luminal casts; (5) renal gene expression of indicators of fibrosis: collagen III, transforming growth factor beta 1 (TGF-β), cluster of differentiation-68 (CD-68), and plasminogen activator inhibitor 1 (PAI-1); and (6) renal tubule levels of TfR1 and DMT1. In rats fed the high-salt, iron-restricted diet, these effects were either absent (e.g., increased renal iron content, urinary iron excretion, scores for renal lesions and indicators of renal fibrosis) or were lessened (e.g., increased blood pressure and renal levels of iron transporters). The authors concluded that the findings suggest the involvement of iron in the

development of hypertensive nephropathy and establish that restriction of iron counteracts the development of salt-induced nephrosclerosis.

In Dahl salt-sensitive rats, the effects of a high-salt diet for 12 weeks on systolic blood pressure, body weight, survival, and histology of the aorta were absent or lessened in rats fed a high-salt, iron-deficient diet (Naito et al. 2011). The high-salt diet was a control rat chow (with ~0.3% NaCl and ~0.003% ferric citrate monohydrate added as a supplement) containing 8% NaCl; the ferric citrate was omitted in the high-salt, iron-restricted diet. The high-salt treatment (compared with values for the control diet group) produced decreased body weight (~25%), decreased survival rate, presumably due to heart failure (~40% vs 100% in controls), and increased systolic blood pressure (~60%), at the beginning and end of a 6-week post-treatment observation period; these effects were essentially absent in the group fed the high-salt, iron-restricted diet. The high-salt treatment also produced aortic vascular hypertrophy, accompanied with: (1) increased aortic gene expression of collagen III, TGF-β, and CD-68; (2) decreased aortic gene expression of activated oxidative stress repair indicators (phosphorylated forms of Akt [also known as protein kinase B], AMP-activated protein kinase, and endothelial nitric oxide synthase); (3) increased urinary excretion of proteins and 8-OHdG; and (4) increased aortic gene expression of TfR1 and subunit H of ferritin. All of these high-salt effects were absent or diminished in the group fed the high-salt, iron-restricted diet. In separate groups fed the high-salt, iron-restricted diet for 6 weeks, treatment with an inhibitor of nitric oxide synthase in drinking water (N^G-nitro-L-arginine methyl ester [L-NAME]) diminished the beneficial effects of iron restriction on systolic blood pressure, urinary protein excretion, and survival. The authors concluded that dietary iron restriction protected against salt-induced hypertension, cardiovascular remodeling, and proteinuria by inhibiting oxidative stress and maintaining activated Akt, AMP-activated protein kinase, and endothelial nitric oxide synthase in the aorta.

Summary. Coupling between iron and sodium membrane transport processes in isolated cells have been examined in many studies and provide evidence that iron uptake into cells is dependent on maintenance of a Na^+/K^+ gradient across cells, but their relevance to the toxicological significance of concurrent oral exposure to excess iron and excess sodium is unclear.

Although no studies were located that examined toxicokinetic, nutritional balance, or toxicological endpoints following repeated oral exposure of humans or laboratory animals to concomitant excess levels of iron and sodium, the studies of the beneficial effect of iron restriction in rat models illustrate complex interactions between elements of iron and sodium homeostasis, and suggest the involvement of iron in the development of hypertensive chronic kidney disease and sodium-salt-induced hypertension and effects on

the kidney and cardiovascular system. These findings raise concerns that repeated oral exposure to high levels of iron and sodium may jointly act to increase the risk for kidney and cardiovascular effects, but it is unknown whether or not joint actions may be additive, less-than-additive, or more-than-additive.

In deriving health guidance values to protect against the development of adverse effects from repeated oral exposure, the critical effect for exposure to high sodium salt is hypertension, whereas the critical effect for exposure to excess iron is gastrointestinal irritation (see Appendices C and F). High acute oral fatal doses of iron (~60 mg/kg) have been associated with fatality due to iron overload, with involvement of the cardiovascular system, central nervous system, kidney, liver, and hematological systems, and genetically determined chronic iron-overload conditions (e.g., thalassemias, congenital atransferrinemia, and aceruloplasminemia) have been associated with multiple other adverse outcomes in humans, including liver cirrhosis, cardiomyopathy, and neurodegeneration (see Appendix C). Oral exposure health guidance values (including TTDs) based on most of these iron overload effects, however, were not derived due to the lack of adequate dose-response data (see Appendix C). In conclusion, there is limited evidence from studies of the beneficial effects of iron restriction in rat models of chronic kidney disease and sodium-salt hypertension associated with kidney and cardiovascular effects that concomitant oral exposure to excess iron and sodium may increase risks for kidney and cardiovascular effects, but it is unknown whether or not the possible joint actions of iron and sodium on these toxicity targets may be additive, less-than-additive, or greater-than-additive.

2.2.15 Iron and Strontium

Extensive research has been conducted at cellular and whole-body levels of organization on possible interactions between calcium and strontium homeostatic elements (see Section 2.2.11) and coupling between calcium and iron homeostatic elements (see Section 2.2.7). From these findings, it is conceivable that oral co-exposure to high levels of iron and strontium might interfere with calcium homeostasis, but no studies directly designed to test this hypothesis at either the cellular or whole-organism level were located. Studies that examine toxicokinetic, nutritional balance, or toxicological endpoints following repeated oral exposure of humans or laboratory animals to concomitant excess levels of iron and strontium would be useful for understanding how repeated oral exposure to excess levels of both may influence each other's toxicity.

In deriving health guidance values to protect against the development of adverse effects from repeated oral exposure, the critical effects of exposure to strontium are skeletal effects, whereas the critical effect

of exposure to excess iron is gastrointestinal irritation (see Appendices C and G). There is no clear evidence in humans or laboratory animals that repeated excess exposure to either of these metallic ions alone results in a common adverse outcome via a common mode of action or adverse effects in a common target organ or tissue (see Appendices C and G). No TTDs for other less sensitive effects from oral exposure to these metallic cations were derived due to inadequate data (see Appendices C and G).

Summary. The available evidence for interactions between iron and strontium is inadequate to conclude whether or not concomitant oral exposure to iron and strontium will modify the potential for strontium to produce skeletal effects or the potential for iron to produce gastrointestinal irritation.

2.2.16 Magnesium and Manganese

Studies with isolated TRPM6 and TRPM7 channels, which are thought to be important components of magnesium cellular and organismal homeostasis, have shown that manganese can permeate through these channels, but the relevance of this possible competitive inhibition is of unknown physiological or toxicological significance (Bouron et al. 2015; Kolisek et al. 2019). Other studies indicate that magnesium and manganese can interact in complex ways with components of calcium homeostasis in isolated cells (see Sections 2.2.8 and 2.2.9), but the physiological and toxicological relevance of findings from this type of research is mostly unclear, especially with regard to making reliable predictions of how combined oral exposure to excess magnesium and excess manganese may influence each other's toxicity.

Possible interactions between magnesium and manganese have been examined in a few oral exposure animal studies, but they do not present a clear understanding of interactions between these metallic cations at the whole-body level of organization. In mice, the short-term intestinal absorption of ^{54}Mn from gavage-delivered solutions containing 1 mM $MnCl_2$ was inhibited by concomitant exposure to relatively high concentrations of $MgCl_2$ (25 mM), but 2-week exposures to a magnesium-depleted diet did not influence short-term gastric absorption of ^{54}Mn (Van Barneveld and Van den Hamer 1984). In contrast, a study of rats fed a magnesium-deficient diet for 70 days reported that manganese absorption, measured as the difference between manganese oral intake and fecal excretion, was increased, compared with rats fed a magnesium-sufficient diet (Sanchez-Morito et al. 1999). It is uncertain if the apparent discrepancy between the two studies with respect to the effect of magnesium deficiency on the apparent absorption of manganese is due to differences in species, nutritional status, methods for measuring absorption, or some other factor, such as differences in metal concentrations in the diets used in the various studies. In other studies, sudden deaths occurred in pigs fed for up to 5 weeks a magnesium-

deficient diet with a high level of manganese, whereas such deaths did not occur in pigs fed a sufficient magnesium diet with the same high manganese level (Miller et al. 2000, 2004). The deaths were associated with myocardial necrosis and mitochondrial swelling not seen in pigs fed sufficient magnesium and high manganese (Miller et al. 2004). No mechanistic studies were located that tested hypotheses related to molecular, cellular or tissue-level changes in manganese homeostatic components influenced by magnesium deficiency.

Summary. Magnesium and manganese can interact with isolated transport proteins associated with magnesium homeostasis and calcium homeostasis, but the physiological and toxicological significance of these interactions is unknown. Other studies suggest that relatively high concentrations of magnesium (compared with manganese concentrations) may inhibit the short-term intestinal absorption of manganese, but do not provide a clear understanding of the effect of magnesium deficiency on intestinal manganese absorption.

No studies were located that examined toxicokinetic, nutritional balance, or toxicological endpoints following repeated oral exposure of humans or laboratory animals to concomitant excess levels of magnesium and manganese; these types of studies would be more useful for understanding how repeated oral exposure to excess levels of both may influence each other's toxicity. There is no clear evidence in humans or laboratory animals that repeated excess exposure to either of these metallic ions alone results in a common adverse outcome via a common mode of action or adverse effects in a common target organ, tissue, or system (see Appendices B and E). In deriving health guidance values to protect against the development of adverse effects from repeated oral exposure, the critical effects for exposure to manganese are neurological, whereas the critical effect of exposure to excess magnesium is gastrointestinal disturbance (mild diarrhea) (see Appendices B and E). TTDs for other less sensitive effects from these metallic cations were not developed due to inadequate data, with the exception of a TTD for kidney effects from magnesium (see Appendices B and E). In conclusion, there is limited evidence that excess magnesium may inhibit gastrointestinal absorption of manganese and thereby may counteract manganese neurotoxicity, but no studies were located that examined neurological endpoints after co-exposure to both cations and compared the responses to responses from exposures to the individual cations alone. The available evidence for interactions between magnesium and manganese is inadequate to conclude whether or not repeated concomitant oral co-exposure will modify the potential for magnesium to induce mild diarrhea.

2.2.17 Magnesium and Sodium

Sodium/magnesium exchange across cellular membranes (sodium influx with magnesium efflux) has long been thought to be an integral part of homeostatic regulation of cellular magnesium. For example, early studies with mammalian cells showed that substitution of extracellular sodium ion by choline inhibited magnesium efflux indicating that magnesium efflux is sodium dependent (Günther et al. 1984). Currently, however, the identity of the membrane transport proteins responsible for the sodium/ magnesium exchange is somewhat controversial. Funato et al. (2018) reviewed evidence that linked dysfunctional CNNM2, a divalent metal cation transporter, to defects in renal reabsorption in mice and zebrafish, and presented evidence that this membrane functions as a sodium/magnesium exchanger. Arjona and de Baaij (2018) presented evidence for an alternative hypothesis that CNNM2 is not the sodium/magnesium exchanger, but rather regulates magnesium influx via TRPM6 and TRPM7 and indirectly affects an independent exchange of magnesium efflux and sodium influx. In a third viewpoint, Kolisek et al. (2019) presented evidence that SLC41A3 plays a role in sodium/magnesium exchange across cellular membranes. Others have provided evidence with isolated cells that the electrogenic Na+-HCO_3^- cotransporter, NBCe1-B, which is involved in regulation of intracellular pH and epithelial HCO_3^- secretion, is regulated by intracellular magnesium concentrations, suggesting another coupling site between magnesium and sodium homeostasis (Yamaguchi and Ishikawa 2008). Regardless of the uncertainty in the molecular details, the available evidence from these types of investigations suggests that there may be coupling between magnesium and magnesium homeostatic mechanisms in at least two cellular sites but this does not explain how repeated oral co-exposure to excess magnesium and sodium may influence each other's toxicity.

In rodent studies, high sodium salt oral intake by rats for 1 week was associated with increased urinary excretion of sodium, calcium, and magnesium along with upregulation of genes for transport proteins for calcium (TRPV5, TRPV6, calbindin-D28K) and magnesium (TRPM6) in the renal distal convoluted tubule (Lee et al. 2012), whereas in another study, high sodium salt oral intake (with normal or low potassium intake) by mice for 4 days was associated with increased urinary excretion of calcium and sodium, but not magnesium, along with renal upregulation of magnesium transporter TRPM6 and calbindin-D28K, down regulation of magnesium transporter TRPM7, and no change in expression of renal genes for calcium transporter TRPV5 (van der Wijst et al. 2018). The apparent differences in the urinary excretion and renal gene expression profiles in the two studies may be due to differences in duration of exposure to the high salt conditions (7 versus 3 days), suggesting time-dependent responses, but differences in species and salt administration (drinking water vs diet) or some other factor could also

be involved. Together, however, the results demonstrate effects of high sodium salt intake on renal handling of both calcium and magnesium and indicate coupling among several homeostatic mechanisms for calcium, magnesium, and sodium.

The development of hypertension is known to be associated with excessive sodium intake in humans (e.g., Galletti and Strazzullo 2016; He et al. 2013; Subasinghe et al. 2016) and laboratory animals (see Appendix F), presumably involving dysfunctional calcium homeostasis (Iwamoto 2005; Khananshvili 2013; McCarron 1985). Chronic magnesium deficiency has also been associated with hypertension (see reviews by Makynen et al. 1995; Swaminathan 2003; Touyz and Sontia 2009).

In various laboratory animal models of hypertension, blood pressure has been negatively correlated with intracellular free magnesium concentrations and positively associated with intracellular free calcium concentrations, and free calcium and free magnesium intracellular concentrations have been inversely correlated with each other (Adachi et al. 1993; Kisters et al. 2001). However, in clinical examinations of hypertensive patients, associations with hypomagnesemia have not been consistently observed. Some studies have reported low indicators of magnesium status (magnesium levels in serum or blood cells) in hypertensive patients (Resnick et al. 1983), others found limited evidence for low magnesium status in hypertensive patients (Cappuccio et al. 1985; Delva et al. 1998; Ferrara et al. 1992; Whang et al. 1982), and another reported high magnesium concentrations in red blood cells from hypertensive patients (Sasaki et al. 2000). In a review of magnesium status and hypertension, Touyz and Sontia (2009) concluded that the evidence indicates that not all hypertensive individuals are magnesium-deficient and not all magnesium-deficient individuals have hypertension.

In epidemiology studies, high oral intake of magnesium (and other minerals like calcium and potassium, in some studies) has been associated with decreased risk for cardiovascular disease or hypertension in most studies (Ascherio et al. 1996; Geleijnse et al. 1996; Joffres et al. 1987; Simons-Morton et al. 1997; Townsend et al. 2005; van Leer et al. 1995; Yang and Chiu 1999), but not in others (e.g., Rosenlund et al. 2005). Several reviews have concluded that the epidemiology evidence for an association of low intake of magnesium with increased risk for hypertension (i.e., high intakes are associated with decreased risk for hypertension) is fairly consistent (Appel et al. 2006; Mizushima et al. 1998; Touyz and Sontia 2009; van Leer et al. 1995; Whelton and Klag 1989).

The evidence that supplemental oral intake of magnesium may prevent or attenuate the development of hypertension in animal models is fairly consistent, but results from clinical trials of magnesium

supplementation as a therapy for hypertension are not consistently positive. Supplemental dietary magnesium counteracted blood pressure increases in various rat models of hypertension in most studies (Berthelot and Esposito 1983; Kh et al. 2000; Laurant et al. 1995; Pamnani et al. 2003; Touyz and Milne 1999), but not in all studies (Makynen et al. 1995). However, evidence from clinical trials of magnesium supplementation as an anti-hypertensive practice has been characterized by several reviews as inconsistent (Appel et al. 2006; Dickinson et al. 2010; Jee et al. 2002; Touyz and Sontia 2009; Whelton and Klag 1989; Widman et al. 1993). In a meta-analysis of 20 clinical trials, Jee et al. (2002) concluded that small dose-dependent blood pressure reductions were detected, but that the relationship needed further confirmation with adequately powered trials with sufficiently high doses of supplemental magnesium. Recommendations from these reviews show some variance. For example, Appel et al. (2006) concluded that the data were insufficient to recommend either supplemental calcium or magnesium as a means to lower blood pressure. Touyz and Sontia (2009) similarly concluded that the therapeutic value of magnesium in the management of hypertension was still unclear but recommended that adequate dietary magnesium is important for hypertension prevention management.

Although magnesium sulfate administered intramuscularly is frequently used to manage pre-eclampsia (a condition associated with increased blood pressure and proteinuria) and eclampsia (the occurrence of one or more convulsions in association with pre-eclampsia) in pregnant women (Lucas et al. 1995; The Eclampsia Trial Collaborative Group 1995; The Magpie Trial Collaborative Group 2002), associations between magnesium biomarkers and pre-eclampsia are not consistent (Touyz and Sontia 2009). For example, one study reported that women with severe pre-eclampsia had increased serum concentrations of magnesium, compared with pregnant women without pre-eclampsia, but levels of magnesium in serum and blood cells were similar in pre-eclamptic and non-pre-eclamptic pregnant women (Sanders et al. 1998). One study reported that no significant differences in magnesium concentrations in serum, red blood cells, or mononuclear blood cells were found among groups of 10–11 normotensive, chronic hypertensive, and pre-eclamptic pregnant women (Frenkel et al. 1994). Another study reported that levels of magnesium in red blood cells in pre-eclamptic women were significantly lower than those in healthy pregnant women (Kisters et al. 1990). Nevertheless, clinical trials comparing anticonvulsant drugs with magnesium have indicated better success with magnesium at reducing convulsions and protecting against maternal mortality in pre-eclamptic pregnant women (Belfort et al. 2003; Lucas et al. 1995; The Eclampsia Trial Collaborative Group 1995; The Magpie Trial Collaborative Group 2002), although the mechanism of therapeutic action does not appear to involve interactions between magnesium and sodium (Greene 2003).

Summary. There is limited, but not consistent, evidence that supplemental oral exposure to magnesium may counteract hypertension, a condition associated with multiple factors including exposure to excess sodium salt. Also, studies with transport proteins in isolated cells, as well as studies with laboratory animals, indicate that linkages exist among homeostatic mechanisms for magnesium and sodium, as well as calcium.

There is no clear evidence in humans or laboratory animals that repeated excess exposure to either of these metallic ions alone results in a common adverse outcome via a common mode of action or adverse effects in a common target organ or tissue (see Appendices D and F). In deriving health guidance values to protect against the development of adverse effects from repeated oral exposure, the critical effect for exposure to sodium is hypertension, whereas the critical effect for exposure to magnesium is mild diarrhea (see Appendices D and F). TTDs for other less sensitive effects from these metallic cations were not developed due to inadequate data, with the exception of a TTD for kidney effects from magnesium (see Appendices D and F). In conclusion, the available evidence for interactions between magnesium and sodium provides limited evidence that repeated oral exposure to supplemental dietary magnesium may counteract sodium salt-associated hypertension and no evidence that excess sodium may modify the potential for magnesium to induce mild diarrhea.

2.2.18 Magnesium and Strontium

Interactions with elements of calcium homeostasis (e.g., calcium channels and calcium-binding regulatory proteins) in isolated cells have been extensively studied with magnesium (Section 2.2.8) and strontium (see Section 2.2.11), owing, at least in part, to shared membership in Group IIA of the periodic table of elements. Both form stable divalent cations. The stokes radii of Ca^{2+} and Sr^{2+} are nearly identical; the Stokes radius of Mg^{2+} is approximately 12% larger than Ca^{2+} and Sr^{2+} (Kadhim and Gamaj 2020). Many studies have also examined interactions of magnesium and strontium with other isolated transport systems including: (1) sodium pumps (Cukierman and Krueger 1990; Gatto et al. 2007); (2) TRP channels (Bouron et al. 2015); (3) calcium-activated BK potassium channels involved in the regulation of neurotransmitter release and neuronal excitability (e.g., Lee and Cui 2010; McLarnon and Sawyer 1993; Zhou et al. 2012); and (4) calcium-activated SK potassium expressed in neurons, smooth muscle, neuroendocrine cells, and hematopoietic cells (e.g., Cao and Houamed 1999). The physiological and toxicological relevance of the findings from this type of research is mostly unclear, especially with regard to making reliable predictions of how combined oral exposure to excess magnesium and strontium may influence each other's toxicity.

No studies that examined the effects of concomitant oral exposure of animals or humans to excess magnesium and excess strontium on toxicokinetic endpoints (e.g., distribution to expected sites of toxicity) or expected toxicity endpoints (e.g., skeletal remodeling) were located. There is no clear evidence in humans or laboratory animals that repeated excess exposure to either of these metallic ions alone results in a common adverse outcome via a common mode of action or adverse effects in a common target organ or tissue (see Appendices D and G). In deriving health guidance values to protect against the development of adverse effects from repeated oral exposure, the critical effect of exposure to excess strontium is increased risk for skeletal effects, whereas the critical effect of exposure to excess magnesium is mild diarrhea (see Appendices D and G). TTDs for other less sensitive effects from these metallic cations were not developed due to inadequate data, with the exception of a kidney TTD for magnesium (Appendices D and G).

Summary. Studies conducted in subcellular systems, isolated cells, and tissues have found evidence for various interactions between magnesium and strontium on membrane transport of ions and neurotransmitters. However, the available evidence for interactions between magnesium and strontium is inadequate to conclude whether or not concomitant oral exposure will modify the potential for strontium to produce skeletal effects or the potential for magnesium to induce diarrhea.

2.2.19 Manganese and Sodium

Interactions between manganese and other divalent cations at binding sites on sodium membrane transport systems in isolated membranes, cells, or tissues have been the subject of research for many years. Conditions under which manganese interacts with sodium transport systems have been described with isolated cell and membrane preparations from tissues including blockage of sodium/calcium exchange in embryonic cardiac cells (Mead and Clusin 1984); blockage of sodium ionic current in single canine cardiac Purkinje cells (Hanck and Sheets 1992; Sheets and Hanck 1992); inhibition of sodium-calcium exchange in sarcolemmal vesicles (Trosper and Philipson 1983) and smooth muscle cells in guinea-pig ureter tissue strips (Aickin et al. 1987); inhibition of exchange currents in guinea-pig heart ventricular myocytes (Kimura et al. 1987); inhibition of calcium uptake and sodium efflux in intact squid giant axons (Allen 1990); inhibition of the K^+-dependent Na^+-Ca^{+2} $^+$exchanger (NCKX2) and NCX1 in cultured Chinese hamster lung cells (CCL-39 cells; Uehara et al. 2005) and inhibition of Na^+,K^+-ATPase in human red blood cell membranes (Sachs 1988). In addition, the use of manganese-enhanced magnetic resonance imaging of the heart has been shown to be dependent on manganese accumulation and

retention in the heart via the sodium-calcium exchanger in isolated perfused rat hearts (Chen et al. 2012). In addition, conditions under which divalent cations, including barium, calcium, and manganese, interact with isolated preparations of the Na+/K+-ATPase sodium pump have been described (Gatto et al. 2007; Robinson 1981).

The physiological and toxicologic relevance of observations of manganese and sodium interactions at binding sites in transport proteins in isolated cell or tissues to environmentally relevant oral exposures to both cations, however, is unclear given the limited understanding of whole-body complex homeostatic systems for these metallic cations. Studies examining toxicokinetic endpoints (such as accumulation in toxicity target tissues), nutritional balance, or toxicological endpoints following repeated oral exposure of humans or laboratory animals to concomitant excess levels of manganese and sodium were not located and would be more useful for understanding how repeated oral exposure to excess levels of both manganese and sodium may influence each other's toxicity.

In deriving health guidance values to protect against the development of adverse effects from repeated oral exposure, the critical effects for exposure to manganese are neurological, whereas the critical effect of exposure to excess sodium is increased blood pressure (see Appendices E and F). Overlapping toxicity targets across a range of exposure conditions have not been clearly identified for manganese and sodium, and TTDs for less sensitive effects from these metallic cations were not derived due to inadequate data (Appendices E and F).

Summary. Studies conducted in subcellular systems, isolated cells, and tissues have found evidence for various interactions between manganese and sodium on membrane transport. However, there is inadequate evidence to make conclusions on whether or not repeated oral exposure to excess manganese and sodium may influence each other's toxicity.

2.2.20 Manganese and Strontium

Years of research have shown that manganese and strontium and other divalent cations can interact in complex ways with isolated membrane transport systems including: (1) components of mammalian calcium homeostatic systems including several types of calcium channels (see Sections 2.2.9 and 2.2.11); (2) sodium pumps (Cukierman and Krueger 1990; Gatto et al. 2007); (3) TRP channels, a diverse family of mammalian cation channels and kinases (see review by Bouron et al. 2015); (4) calcium-activated BK potassium channels involved in the regulation of neurotransmitter release and neuronal excitability (e.g.,

Lee and Cui 2010; McLarnon and Sawyer 1993; Zhou et al. 2012); and (5) calcium-activated SK potassium channels expressed in neurons, smooth muscle, neuroendocrine cells, and hematopoietic cells (e.g., Cao and Houamed 1999). Studied points of divalent cation interaction with these transport proteins include inhibition of, and competitive permeation through, the ion conducting channels and modulation of activity through binding sites that cause conformational changes in protein three-dimensional structure. The study of these interactions of divalent cations on transport proteins has been important in understanding how the systems work in isolation, but the physiological and toxicological relevance of the findings from this type of research is mostly unclear, especially with regard to making reliable predictions of how combined oral exposure to any pair of metallic cations that are the subject of this profile may influence their individual toxicity.

No studies were located that examined toxicokinetic, nutritional balance, or toxicological endpoints following repeated oral exposure of humans or laboratory animals to concomitant excess levels of manganese and strontium; these types of studies would be more useful for understanding how repeated oral exposure to excess levels of both may influence each other's toxicity. There is no clear evidence in humans or laboratory animals that repeated excess exposure to either of these metallic ions alone results in a common adverse outcome via a common mode of action or adverse effects in a common target organ or tissue (see Appendices E and G). In deriving health guidance values to protect against the development of adverse effects from repeated oral exposure, the critical adverse effects observed in humans and laboratory animals excessively exposed to manganese are neurological, whereas the critical effect of repeated exposure to excess strontium is skeletal effects (see Appendices E and G). Overlapping toxicity targets across a range of exposure conditions have not been clearly identified and TTDs for other less sensitive effects were not developed for manganese and strontium due to inadequate data (Appendices E and G).

Summary. Studies conducted in subcellular systems, isolated cells, and tissues have found evidence for various interactions between manganese and strontium on membrane transport of ions and neurotransmitters. However, there is inadequate evidence to make conclusions whether or not repeated oral exposure to excess manganese and strontium may influence each other's toxicity.

2.2.21 Sodium and Strontium

Interactions have been described in which sodium and strontium affect functions of isolated transport proteins, but the relevance of these interactions to how concomitant oral exposure to excess sodium and

strontium may influence each other's toxicity is unknown. A few examples of reports of these types of interactions include competitive binding between calcium, strontium, and sodium at specific sites in an isolated sodium-calcium exchanger (Liao et al. 2016); inhibition of Na/K pump activity in isolated rat peritoneal mast cells by extracellular divalent cations including calcium, strontium, magnesium, and barium (Knudsen 1995); and modulation of gating activity of isolated sodium channels in lipid bilayers by divalent cations including calcium, barium, strontium, and magnesium (Cukierman and Krueger 1990).

Evidence for complex interactions between sodium and strontium have been demonstrated in a study that examined retention of sodium, phosphorus, and strontium in chickens provided varying levels of strontium and vitamin D3 (D3, Browning and Cowieson 2015). The results showed that supplemental strontium (500 or 1,000 mg Sr/kg diet) increased phosphorus, sodium, and strontium retention in birds fed 2,500 IU D3/kg diet kg, but reduced phosphorus, sodium, and strontium retention in birds fed 5,000 IU D3/kg diet. Although these results are consistent with strontium influencing the whole-body retention of sodium, their relevance to scenarios of co-exposure to excess sodium and strontium is unclear.

No studies were located that examined toxicokinetic, nutritional balance, or toxicological endpoints following repeated oral exposure of humans or laboratory animals to concomitant excess levels of sodium and strontium; these types of studies would be more useful for understanding how repeated oral exposure to excess levels of both may influence each other's toxicity. There is no clear evidence in humans or laboratory animals that repeated excess exposure to either of these metallic ions alone results in a common adverse outcome via a common mode of action, but skeletal effects have been associated with high sodium salt intake (osteoporosis; Appendix F) and excess strontium intake (skeletal abnormalities in children with poor diets (vitamin D, calcium, and protein deficiencies; Appendix G). In deriving health guidance values to protect against the development of adverse effects from repeated oral exposure, the critical adverse effect for exposure to sodium is hypertension, whereas the critical effects of repeated exposure to strontium are skeletal effects (see Appendices F and G). TTDs for skeletal effects from high sodium salt intakes and for other less sensitive effects from these metallic cations alone were not developed due to inadequate data (see Appendices F and G).

Summary. Studies conducted in subcellular systems, isolated cells, and tissues have found evidence for various interactions between sodium and strontium on membrane transport. However, the available interaction data are inadequate to conclude whether or not repeated concomitant oral exposure to sodium and strontium will influence each other's critical toxic effects. Additional studies of the effects of co-

exposure to excess sodium and strontium on skeletal endpoints could provide better information on how they may jointly act to produce adverse skeletal effects.