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 considering 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.