Errata for Alberta Biomonitoring Program: Chemicals in serum of children in southern Alberta 2004–2006 (Phase two)

Influence of age and comparison to pregnant women

Alberta

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Errata for Alberta Biomonitoring Program: Chemicals in serum of children in southern Alberta 2004-2006 (Phase Two) Influence of age and comparison to pregnant women | Alberta Health

Contents

Summary

The serum pools analyzed for environmental chemicals in this study were created from leftover blood serum collected from children presenting for elective surgeries. Red top glass tubes were used for these collections These tubes are not recommended for trace metals analysis. Therefore, it is possible the results for some metals, metalloids, and micronutrients in this report may be artificially inflated or reduced as a result of the use of these tubes and other sample collection and storage devices as background contamination by these elements was not assessed.

Several of the limits of detection/quantification (LOD/LOQs) provided for the metals and micronutrients in the original report were incorrect.

Please note the units for the perfluorinated compounds throughout the report should be ng/mL.

Errata Table 1 below lists the errata for this report. The Appendix provides the rationale for the metals and micronutrients recommendations.

ERRATA TABLE 1: CORRECTIONS BY PAGE NUMBER

Page	Paragraph, table	Section, line	Correction
12	List of Chemicals Table	Perfluorinated Chemicals	Units: ng/mL serum
12	List of Chemicals Table	Metals and Micronutrients/Antimony	LOD/LOQ: 0.1 µg/L
		Metals and Micronutrients/Arsenic	LOD/LOQ: 0.2 µg/L
		Metals and Micronutrients/Boron	LOD/LOQ: 20 µg/L with a caveat that the inaccuracy may be up to 30% with this revised LOD/LOQ
13		Metals and Micronutrients/Copper	LOD/LOQ: 50 µg/L
		Metals and Micronutrients/Iron	LOD/LOQ: 50 µg/L
		Metals and Micronutrients/Lead	LOD/LOQ: 5 µg/L
		Metals and Micronutrients/Zinc	LOD/LOQ: 50 µg/L
		Metals and Micronutrients/Manganese	LOD/LOQ: 0.5 µg/L
		Metals and Micronutrients/Nickel	LOD/LOQ: 0.5 µg/L
		Metals and Micronutrients/Selenium	LOD/LOQ: 50 µg/L
		Metals and Micronutrients/Silver	LOD/LOQ: 0.1 µg/L
		Metals and Micronutrients/Cesium	LOD/LOQ: 0.1 µg/L
39–40	Figures 14–17	Y-axis titles	Units: ng/mL serum
74	2	1	Sentence should read, “All analytes except for aluminum, beryllium, lead, platinum, thallium, uranium, and tungsten were detected in at least 25% of the serum pools. Antimony and barium were detected, but not reported due to background levels of these analytes in quality control samples. Cadmium was detected in two sample pools (33%), but not reported because the means in both age groups were below the LOD.”

Page	Paragraph, table	Section, line	Correction
74–92		Metals and Micronutrients Sections	Detailed results added for three metals (arsenic, manganese, and mercury). Refer to the Detailed analytical results section below.
		Metals and Micronutrients Sections	Units on y-axis of the figures and in the discussions are ng/mL. This is equivalent and directly comparable to µg/L.
76	3	1–3	Concentrations were measured in serum collected in red top glass tubes, which are not recommended by clinical reference laboratories for cesium analysis. Background contamination is possible.
77	Figure 49	Cesium	Maternal LOD is 0.2 µg/L; Children's LOD is 0.1 µg/L as shown in Figure E49 below.
78	3	1–3	Concentrations were measured in serum collected in red top glass tubes, which are not recommended by clinical reference laboratories for chromium analysis. Background contamination is highly likely.
81	Figure 51	Silver	Maternal LOD is 0.2 µg/L; Children's LOD is 0.1 µg/L as shown in Figure E51 below.
82	3	1–3	Concentrations were measured in serum collected in red top glass tubes, which are not recommended by clinical reference laboratories for vanadium analysis. Background contamination is possible.
85	1	1–2	Concentrations were measured in serum collected in red top glass tubes, which are not recommended by clinical reference laboratories for boron analysis. Contamination is highly likely.
85	Figure 53	Boron	Maternal LOD is 2 µg/L; Children's LOD is 20 µg/L as shown in Figure E53 below.
87	1	1–4	Concentrations were measured in serum collected in red top glass tubes, which are not recommended by clinical reference laboratories for copper analysis. Background contamination is possible.
87	Figure 55	Copper	Maternal LOD is 0.2 µg/L; Children's LOD is 50 µg/L as shown in Figure E55 below.
88	Figure 56	Iron	Maternal LOD is 10 µg/L; Children's LOD is 50 µg/L as shown in Figure E56 below.
89	1	1–2	Concentrations were measured in serum collected in red top glass tubes, which are not recommended by clinical reference laboratories for molybdenum analysis. Background contamination is possible.
90	1	1–2	Concentrations were measured in serum collected in red top glass tubes, which are not recommended by clinical reference laboratories for nickel analysis. Background contamination is possible.
90	Figure 58	Nickel	Maternal LOD is 0.2 µg/L; Children's LOD is 0.5 µg/L as shown in Figure E58 below.
91	1	1–2	Concentrations were measured in serum collected in red top glass tubes, which are not recommended by clinical reference laboratories for selenium analysis. Background contamination is possible.
91	Figure 59	Selenium	Maternal LOD is 0.5 µg/L; Children's LOD is 50 µg/L as shown in Figure E59 below.

Page	Paragraph, table	Section, line	Correction
92	1	1–2	Concentrations were measured in serum collected in red top glass tubes, which are not recommended by clinical reference laboratories for zinc analysis. Background contamination is possible.
92	Figure 60	Zinc	Maternal LOD is 5 µg/L; Children's LOD is 50 µg/L as shown in Figure E60 below.

Detailed analytical results

Arsenic (As)*

General information

Sources

Arsenic is a naturally occurring element widely distributed in the earth's crust. In the environment, arsenic can combine with other elements to form inorganic arsenic compounds or it can combine with carbon and hydrogen to form organic arsenic compounds [1]. Inorganic arsenic is mainly used to preserve wood. Copper chromated arsenic is used to make pressure-treated lumber but is no longer used for residential purposes; however, it is still used for industrial applications. Organic arsenic compounds can be used as pesticides, primarily on cotton plants. Arsenic is released into the environment from several industrial processes, predominately during the generation of power from coal-fired furnaces. Arsenic compounds are also widely used in agricultural and silvicultural products, and small quantities are utilized as a feed additive to boost immune systems and assure rapid disease-free growth [2].

There are numerous ways in which a person may become exposed to low levels of arsenic. A person normally takes in small amounts of arsenic via the air, water, and food. Food is the major source of intake with total arsenic concentrations being highest in seafood. Several organic arsenicals, generally felt to be essentially nontoxic, accumulate in fish and seafood, and are sometimes referred to as 'fish arsenic' [1,3]. Marine fish, fresh water fish, and canned fish contribute substantially to the total arsenic intake; however, this is contributing mostly organic and essentially nontoxic forms of arsenic to the diet [4]. Certain geographic areas may naturally contain higher levels of arsenic in rock resulting in higher concentrations in soil or water. Exposure may occur through the occupational environment or during home renovations where arsenic-treated wood sawdust may be released [5].

Possible health effects

Inorganic arsenic has been associated with human toxicity [1]. Ingestion may result in gastrointestinal irritation and decreased production of red and white blood cells. Inhalation may result in a sore throat and irritated lungs. A characteristic effect of long-term arsenic exposure is a pattern of skin changes such as patches of darkened skin and the appearance of small corns or warts on the palms, soles, and torso [5]. Arsenic toxicity symptoms also include hyperkeratosis, Blackfoot disease, myocardial ischemia, liver dysfunction, epithelioma, hypertension, and death. The current study utilizes serum concentrations of arsenic and, as such, direct comparison of this study to other studies which utilize whole blood concentrations of arsenic may not be accurate.

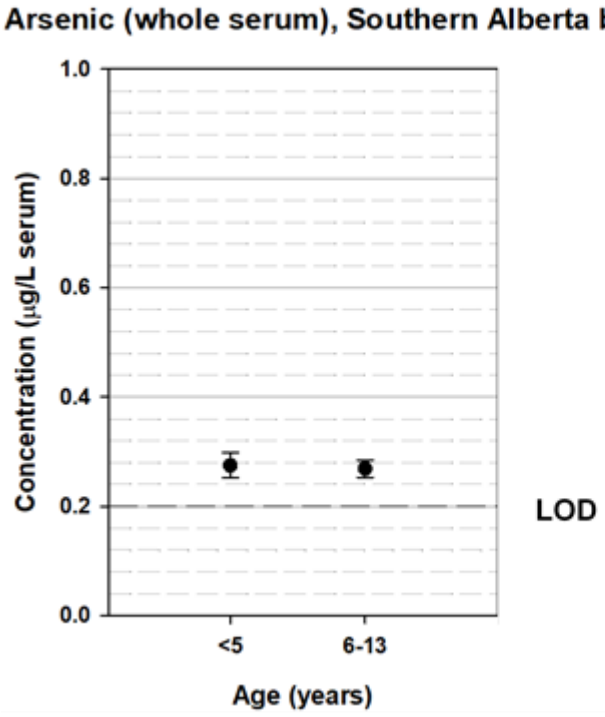
Blood serum concentrations in children in Alberta

Concentrations and trends

Mean concentrations of arsenic in the six serum pools ranged from 0.25 to 0.29 µg/L. Concentrations of arsenic are not significantly different between the <5 and the 6–13 year groups (Errata Figure 1). More than 25% of the serum pools in the southern Alberta pregnant women had arsenic levels below the limit of detection so descriptive statistics were not calculated for those results.

Concentrations of serum or plasma arsenic in studies of children around the world were found to be higher than those detected in the children in Alberta. One study found a mean plasma concentration of arsenic in 9-year-old Bangladeshi

children to be 1.9 (±2.7 [SD]) µg/L. These samples were collected between 2003–2004. The concentrations of arsenic in this cohort ranged from 0.0080–20 µg/L. The concentrations in the Bangladeshi children were generally higher than those detected in the serum of the Alberta children and had significantly more variation [6]. A Russian study published in 2017 found a mean serum arsenic concentration in a healthy control group of 48 children with an average age of 6.5 years to be 1.9 (±0.8 [SD]) µg/L, which is higher than the serum arsenic concentration in the Alberta children [7]. An Italian study published in 2011 noted a mean plasma arsenic concentration in 28 healthy children aged 2–6 years to be 13.72 (±6.57 [SD]) ng/g [8]. A Brazilian study published in 2015 detected mean serum arsenic levels of 14.0 (±2.6 [SD]) µg/L in 33 rural children and 25.0 (±4.0 [SD]) µg/L in 20 urban children [9].



Errata Figure 1

***** Serum used for this testing was collected in red top glass tubes, which are not the recommended tubes for trace metals testing. Background contamination is possible.

Manganese (Mn)*

General information

Sources

Manganese is naturally occurring and most often found in rocks and soils; however, it does not occur in the environment as a pure metal. It is usually combined with oxygen, sulphur, or chlorine. It is principally used in steel production to improve hardness, stiffness, and strength. It can also be used in fireworks, dry cell batteries, paints, as a medical imaging agent, and in cosmetics [10].

The general population is exposed through food, water, air, and consumer products containing manganese. Manganese is an essential nutrient required as a cofactor for a variety of enzymes [10]. The highest concentrations are found in grains, nuts, legumes, and fruit. The extent of absorption is a function of particle size, which determines where manganese will be deposited. The amount of manganese absorbed across the gastrointestinal tract is variable but typically averages 3–5%. Adults maintain stable tissue levels of manganese through the regulation of gastrointestinal absorption and hepatobiliary excretion. Absorbed manganese is widely distributed throughout the body, with higher levels found in the liver, pancreas, and kidney. The primary route of excretion is through the feces.

Possible health effects

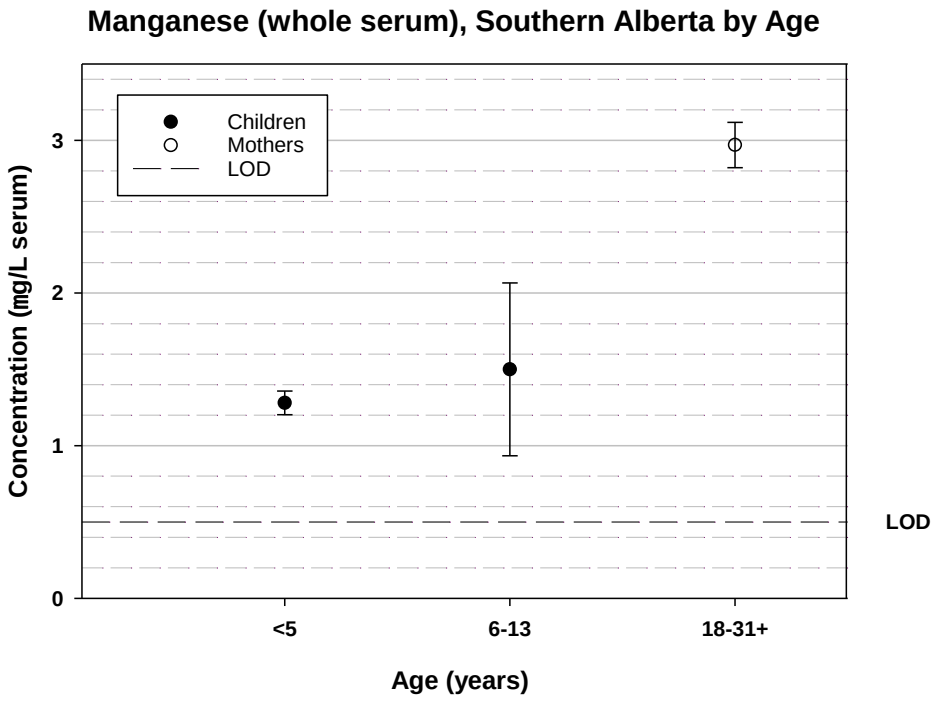
Health effects will depend on the dose, duration, and route of exposure. As an essential nutrient, it is involved in the formation of bone, in cellular protection from free radical damage, and in amino acid, cholesterol, and carbohydrate metabolism [10]. Manganese deficiency is rare, but excessive exposure can cause neurological effects. Inhaled manganese can be transported directly to the brain and can result in a permanent neurological disorder, known as manganism, with symptoms that include tremors, difficulty walking, and facial muscle spasms. Exposure to high or very high levels of manganese, such as those in accidental or occupational exposures, can result in lung inflammation and impaired lung function [10].

Blood serum concentrations in children in Alberta

Concentrations and trends

Mean concentrations of manganese in the six serum pools ranged from 1.18–2.08 µg/L. Concentrations of manganese are not significantly different between the <5 and the 6–13 year groups (Errata Figure 2). However, the concentration of manganese in the children's serum is lower than in the pregnant women from southern Alberta.

The serum mercury concentrations in the Alberta children were within the range of those detected in the plasma of 99 healthy children in France. The range (5^{th}–95^{th} percentile) of plasma concentrations in the French study was 0.53–2.21 µg/L [11]. A Brazilian study published in 2015 detected mean serum manganese levels of 2.0 (±0.8 [SD]) µg/L in 33 rural children and 15.0 (±1.0 [SD]) µg/L in 20 urban children [9]. The rural concentrations were comparable to the Alberta children's serum concentrations while the urban concentrations were significantly higher. The manganese concentration in children's serum in Alberta was lower than most other studies in children. A Russian study published in 2017 found a mean serum manganese concentration in a healthy control group of 48 children with an average age of 6.5 years to be 2.2 (±0.6 [SD]) µg/L [7], which is slightly higher than the mean concentration in the Alberta study. An Italian study published in 2011 noted a mean plasma manganese concentration in 28 healthy children aged 2–6 years to be 3.56 (±3.54 [SD]) ng/g [8]. A Turkish study published in 2011 found a mean serum manganese concentration of 0.55 (±0.41 [SD]) µg/L in the control group of 33 children (mean age of 10.7 years) [12]. A United States study using NHANES data from 2011–2014 determined a mean serum manganese concentration in 4021 male children aged 6–19 years to be 10.69 (3.21–58.86 [range]) µg/L [13].



Errata Figure 2

***** Serum used for this testing was collected in red top glass tubes, which are not the recommended tubes for trace metals testing. Background contamination is possible.

Mercury (Hg)

General information

Sources

Mercury is a naturally occurring chemical element that is widely distributed around the earth in its elemental, inorganic, and organic forms [14–18]. It is the only metal that is liquid at room temperature. Elemental and inorganic mercury compounds are used or found in a wide variety of industrial, commercial, and medicinal products such as electrical instruments, thermostats, switches, thermometers, batteries, antiseptics, fungicides, preservatives, and dental fillings. Its use has been greatly reduced or phased out of most products [19,20], but is still present in many lamps and lights, including fluorescent lamps, mercury vapour lamps, and compact fluorescent bulbs [20].

Mercury enters the environment from natural processes such as weathering of rocks and minerals and volcanic activity. Inorganic and elemental mercury can also be released from anthropogenic activities such as the combustion of fossil fuels (mainly coal), mining, smelting, and other industrial processes. Mercury is not commonly found in water as it generally binds to soil and sediment; however, it may enter the water system from spills, industrial effluent, irrigation run off, or drainage from areas in which agricultural pesticides are in use [21]. High mercury levels can be found in the Arctic regions as a result of global atmospheric circulation and long-range transboundary transport. Depending on the form of mercury, bioaccumulation and biomagnification can occur, particularly with organic mercury.

There are many ways in which humans can be exposed to mercury, including ingestion of food, water, soil, and inhalation of air containing trace concentrations of mercury. Total blood mercury concentrations in the general population are due primarily to the dietary intake of forms of organic mercury [17]. Food is the main source of mercury exposure in populations that are not exposed to mercury occupationally [16]; however, occupational settings where mercury-containing products are manufactured or used may cause people to be exposed to higher mercury concentrations than the general public. Other routes of exposure include inhalation of mercury particles or vapor, dental fillings, and ingestion of drinking water. Approximately 80% of inhaled inorganic mercury is absorbed into blood making it the most significant route of exposure leading to internal doses of inorganic mercury [16,17]. Inorganic mercury is absorbed poorly from the gastrointestinal tract with less than 15% of total exposed mercury actually being absorbed [17]. Inorganic mercury is widely distributed throughout the body with the highest concentrations occurring in the kidneys. Excretion occurs primarily through the urine.

Possible health effects

Health effects associated with exposure to mercury are dependent upon the length of exposure, absorbed dose, and the form of mercury [17]; however, areas of the body such as the brain and kidneys are particularly sensitive to the effects of mercury [14]. Exposure to inhaled elemental mercury can result in health effects such as pneumonitis, tremors, depression, fatigue, sleep disturbances, and neurocognitive and behavioral disturbances. Ingestion of inorganic mercury can result in irritation of the gastrointestinal tract, and once absorbed can lead to effects on the kidneys such as renal tubular necrosis. The IARC has classified methylmercury as a possible human carcinogen and inorganic mercury as unclassifiable [17]. Under a high dose long-term exposure scenario, elemental and inorganic mercury may cause adverse health effects such as general weakness, nausea, vomiting, skin rash, eye irritation, and brain and kidney effects [14]. In 2004, Health Canada established a total mercury blood guidance value of 20 µg/L for adults [22].

The current study utilizes serum concentrations of mercury and, as such, direct comparison of this study to other studies that utilize whole blood concentrations of mercury may not be accurate.

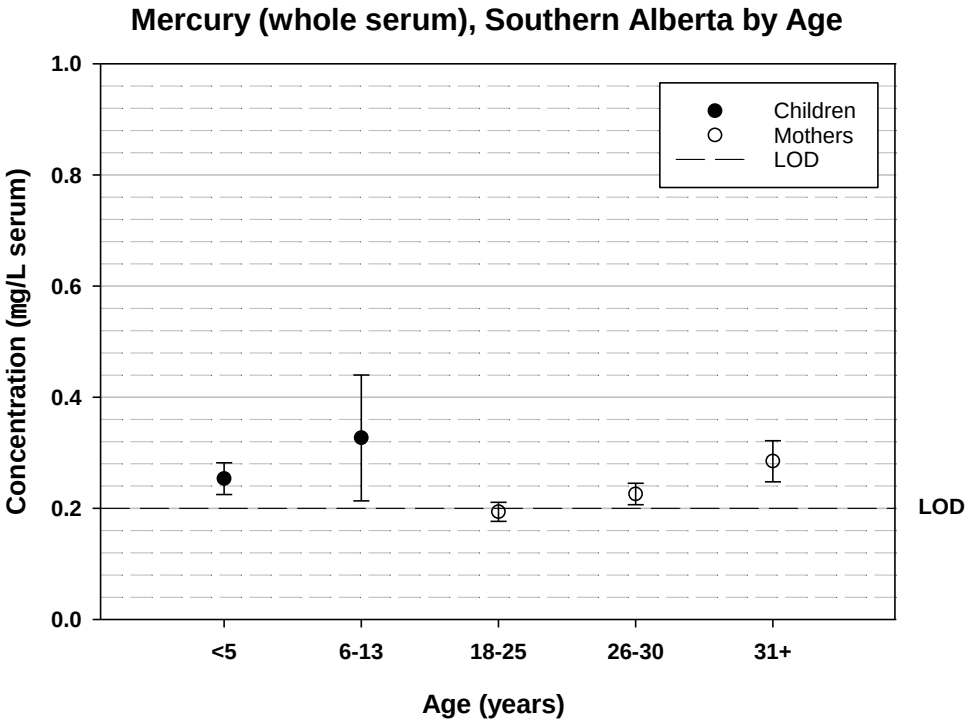
Blood serum concentrations in children in Alberta

Concentrations and trends

Mean concentrations of mercury in the six serum pools ranged from 0.23 to 0.44 µg/L. Concentrations of mercury were not significantly different between the <5 and the 6–13 year groups (Errata Figure 3). However, the concentration of mercury in the serum from the <5 year group is greater than in the 18–25 year pregnant women from southern Alberta.

The serum mercury concentrations in the Alberta children were significantly lower than those found in a study published in 2016 and carried out in Egypt. This study found a mean plasma concentration of mercury in forty 3–6 year old children in a

control group to be 12.08 (±4.5 [SD]) µg/L [23]. Serum mercury concentrations in the Alberta children were also lower than those found in two Asian studies. A Korean study that used data from a study conducted in 2010 found a median serum mercury level in 311 elementary school children of 2.19 (1.66–2.87 [interquartile range 25–75%]) µg/L [24]. A Chinese study conducted between 2008–2009 found mean serum mercury concentrations of 3.02 (2.32 [SD]) µg/L in a group of 110 children with eczema (mean age 9.9 years) and 2.66 (2.03 [SD]) µg/L in a group of 41 children with miscellaneous skin conditions (mean age 11.5 years) [25]. The serum mercury concentration in the Alberta children was also lower than that determined in a Slovenian study. This study, which was published in 2014, found a mean serum mercury concentration of 1.55 (±0.56)[SD]) µg/L in the control group of fourteen 1–16 year olds [26]. The serum mercury concentrations in the Alberta children were similar to two studies in Europe and one in the United States. A Turkish study published in 2011 found a mean serum mercury concentration of 0.12 (±0.66 [SD]) µg/L in the control group of 33 children (mean age of 10.7 years) [12]. A study published in 1998 examining children from an industrial area of Poland detected a mean serum mercury concentration of 0.44 (0.14–3.0 [range]) µg/L in 68 4–14 year olds [27]. A United States study using NHANES data from 2011–2014 determined a mean serum mercury concentration in 4021 male children aged 6–19 years to be 0.65 (0–13.71 [range]) µg/L [13]. The serum mercury concentrations in the Alberta children were slightly higher than the mean serum mercury concentration in an Iranian study. This study, published in 2014, detected a mean serum mercury level in one hundred and sixty 10–18 year olds in the control group of 0.10 (±0.08 [SD]) µg/L [28].



Errata Figure 3

Adjusted figures

All error bars indicate the 95% confidence intervals.

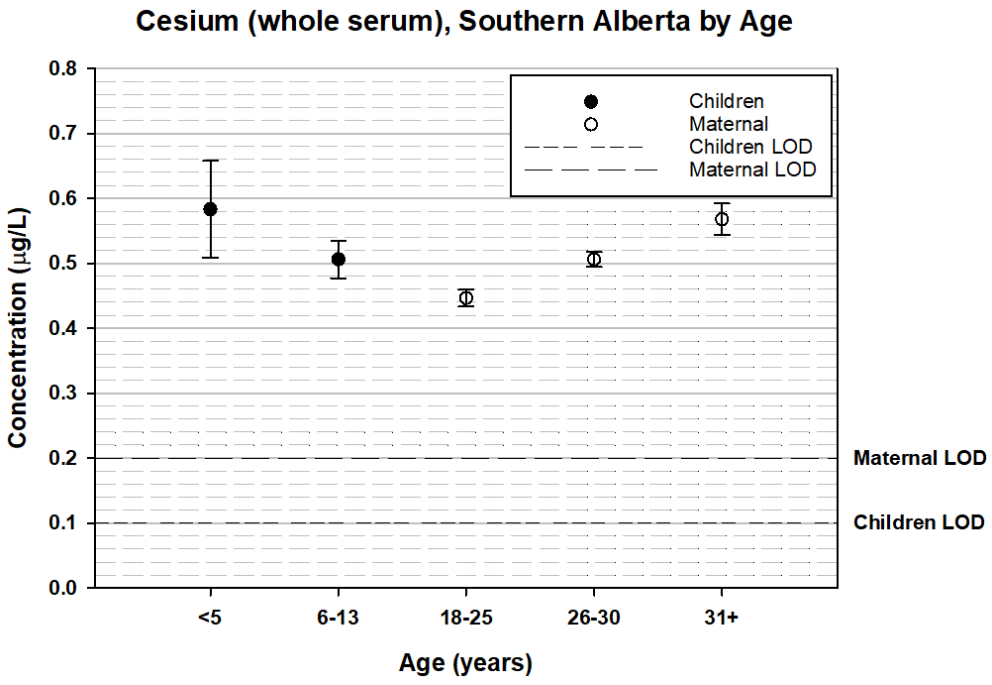


Figure E49

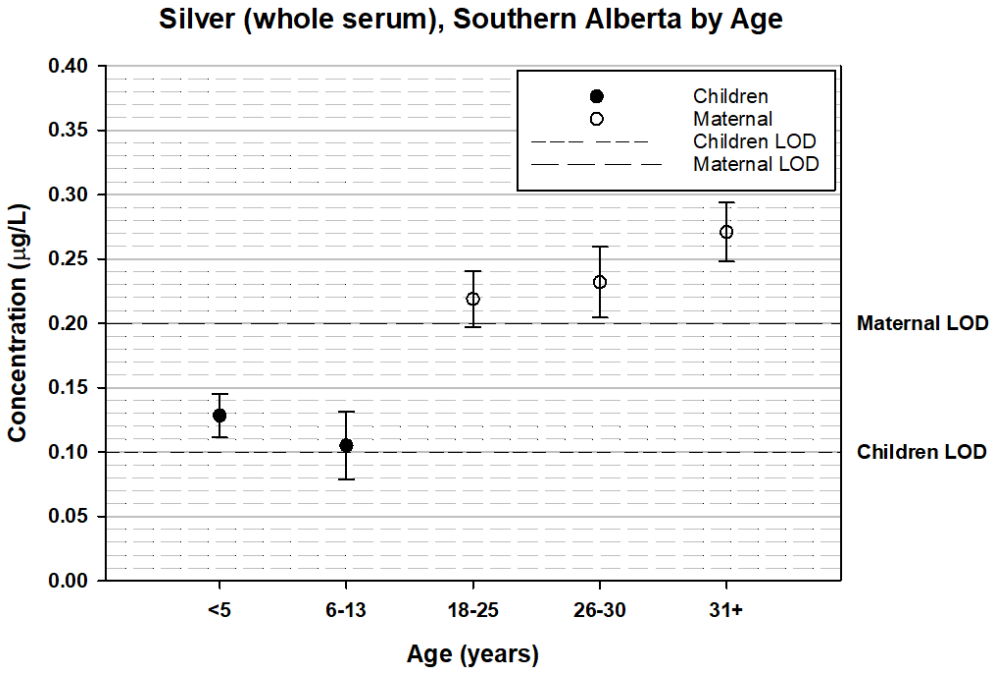


Figure E51

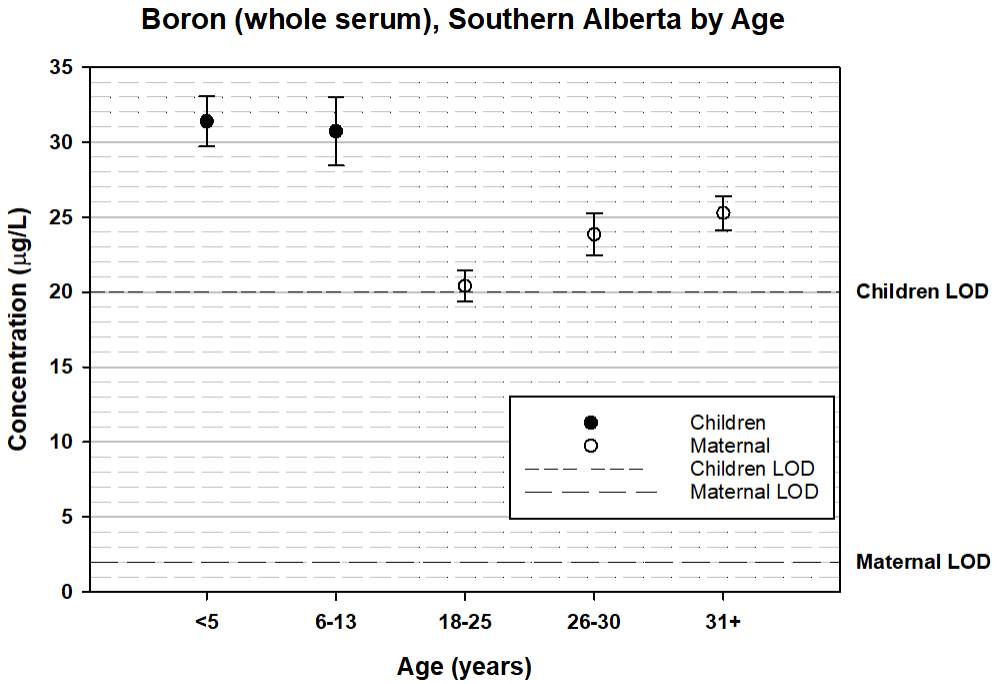
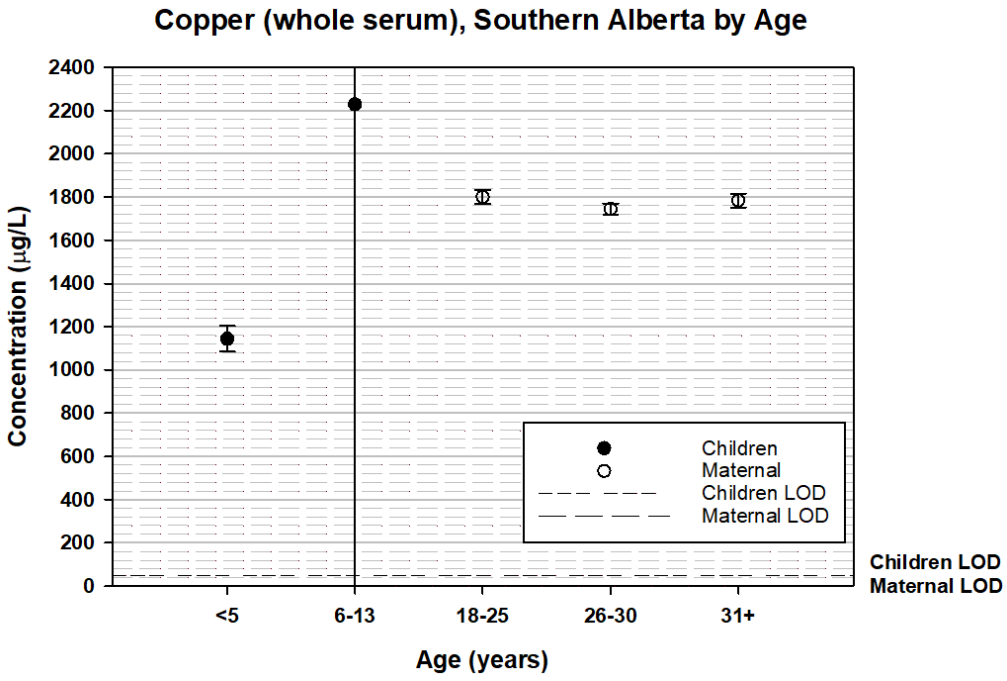


Figure E53



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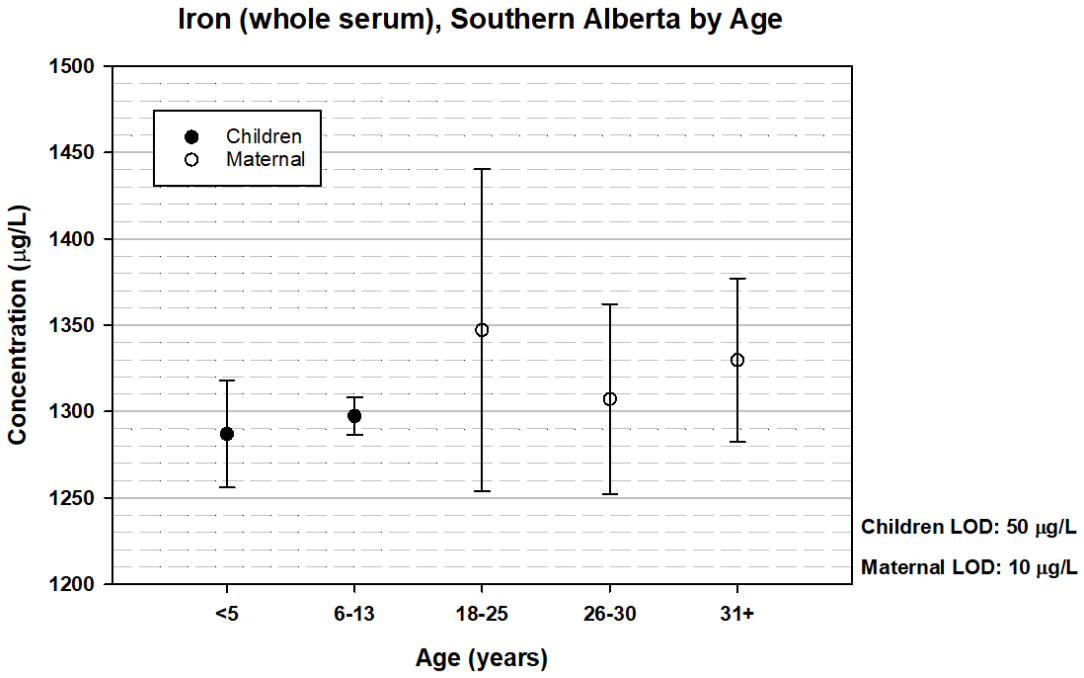

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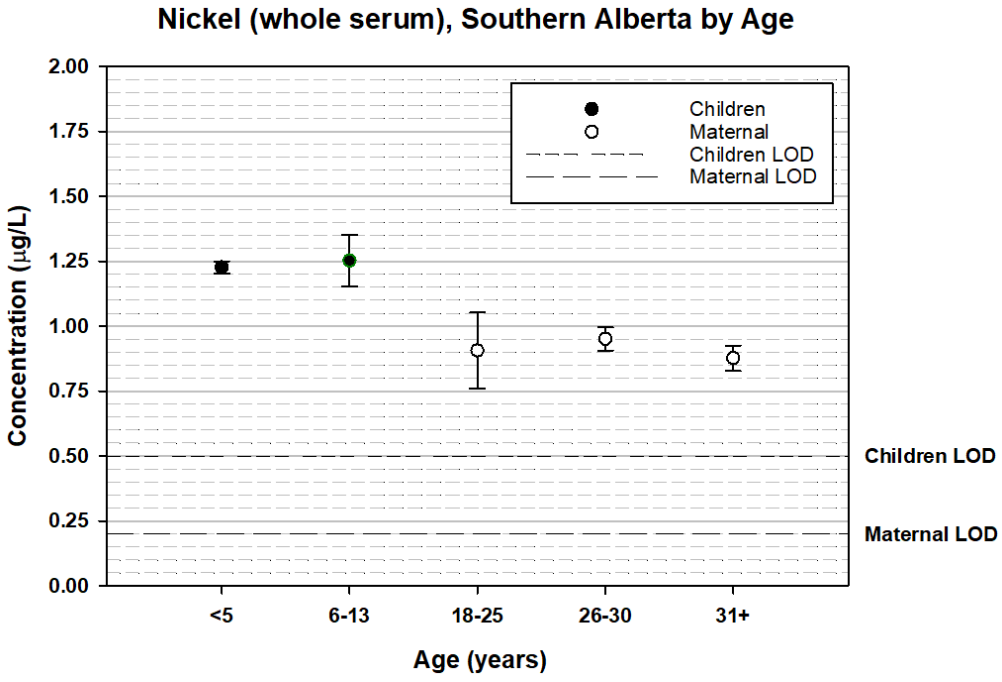

Figure E58

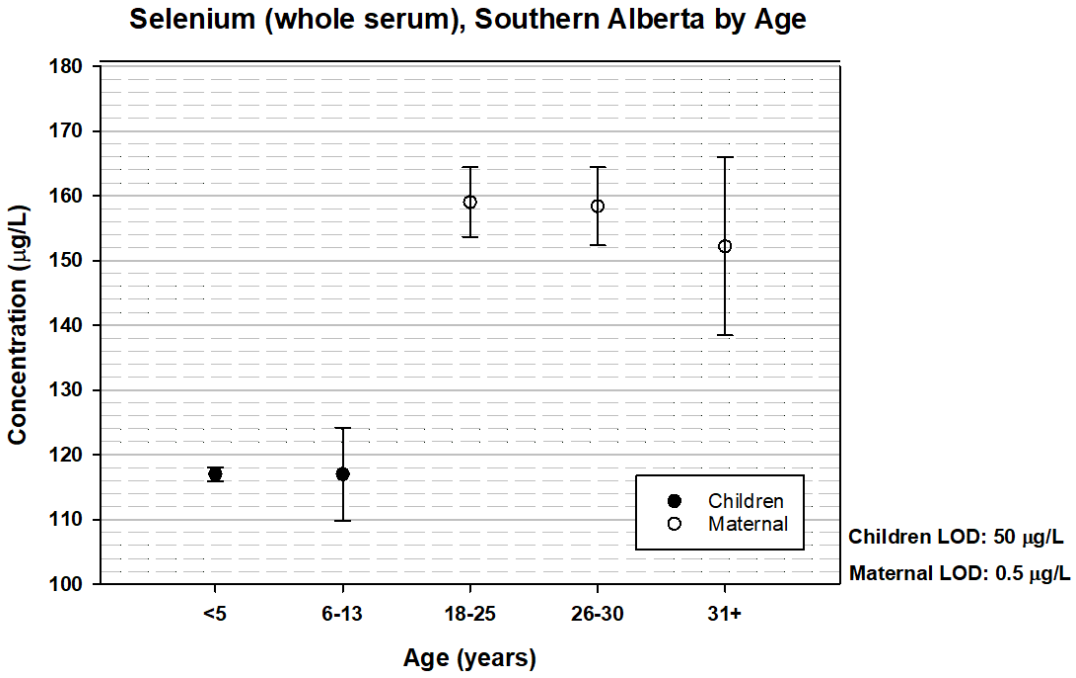

Figure E59

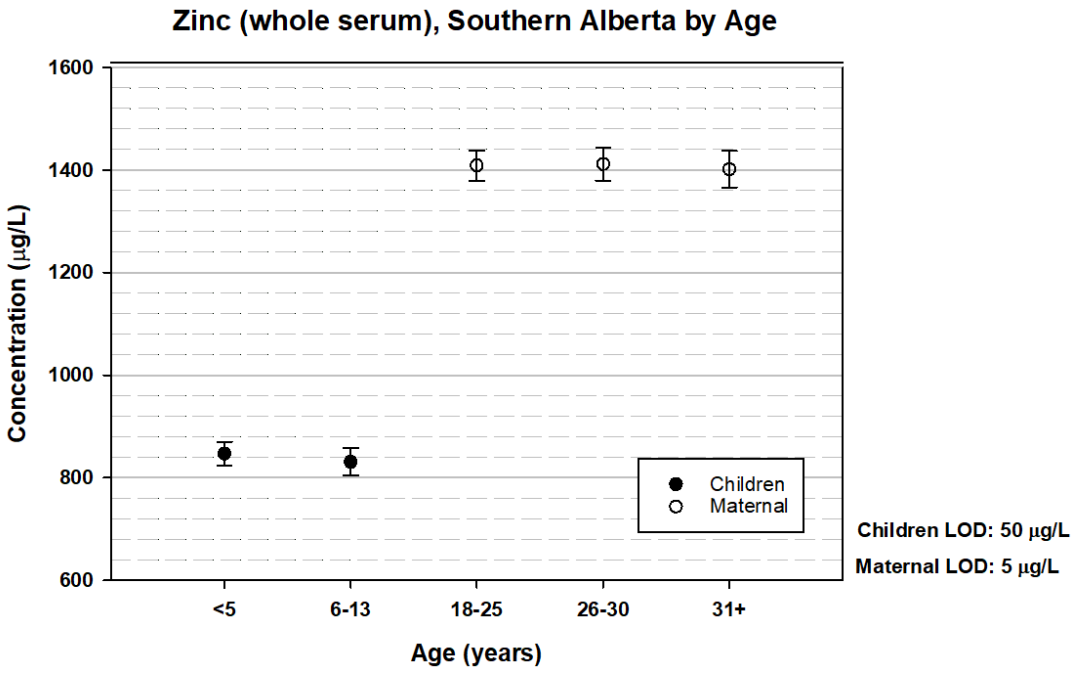

Figure E60

Appendix

Rationale for metals, metalloids, and micronutrients recommendations for Alberta Biomonitoring Program: Chemicals in serum of children in southern Alberta 2004-2006 (Phase Two)

The metals, metalloids, and micronutrients results were evaluated based on comparison to other serum or plasma general reference ranges, a background contamination study performed on red top glass tubes prior to sample collection for Phase Three of the Alberta Biomonitoring Program, general population reference ranges, and cautions provided for each specific test in serum on the Mayo Clinic website (where available). The Alberta Biomonitoring Program's Phase One as well as the Northern Saskatchewan Prenatal Biomonitoring Study's results are provided for comparison. Serum samples used in Phase One and Saskatchewan (SK) were collected in serum separator tubes (SSTs). Phase Two serum samples were collected in red top glass tubes with no additive. The recommendations are based on the criteria below:

- If two or more of the following criteria were met, the comment "contamination is highly likely" was assigned:
  - Metal/micronutrient concentration detected in red top glass tubes in background contamination study (for Phase Three) was greater than the mean concentration plus two standard deviations detected in blank bovine serum;
  - Metal/micronutrient detected above normal in at least 50% of reference ranges;
  - At least one warning of contamination issues from a reference laboratory or in the literature.
- If one of the above criteria was met, "contamination is possible" was assigned.
- If none of the above criteria were met, no caveat was assigned.

ERRATA TABLE A: RATIONALE FOR METALS, METALLOIDS, AND MICRONUTRIENTS RECOMMENDATIONS

Element	Overall mean (±95%CI) (µg/L)			Reference range serum concentration comparison (µg/L)	Literature cautions and findings	Comments and recommendations
	Phase Two	Phase One	SK			
Arsenic	0.27 (±0.01)	<LOD	<LOD	4.4–14.2 [29] 1.55–7.58 [30] <0.5–3.6 [31] Children's plasma 1.43–3.12 [11]		**It is recommended that arsenic data be reported with the caveat for Phase Two: the serum used for this testing was collected in red top glass tubes, which are not the recommended tube for arsenic analysis and have shown some background levels in a contamination study. Background contamination is possible.**
Manganese	1.4 (±0.3)	2.9 (±0.3)	3.5 (±0.4)	0.6–2.3 [32] 0.63–2.26 [29] 0.35–1.08 [30] 0.3–1.04 [31] 0–2.0 [33] Children's plasma 0.53–2.21 [11]	The Mayo Clinic website states: Specimens collected from healthy, unexposed adults have extremely low levels of manganese (Mn). Because of the high environmental concentration of Mn, contamination is always a possibility when considering elevated results. Precautions must be taken to ensure the specimen is not contaminated. Metal-free serum collection procedures must be followed and centrifuged serum must be aliquoted into an acid-washed Mayo metal-free vial [32].	**It is recommended that manganese data be reported with the caveat for Phase Two: the serum used for this testing was collected in red top glass tubes, which are not the recommended tube for manganese analysis. Background contamination is possible.**
Mercury	0.29 (±0.06)	0.25 (±0.01)	0.38 (±0.14)	0.09–1.01 [30] 0.21–1.3 [31]		Phase Two mean within the two reference ranges. **It is recommended that mercury data be reported with NO CAVEAT for Phase Two as there was no mercury was detected above the mean +2SD blank bovine serum concentrations in serum passed through any of the collection devices that may have been used in Phase Two.**

Element	Overall mean (±95%CI) (µg/L)			Reference range serum concentration comparison (µg/L)	Literature cautions and findings	Comments and recommendations
	Phase Two	Phase One	SK			
Chromium	0.92 (±0.11)	1.5 (±0.1)	<LOD	<0.29–0.79 [29] 0.05–0.48 [31] <0.3 [34] ≤5 [35] Children's plasma <0.29–1.39 [11]	The Mayo Clinic website states the <0.3 µg/L reference range applies to samples collected by a phlebotomist trained in ultra-clean collection techniques. The majority of specimens submitted to their facility range from 0.3–0.9 µg/L for unexposed individuals. Chromium concentrations in samples collected in non-trace metal tubes range from 2–5 µg/L [34]. Reports of increased serum chromium could be due to external contamination [34].	It is likely the phlebotomist who drew the samples was not concerned with ultra-clean collection techniques as the original purpose for the samples was not trace metal analysis. **It is recommended that chromium data be reported with the caveat for Phase Two: the serum used for this testing was collected in red top glass tubes, which are not the recommended tube for chromium analysis and have shown some background levels in a contamination study. Therefore, contamination is highly likely.**
Cesium	0.54 (±0.49)	0.48 (±0.01)	0.85 (±1.0)	0.45–0.82 [31]		Phase Two mean within reference range concentrations. **It is recommended that cesium data be reported with the caveat for Phase Two: the serum used for this testing was collected in red top glass tubes, which are not the recommended tube for cesium analysis. Cesium was detected above the mean blank bovine serum concentration in butterfly needles, which may have been used for serum collection in some situations. Therefore, background contamination is possible.**
Silver	0.12 (±0.02)	0.27 (±0.01)	0.22 (±0.03)	<0.06–0.80 [30] 0.062–0.24 [31] 0.11–0.17 [31] Children's plasma <0.57 [11]		Phase Two mean within four ranges. **It is recommended that silver data be reported with NO CAVEAT for Phase Two as there was no silver detected above the mean +2SD blank bovine serum concentrations in serum passed through any of the collection devices that may have been used in Phase Two.**
Vanadium	0.31 (±0.02)	<LOD	<LOD	<0.21–0.38 [30] 0.015–0.106 [31] Children's plasma <0.21–0.41 [11]		**For Phase Two, it is recommended that reporting the vanadium data with the following caveat: the serum used for this testing was collected in red top glass tubes, which are not the recommended tube for vanadium analysis. Background contamination is possible.**

Element	Overall mean (±95%CI) (µg/L)			Reference range serum concentration comparison (µg/L)	Literature cautions and findings	Comments and recommendations
	Phase Two	Phase One	SK			
Boron	31 (±1)	22 (±1)	17 (±3)	<100 [36] 19–79 [29] 7–19 [31]	The Mayo Clinic website states: Specimens for elemental testing should be collected in certified metal-free containers. Elevated results for elemental testing may be caused by environmental contamination at the time of specimen collection and should be interpreted accordingly [36].	Phase Two mean is within two of three general ranges. **It is recommended that boron data be reported with the caveat for Phase Two: the serum used for this testing was collected in red top glass tubes, which are not the recommended tube for boron analysis. Background contamination is highly likely.**
Cobalt	<LOD	0.33 (±0.05)	0.45 (±0.03)	0–0.9 [37] 0.3–1.02 [29] 0.24–0.59 [30] 0.03–0.18 [31] ≤1 [38] Children's plasma 0.31–0.79 [11]	"Specimen collection procedures for cobalt require special specimen collection tubes, rigorous attention to ultraclean specimen collection and handling procedures, and analysis in an ultraclean facility. Unless all of these precautions are taken, elevated serum cobalt results may be an incidental and misleading finding." [37]	**It is recommended that cobalt data be reported with the caveat for Phase Two: the serum used for this testing was collected in red top glass tubes, which are not the recommended tube for cobalt analysis. Background contamination is possible.**
Copper	1686 (±1110)	1851 (±18)	1960 (±116)	400–1800 [39] 794–2023 [29] 627–1659 [30] 740–1300 [31] 800–1550 [40] Children's plasma 705–1776 [11]		Phase Two mean is higher than three of the six general reference ranges. **It is recommended that copper data be reported with the caveat for Phase Two: the serum used for this testing was collected in red top glass tubes, which are not the recommended tube for copper analysis. Background contamination is possible.**
Iron	1292 (±15)	1241 (±25)	1070 (±82)	350–1500 [41] 550–1200 [31] 300–1600 [42] 280–1620 [43]		Phase Two mean within three general reference ranges. **It is recommended that iron data be reported with NO CAVEAT for Phase Two as there was no iron detected above the mean +2SD blank bovine serum concentrations in serum passed through any of the collection devices that may have been used in Phase Two.**

Element	Overall mean (±95%CI) (µg/L)			Reference range serum concentration comparison (µg/L)	Literature cautions and findings	Comments and recommendations
	Phase Two	Phase One	SK			
Molybdenum	3.2 (±0.3)	1.4 (±0.1)	1.2 (±0.1)	0.3–2 [44] 0.67–1.68 [29] 0.36–1.15 [30] 0.27–0.85 [31] Children's plasma 0.55–1.98 [11]	The Mayo Clinic website states: Increased serum molybdenum may be seen in acute viral hepatitis, chronic active hepatitis, alcoholic liver disease, and other forms of liver inflammation [44].	Phase Two mean is higher than the reference ranges. **It is recommended that molybdenum data be reported with the caveat for Phase Two: the serum used for this testing was collected in red top glass tubes, which are not the recommended tube for molybdenum analysis. Background contamination is possible.**
Nickel	1.2 (±0.1)	0.88 (±0.08)	0.76 (±0.57)	<2 [45] 0.04–5.31 [29] 0.44–1.26 [30] 0.13–0.55 [31] ≤10 [46] Children's plasma 0.64–2.51 [11]	The Mayo Clinic website (in serum section, but refers to urinary values) states: Specimen collection procedures for nickel require special collection containers, rigorous attention to ultraclean specimen collection and handling procedures, and analysis in an ultraclean facility. Unless all of these procedures are followed, increased urinary nickel results may be an incidental and misleading finding [45].	Phase Two mean is within five of six reference ranges. **It is recommended that nickel data be reported with the caveat for Phase Two: the serum used for this testing was collected in red top glass tubes, which are not the recommended tube for nickel analysis. Background contamination is possible.**
Selenium	117 (±3)	154 (±3)	118 (±5)	45–150 [47] 79–141 [29] 73–110 [30] 74–90 [31] 23–190 [48] Children's plasma 55–90 [11]	The Mayo Clinic website states: Selenium is quite volatile; therefore, careful specimen collection is necessary to ensure accurate results [47].	Phase Two mean higher than three reference ranges. **It is recommended that selenium data be reported with the caveat for Phase Two: the serum used for this testing was collected in red top glass tubes, which are not the recommended tube for selenium analysis. Background contamination is possible.**
Zinc	839 (±17)	1391 (±14)	1410 (±66)	600–1200 [49] 551–925 [29] 510–809 [30] 420–710 [31] 600–1200 [50] Children's plasma 508–1017 [11]	The Mayo Clinic website states: Hemolyzed specimens will cause false elevation of serum zinc levels. It is essential that the specimen is collected following the trace metals collection procedure (metal-free collection tubes) [49].	Phase Two mean above two reference ranges. **It is recommended that zinc data be reported with the caveat for Phase Two: the serum used for this testing was collected in red top glass tubes, which are not the recommended tube for zinc analysis. Background contamination is possible.**

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