Errata for:

Northern Saskatchewan Prenatal Biomonitoring Study

Saskatchewan

Saskatchewan Health Authority

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Errata for

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Errata for:

Northern Saskatchewan Prenatal Biomonitoring Study Technical Report

Summary

The levels of some metals, micronutrients and phthalate metabolites described in this report may be inflated by contamination introduced from the type of blood collection tubes used. The serum pools analyzed for environmental chemicals in this study and the previous 2005 Alberta Biomonitoring Program study were created from blood serum specimens collected specifically for prenatal screening. Serum separator tubes (STTs) were used for these collections which are standard for prenatal screening. SSTs are not recommended for trace metal analysis. Therefore, it is possible the results for some metals, metalloids, and micronutrients in this report may be artificially inflated or reduced by the use of these tubes and other sample collection and storage devices as background contamination by these elements was not assessed.

Listed below are the errata for the metals, metalloids, micronutrients and phthalate metabolite data for the Northern Saskatchewan Prenatal Biomonitoring Study Technical Report. Appendix A and B provide the rationale for these recommendations. Similar errata have been developed for the Alberta Biomonitoring Program's data on pregnant women (2005) and children (2004-2006), which has been used as comparative data for the Saskatchewan report.

Metals and Micronutrients

Blood samples were collected in serum separator tubes (SSTs), which are not recommended for trace metals analysis. As a result of the use of these tubes it is possible the results for some metals and micronutrients in this report may be artificially inflated through metals or micronutrients leaching from the separator gel or reduced through adsorption onto or absorption into the gel. There is less evidence of the potential to the SSTs to reduce the concentration but this is theoretically possible. The classifications below are detailed further in Appendix A.

The metals most likely to be affected by contamination include:

- Aluminum
- Antimony
- Barium
- Boron
- Manganese
- Nickel

Metals that are less likely, but **may possibly** be affected by background contamination include:

- Lead
- Cobalt
- Selenium
- Strontium
- Zinc

Metals for which it is **unknown** if SST collection may affect concentrations include:

- Cesium
- Cadmium
- Mercury
- Silver
- Copper
- Iron
- Magnesium
- Methyl mercury
- Molybdenum
- Uranium

Phthalate Metabolites

To investigate the potential for contamination by phthalate diesters, an experiment was conducted. Bovine serum was substituted for human serum, to evaluate background levels of phthalate metabolites in the SSTs used for collection and the cryovials used for storage of the serum samples. In the presence of phthalate diesters (parent phthalate compounds), some of which are ubiquitous in the environment, esterases in the serum can convert the diesters to the primary metabolites. This results in elevated levels of the primary metabolites. Therefore, there is a risk that serum levels of some of the primary metabolites may be falsely elevated if contamination of samples with the primary diesters occurred during or after collection.

The primary phthalate metabolite MiBP (monoisobutylphthalate) was detected in bovine serum stored in the collection tubes and containers at levels greater than the mean plus two standard deviations of the concentration detected in the blank bovine serum samples. The parent phthalate diester, DBP (dibutylphthalate), is used in some medical devices and is ubiquitous in the environment, leading to a higher background contamination risk than some other phthalates. Therefore, the MiBP results in this report may be falsely elevated due to background contamination and should be interpreted with caution. Appendix B provides further details on the phthalate metabolite recommendations.

Errata Table 1 below lists the specific errata for this report (Northern Saskatchewan Prenatal Biomonitoring Survey: Technical Report). The Appendix A provides the rationale for the metals, metalloids, and micronutrients recommendations. Appendix B provides the rational for the phthalate metabolite recommendations.

ERRATA TABLE 1: CORRECTIONS BY PAGE NUMBER

Page	Paragraph, Table, Figure	Section	Correction
130-131	p.130: paragraph 3 & p.131: Figure 101	Methyl Mercury	Concentrations were measured in serum collected in SSTs, which are not recommended by clinical reference laboratories for methyl mercury analysis as it is unknown how the SSTs can contribute or reduce the serum methyl mercury concentration.
133-134	p.133: paragraph 4 & Table 21 & p.134: Figure 102	Monoisobutyl phthalate (MIBP)	As the parent phthalate diester, DBP, is used in some medical devices and is ubiquitous in the environment, MIBP results may be falsely elevated due to background contamination and should be interpreted with caution.
139	p.139: paragraph 2 & Figure 107	Aluminum	Concentrations were measured in serum collected in SSTs, which are not recommended by clinical reference laboratories for aluminum analysis as contamination is highly likely.
141	p.141: paragraph 2 & Figure 108	Antimony	Concentrations were measured in serum collected in SSTs, which are not recommended by clinical reference laboratories for antimony analysis as contamination is highly likely.
143	p.143: paragraph 1 & Figure 109	Arsenic	Concentrations were measured in serum collected in SSTs, which are not recommended by clinical reference laboratories for arsenic and it is unknown how the SSTs may affect concentration.
145–146	p.145: paragraph 4 p.146: Figure 110	Barium	Concentrations were measured in serum collected in SSTs, which are not recommended by clinical reference laboratories for barium analysis as contamination is highly likely.
148	p.148: paragraph 4	Cadmium	Concentrations were measured in serum collected in SSTs, which are not recommended by clinical reference laboratories for cadmium and it is unknown how the SSTs may contribute to or reduce the serum cadmium concentration.
151–152	p.151: paragraph 4 p.152: Figure 111	Cesium	Concentrations were measured in serum collected in SSTs and it is unknown how the SSTs may contribute to or reduce the serum cesium concentration.
154	p.154: paragraph 3 & Figure 112	Chromium	Concentrations were measured in serum collected in SSTs, which are not recommended by clinical reference laboratories for chromium analysis as contamination is highly likely.
157-158	p.157: paragraph 3 & p.158 Figure 113	Lead	Concentrations were measured in serum collected in SSTs, which are not recommended by clinical reference laboratories for lead analysis as background contamination is possible.

Page	Paragraph, Table, Figure	Section	Correction
160	p.160: paragraph 1 & Figure 114	Mercury	Concentrations were measured in serum collected in SSTs which are not recommended by clinical reference laboratories for mercury as it is unknown how the SSTs may contribute to or reduce the serum mercury concentrations.
162-163	p.162: paragraph 4 & p.163: Figure 115	Strontium	Concentrations were measured in serum collected in SSTs, which are not recommended by clinical reference laboratories for strontium analysis as it is unknown how the SSTs may contribute or reduce the serum strontium concentration.
164	p.164: paragraph 1	Uranium	Concentrations were measured in serum collected in SSTs, which are not recommended by clinical reference laboratories for uranium analysis as it is unknown how the SSTs may contribute to or reduce the serum uranium concentration.
166-167	p.166: paragraph 5 & p.167: Figure 116	Boron	Concentrations were measured in serum collected in SSTs, which are not recommended by clinical reference laboratories for boron analysis as environmental contamination is highly likely.
168-169	p.168: paragraph 4 & p.169: Figure 117	Cobalt	Concentrations were measured in serum collected in SSTs, which are not recommended by clinical reference laboratories for cobalt analysis as contamination is possible.
171-172	p.171: paragraph 5 & p.172: Figure 118	Copper	Concentrations were measured in serum collected in SSTs, which are not recommended by clinical reference laboratories for copper analysis as it is unknown how the SSTs may contribute to or reduce the serum copper concentration.
174	p.174: paragraph 3 & Figure 119	Iron	Concentrations were measured in serum collected in SSTs, and it is unknown how the SSTs may contribute to or reduce the serum iron concentration.
176	p.176: paragraph 1 & Figure 120	Manganese	Concentrations were measured in serum collected in SSTs, which are not recommended by clinical reference laboratories for manganese analysis as environmental contamination is highly likely.
177-178	p.177: paragraph 5 & p.178: Table 121	Magnesium	Concentrations were measured in serum collected in SSTs, and it is unknown how the SSTs may contribute to or reduce the serum magnesium concentration.
179	p.179: paragraph 4 & Figure 122	Molybdenum	Concentrations were measured in serum collected in SSTs, which are not recommended by clinical reference laboratories for molybdenum analysis as it is unknown how the SSTs may contribute to or reduce the serum molybdenum concentration.
181-182	p.181: paragraph 5 & p.182: Figure 123	Nickel	Concentrations were measured in serum collected in SSTs, which are not recommended by clinical reference laboratories for nickel analysis as contamination is highly likely.

Page	Paragraph, Table, Figure	Section	Correction
184	p.184: paragraph 1 & Figure 124	Selenium	Concentrations were measured in serum collected in SSTs which are not recommended by clinical reference laboratories for selenium analysis as contamination is highly likely.
185-186	p.185: paragraph 5 & p.186: Figure 125	Silver	Concentrations were measured in serum collected in SSTs which are not recommended by clinical reference laboratories for silver analysis as background contamination is possible.
188-189	p.188: paragraph 4 & p.189: Figure 126	Zinc	Concentrations were measured in serum collected in SSTs, which are not recommended by clinical reference laboratories for zinc analysis as contamination is possible.

Appendix A: Rationale for metals, metalloids, and micronutrient recommendations for Northern Saskatchewan Prenatal Biomonitoring Study Technical Report, Technical Summary Report and General Summary Report

The metals, metalloids, and micronutrients results were evaluated based on comparison to other serum or plasma general reference ranges, articles specifically examining serum/plasma levels in pregnant women, an article examining metals concentrations in leachate collected from serum separator tubes (SSTs), and cautions provided for each specific test in serum on the Mayo Clinic website (where available). The Alberta Biomonitoring Program's Phase One (pregnant women, 2005) and Two (children, 2004-2006) results are provided for comparison. Serum samples used in Phase One and Saskatchewan (SK) were collected in 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 possible" was assigned:
    - Metal/metalloid/micronutrient concentration detected in SSTs by Rodushkin & Ödman (2001) [1] was greater than Saskatchewan limit of detection (LOD);
    - Metal/metalloid/micronutrient detected in the Saskatchewan data at levels 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, "unknown how the SSTs may affect concentration" was assigned.
- Exceptions:
    - Cesium (Cs) listed as unknown as only two reference ranges were available and the SK mean concentration was only 3.5% higher than one range and within the other range.
    - Copper (Cu) is known to increase during pregnancy, which may contribute to the concentrations being higher than the non-maternal reference ranges. Although Cu was detected above LOD in the SSTs [1]the concentration was 1400x lower than the mean SK concentration.
    - Zinc (Zn): although Zn met two criteria, it may be higher than the non-maternal reference ranges due to supplementation (contained in many prenatal vitamins) so it is assigned the statement of "contamination is possible"

Element	Overall Mean (±95% CI) (µg/L)			Reference Range/Maternal Serum Concentration Comparison (µg/L)	Literature Cautions and Findings	Comments and Recommendation
	SK	Phase One (AB)	Phase Two (AB)			
Aluminum	9.0 (±2.7)	22 (±0.9)	<LOD	0 - 6 [2] 1.2 – 17.3 [3] 0.8 – 5.9 [4] 0 - 15 [5] Maternal serum: 69.27 (median) [6]	Failure to pay attention to proper specimen collection procedures can cause abnormal results due to specimen contamination, which can lead to misinterpretation and misdiagnosis [2]. Special evacuated blood collection tubes are required for aluminum testing. These tubes are readily available and should always be used [2]. Most of the common evacuated blood collection devices have rubber stoppers that are comprised of aluminum-silicate. Simple puncture of the rubber stopper for blood collection is sufficient to contaminate the specimen with aluminum. Typically, blood drawn in standard evacuated blood tubes will be contaminated by 20 to 60 ng/mL aluminum [2].	SK mean is higher than two of the four reference ranges while AB Phase One is higher than four reference ranges SK and AB Phase One means are lower than the median maternal serum concentration in Liang *et al.* (2019) [6], but they do not provide details of their blood sample collection procedure. **We recommend aluminum data be reported with the caveat for SK that the serum used for this testing was collected in SSTs, which are not recommended by clinical reference laboratories for aluminum analysis as contamination is highly likely.**

Element	Overall Mean (±95% CI) (µg/L)			Reference Range/Maternal Serum Concentration Comparison (µg/L)	Literature Cautions and Findings	Comments and Recommendation
	SK	Phase One (AB)	Phase Two (AB)			
					Rodushkin and Ödman (2001) noted significant leaching of aluminum from SST tubes into a 0.05 nitric acid solution,which they state to be a moderately strong leaching agent. A higher concentration of metal/micronutrient would be expected to leach into acid than serum, but the results suggests that a detectable level of aluminum is likely to leach into serum [1].	
Antimony	3.5 (±0.2)	3.8 (±0.2)	<LOD	0.03-0.15 [3] 0.027-0.063 [4] 0.070-0.11 [4] <1 [7] Maternal serum: <0.36 – 1.7 [8]	Filella *et al*. 's (2013) review on antimony in human blood states "the risk of contamination is high because sampling is often performed in hospitals by persons not necessarily aware of all the risks of contamination in relation to trace element analysis and it includes many manipulations and the use of a variety of materials" [7]. Filella *et al*. (2013) also note it is widely recommended by the manufacturers of the blood collection tubes (Becton Dickinson [BD])to use a glass green top sodium heparin tube due to the presence of high concentrations of antimony in some plastic tubes [7]. Rodushkin and Ödman (2001) noted leaching of antimony from SSTs into a 0.05M nitric acid solution [1].	SK and AB Phase One means are significantly higher than the reference ranges and the maternal serum concentration range in Krachler *et al.* (1999) [8]. **We recommend antimony data be reported with the caveat for SK: the serum used for this testing was collected in SSTs, which are not recommended by clinical reference laboratories for antimony analysis as contamination is highly likely.**
Arsenic	0.11	NA	NA	1.55 – 7.58 [9] < 0.05 – 3.6 [4] 4.4 – 14.2 [3] Maternal serum: 1.74 (mean) [6]	Rodushkin and Ödman (2001) noted leaching of arsenic from SSTs into a 0.05M nitric acid solution but at levels lower than SK LOD [1].	**We recommend arsenic data be reported with the caveat for SK that the serum used for this testing was collected in SSTs, which are not recommended by clinical reference laboratories for arsenic and it is unknown how the SSTs may affect concentration.**
Barium	3.2 (±0.3)	8.5 (±0.4)	109 (±3)	90-154 [3] 0.22-1.22 [9] 0.4-1.7 [4] Maternal serum: 41.43 (median) [6] 0.6 – 26 [8]	Oskarrson (2014) states reference ranges vary from 1 – 60 ug/L and concentrations increase with age, especially in women [10]. Poddalgoda *et al.* (2017) state that urinary values have not changed significantly in NHANES data across the years, but serum barium concentrations are lower in more recent studies than in older studies in similar populations [11]. Rodushkin and Ödman (2001) noted significant leaching of barium from SSTs into a 0.05M nitric acid solution [1].	SK and AB Phase One means are higher than two general reference ranges. SK and AB Phase One means are lower or within the range of the maternal serum concentrations in other studies. The decrease in serum barium levels in recent years could possibly be due to more stringent sample collection protocols upon recognizing the ease of contamination of serum samples with barium. **We recommend barium data be reported with the caveat for SK and AB Phase One: the serum used for this testing was collected in SSTs, which are not recommended by clinical reference laboratories for barium analysis as contamination is highly likely.**

Element	Overall Mean (±95% CI) (µg/L)			Reference Range/Maternal Serum Concentration Comparison (µg/L)	Literature Cautions and Findings	Comments and Recommendation
	SK	Phase One (AB)	Phase Two (AB)			
Cadmium	< 0.05	NA	NA	< 0.03 – 0.04 [9] 0.013 – 0.074 [4] 0.01 – 0.05 [3] Maternal serum: 0.067 (mean) [6] <0.08 – 1.3 [8] 0.21 (mean) [12]	Rodushkin and Õdman (2001) detected cadmium in 0.05M nitric acid solution stored in SSTs [1], but the concentration is below the SK LOD.	The SK concentrations are below the detection level. **We recommend the caveat that the serum used for this testing was collected in SSTs, which are not recommended by clinical reference laboratories for cadmium analysis and it is unknown how the SSTs may contribute to or reduce the serum cadmium concentration.**
Cesium	0.85 (±1.0)	0.48 (±0.01)	0.54 (±0.49)	0.45 - 0.82 [4] Maternal serum: 0.3-1.8 (range) [8] 0.40 (mean) [12]	Rodushkin and Ödman (2001) detected cesium in 0.05M nitric acid solution stored in SSTs [1], but the concentration is below the the SK LOD.	SK mean is slightly higher than the reference range, within the maternal serum range, and the SK and AB Phase One means are higher than the maternal serum mean from another study. **We recommend cesium data be reported with the caveat for SK and AB Phase One: the serum used for this testing was collected in SSTs, which are not recommended by clinical reference laboratories for cesium analysis. It is unknown how the SSTs may contribute to or reduce the serum cesium concentration.**
Lead	0.48 (±0.10)	<LOD	Not reported (NR) due to background levels in QCs	0.014 – 0.25 [3] 0.12 – 0.51 [4] Maternal serum: 0.94 (median) [6] <0.12 – 2.1 [8]	Rodushkin and Ödman (2001) detected lead that had leached from SSTs into a 0.05M nitric acid solution [1].	The SK mean is higher than one general reference range and at the high end of the second general range, but similar or lower than concentrations in maternal serum. **We recommend lead data be reported with the caveat for SK: the serum used for this testing was collected in SSTs, which are not recommended by clinical reference laboratories for lead analysis as background contamination is possible.**
Methyl mercury	< 0.05 – 0.3	0.04 – 0.2				Many studies determine methyl-mercury by analysis of total mercury or may determine it by subtracting inorganic mercury from total mercury. **We recommend methyl-mercury data be reported with the caveat that the serum used for this testing was collected in SSTs, which are not recommended by clinical reference laboratories. It is unknown how the SSTs may contribute to or reduce the serum mercury concentration.**
Mercury	0.38 (±0.14)	0.25 (±0.01)	0.29 (±0.06)	0.09 - 1.01 [9] 0.21 – 1.3 [4] Maternal serum: 0.01 – 1.65 [13] (SSTs) 0.46 (median) [6]	Rodushkin and Ödman (2001) detected mercury in 0.05M nitric acid solution stored in SSTs [1], but the concentration is below the Phase One and SK LOD.	SK and AB Phase One means are within the reference ranges and lower than the median maternal concentration in Liang *et al.* (2019) [6]. **We recommend mercury data be reported with the caveat for SK and AB Phase One?: the serum used for this testing was collected in SSTs, which are not recommended by clinical reference laboratories for mercury analysis. It is unknown**

Element	Overall Mean (±95% CI) (µg/L)			Reference Range/Maternal Serum Concentration Comparison (µg/L)	Literature Cautions and Findings	Comments and Recommendation
	SK	Phase One (AB)	Phase Two (AB)			
						how the SSTs may contribute to or reduce the serum mercury concentration.
Silver	0.22 (±0.03)	0.27 (±0.01)	0.12 (±0.02)	<0.06 – 0.80 [9] 0.062 - 0.24 [4] 0.11 – 0.17 [4]	Rodushkin and Ödman (2001) noted silver was not detectable in leachate from SSTs [1].	SK mean is within two reference ranges and higher than a third rangewhile theAB Phase One mean is within one reference range and higher than two others. **We recommend silver data be reported with the caveat for SK: the serum used for this testing was collected in SSTs, which are not recommended by clinical reference laboratories for silver analysis. It is unknown how the SSTs may contribute to or reduce the serum silver concentration. The AB Phase One data was recommended to be reported with the caveat that the serum used for this testing was collected in SSTs, which are not recommended by clinical reference laboratories for silver analysis as background contamination is possible.**
Strontium	27 (±6)	NA	NA	19-96 [14] 18-75 [3] Maternal serum: 11.7 - 49.4 [8] 0.3	Rodushkin and Ödman (2001) noted leaching of strontium from SSTs into a 0.05M nitric acid solution [1].	**We recommend strontium data be reported with the caveat for SK: the serum used for this testing was collected in SSTs. It is unknown how the SSTs may contribute to or reduce the serum strontium concentration.**
Uranium	< 0.05	NA	NA	<0.002 – 0.006 [9] 0.004 – 0.011 [3] 0.0005 – 0.019 [4] 0.014 – 0.015 [4]	Rodushin and Ödman (2001) noted leaching of uranium from SSTs into a 0.05M nitric acid solution [1], but the concentration is below the SK LOD.	The SK results were all below the level of detection. **We recommend uranium data be reported with the caveat that serum used for this testing was collected in SSTs which are not recommended by clinical reference laboratories and it is unknown how the SSTs may contribute to or reduce the serum uranium concentration.**
Boron	17 (±3)	22 (±1)	31 (±1)	<100 [15] 19 - 79 [3] 7 - 19 [4] Range for maternal serum: 8.4 – 25.4 [16]	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 [15]. Rodushkin and Ödman (2001) noted significant leaching of boron from SSTs into a 0.05M nitric acid solution [1].	The SK mean is within all three general reference ranges and the maternal serum range while the AB Phase One data mean is within two of the general reference ranges and the maternal serum concentration range. **We recommend boron data be reported with the caveat for SK and AB Phase One: the serum used for this testing was collected in SSTs, which are not recommended by clinical reference laboratories for boron analysis as environmental contamination is highly likely.**
Cobalt	0.45 (±0.03)	0.33 (±0.05)	<LOD	0 – 0.9 [17] 0.3 – 1.02 [3] 0.24 – 0.59 [9] 0.03 – 0.18 [4] ≤1 [18]	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	SK and AB Phase One means are within four general reference ranges and the maternal serum range; elevated above one general reference range and the median in Liang *et al.* (2019) [6].

Element	Overall Mean (±95% CI) (µg/L)			Reference Range/Maternal Serum Concentration Comparison (µg/L)	Literature Cautions and Findings	Comments and Recommendation
	SK	Phase One (AB)	Phase Two (AB)			
				Maternal serum: 0.17 (median) [6] 0.5 – 4.0 [8]	taken, elevated serum cobalt results may be an incidental and misleading finding." [17] Rodushkin and Ödman (2001) detected cobalt in 0.05M nitric acid solution stored in SSTs [1], but the concentration is below the Phase One LOD.	**We recommend cobalt data be reported with the caveat for SK and AB Phase One: the serum used for this testing was collected in SSTs, which are not recommended by clinical reference laboratories for cobalt analysis as contamination is possible.**
Copper	1960 (±116)	1851 (±18)	1686 (±1110)	400 – 1800 [19] 794 – 2023 [3] 627 – 1659 [9] 740 – 1300 [4] 800 – 1550 [20] Maternal serum: 1530 (median) [6] 1610 (mean) [12] 400-2950 [8]	Serum copper is known to increase during pregnancy [21]. Rodushkin and Ödman (2001) detected leaching of copper from SSTs into a 0.05M nitric acid solution [1], but the level was significantly lower (>1000x lower) than the detected levels in Alberta and Saskatchewan.	SK mean is higher than or at the high end of the general reference ranges, higher than the two central measures of maternal serum copper concentration, and within the maternal serum range provided. There is no significant difference with the AB Phase One data. **We recommend copper data be reported with the caveat for SK and AB Phase On: the serum used for this testing was collected in SSTs, which are not recommended by clinical reference laboratories for copper analysis. It is unknown how the SSTs may contribute to or reduce the serum copper concentration.**
Iron	1070 (±82)	1241 (±25)	1292 (±15)	350 – 1500 [22] 550 – 1200 [4] 300 – 1600 [23] 280 – 1620 [24] Maternal serum: 1530 (median) [6] 871 (mean) [12]		SK and AB Phase One means are within three general reference ranges; higher than the maternal serum mean, but lower than the median from another maternal study. **We recommend iron data be reported with the caveat for SK and AB Phase One: the serum used for this testing was collected in SSTs, and it is unknown how the SSTs may contribute to or reduce the serum iron concentration.**
Magnesium	18600 (±685)	NA	NA	17000-23000 [25] Maternal serum: 20430 (mean) [6] 13200 - 22900 [8]		**We recommend magnesium data be reported with the caveat for SK: the serum used for this testing was collected in SSTs. It is unknown how the SSTs may contribute to or reduce the serum magnesium concentration.**
Manganese	3.5 (±0.4)	2.9 (±0.3)	1.4 (±0.3) (not included in Phase Two report)	0.6 – 2.3 [26] 0.63 – 2.26 [3] 0.35 – 1.08 [9] 0.3 – 1.04 [4] 0 – 2.0 [27] Maternal serum: 2.76 (median) [6] 2.37 (mean) [12] 0.6 – 7.2 [8]	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 [26]. Rodushkin and Ödman (2001) detected leaching of manganese	SK and AB Phase One means are higher than all five general reference ranges and the central measures in maternal serum. **We recommend manganese data be reported with the caveat for SK and AB Phase One: the serum used for this testing was collected in SSTs, which are not recommended by clinical reference laboratories for manganese analysis as environmental contamination is highly likely.**

Element	Overall Mean (±95% CI) (µg/L)			Reference Range/Maternal Serum Concentration Comparison (µg/L)	Literature Cautions and Findings	Comments and Recommendation
	SK	Phase One (AB)	Phase Two (AB)			
					from SSTs into a 0.05M nitric acid solution [1].	
Molybdenum	1.2 (±0.1)	1.4 (±0.1)	3.2 (±0.3)	0.3 – 2 [28] 0.67 – 1.68 [3] 0.36 – 1.15 [9] 0.27 – 0.85 [4] Maternal serum: 0.94 (median) [6] 0.3 -1.4 [8]	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 [28]. Rodushkin and Ödman (2001) detected molybdenum in 0.05M nitric acid solution stored in SSTs [1], but the concentration is below the Phase One LOD.	SK and AB Phase One means ares within two reference ranges and higher than two other reference ranges; within the range of the maternal serum concentrations and similar to the maternal serum median. **We recommend molybdenum data be reported with the caveat for SK (AB): the serum used for this testing was collected in SSTs, which are not recommended by clinical reference laboratories for molybdenum analysis. It is unknown how the SSTs may contribute to or reduce the serum molybdenum concentration.**
Nickel	0.76 (±0.57)	0.88 (±0.08)	1.2 (±0.1)	<2 [29] 0.04 – 5.31 [3] 0.44 – 1.26 [9] 0.13 - 0.55 [4] ≤10 [30] Maternal serum: 0.72 (median) [6]	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 [29]. Rodushkin and Ödman (2001) detected nickel in a 0.05M nitric acid solution stored in SSTs [1].	SK and AB Phase One means are within four reference ranges and higher than the fifth; similar to the maternal serum median. **We recommend nickel data be reported with the caveat for SK and AB Phase One: the serum used for this testing was collected in SSTs, which are not recommended by clinical reference laboratories for nickel analysis as contamination is highly likely.**
Selenium	118 (±5)	154 (±3)	117 (±3)	45 – 150 [31] 79 – 141 [3] 73 – 110 [9] 74 – 90 [4] 23 – 190 [32] Maternal serum: 72.83 (median) [6] 78.96 (mean) [12]	Mayo Clinic website states: Selenium is quite volatile; therefore, careful specimen collection is necessary to ensure accurate results [31].	SK mean within three ranges and higher than two ranges; higher than both maternal serum concentrations while the AB Phase One mean is higher than all reference ranges and maternal serum median/means. **We recommend selenium data be reported with the caveat for SK: the serum used for this testing was collected in SSTs, which are not recommended by clinical reference laboratories for selenium analysis as contamination is possible. The AB Phase One data was recommended to be reported with the caveat that the serum used for this testing was collected in SSTs, which are not recommended by clinical reference laboratories for silver analysis as background contamination is highly likely.**
Zinc	1410 (±66)	1391 (±14)	839 (±17)	600 – 1200 [33] 551 – 925 [3] 510 – 809 [9] 420 – 710 [4] 600 – 1200 [34] Maternal serum: 1020 (median) [6] 256 (mean) [12]	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) [33].	SK and AB Phase One means are above all reference ranges and maternal serum concentrations and ranges. **We recommend zinc data be reported with the caveat for SK and AB Phase One: the serum used for this testing was collected in SSTs,**

Element	Overall Mean (±95% CI) (µg/L)			Reference Range/Maternal Serum Concentration Comparison (µg/L)	Literature Cautions and Findings	Comments and Recommendation
	SK	Phase One (AB)	Phase Two (AB)			
				440 – 1290 [8]	Rodushkin and Ödman (2001) detected zinc in a 0.05M nitric acid solution stored in SSTs, but the concentration was below the Phase One LOD [1].	**which are not recommended by clinical reference laboratories for zinc analysis as contamination is possible.**

Appendix B: Rationale for phthalate metabolite recommendations for Northern Saskatchewan Prenatal Biomonitoring Study Technical Report, Technical Summary Report and General Summary

Metabolite	Overall Mean (ng/mL)			Background Levels in Bovine Serum (ng/mL)	Maternal Serum Concentrations (µg/L) in the Literature	Literature Findings	Comments and Recommendation
	SK	Phase Three (mat)	Phase Three (cord)				
MEHP (mono – ethylhexyl-phthalate)	Not reported due to contamination concerns	4500	4460	Max of 10 blanks: 41.2 Max in collection and/or storage devices: 123	<0.98 – 10.3 [35]1.14 (median) [36] 3.07 (median) [37] 3.73 (median) [38] 3.69 (median) [38] 3.59 (median) [38] <0.0253 – 21.2 [39]	DEHP is very commonly used as a plasticizer in medical devices [40][41][42][43][44].	**Based on the concentrations being detected in blank bovine serum and bovine serum passed through or stored in collection materials, it was decided to not report the MEHP data for the Saskatchewan report.**
MBzP (mono-benzyl-phthate)	1.48	1.80	1.32	Max of 10 blanks: Not detected Max in collection and/or storage devices: Not detected	<1.0 - 3.7 [35] <LOD (median) (LOD=0.40) [36] <LOD (median) (LOD=0.57) [37] <LOD (median) (LOD=0.26) [38] <LOD (range) (LOD=0.372) [39]	DBzP, the parent diester of MBzP, is less commonly used in various applications than other phthalates [45]; therefore, its presence in the environment is less common. Henricksen *et al*. (2020) suggest serum be used for phthalate exposure measurements in historical cohorts before regulations were put in place and where urine samples are not available [38]. There is potential to use the phthalate serum measurements from historical cohorts to assess potential long-term harmful effects of prenatal exposure. According to Henricksen *et al.* (2020), if associations are found, they are	**As MBzP was not detected in bovine serum blanks or bovine serum stored in or passed through sample collection devices, DBzP is a less commonly used in medical devices than other phthalate, MBzP can be reported with no further caveat.**

Metabolite	Overall Mean (ng/mL)			Background Levels in Bovine Serum (ng/mL)	Maternal Serum Concentrations (µg/L) in the Literature	Literature Findings	Comments and Recommendation
	SK	Phase Three (mat)	Phase Three (cord)				
						likely true and deserve further corroboration.	
MEP (mono-ethyl-phthalate)	4.4	2.63	2.05	Max of 10 blanks: Not detected Max in collection and/or storage devices: Not detected	<1.0 – 89.3 [35] 1.56 (median) [36] 3.11 (median) [37] 2.91 (median) [38] 2.37 (median) [38] <0.07 -3.73 [39]	DEP has been detected in medical devices, but to a lesser extent than DEHP [41][42].	**As MEP was not detected in any bovine blank serum or bovine serum stored in or passed through sample collection devices, MEP can be reported with no further caveat.**
MiBP (monoiso-butyl-phthalate)	14.2	41.9	48.4	Max of 10 blanks: 0.69 Max in collection and/or storage devices: 1.42	<1.04 – 7.6 [35] 3.78 (median) [36] 1.27 (median) [37] 1.19 (median) [38] 2.12 (median) [38] <0.384 – 75.5 [39]	DiBP has been detected in medical devices, but to a lesser extent than DEHP [42]. DiBP is used in a large number of products and is ubiquitous in the environment, leading to a higher contamination risk than some other phthalates [45].	**As MiBP was detected in both blank bovine serum and bovine serum stored in or passed through collection devices, it is recommended to interpret the MiBP data with caution.**

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Reference Notes on Collection Tubes

Mayo Clinic and ARUP references: do not mention type of tube used, but their trace metals collection protocol states that trace metal specimen vials should be used.
J.-P. Goullé *et al*: heparanized Vacutainer tubes specifically for trace elements
ALS reference (7 within this reference): collection materials were acid-washed
C. Liang *et al*: metal-free polypropylene tubes
M. Filella *et al*: reference range of <1 □g/L based on review of results over time
M. Krachler and E. Rossipal: "uncontaminated polyethylene tubes"
A. Cesbron *et al*: BD vautainer tubes with EDTA specifically for trace element testing
Osada *et al*: polyethylene terephthalate tubes that were checked for backround trace metal contamination
Yau *et al*: SSTs
Caglar *et al*: non-heparinized tubes
Clinical Laboratory reference values (Royal College of Physicians & Surgeons of Canada): do not mention type of tube used