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