INTERACTION PROFILE FOR SELECTED METALLIC IONS IDENTIFIED IN WASTE WATER FROM UNCONVENTIONAL OIL AND GAS EXTRACTION ACTIVITIES:

BARIUM, CALCIUM, IRON, MAGNESIUM, MANGANESE, SODIUM, AND STRONTIUM

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PREFACE

The Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) mandates that the Agency for Toxic Substances and Disease Registry (ATSDR) shall assess whether adequate information on health effects is available for the priority hazardous substances. Where such information is not available or under development, ATSDR shall, in cooperation with the National Toxicology Program, initiate a program of research to determine these health effects. The Act further directs that where feasible, ATSDR shall develop methods to determine the health effects of substances in combination with other substances with which they are commonly found.

To carry out these legislative mandates, ATSDR's Office of Innovation and Analytics, Toxicology Section has developed and coordinated a mixtures program that includes trend analysis to identify the mixtures most often found in environmental media, *in vivo* and *in vitro* toxicological testing of mixtures, quantitative modeling of joint action, and methodological development for assessment of joint toxicity. These efforts are interrelated. For example, the trend analysis suggests mixtures of concern for which assessments need to be conducted. If data are not available, further research is recommended. The data thus generated often contribute to the design, calibration, or validation of the methodology. This pragmatic approach allows identification of pertinent issues and their resolution as well as enhancement of our understanding of the mechanisms of joint toxic action. All of the information obtained is thus used to enhance existing or developing methods to assess the joint toxic action of environmental chemicals. Over a number of years, ATSDR scientists, in collaboration with mixtures risk assessors and laboratory scientists, have developed approaches for the assessment of the joint toxic action of chemical mixtures. As part of the mixtures program a series of documents, Interaction Profiles, are being developed for certain priority mixtures that are of special concern to ATSDR.

The purpose of an Interaction Profile is to evaluate data on the toxicology of the "whole" priority mixture (if available) and on the joint toxic action of the chemicals in the mixture in order to recommend approaches for the exposure-based assessment of the potential hazard to public health. Joint toxic action includes additivity and interactions. A weight-of-evidence approach is commonly used in these documents to evaluate the influence of interactions in the overall toxicity of the mixture. The weight-of-evidence evaluations are qualitative in nature, although ATSDR recognizes that observations of toxicological interactions depend greatly on exposure doses and that some interactions appear to have thresholds. Thus, the interactions are evaluated in a qualitative manner to provide a sense of what influence the interactions may have when they do occur.

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PEER REVIEW

A peer review panel was assembled for the first draft of the profile in 2015. The panel consisted of the following members:

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A second peer review panel was assembled for the second draft of the profile in 2019. The panel consisted of the following members:

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These experts collectively have knowledge of toxicology, chemistry, and/or health effects. All reviewers were selected in conformity with Section 104(I)(13) of the Comprehensive Environmental Response, Compensation, and Liability Act, as amended.

ATSDR scientists review peer reviewers' comments and determine whether changes will be made to the profile based on comments. The peer reviewers' comments and responses to these comments are part of the administrative record for this compound.

The citation of the peer review panel should not be understood to imply its approval of the profile's final content. The responsibility for the content of this profile lies with the ATSDR.

SUMMARY

The purpose of this profile is to investigate the possible joint toxic actions of elevated groundwater levels of selected metallic ions (barium [Ba], calcium [Ca], iron [Fe], magnesium [Mg], manganese [Mn], sodium [Na], and strontium [Sr]) that may occur near unconventional oil and gas (UOG) extraction activities, and potential impacts on human health. Health hazards associated with excess oral exposure to these cations show variability with respect to the most sensitive toxicity targets associated with repeated oral exposure: barium (kidney effects), calcium (kidney effects), iron (gastrointestinal disturbances), magnesium (gastrointestinal disturbances), manganese (nervous system effects), sodium (blood pressure effects), and strontium (bone development effects in children). The focus of this profile is not intended to minimize the importance of understanding and assessing the potential individual and combined (mixtures) effects from other ions and chemicals that may also be present in groundwater contaminated with UOG waste waters. In assessing the available information on possible interactions among these metallic cations, this profile concludes with recommendations for conducting screening-level assessments of public health impacts from repeated joint exposure to mixtures of these seven metallic cations in potentially contaminated groundwater near UOG extraction activities.

ATSDR recommends a component-based approach assuming dose addition for screening-level, exposure-based assessments of potential hazards to public health from repeated oral exposure to mixtures containing barium, calcium, iron, magnesium, manganese, sodium, and strontium. The recommendations include the estimation of an overall screening-level hazard index for all compounds (with no grouping by common adverse outcomes) for an initial Tier 0 human health assessment. In a subsequent Tier 1 analysis, calculation of separate screening-level hazard indexes is recommended for cardiovascular (hypertensive) effects from barium and sodium, neurological effects from barium and manganese, kidney effects from barium, calcium, and magnesium, and gastrointestinal effects from iron and magnesium. Data regarding potential interactions between members of the 21 pairs of cations were assessed in this document. Evidence for coupling of homeostatic mechanisms at the cellular level was available for all 21 pairs of the selected metallic cations; evidence for coupling at the whole-body level of organization was available for 12 pairs. In contrast to the relative wealth of evidence for homeostatic coupling among the seven metallic cations, limited evidence for how repeated oral co-exposure may influence toxic responses was available for only 11 pairs. The evidence for interactions was adequate to suggest possible influences on the critical-effect toxicity of barium and calcium (kidney effects), manganese (neurotoxic effects), sodium (cardiovascular effects), and strontium (skeletal effects).

Hazard index approaches for exposure to mixtures with the subject metals and utilizing a hazard quotient for manganese should be accompanied with qualitative statements about the likely susceptibility of iron-deficient individuals to manganese neurotoxicity, the possible joint toxic action of excess iron and excess manganese on neurological endpoints, and the possible, but uncertain, protective effects of concurrent exposure to excess calcium or magnesium. Calculation of a neurological target toxicity hazard index with hazard quotients for barium and manganese should be accompanied by qualitative statements that: (1) available interaction data for barium and manganese are inadequate to assess whether the joint action of these metals on neurotoxic endpoints may be dose-additive, greater-than-dose-additive, or less-than-dose-additive and (2) accumulation of iron, manganese, and other metals in the brain may jointly act to produce neurological impairment that may not be accounted for in a neurological hazard index based only on barium and manganese.

A hazard quotient for sodium-induced hypertension should be accompanied with a qualitative statement about the possible, but uncertain, protective actions of concomitant high exposure levels to calcium and magnesium against sodium-induced hypertension. Calculation of a cardiovascular hazard index with hazard quotients for barium and sodium should be accompanied by qualitative statements that available interaction data for barium and sodium are inadequate to assess whether the joint action of these metals to produce cardiovascular effects may be dose-additive, greater-than-dose-additive, or less-than-dose-additive and that possible contributions to effects on blood pressure from excess iron are not accounted for in the hazard index due to the lack of adequate data for toxicity target dose (TTD) development.

A hazard quotient for adverse skeletal effects from strontium should be accompanied by qualitative statements about: (1) the uncertainty that excess calcium may counteract the development of strontium-induced skeletal effects and (2) skeletal effects from excess sodium are also possible, but available data are inadequate for TTD development. A hazard quotient for adverse skeletal effects from strontium should also be accompanied with a qualitative statement about the potential beneficial effects of strontium in inhibiting bone resorption and stimulating bone formation in osteoporotic animals and humans presumably via interactions with the calcium-sensing receptor (CaSR).

A hazard quotient for calcium based on kidney stone formation should be accompanied by qualitative statements of the uncertainties associated with calcium's potential to induce kidney stones in humans and magnesium's potential to protect against kidney stone formation in humans. Calculation of a kidney hazard index with hazard quotients for barium, calcium, and magnesium should be accompanied by qualitative statements that available interaction data are inadequate to assess whether the joint toxic action

may be dose-additive, greater-than-dose-additive, or less-than-dose-additive and that possible contributions to adverse kidney effects from excess iron and excess sodium would not be captured in the hazard index due to inadequate data for kidney TTDs for these metallic cations.

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LIST OF ACRONYMS, ABBREVIATIONS, AND SYMBOLS

8OHdG	8-hydroxy-2'-deoxyguanosine
ACGIH	American Conference of Governmental Industrial Hygienists
AI	adequate intake
ATSDR	Agency for Toxic Substances and Disease Registry
Ba	barium
BINWOE	binary weight of evidence
BMDL	benchmark dose limit
Ca	calcium
CaSR	calcium-sensing receptor
CASRN	Chemical Abstracts Service Registry Number
CD	cluster of differentiation-68
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
CI	confidence interval
DMT-1	divalent metal transporter 1
DNA	deoxyribonucleic acid
DWEL	drinking water equivalent level
EFSA	European Food Safety Authority
EPA	U.S. Environmental Protection Agency
FDA	Food and Drug Administration
Fe	iron
GABA	gamma-aminobutyric acid
IARC	International Agency for Research on Cancer
IRIS	Integrated Risk Information System
L-NAME	N^G-nitro-L-arginine methyl ester
LOAEL	lowest-observed-adverse-effect level
Mg	magnesium
Mn	manganese
MRL	Minimal Risk Level
mRNA	messenger ribonucleic acid
Na	sodium
NAS	National Academy of Sciences
NICNAS	National Industrial Chemicals Notification and Assessment Scheme
NIOSH	National Institute for Occupational Safety and Health
NOAEL	no-observed-adverse-effect level
NTP	National Toxicology Program
OSHA	Occupational Safety and Health Administration
PAI-1	plasminogen activator inhibitor 1
PBPK/PD	pharmacokinetic/pharmacodynamic
PEL	permissible exposure limit
PND	postnatal day
POD	point of departure
PRI	Population Reference Intake
PTH	parathyroid hormone
REL	recommended exposure limit
RDA	recommended dietary allowance
RfC	reference concentration
RfD	reference dose

SHRSP	spontaneously hypertensive rats, stroke prone
Sr	strontium
TfR	transferrin receptor
TGF-β	transforming growth factor beta 1
TLV	Threshold Limit Value
TRP	transient receptor potential
TRPC	transient receptor potential channel
TTD	target-organ toxicity
TUNEL	terminal dUTP nick end labeling
TWA	time-weighted average
UL	tolerable upper intake level
INII	Unique Ingredient Identifier
UOG	unconventional oil and gas
U.S.	United States
VSMC	vascular smooth muscle cell
WHO	World Health Organization

1. Introduction

The primary purpose of this *Interaction Profile for Selected Metallic Ions Identified in Waste Water from Unconventional Oil and Gas Extraction Activities* is to evaluate data on the toxicology of the "whole" mixture and the joint toxic action of the chemicals in the mixture in order to recommend approaches for assessing the potential hazard of mixtures of these metals to public health. To this end, the profile evaluates the whole mixture data (if available), focusing on the identification of health effects of concern, adequacy of the data as the basis for a mixture health guidance value, and adequacy and relevance of physiologically-based pharmacokinetic/pharmacodynamic (PBPK/PD) models for the mixture. The profile also evaluates the evidence for joint toxic action—additivity and interactions—among the mixture components. A weight-of-evidence approach is commonly used in these profiles to evaluate the influence of interactions in the overall toxicity of the mixture. The weight-of-evidence evaluations are qualitative in nature, although the Agency for Toxic Substances and Disease Registry (ATSDR) recognizes that observations of toxicological interactions depend greatly on exposure doses and that some interactions appear to have thresholds. Thus, the interactions are evaluated in a qualitative manner to provide a sense of what influence the interactions may have when they do occur. The profile provides environmental health scientists with ATSDR Office of Innovation and Analytics, Toxicology Section recommended approaches for the incorporation of the whole mixture data or the concerns for additivity and interactions into an assessment of the potential hazard of this mixture to public health. These approaches can then be used with specific exposure data from hazardous waste sites or other exposure scenarios.

ATSDR has received comments from the public expressing concern for possible health effects from exposure to drinking water containing methane, organic chemicals, and metals, potentially from or exacerbated by unconventional oil and gas (UOG) extraction activities in Pennsylvania and West Virginia. In response to these concerns and the rapid expansion of natural gas extraction from shale formations using horizontal drilling and hydraulic fracturing in this and other regions of the United States, ATSDR initially considered the preparation of an Interaction Profile on repeated combined exposure to methane and metallic ions in drinking water but settled on a focus on interactions among metallic cations. Methane, although presenting a flammability and explosive hazard, is generally considered to be inert, and available scientific data are inadequate to establish a specific health hazard of concern for methane (other than as an asphyxiant gas) or evaluate whether co-exposure to metals may affect the expression of methane's toxicity or whether methane may affect the toxicity of metallic cations, as further explained in the following paragraphs. Cations discussed in this profile are accompanied by anions, which also influence the toxicity of the mixture. For some metals, the anion that accompanies the cation could be of

greater toxicological concern than the cation (e.g., nitrate, nitrite, cyanide, chromate). Anions in the UOG extraction waste water may will influence the toxicity of the soluble mixture. Dietary mineral intakes may confound the impact of barium, calcium, iron, magnesium, manganese, sodium, and strontium from the waste water when these minerals are also present in the conventional daily diet, especially for those minerals that are essential nutrients.

The reader is referred to reports developed by EPA (EPA 2016a, 2016b, 2016c) for more detailed information about potential impacts of UOG extraction activities on groundwater and drinking water and factors that can affect variability in the mineral composition of UOG waste water. Processes by which UOG extraction activities can result in waste water being released into groundwater and surface water are complex and, in general, include the following (EPA 2016 a, 2016b, 2016c):

- Waste water from drilling activities is typically managed via disposal in injection wells or evaporation ponds, application to fields, spreading on roads, and/or treatment and reuse for future oil and gas operations.
- Produced water is often disposed of by injecting it into deep geologic formations via wells that are specifically designed for that purpose. In some cases, produced water can be treated and reused to hydraulically fracture another well.
- The water used for fracking—a mixture of water, sand, and chemicals—is pumped underground at high pressure and wedges rocks apart. The sand stays put in the cracks, creating pathways for oil and gas to travel towards the well. About 40% of the water and chemicals flow back to the surface.

Exposure to drinking water contaminated with methane and metallic ions is possible and can impact water quality, although groundwater contamination can be difficult to attribute to stray gas and metals specifically from natural gas extraction activities. From 2008 through March 2019, the Pennsylvania Department of Environmental Protection (PA DEP 2019) documented 339 cases where the state determined a private water supply was impacted by oil and gas activities in Pennsylvania. These oil and gas activities include “operations associated with both conventional and unconventional drilling activities that either resulted in a water diminution event or an increase in constituents above background conditions.” Another possible hazard associated with methane in drinking water is the initiation of chemical and biological reactions which could result in reductive dissolution of iron and manganese (EPA 2016a).

In studies of Pennsylvania private drinking water wells, elevated levels of methane, ethane, or propane, but not salts, metals, or radioactivity, were found in a subset of drinking water wells within a 1-km radius of shale gas extraction sites (Jackson et al. 2013a; Osborn et al. 2011). Another study identified subsets of drinking water wells in Pennsylvania and Texas (overlying the Marcellus and Barnett Shales, respectively) in which methane, ethane, or propane concentrations were increasing with time (Darrah et al. 2014). Isotope and compositional data for noble and hydrocarbon gases suggested that contamination in these subsets of wells, located <1 km from gas extraction sites, was associated with deficiencies in gas well casings or cements (Darrah et al. 2014). Other studies have hypothesized that natural gas in shallow aquifers occurs naturally from microbial methane production in aquifers or from gas-bearing geological formations of intermediate depth between aquifers and underlying shale formations, and is not related to gas extraction activities (Baldassare et al. 2014; Kornacki and McCaffrey 2011; Molofsky et al. 2013).

Waste fluids from hydraulic fracturing shale gas extraction activities (including drill cuttings, flowback water, and produced water) are known to contain relatively high levels of salinity, metals, and naturally occurring radioactivity, compared with surface streams (Barbot et al. 2013; Brown 2014; EPA 2015; Haluszczak et al. 2013; Jackson et al. 2013b; Lampe and Stolz 2015; Warner et al. 2013). Appendix I Tables I-1, I-2, and I-3 list concentrations of inorganic ions in waste water samples from several shale formation UOG extraction sites in the United States (Barbot et al. 2013; Haluszczak et al. 2013; Jackson et al. 2013b). Metallic cations detected in the waste water samples and listed in approximate order of decreasing average concentrations in mg/L are: sodium (Na, ~5,000–24,000) > calcium (Ca, ~2,000–11,000) > strontium (Sr, ~20–2,300) = barium (Ba, ~1–2,000) > magnesium (Mg, ~75–630) > iron (Fe, ~25–75) > manganese (Mn, ~4–45). These data emphasize the possibility that metals in UOG waste water could contaminate drinking water, if they enter into surface waters or underground aquifers. Confirming empirical data for this possibility, however, are not available.

Disposal options for UOG activities include recycling of waste fluids for shale gas operations, injection into deep disposal wells, and treatment in publicly owned or commercially operated waste water treatment plants before release into surface streams (Warner et al. 2013). Waste water treatment operations can include addition of Na_2SO_4 to remove metals (and salts) as solid precipitates, which are subsequently placed in landfills (Warner et al. 2013). Barium and radium levels in effluent from a western Pennsylvania treatment plant were substantially decreased by about 90%, compared with the waste water, but ^{226}Ra levels in stream sediments at the point of discharge were about 200 times higher than background and upstream sediment levels (Warner et al. 2013). In addition, elevated concentrations of bromide and chloride concentrations were found in stream samples collected >300 m downstream,

compared with upstream and background samples (Warner et al. 2013). Because Pennsylvania geology generally is not compatible with deep injection wells, UOG waste water has been transported to Ohio for deep injection disposal (Brown 2014). Based on Brown (2014), Pennsylvania had 6 deep injection wells, whereas Ohio had 177 and Texas had 50,000. It is unlikely that UOG extraction waste water components will migrate from deep injection wells to shallow aquifers, but casing issues/failures and resulting spill events at gas well sites, at deep injection sites, and during transport are possible ways by which UOG extraction waste water components could enter into surface streams or shallow aquifers.

ATSDR decided that including methane in an Interaction Profile for potential mixtures in waste water generated from gas extraction activities would not be useful, because the human health hazards associated with repeated exposure to methane gas by inhalation or methane gas dissolved in water are not well characterized. Associations between repeated exposure of humans or laboratory animals to methane and specific health hazards have not been established. Thus, even though there is public concern for possible health hazards from methane gas in drinking water, available scientific data are inadequate to establish a specific health hazard of concern or evaluate whether co-exposure to metals may affect the expression of methane's toxicity. Conversely, available scientific data are inadequate to determine whether the presence of methane in groundwater may influence potential toxic effects of metals or water chemistry (e.g., solubility of metals). Methane is considered to be relatively inert (although of high flammability), and its toxic effects are thought to be restricted to its action as an asphyxiant gas. The presence of methane in waste water from UOG extraction activities may alter dissolution and solubility of metals and these changes could affect exposures to those metals (EPA 2016a; Konrad and Lankau et al. 2005; Loomer et al. 2018; Vengosh et al. 2014).

ATSDR decided that preparing an Interaction Profile on repeated combined drinking water exposure to metals potentially elevated in groundwater near UOG extraction activities would be more useful, because: (1) elevated concentrations of metallic cations in UOG waste fluids have been measured in several studies; (2) the toxicities of repeated exposure to several metals identified in UOG extraction waste waters are well characterized; and (3) samples of UOG extraction waste waters from the Marcellus Shale in Pennsylvania were cytotoxic to cultured human BEAS-2B cells and transformed them to carcinogenic cells that produced tumors in mice following subcutaneous injection (Yao et al. 2015). Radioactivity levels were below detection limits (by beta detectors and gamma scintillation counters) in the waste water samples used in the BEAS-2B studies, and the samples were filtered through 0.22 μm polyethersulfone membrane filters to remove possible biotic materials and large organic molecules and allow small particles containing metals to remain in the test mixtures of chemicals. Yao et al. (2015) proposed that

metallic cations present in the test substance filtrates could account for a considerable fraction of the observed *in vitro* toxicity but noted that they could not discern if the "transformation activity was solely from Ba, Sr, or other metals that have been detected in the flow back waters at lower levels."

For the focus of this Interaction Profile, ATSDR selected the seven metallic cations that have been detected in UOG extraction waste water samples at the highest elevated concentrations: barium, calcium, iron, magnesium, manganese, sodium, and strontium (see Appendix I). Relative toxicity was not considered in the selection of the metallic cations. ATSDR believed that it was important to evaluate evidence for interactions among each of the metallic cations, even though reported concentrations for cations with the highest values (calcium and sodium) were at least an order of magnitude higher than those metallic cations with the lowest concentrations in UOG extraction waste water samples (iron and manganese) (see Appendix I). The toxicities of several of these cations are well characterized (e.g., barium, manganese, sodium, and strontium), and several of these cations are essential elements that are only toxic at very elevated intakes (i.e., calcium, iron, and magnesium). The oral route was selected as the focus, because this would be the primary route of exposure if groundwater or drinking water wells become contaminated from repeated spills or leakage from waste water holding facilities, and oral toxicity values for the relatively toxic metallic cations are available. Thus, the exposure scenario of greatest concern for this mixture and interaction profile is chronic-duration, environmentally relevant oral exposure to contaminated drinking water. Environmentally relevant concentrations of the subject cations in groundwater and drinking water wells are expected to be lower than concentrations detected in UOG extraction waste water samples (see Appendix I), due to dilution and ground filtering. Noncancer effects from these cations were the focus of this profile because none of them have cancer weight-of-evidence determinations or cancer slope factors, which would be used in public health assessments (see Appendices A–G).

ATSDR recognizes that exposure while bathing or showering is possible via the skin or inhaled via respirable droplets, but assumes that such exposure scenarios will be secondary to oral exposure to metallic cations. It is also recognized that any assessment of the possible public health impacts of metallic cations present at elevated levels in UOG extraction waste waters should acknowledge possible toxicity contributions of other components in these fluids; it should not be assumed that the mixture of these five chemicals is representative of the full hydraulic fracturing waste fluid mixture.

As discussed in Appendix J, a literature search was conducted to identify noncancer and cancer toxicity, toxicokinetic, and interaction data from studies of humans and laboratory animals, as well as mechanistic

studies using tissue, cell, or *in vitro* systems. The search targeted studies mentioning two or more of the metals of interest. The toxicologist selected interaction studies with whole-body exposure scenarios with mammals, but did not exclude interaction studies conducted with isolated cell components, cells, or tissues.

Health guidance values for repeated oral exposure were identified by searching ATSDR (http://www.atsdr.cdc.gov/toxprofiles/index.asp), U.S. Environmental Protection Agency (EPA) Integrated Risk Information System (IRIS) (http://www.epa.gov/iris/index.html), and the National Academy of Sciences (NAS) (https://www.nap.edu) for minimal risk levels (MRL), reference doses/ concentrations (RfD/RfC), and tolerable upper intake limits (UL), respectively. None of the subject metallic cations have been assessed for carcinogenicity by the International Agency for Research on Cancer (IARC; https://monographs.iarc.fr/agents-classified-by-the-iarc/) or the National Toxicology Program (NTP; https://ntp.niehs.nih.gov/whatwestudy/assessments/cancer/roc/index.html), and no cancer slope factors are available (see Appendices A–G). The EPA IRIS classified barium, manganese, and strontium in cancer Group D, *not classifiable as to human carcinogenicity*, and has not assessed the carcinogenicity of calcium, iron, magnesium, or sodium. Critical endpoints (i.e., the most sensitive effects) forming the basis of ATSDR MRL health guidance values for noncancer toxic effects from repeated oral exposure are tabulated in Table 1. Appropriate ATSDR oral MRL values were not available for some of the metallic cations: calcium, iron, magnesium, manganese, and sodium. For the first four cases of essential metals, health guidance values for repeated oral exposure derived by the NAS (ULs) were used to identify critical effects. For manganese, EPA's oral RfD was used (see Appendices A–G for additional details). The following critical effects were identified for repeated oral exposure to each metal of concern: barium (kidney effects), iron (gastrointestinal effects), calcium (kidney stones), magnesium (diarrhea)manganese (neurobehavioral effects), sodium (hypertension), and strontium (skeletal effects).

Table 1. Noncancer Health Guidance Values and Critical Endpoints for the Selected Metallic Cations[a]

	Inhalation (mg/m^3)[b]			Oral (mg/kg/day)[b]			
	Intermediate MRL	Chronic MRL	RfC	Intermediate MRL	Chronic MRL	RfD	UL[c]
Barium	–	–	–	0.2 (Kidney)	0.2 (Kidney)	0.2 (Kidney)	–
Calcium	–	–	–	–	–	–	36 (Kidney)
Iron	–	–	–	–	–	–	0.6 (GI)

Table 1. Noncancer Health Guidance Values and Critical Endpoints for the Selected Metallic Cations[a]

	Inhalation (mg/m^3)[b]			Oral (mg/kg/day)[b]			
	Intermediate MRL	Chronic MRL	RfC	Intermediate MRL	Chronic MRL	RfD	UL[c]
Magnesium	–	–	–	–	–	–	5 (GI)
Manganese	–	0.003 (Neuro)	0.0005 (Neuro)	–	–	0.14 (Neuro)[d]	0.16 (Neuro)
Sodium	–	–	–	–	–	–	33 (Cardio)
Strontium	–	–	–	2 (Skeletal)	–	0.6 (Skeletal)	–

[a]See Appendix H for more details.
[b]No acute MRLs were derived for any of the metals of concern.
[c]Doses were converted from mg/day to mg/kg/day using an assumed body weight of 70 kg.
[d]ATSDR did not derive oral MRLs for manganese. The EPA RfD and the NAS UL are similarly based on a lack of adverse neurological effects associated with average manganese intake levels in Western diets (see Appendix E).

Cardio = cardiovascular; GI = gastrointestinal; MRL = Minimal Risk Level; Neuro = neurotoxicity; RfC = reference concentration; RfD = reference dose; UL = tolerable upper intake level

Based on the reviews in the ATSDR or NAS documents deriving the health guidance values, some biological systems are adversely affected by repeated exposure to more than one of the subject metallic cations (see Appendices A–G). Table 2 (prepared from information in Appendices A–G) describes common targets from repeated oral exposure (not necessarily the critical effects) as the cardiovascular system for barium, sodium, and potentially iron, the nervous system for barium, iron, and manganese, the gastrointestinal system for barium, iron, and magnesium, and the kidney for barium, calcium, magnesium, and potentially iron. Additional information on these toxic effects, as well as toxicokinetic and mechanistic considerations, are presented in Appendices A–G. In addition, Toxicity Target Doses (TTDs) for less sensitive effects (i.e., effects occurring at doses higher than those associated with the critical effect for the health guidance value) were derived, if available dose-response data in the review documents were sufficient for derivations. Individuals at risk for primary or secondary iron overload due to altered iron absorption/uptake/excretion because of genetic or disease factors or extremely high oral intakes may develop systemic toxicity involving many organs, particularly the liver and heart, but available dose-response data were insufficient to derive TTDs for these high-dose iron effects (see Appendix C). Information included in the appendices is intended to provide public health assessors with a broad, accurate overview of the toxicity of individual compounds, and is based primarily on existing

Table 2. Noncancer Targets of Subchronic and Chronic Oral Toxicity of Selected Metallic Cations[a]

	Affected by:						
Endpoint	Barium	Calcium	Iron	Magnesium	Manganese	Sodium	Strontium
Cardiovascular	Yes	No	With overload[b]	No	No	***Yes, cUL[a]***	No
Neurological	Yes	No	With overload	No	***Yes, cRfD, UL[c]***	No	No
Skeletal	No	No	No	No	No	Limited evidence	***Yes, cMRL, RfD***
Kidney	***Yes, cMRL, RfD***	***Yes, cUL***	With overload	Yes	No	Secondary to cardiovascular	No
Gastrointestinal	No	No	***Yes, cUL***	***Yes, cUL***	No	No	No
Respiratory	No	No	No	No	No	Limited evidence	No
Liver	No	No	With overload	No	No	No	No
Reproductive	No	No	With overload	No	Yes	No	No

[a]Critical effects for derivation of health guidance values (MRLs, RfDs, or ULs) are bolded and italicized. Additional information on other health effects associated with higher exposures to the subject metallic ions is presented in Appendices A-G. Available data were adequate to derive TTDs for cardiovascular and neurological effects from repeated oral exposure to barium (Appendix A). For iron, data were inadequate to derive a TTD for neurotoxicity. For magnesium, data were adequate to derive a TTD for kidney effects (Appendix D). For manganese, data were adequate to derive a TTD for reproductive effects, but the resultant value was equivalent to the RfD. For the other cations, data were inadequate to derive oral TTDs for less sensitive effects occurring above the critical effect for the MRL, RfD, or UL (see Appendices A–G).
[b]Individuals susceptible to primary or secondary iron overload due to genetic disorder/polymorphism or disease state can develop systemic iron toxicity (cardiovascular, neurological, kidney, liver, or reproductive); limited evidence exists for iron overload symptoms in some populations with unusually high dietary intakes in food or drinking water. Available dose-response data were inadequate to derive TTDs for high-dose effects from iron (Appendix C).
[c]ATSDR (2012) did not derive a chronic or intermediate-duration oral MRL for manganese. The RfD for manganese (0.14 mg/kg/day) is recommended for ATSDR public health assessments in the absence of a chronic oral MRL. The RfD is similar in value to the NAS UL for manganese (0.16 mg/kg/day) and is similarly based on a lack of adverse effects associated with average manganese intake levels in Western diets (Appendix E).

cMRL = chronic-duration Minimal Risk Level (Agency for Toxic Substances and Disease Registry); cRfD = chronic-duration reference dose (U.S. Environmental Protection Agency); cUL = chronic tolerable upper intake limit (National Academy of Sciences)

ATSDR Toxicological Profiles with supplementation information from other public health, occupational health, or regulatory agency guidance documents (e.g., NAS, European Food Safety Authority [EFSA], IRIS, American Conference of Governmental Industrial Hygienists [ACGIH]). In instances where guidance documents were limited to older NAS documents (sodium, iron) and/or specific information was not included in available public health guidance documents (e.g., mechanisms of toxicity), reviews and meta-analyses from the peer-reviewed literature were included as appropriate. Appendices are not intended to provide a comprehensive review of the primary literature. Primary literature reviewed for this document was limited to studies focusing on possible interactions among the subject metallic cations.

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 for understanding 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 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 deriving 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 others' 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 anaesthetized 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 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 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 waste water. 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 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 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 waste water.

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 channel 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 to 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.

3. Recommendation for Exposure-Based Assessment of Joint Toxic Action of Mixtures with Barium, Calcium, Iron, Magnesium, Manganese, Sodium, and Strontium

3.1 Recommendations for Public Health Assessment Approaches

As discussed in Chapter 1 (Introduction), mixtures of barium, calcium, iron, magnesium, manganese, sodium, and strontium were chosen as the subject for this interaction profile because these metallic cations are frequently found at high concentrations in waste water from UOG extraction activities. The exposure scenario of greatest concern for this mixture is chronic-duration, low-level oral exposure to contaminated drinking water.

To conduct exposure-based assessments of possible human health hazards from oral exposures from mixtures of barium, calcium, iron, magnesium, manganese, sodium, and strontium in waste water from UOG extraction activities, ATSDR recommends the use of a component-based approach, because there are no direct data available to characterize health hazards (and dose-response relationships) from exposure to mixtures of these metallic cations. In addition, "interaction" PBPK/PD models have not yet been developed that would predict appropriate target doses of the components.

The major health endpoints identified for repeated oral exposure to the individual metallic cations are shown in Table 2 (see Introduction). The toxicity target of the most sensitive critical effect used to derive health guidance values for repeated oral exposure (MRLs, RfDs, or ULs) is different for each metallic cation in the mixture of concern, with the exception of the kidney, which is the most sensitive target for barium and calcium, and the gastrointestinal tract, which is the most sensitive target for iron and magnesium. Available data from sources used to prepare Appendices A–G were adequate to derive only a few repeated oral exposure TTDs for effects occurring at doses higher than those associated with the respective critical effects for MRLs, RfDs or ULs: TTDs for cardiovascular and neurological effects from barium exposure (Appendix A), kidney effects from magnesium exposure (Appendix D), and reproductive effects from manganese exposure (Appendix E).

Following ATSDR (2004a, 2018) guidelines, a component-based hazard index approach is recommended assuming dose additivity for preliminary screening-level public health assessments. In the first tier of analysis (Tier 0), there is no grouping based on common toxicity targets or common adverse outcomes with common modes of action. In this approach, the ratios of exposure levels to health guidance values

(hazard index) for each substance affecting a particular endpoint (hazard quotients) are summed to provide a measure of hazard for the whole mixture (see formulas for hazard quotients and hazard index below). Because it assumes dose addition, the hazard index is most appropriately applied to components that cause the same effect by the same mechanism of action (i.e., elicit a common adverse outcome via a common mode of action). However, ATSDR (2004a, 2018) recommends (in the absence of hazard identification and dose-response data for a mixture of concern or a sufficiently similar mixture) using the hazard index approach for all components in a mixture regardless of toxicity target in an initial Tier 0 preliminary screening-level approach, followed by more complex tiers of analysis, involving progressive grouping of components based on common adverse outcomes (i.e., toxicity target) alone or common adverse outcomes via a common mode of action as indicated by available data on the components. The recommended dose-addition approach is a default approach for screening-level assessments that is supported by results from studies of cultured cells and laboratory animals exposed to various chemical mixtures indicating that deviations from dose addition (also known as concentration addition) have been relatively small from a risk assessment perspective (ATSDR 2018).

ATSDR (2004a, 2018) recommends that hazard indexes be calculated if two or more of the individual components have hazard quotients ≥0.1; if only one or if none of the mixture components has a hazard quotient of this magnitude, then no further assessment of the joint toxic action is needed because additivity and/or interactions are unlikely to result in significant health hazard. Although there is no direct quantitative relationship between hazard quotient or hazard index and risk, concern for the possibility of a health hazard increases with increasing values of individual hazard quotients or the mixture hazard index >1. As discussed by ATSDR guidelines (2004a, 2018), this exposure-based assessment of potential health hazard is a screening approach, to be used in conjunction with biomedical judgment, community-specific health outcome data, and community health concerns to assess the degree of public health hazard.

A screening-level hazard index for all adverse effects from chronic oral exposure (E) to a mixture of barium, calcium, iron, magnesium, manganese, sodium, and strontium, related to UOG activities would be calculated as follows:

$$HI_{mixture} = \frac{E_{barium}}{MRL_{barium}} + \frac{E_{calcium}}{UL_{calcium}} + \frac{E_{iron}}{UL_{iron}} + \frac{E_{magnesium}}{UL_{magnesium}} + \frac{E_{manganese}}{RfD_{manganese}} + \frac{E_{sodium}}{UL_{sodium}} + \frac{E_{strontium}}{RfD_{strontium}}$$

In the absence of chronic oral MRLs for several of the essential metals, the NAS ULs are recommended for calcium, iron, magnesium, and sodium, and the RfD for manganese is recommended to be used in calculating the hazard index (see Appendices B, C, D, and F). The values of the NAS UL and the EPA RfD for manganese are similar in value and are similarly based on the absence of neurological effects in the general population with normal manganese dietary intakes (see Appendix E).

For this assessment, there is no evidence for a common adverse outcome via a common mode of action for most of the pairs of metallic cations; the only pair with evidence for a shared mode of action is iron and manganese, each of which may damage tissue via reactive oxygen species generation (see Appendices C and E). However, some common targets were adversely affected by more than one metallic cation. Common targets from repeated oral exposures are the cardiovascular system (elevated blood pressure) for barium, sodium, and potentially iron; the kidney for barium, calcium, iron, magnesium, and sodium; the nervous system for barium, iron, and manganese; the gastrointestinal system for iron and magnesium; and the skeletal system for strontium and potentially sodium (see Table 2 in the Introduction). Adverse neurological effects from iron and manganese have been proposed to involve, at least in part, the generation of tissue damaging reactive oxygen species (see Section 2.2.13 and Appendix C), but dose-response data for neurotoxic and other effects (cardiovascular, liver, kidney, reproductive) from oral exposure producing high iron tissue levels were inadequate to derive TTDs (see Appendices C and E). Common-target hazard indexes are recommended for the effects with adequate dose-response data to derive TTDs, which include neurological and cardiovascular effects (see Table 3). Any calculation of these common-target hazard indices should be accompanied with qualitative statements about evidence for possible interactions among the components in the calculation (see Section 3.2 for further discussion).

A screening-level hazard index for adverse cardiovascular effects (hypertension) from chronic oral exposure to barium and sodium related to UOG activities would be calculated as follows:

$$HI_{Cardiovascular} = \frac{E_{barium}}{TTD_{(cardio)barium}} + \frac{E_{sodium}}{UL_{sodium}}$$

Calculation of this cardiovascular hazard index also should be accompanied with a qualitative statement that exposure to excess iron has also been associated with hypertension, but available dose-response data were inadequate to derive a cardiovascular TTD for iron and thus a hazard quotient for cardiovascular effects from iron (see Appendix C and Section 2.2.14).

Table 3. Noncancer Health Guidance Values and TTDs for Intermediate or Chronic Oral Exposure to Chemicals of Concern[a]

	Chemical (mg/kg/day)						
Toxicity target	Barium	Calcium	Iron	Magnesium	Manganese	Sodium	Strontium
Cardiovascular	0.21 (TTD)	NA	NA	NA	NA	33 (UL)	NA
Neurological	0.4 (TTD)	NA	NA	NA	0.14 (RfD) 0.16 (UL)	NA	NA
Kidney	0.2 (RfD, MRL)	36 (UL)	NA	6 (TTD)	NA	NA	NA
Gastrointestinal tract	NA	NA	0.6 (UL)	5 (UL)	NA	NA	NA

[a]Refer to Appendices A, B, D, E, F, and G for more details.

MRL = Minimal Risk Level; NA = not available (RfD, MRL, TTD, or UL not available for this toxicity target); RfD = reference dose; TTD = target-organ toxicity dose; UL = tolerable upper intake limit

A screening-level hazard index for adverse neurological effects from chronic oral exposure to barium and manganese related to UOG activities would be calculated as follows:

$$HI_{Neurological} = \frac{E_{barium}}{TTD_{(neuro)barium}} + \frac{E_{manganese}}{RfD_{manganese}}$$

This neurological hazard index also should be accompanied with a qualitative statement that exposure to excess iron has been associated with neurological effects, but available dose-response data were inadequate to derive a neurological TTD for iron and thus a neurological hazard quotient for iron (see Appendix C).

Common-target screening level hazard indices could be similarly calculated for:

1. kidney effects (with hazard quotients for calcium using the calcium UL, for barium using the barium MRL, for magnesium using the kidney TTD, and a statement of possible kidney damage from excess iron and excess sodium and the lack of adequate dose-response data for TTD development); and
2. gastrointestinal effects (with hazard quotients using the iron UL and the magnesium UL).

An additional issue of uncertainty to be discussed in applying this common-target screening-level approach is the inability to develop skeletal TTDs for sodium due to inadequate data and thereby calculate a hazard index for skeletal effects from co-exposure to sodium and strontium (see Appendix F).

Because the kidney plays a key role in whole-body homeostasis for most of these metallic cations, people with kidney functional abnormalities or disease are expected to be especially susceptible to their toxicities. Use of the recommended hazard index approaches in public health assessments should be accompanied with qualitative statements that the assessments may not be protective for such individuals.

3.2 Evaluation of Interaction Data and Recommendations

Use of the recommended approaches should be accompanied with qualitative descriptions of uncertainties associated with the exposure assessment and hazard assessment. A key uncertainty associated with the use of the hazard index approach is the lack of data to assess whether or not dose addition provides an accurate prediction of toxic noncancer responses to mixtures with the selected metallic cations. Studies

with other mixtures of chemicals indicate that deviations from dose addition, when found, were small (see ATSDR 2018), but pertinent studies have not been conducted to assess the combined toxic action of repeated oral exposure to mixtures with barium, calcium, iron, magnesium, manganese, sodium, and strontium. In addition, studies were not located examining effects on toxicokinetic or toxicological endpoints in humans or laboratory animals exposed to mixtures containing more than two of the subject metallic cations, compared with responses to sole exposure to the individual cations.

For this document, data on potential interactions between pairs of the selected metallic cations were identified and evaluated to assess evidence that could qualitatively modify public health assessments (using component-based hazard index approaches) for mixtures with the metallic cations of concern. A summary of this analysis (presented in Chapter 2) follows. This binary approach is acknowledged to be a practical approach with inherent uncertainty due to evidence that coupling of metallic cation homeostatic mechanisms is complex and can overlap for two or more metals. As such, changes in tissue distribution of metals, and subsequent toxic responses, seen with co-exposure to two metals may not be the same as those found with simultaneous exposure to the same two metals plus additional metals (see Section 2.1). Another area of uncertainty is that metal-metal interactions observed in laboratory animal studies may not be directly applicable to humans because diets for laboratory animals may often be more heavily supplemented with essential metals than human diets.

Table 4 summarizes evidence for potential interactions among the 21 pairs of metallic cations with repeated oral exposure (see "A + B pairs" in the first column). The second column of Table 4 indicates weights of evidence for coupling of homeostatic mechanisms: (1) coupling at one homeostatic mechanism/process from studies of isolated membranes, cells, or tissues (+); (2) coupling at two mechanisms/processes from studies of isolated membranes, cells, or tissues, and *in vivo* studies (++); and (3) coupling at more than two mechanisms/processes from studies of isolated membranes, cells, or tissues, and *in vivo* studies (+++). Table 4 also summarizes (in the third and fourth columns) availability of data and evidence assessing whether or not concomitant repeated oral exposure to both components of the pair at elevated oral exposure levels may influence the toxicity of each member of the pair: (+) = evidence for toxicity enhancement; (–) = evidence for toxicity counteracted, (0) = evidence for no influence. Pairs with evidence for influence on expression of toxicity are bolded in Table 4.

Table 4. Summary of Evidence for Interactions Between Pairs of Metallic Cations of Concern with Repeated Oral Exposure

A + B pair	Evidence for coupling of homeostatic mechanisms[a]	Evidence that A + B influences B toxicity[b]	Evidence that A + B influences A toxicity[b]
Ba + Ca	+	ID	ID
Ba + Fe	+	ID	ID
Ba + Mg	+	ID	ID
Ca + Mg	**+++**	**ID**	**Limited (–)**
Fe + Ca	+++	ID	ID
Fe + Mg	+++	ID	ID
Mn + Ca	**+++**	**ID**	**Limited (0)**
Mn + Fe	**+++**	**ID**	**Limited (+)**
Mn + Mg	**+++**	**ID**	**Limited (–)**
Mn + Ba	+	ID	ID
Mn + Na	+	ID	ID
Mn + Sr	+	ID	ID
Na + Ca	**+++**	**ID**	Limited (–)
Na + Fe	**+++**	**Limited (+)**	**Limited (+)**
Na + Mg	**+++**	**ID**	**Limited (–)**
Na + Ba	+	ID	ID
Na + Sr	++	ID	ID
Sr + Ba	**+**	**ID**	**Limited (0)**
Sr + Ca	**+++**	**ID**	**Limited (–)**
Sr + Fe	+++[c]	ID	ID
Sr + Mg	+	ID	ID

[a]**+** = Evidence from studies of isolated membranes, cells, or tissues for potential coupling of at least one homeostatic mechanism/process.
++ = Evidence from studies of isolated membranes, cells, or tissues, and *in vivo* studies for potential coupling of at least two homeostatic mechanisms/processes.
+++ = Evidence from studies of isolated membranes, cells, and *in vivo* studies for coupling of more than two homeostatic mechanisms/processes

[b]Evidence for concomitant exposure to A + B influencing critical effect toxicity of B or A:
ID = inadequate data
(+) = toxicity enhanced
(-) = toxicity counteracted
(0) = toxicity not influenced

[c]No evidence was found for direct homeostatic coupling for iron and strontium, but there is evidence that both may influence calcium homeostasis at several levels of biological organization (see Sections 2.2.7 and 2.2.11).

Evidence for coupling of homeostatic mechanisms was available for all pairs of the selected metallic cations. Eleven pairs were the most heavily studied, and had evidence from studies of isolated membranes, cells, or tissues, and *in vivo* studies for potential coupling at more than two homeostatic mechanisms/processes (Ca+Mg, Fe+Ca, Fe+Mg, Mn+Ca, Mn+Fe, Mn+Mg, Na+Ca, Na+Fe, Na+Mg, Sr+Ca, and Sr+Fe) (Table 4). One pair (Na+Ba) had evidence from studies of isolated membranes, cells, or tissues and *in vivo* studies for potential coupling of at least two homeostatic mechanisms/processes. The remaining nine pairs (Ba+Ca, Ba+Fe, Ba+Mg Mn+Ba, Mn+Na, Mn+Sr, Sr+Ba, Sr+Mg, and Na+Sr,)

had evidence for homeostatic coupling only from studies using isolated membranes, cells or tissues and lacked evidence from *in vivo* studies (Table 4).

In contrast to the relative wealth of evidence for homeostatic coupling among the seven metallic cations, limited evidence for how repeated oral co-exposure may influence toxic responses was available for only a few pairs (see Table 4 and sections noted below):

1. Limited evidence from one study suggesting that repeated gavage co-exposure of rats to barium and strontium did not affect strontium distribution to the bone, compared with exposure to barium or strontium alone, and thus may have no influence on possible skeletal effects from excess strontium (Section 2.2.6).
2. Limited, but inconsistent, evidence from human clinical trials that supplemental magnesium may counteract calcium-induced kidney stones (Section 2.2.8).
3. Limited evidence that calcium co-exposure may not influence the neurotoxic effects of manganese (Section 2.2.9).
4. Limited, but inconsistent, evidence that supplemental calcium (Section 2.2.10) or magnesium (Section 2.2.17) may counteract excess sodium's effects on blood pressure.
5. Limited evidence from dietary studies in animals that excess calcium may protect against strontium-induced skeletal effects and that excess strontium may stimulate bone formation in osteoporotic animals and humans (Section 2.2.11).
6. Limited evidence that iron co-exposure may enhance or add to the neurotoxic effects of manganese (Section 2.2.13).
7. Limited evidence that co-exposure to excess iron and sodium may increase risks for kidney and cardiovascular effects, compared with exposure to excess sodium alone, but it is unknown whether or not the possible joint action may be additive, less-than-additive, or greater-than-additive (Section 2.2.14).
8. Limited evidence that magnesium co-exposure may counteract the neurotoxic effects of manganese (Section 2.2.16).

Potential Influences on Manganese Neurotoxicity and Possible Combined Neurotoxic Effects.
Available evidence suggests that: (1) iron-deficiency or fortification in laboratory animals may enhance the neurotoxic effects of manganese via enhanced distribution of manganese to the brain (Section 2.2.13); (2) brain accumulation of iron and manganese and other metals is associated with neurodegenerative diseases, indicative of possible joint neurotoxic actions of iron, manganese, and other metals involving

neuronal damage from reactive oxygen species (Section 2.2.13); (3) dietary calcium supplementation may be without effect on manganese balance in human balance studies (Section 2.2.9); and (4) magnesium at relatively high dietary levels (compared with manganese levels) inhibited short-term gastrointestinal absorption of manganese in mice and prevented deaths in pigs fed diets with inadequate magnesium levels and high levels of manganese (Section 2.2.16). A clear and logical qualitative extrapolation of these contrasting interaction data to cases when oral exposure levels of iron, calcium, magnesium, and manganese are elevated is not available, but additional studies involving elevated exposure to barium, iron, and manganese with or without supplemental calcium and magnesium may inform whether or not supplemental calcium or magnesium may counteract the enhancement of manganese distribution to the brain caused by iron deficiency or fortification and the possible enhancement of neurological effects.

Currently, the hypothetical protective actions of concurrent exposure to high levels of calcium or magnesium against manganese neurotoxicity are not supported by consistent evidence. For example, in longer-term human balance studies, dietary supplementation with calcium was reported to cause small negative manganese balance in some studies, but no effect on manganese balance in others (Section 2.2.9). Similar human studies examining manganese balance during dietary supplementation with magnesium were not available (Section 2.2.16). Hazard index approaches for exposure to mixtures containing manganese, iron, calcium, and magnesium and utilizing a hazard quotient for manganese should be accompanied with qualitative statements about the likely susceptibility of iron-deficient individuals to manganese neurotoxicity, the possible joint toxic action of excess iron and excess manganese on neurological endpoints, and the possible, but uncertain, protective effects of concurrent exposure to excess calcium or magnesium.

Results from studies of laboratory animals indicate that elevated barium intakes were associated with neurological effects, and a TTD for neurological effects from barium was derived based on these results (see Appendix A). In contrast, although there is evidence that excess iron may contribute to neurological degenerative diseases through neuronal damage from reactive oxygen species (a common mode of action with manganese but not barium), available dose-response data are inadequate to derive a neurological TTD for iron (Appendix C and Section 2.2.13). Calculation of a neurological hazard index with hazard quotients for barium and manganese should be accompanied by qualitative statements that: (1) available interaction data for barium and manganese are inadequate to assess whether the joint action of these metals may be dose-additive, greater-than-dose-additive, or less-than-dose-additive (Section 2.2.4); and (2) accumulation of iron, manganese, and other metals in the brain may jointly act to produce

neurological impairment that may not be accounted for in a neurological hazard index based only on barium and manganese.

Although acute exposure to magnesium at levels producing serum magnesium levels higher than the normal range of 0.7–1.1 mmol/L is thought to produce neurological impairment via magnesium inhibiting calcium entry and preventing the release of neurotransmitters from pre-synaptic sympathetic and neuromuscular nerve junctions, potential neurological impairments from repeated oral exposure to excess magnesium are uncharacterized and data are inadequate to derive a neurological TTD for repeated oral exposure to magnesium (see Appendix D).

***Potential Influences on Sodium-induced Hypertension and Possible Combined Cardiovascular Effects*.** Sodium homeostasis is coupled to both calcium (Section 2.2.10) and magnesium (Section 2.2.17) homeostatic mechanisms/processes, and evidence from laboratory animal studies suggests that dietary supplementation with either calcium or magnesium may counteract the development of sodium-salt induced hypertension. Evidence in human clinical trials, however, has been mixed, with some studies obtaining positive evidence of counteraction and others reporting no counteractive effect (Sections 2.2.10 and 2.2.17). In summary, the available evidence provides no evidence that high levels of calcium or magnesium may enhance hypertension, a condition in humans associated with high sodium salt intake and other factors, and some inconsistent evidence of protective action against the development of hypertension. Hazard index approaches utilizing a hazard quotient for sodium-induced hypertension should be accompanied with qualitative statements about the possible, but uncertain, protective actions of concomitant high exposure levels to calcium and magnesium against sodium-induced hypertension.

Results from studies of laboratory animals indicate that elevated barium intakes were associated with hypertension, and a TTD for hypertensive effects from barium was derived based on these results (Appendix A). Excess iron tissue accumulation also has been associated with increased blood pressure, but available data were inadequate for TTD development (Appendix C). Calculation of a cardiovascular hazard index with hazard quotients for barium and sodium should be accompanied by qualitative statements that available interaction data for barium and sodium are inadequate to assess whether the joint action of these metals to produce cardiovascular effects may be dose-additive, greater-than-dose-additive, or less-than-dose-additive (Section 2.2.5) and that possible contributions to effects on blood pressure from excess iron are not accounted for in the hazard index due to the lack of adequate data for TTD development (Appendix C and Section 2.2.14).

Potential Influences on Strontium-induced Skeletal Effects and Possible Combined Skeletal Toxicity. Available evidence from studies of laboratory animals suggest that strontium can disrupt calcium homeostasis, particularly when strontium exposure levels are higher than calcium exposure levels (Section 2.2.11). The available evidence for interactions between calcium and strontium is inadequate to conclude whether or not concomitant exposure to excess calcium and excess strontium will modify the potential for calcium to induce kidney stones, but there is some evidence that excess calcium can counteract the potential for strontium to induce adverse skeletal effects. The evidence comes from studies showing that excess calcium can protect against strontium-induced skeletal effects in animals and that excess strontium may stimulate bone formation in osteoporotic animals and humans (Section 2.2.11). For hazard index approaches utilizing a hazard quotient for adverse skeletal effects from strontium, the hazard quotient should be accompanied by qualitative statements about: (1) the uncertain possibility that excess calcium may counteract the development of strontium-induced skeletal effects and (2) skeletal effects from excess sodium are also possible, but available data are inadequate for TTD development (Appendix F). The hazard quotient for adverse skeletal effects from strontium should also be accompanied with a qualitative statement about the potential beneficial effects of strontium in inhibiting bone resorption and stimulating bone formation in osteoporotic animals and humans presumably via interactions with the CaSR (Section 2.2.11).

Potential Influences on Calcium-induced Kidney Stones and Possible Combined Kidney Toxicity. Magnesium has been shown to be an effective inhibitor of calcium oxalate stones *in vitro*, but results from clinical trials of dietary supplementation with magnesium as therapy against kidney stone recurrence in humans are mixed: some trials suggested a protective effect and others reported no effect (Section 2.2.8). The designated "limited evidence" for a protective effect of magnesium against calcium-induced kidney stones in Table 4 reflects the positive results obtained in these studies, but should be regarded as highly uncertain because the formation of calcium oxalate kidney stones is thought to be influenced by multiple factors (including age, sex, fluid intake, obesity, diabetes, citrate intake, extent of binding of oxalate in the intestine, urinary oxalate excretion, and potassium intake), and there is controversy on how to interpret the inconsistent evidence for increased calcium intake as a risk factor for kidney stone formation in humans (see Section 2.2.8 and Appendix B). For example, 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. NAS (2011) based their value on a putative human "LOAEL" value, whereas EFSA (2012) determined that there was no reliable human "LOAEL" within the database for dietary calcium supplementation (see Appendix B). Emerging evidence indicates that coupling of homeostatic processes for calcium and magnesium exists at several sites (e.g., TRPV5-mediated calcium reabsorption in the renal distal tubule, CaSR, PTH secretion

from the parathyroid), but current understanding of the complex and interacting homeostatic processes for calcium and magnesium 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 waste water from UOG activities. Hazard index approaches utilizing a hazard quotient for calcium based on kidney stone formation should be accompanied by qualitative statements of the uncertainties associated with calcium's potential to induce kidney stones in humans and magnesium's potential to protect against kidney stone formation in humans.

As discussed in Appendix A, kidney effects from repeated oral exposure to barium are the basis of the oral MRL. In addition, a kidney TTD has been derived for high intakes of magnesium (Appendix D). Calculation of a kidney hazard index with hazard quotients for barium, calcium, and magnesium should be accompanied by qualitative statements that available interaction data for barium, calcium, and magnesium are inadequate to assess whether the joint toxic action may be dose-additive, greater-than-dose-additive, or less-than-dose-additive (Section 2.2.1) and that possible contributions to kidney adverse effects from excess iron and excess sodium would not be captured in the hazard index due to inadequate data for kidney TTDs for these metallic cations (Appendices C and F and Section 2.2.14).

3.3 Data Needs

The recommended component-based hazard index approach and binary evaluation of interaction data is acknowledged to be a practical approach with inherent uncertainty due to evidence that coupling of metallic cation homeostatic mechanisms is complex and can overlap for two or more metals. New studies comparing effects on pertinent endpoints for possible shared toxicity targets (e.g., neurological, cardiovascular, kidney) in laboratory animals orally exposed to mixtures of three or more of the subject metallic cations, with effects from exposure to the individual components alone may lead to better characterization of joint toxic actions on the selected endpoints and help to improve public health assessments for oral exposure to mixtures containing the metallic cations. Designs for these studies with such mixtures should consider the relative proportion of the metals in UOG extraction waste water and expectations on how those proportions may change after release into the environment.

Although no mechanistic, toxicokinetic, or toxicity data were identified for mixtures of three or more of the selected metallic cations, Yao et al. (2015) reported that samples of UOG waste fluids were cytotoxic to cultured human BEAS-2B cells and 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. Studies of additional toxicity endpoints in cells, tissues, or whole animals repeatedly exposed to multiple doses of other samples of UOG waste fluids may help to better define potential toxicity targets of concern and dose-response relationships for exposure to water contaminated with UOG waste fluids. Endpoints in such studies would be most useful if they were associated with putative shared toxicity targets among the subject metallic cations, including the nervous system, cardiovascular system, and the kidney. Supplemental toxicity studies of fractions of UOG extraction waste fluid samples (e.g., metallic cations, radioactive chemicals, nonvolatile and volatile organic chemicals) would lead to better understanding of the relative contributions of different fractions to the toxicity of UOG extraction waste fluids complex mixtures.

Other new studies aimed at better describing dose-response relationships for less sensitive effects occurring at oral exposure levels to the individual metallic cations above the doses associated with the critical effects of health guidance values could decrease uncertainty in common-target hazard indices, especially for the various effects associated with high iron tissue accumulation (liver, kidney, nervous system, and cardiovascular endpoints) and for possible skeletal, neurological, and kidney effects from excess sodium salt.

Better assessment of neurological hazards is likely with results from appropriately designed studies examining brain tissue concentrations and neurological endpoints in laboratory animals orally exposed to mixtures of excess barium, iron, and manganese and comparing the responses to responses to those from sole exposure to the individual cations. Similarly designed studies may better inform joint toxic actions of barium, iron, and sodium in inducing hypertension; barium, calcium, iron, and magnesium in inducing kidney effects; and iron and magnesium in inducing gastrointestinal effects. Additional studies with laboratory animals may better inform whether or not supplemental calcium or magnesium may counteract sodium-salt induced hypertension, combined hypertension effects for barium, iron, and sodium, or combined neurotoxic effects from excess barium, iron, and manganese.

The potential for exposure of people living close to gas-extraction sites and waste-fluid holding facilities to toxic components in waste fluids could be better characterized by additional monitoring studies of suspected toxic components in groundwater and drinking water wells in the vicinity of gas-extraction sites and waste-fluid holding facilities. Supplemental air monitoring studies for expected gases, aerosols, and dusts released from gas extraction activities could better characterize the inhalation exposure potential for people living close to gas-extraction sites and waste-fluid holding facilities. Data are not sufficient to

fully characterize the impact of diet on toxicity of UOG waste water contaminants. Dietary mineral intakes may confound the impact of sodium, strontium, barium, iron, manganese, calcium, and magnesium from the waste water when these minerals are also present in the conventional daily diet, especially for those minerals that are essential nutrients.

4. Conclusions

A component-based approach assuming dose additivity is recommended for preliminary screening-level, exposure-based assessments of potential hazards to public health from chronic oral exposure to mixtures containing barium, calcium, iron, magnesium, manganese, sodium, and strontium. These metals have been found at elevated concentrations in waste fluids from UOG extraction activities. The recommendations include the estimation of an overall screening-level hazard index for all compounds (with no grouping by common adverse outcomes via a common mode of action or common toxicity targets) for an initial Tier 0 human health assessment. As a Tier 1 assessment, calculation of separate common toxicity target screening-level hazard indexes is recommended for cardiovascular effects from barium and sodium, neurological effects from barium and manganese, kidney effects from barium and calcium, and gastrointestinal effects from iron and magnesium. The hazard index approach is appropriate when the hazard quotients of at least two of the components are ≥0.1 (ATSDR 2004a, 2018). The hazard index approach includes making qualitative statements about associated uncertainties including: (1) evidence about possible interactions among the components of the mixtures that may deviate from the hazard predicted by dose addition and (2) the likelihood that estimates of hazard using this approach may not be protective for people with compromised kidney function, important for the normal, whole-body homeostasis of the five metals under consideration which are essential elements. The recommended hazard index approaches with their accompanied qualitative statements of uncertainty are default approaches supported by results from studies of cultured cells and laboratory animals exposed to various chemical mixtures indicating that deviations from dose addition have been small in these cases (ATSDR 2018). When the screening criteria are exceeded (hazard index >1), further evaluation is needed, using biomedical judgment and community-specific health outcome data and taking into account community health concerns (ATSDR 2004a, 2018).

Data on potential interactions between pairs of the selected metallic cations were identified and evaluated in Chapters 2 and 3 to assess evidence that could qualitatively modify public health assessments using component-based hazard index approaches for mixtures with the metallic cations of concern. The binary approach is acknowledged to be a practical approach with inherent uncertainty due to evidence that coupling of metallic cation homeostatic mechanisms is complex and can overlap for two or more metals.

Evidence for coupling of homeostatic mechanisms at the cellular level was available for all 21 pairs of the selected metallic cations; evidence for coupling at the whole-body level of organization was available for 12 pairs. The physiological and toxicological relevance of the findings from *in vitro* studies is not always

clear, especially with regard to making reliable predictions of how repeated combined oral exposure to any pair of metallic cations may influence their individual toxicity or indicate how they may jointly act on a shared toxicity target. For binary weights of evidence (BINWOEs) making a directional prediction, a small fraction (5%) was explained by *ex vivo* interactions (Pryzbyla et al. 2021). In contrast to the relative wealth of evidence for homeostatic coupling among the seven metallic cations, limited evidence for how repeated oral co-exposure may influence toxic responses was available for only 11 pairs. The evidence for interactions was adequate to suggest possible influences on the critical-effect toxicity of barium and calcium (kidney effects), manganese (neurotoxic effects), sodium (cardiovascular effects), and strontium (skeletal effects).

Hazard index approaches for exposure to mixtures with the subject metals and a hazard quotient for manganese should be accompanied with qualitative statements about the likely susceptibility of iron-deficient individuals to manganese neurotoxicity, the possible joint toxic action of excess iron and excess manganese on neurological endpoints, and the possible, but uncertain, protective effects of concurrent exposure to excess calcium or magnesium. Calculation of a neurological target toxicity hazard index with hazard quotients for barium and manganese should be accompanied by qualitative statements that: (1) available interaction data for barium and manganese are inadequate to assess whether the joint action of these metals on neurotoxic endpoints may be dose-additive, greater-than-dose-additive, or less-than-dose-additive; and (2) accumulation of iron, manganese, and other metals in the brain may jointly act to produce neurological impairment that may not be accounted for in a neurological hazard index based only on barium and manganese.

Hazard index approaches using a hazard quotient for sodium-induced hypertension should be accompanied with qualitative statements about the possible, but uncertain, protective actions of concomitant high exposure levels to calcium and magnesium against sodium-induced hypertension. Calculation of a cardiovascular hazard index with hazard quotients for barium and sodium should be accompanied by qualitative statements that available interaction data for barium and sodium are inadequate to assess whether the joint action of these metals to produce cardiovascular effects may be dose-additive, greater-than-dose-additive, or less-than-dose-additive and that possible contributions to effects on blood pressure from excess iron are not accounted for in the hazard index due to the lack of adequate data for TTD development.

Hazard index approaches utilizing a hazard quotient for adverse skeletal effects from strontium should be accompanied by qualitative statements about: (1) the uncertainty that excess calcium may counteract the

development of strontium-induced skeletal effects and (2) skeletal effects from excess sodium are also possible, but available data are inadequate for TTD development. The hazard quotient for adverse skeletal effects from strontium should also be accompanied with a qualitative statement about the potential beneficial effects of strontium in inhibiting bone resorption and stimulating bone formation in osteoporotic animals and humans presumably via interactions with the CaSR.

Hazard index approaches utilizing a hazard quotient for calcium based on kidney stone formation should be accompanied by qualitative statements of the uncertainties associated with calcium's potential to induce kidney stones in humans and magnesium's potential to protect against kidney stone formation in humans. Calculation of a kidney hazard index with hazard quotients for barium, calcium, and magnesium should be accompanied by qualitative statements that available interaction data for barium, calcium, and magnesium are inadequate to assess whether the joint toxic action may be dose-additive, greater-than-dose-additive, or less-than-dose-additive and that possible contributions to kidney adverse effects from excess iron and excess sodium would not be captured in the hazard index due to inadequate data for kidney TTDs for these metallic cations.

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Appendix A. Background Information for Barium

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

A.1 Toxicokinetics

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

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

A.2 Health Effects

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

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

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

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

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

A.3 Mechanisms of Action

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

A.4 Health Guidelines

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

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

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

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

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

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

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

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

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Appendix B. Background Information for Calcium

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

B.1 Toxicokinetics

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

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

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

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

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

B.2 Health Effects

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

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

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

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

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

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

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

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

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

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

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

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

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

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

B.3 Mechanisms of Action

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

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

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

B.4 Health Guidelines

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

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

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

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

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

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

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

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

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

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

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

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

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Appendix C. Background Information for Iron

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

C.1 Toxicokinetics

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

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

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

C.2 Health Effects

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

C.3 Mechanisms of Action

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

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

C.4 Health Guidelines

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

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

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

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

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

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

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

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

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

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

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

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

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

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Appendix D. Background Information for Magnesium

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

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

D.1 Toxicokinetics

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

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

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

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

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

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

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

D.2 Health Effects

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

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

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

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

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

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

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

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

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

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

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

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

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

D.3 Mechanisms of Action

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

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

D.4 Health Guidelines

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

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

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

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

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

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

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

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

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

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

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

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Appendix E. Background Information for Manganese

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

E.1 Toxicokinetics

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

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

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

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

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

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

E.2 Health Effects

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

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

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

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

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

E.3 Mechanisms of Action

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

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

E.4 Health Guidelines

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

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

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

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

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

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

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

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

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Appendix F. Background Information for Sodium

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

F.1 Toxicokinetics

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

F.2 Health Effects

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

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

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

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

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

F.3 Mechanisms of Action

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

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

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

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

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

F.4 Health Guidelines

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

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

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

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

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

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Appendix G. Background Information for Strontium

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

G.1 Toxicokinetics

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

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

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

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

G.2 Health Effects

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

G.3 Mechanisms of Action

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

G.4 Health Guidelines

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

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

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

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

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

G.6 References

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Appendix H. Noncancer Health Guidance Values for Selected Metals

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

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

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

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

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

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

H.1 References

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

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

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

Source: Barbot et al. 2013

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

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

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

Source: Jackson et al. 2013b

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

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

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

I.1 References

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

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

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

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

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

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

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

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

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

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

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

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

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

Table J-2. Database Query Strings

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

Table J-2. Database Query Strings

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

Table J-2. Database Query Strings

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

Table J-2. Database Query Strings

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

Table J-2. Database Query Strings

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

Table J-2. Database Query Strings

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

Table J-2. Database Query Strings

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

Table J-2. Database Query Strings

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

Table J-2. Database Query Strings

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

Table J-2. Database Query Strings

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

Table J-2. Database Query Strings

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

Table J-2. Database Query Strings

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

Table J-2. Database Query Strings

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

Table J-2. Database Query Strings

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

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

Table J-2. Database Query Strings

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

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

Table J-2. Database Query Strings

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

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

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

Table J-2. Database Query Strings

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

Table J-2. Database Query Strings

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

Table J-3. Additional Electronic Screening Keywords

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