



## Short Communication

# Meconium identifies high levels of metals in newborns from a mining community in the U.S.



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## HIGHLIGHTS

- We collected meconium specimens from a small sample of newborns in Columbia South Carolina and in Butte Montana. The concentrations found in Columbia were in the low  $\mu\text{g kg}^{-1}$  range (or less), and were similar to the low levels that have been identified in other studies of meconium.
- The magnitude of the differences in concentrations found in Butte compared to Columbia was 1650-1900 times higher for Cu, Mn, and Zn.
- Using meconium to measure metal exposure of newborns has implications for risk assessment in a mining-exposed population.
- This approach was inexpensive and mothers we positively disposed to consent to collection of diaper excrement.

## GRAPHICAL ABSTRACT



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## ABSTRACT

**Background:** This pilot study was conducted to determine if we could identify intrauterine exposure to metals in meconium, as a measure of exposure for mother-child pairs living in proximity to a mining operation.

**Objectives:** We used meconium as a means to measure metal exposure in utero. We set out to quantify the exposure to selected metals that are currently being mined and also are found in the Superfund site in Butte, Montana, and to compare it to that of Columbia, South Carolina, US, where mining is not occurring.

**Methods:** This cross-sectional study was conducted between May and November 2018. We received Institutional Review Board approval and we consented women following the birth of their newborns, and collected meconium within 24 h of birth, without any identifiers. Each laboratory used the same protocol for collection, transport, and storage; and the same laboratory protocol was used for the analysis of all samples. Samples were digested using standard acid/peroxide digestion methods and measured by inductively coupled plasma mass spectroscopy.

**Results:** We collected meconium specimens from 17 infants in Columbia, South Carolina and 15 infants in Butte, Montana. The concentrations found in Columbia were in the low  $\mu\text{g kg}^{-1}$  range (or less) and were similar to the low levels that have been identified in other studies of meconium. The magnitude of the

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differences in concentrations found in Butte compared to Columbia was 1792 times higher for Cu, 1650 times higher for Mn, and 1883 times higher for Zn.

**Conclusion:** Using meconium to measure exposure of newborns has implications for risk assessment in a mining-exposed population. This approach was inexpensive and thorough. The magnitude of the differences in the metal levels identified from the two study sites suggests there is an urgent need for further research to learn if there are health consequences to these highly exposed infants.

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## 1. Introduction

Exposure to lead (Pb), manganese (Mn), arsenic (As), mercury (Hg) and other metals through inhalation, dermal absorption, and ingestion of contaminated foods, soils, and dust significantly increases the risk for neurodevelopmental disabilities in children exposed in utero or during early childhood (Aschner and Costa, 2011–2018; McDermott et al., 2014; Al-Saleh et al., 2014; McDermott et al., 2011; Liu et al., 2010; Claus Henn et al., 2017; Rodríguez-Barranco et al., 2013; Ciesielski et al., 2012). Superfund sites are designated by the US Environmental Protection Agency (EPA) as contaminated waste sites that require cleanup because they present risks to human health or to the health of the environment. About 15 million people in the U.S., including about 3.5 million children, live within one mile of a federal Superfund site (US Environmental Protection Agency, n.d.-a). One of the largest Superfund sites in the U.S. is located in and around Montana's fifth largest city, Butte. This Superfund site designation followed 150 years of mining and smelting in a two-county area. Currently, Butte has a large active open pit mine for copper [Cu] and molybdenum [Mo], which has been operated by Montana Resources/Atlantic Richfield from 1986 to the present (The City-County of Butte Silver Bow, n.d.).

Mining for copper in Butte has been ongoing since the late-19th century (The City-County of Butte Silver Bow, n.d.), and large quantities of waste from mining, milling, and smelting have been directly deposited into air, water, and soils (US Environmental Protection Agency, n.d.-b). The EPA designated the Butte Area Superfund Site in 1983, and listed arsenic (As), cadmium (Cd), lead (Pb), and mercury (Hg) as metal contaminants of concern for residents living near it (US Environmental Protection Agency, n.d.-b). The only recent human monitoring by the EPA has been of school children, and the only metals that have been tested were Pb in blood (ATSDR Press Release About Exposure Investigation in Anaconda, MT, n.d.), and to a much lesser extent, As in urine (The Butte-Silver Bow County Environmental Health Lead Study, Final Report, 1992).

For the analysis of prenatal exposure of infants to metals, meconium provides an ideal matrix, since collection is non-invasive and easily achieved. Meconium is the first stool passed by a newborn, usually during the first 24 h of life. Meconium contains elements that were acquired by the fetus from the twelfth week of gestation and accumulating throughout the remainder of the pregnancy. During gestation, xenobiotics, including metals, are principally deposited in meconium either directly from bile secretion or from the fetus swallowing amniotic fluid containing toxicants (Ortega García et al., 2006). There is substantial literature about fetal exposure to metals, using a variety of biologic samples (Basu et al., 2014; Cooke, 2014; Ozel et al., 2019; Arbuckle et al., 2016; Cerrillos et al., 2019; Manduca et al., 2019). A study comparing the effectiveness of maternal blood, maternal hair, infant hair, cord blood, and meconium to detect exposure to various toxins, found a higher percentage of exposure by meconium analysis (Ostrea et al., 2008). Thus, the purpose of this pilot study was to use meconium to quantify the prenatal metal exposure of newborns to learn if differences were present between those born near the mining- and Superfund-impacted site and the non-impacted site, and also to compare the findings to babies from other locations, as reported in the literature.

## 2. Methods

Our cross-sectional pilot study was performed in Butte, MT, and Columbia, SC. Columbia was chosen for comparison, since it is an area that has not been exposed to metal mining. We collected a convenience sample of meconium from 17 infants in Columbia, South Carolina and 15 infants in Butte, Montana, during the first 24 h of life, while the babies were still in the postpartum unit of two hospitals. We only included full term infants, with uncomplicated births. No individual identifiers were collected, and the study was deemed exempt by the Columbia hospital's Institutional Review Board (IRB) and expedited by the Butte hospital's IRB. The IRB approval was contingent upon limiting data collection to the meconium samples without linkage to child or maternal characteristics. The mothers verbally consented to the collection of the meconium with the understanding that the study was designed to determine if metals could be identified in the first bowel movement of newborns.

We collected meconium specimens from 17 infants in Columbia and 15 infants in Butte, using the same protocol for collection, transport, and storage. The study protocol required recruitment of women and their newborns who were receiving obstetric and pediatric care at the designated hospitals. An authorized recruiter, under the supervision of the Study Physician at each site, spoke to the mothers in the postnatal unit. The Recruiter described the details of the study, answered questions, and obtained verbal assent to collect their newborn's meconium in a specially prepared, metal free, diaper insert. Women and their newborns were excluded if they did not speak English, if the meconium had already been passed at the time of the Recruiter visit, or if the baby required care in the Neonatal Intensive Care Unit (NICU). The Recruiter was called to the mother's room or the newborn nursery by an attending nurse or physician, as soon as the meconium was collected. The Recruiter retrieved it, and labeled it with a study number (e.g., Sample 1, Sample 2). The Recruiter placed all samples in a cooler under her control transferred all samples to the designated laboratory. At each site's laboratory, a technician processed, stored, and analyzed the samples for metals.

All meconium samples collected in Butte, MT were digested and analyzed in chemistry and analytical laboratories in MT. All samples collected in Columbia, SC were digested and analyzed in Columbia laboratories. While digestion and analytical protocols were the same at each site, there are slight differences in limits of detection between the two sets of analyses. Limits of detection for the Columbia site samples were As = 1.4, Cu = 0.5, Mn = 0.5, Mo = 0.7, Pb = 0.6, Zn = 1.8, while they were As = 5.0, Cu = 5.0, Mn = 5.0, Mo = 0.1, Pb = 0.1, Zn = 5.0 for the Butte site (units of  $\mu\text{g kg}^{-1}$ ). A Perkin Elmer Nexion 350 ICP-MS was used in Columbia and a Perkin-Elmer ELAN DRC II ICP-MS was used in Butte. It is possible that other slight differences lead to different limits of detection for the elements analyzed. These different limits of detection are noted in Table 1 of the results.

Total digestion of the samples was effected by following Tekran application note N2600–10. Briefly, one gram of meconium was placed in a Teflon digester tube. Three milliliters of concentrated nitric acid and 1.5 mL of ultra-pure water was placed in the tube with the meconium, and the samples were heated at 85 °C for 4 h. At the end of the first heating, samples were removed from the heat block, cooled to room

**Table 1**Meconium Metal Detection (Units:  $\mu\text{g kg}^{-1}$ ) in Newborns in Butte Montana (MT) and Columbia South Carolina (SC).

	Butte, MT N = 15	Columbia, SC N = 17	Wilcoxon rank sum test p-Value	t-Test p-Value
	Units: $\mu\text{g kg}^{-1}$ Median (minimum, maximum) Mean (standard deviation)			
<b>As</b>	Median 32 Min 16, Max 49 Mean 35 Std Dev. 10	<LoD <LoD	<0.0001	<0.0001
<b>Cu</b>	Median 26,311 Min 11,006, Max 47,270 Mean 28,134 Std Dev. 10,411	Median 14.68 Min 2.40, Max 27.42 Mean 14.75 Std Dev. 7.68	<0.0001	<0.0001
<b>Mn</b>	Median 5364 Min 388, Max 18,120 Mean 6807 Std Dev. 5726	Median 3.25 Min 0.20, Max 12.83 Mean 4.67 Std Dev. 4.48	<0.0001	<0.0001
<b>Mo</b>	Median 59 Min 24, Max 105 Mean 64 Std Dev. 22	<LoD <LoD	<0.0018	<0.0018
<b>Pb</b>	Median # Mean 5 Std Dev. 5	<LoD <LoD	<0.0001	<0.0001
<b>Zn</b>	Median 81,642 Min 22,120, Max 312,695 Mean 109,154 Std Dev. 82,772	Median 43.34 Min 12.17, Max 117.25 Mean 53.74 Std Dev. 36.16	<0.0001	<0.0001

Limits of Detection (LoD) for MT samples:

As = 5.0, Cu = 5.0, Mn = 5.0, Mo = 0.1, Pb = 0.1, Zn = 5.0

Limits of Detection (LoD) for SC samples:

As = 1.4, Cu = 0.5, Mn = 0.5, Mo = 0.7, Pb = 0.6, Zn = 1.8

# only one sample was above the limit of detection

Limits of Detection (LoD) for MT samples: As= 5.0, Cu= 5.0, Mn= 5.0, Mo= 0.1, Pb= 0.1, Zn=5.0.

Limits of Detection (LoD) for SC samples: As= 1.4, Cu= 0.5, Mn= 0.5, Mo= 0.7, Pb= 0.6, Zn= 1.8.

# Only one sample was above the limit of detection.

temperature, and 1.0 mL of 30%  $\text{H}_2\text{O}_2$  was added to each. Samples were then returned to the heat block at 70 °C for 30 min. At the end of the second incubation, samples were removed from the heat blocks and cooled to room temperature. Samples were diluted to 50 mL with ultra-pure  $\text{H}_2\text{O}$ , and then filtered and analyzed by ICP-MS for the six metals of interest. Sorptive losses and contamination were minimized and quantified. The technique and preparation methods provided low detection limits, a large linear range, and high precision that allowed us to measure low concentrations accurately.

This data was generated from a preliminary, proof of concept study, to determine if a) metals were detectable in the meconium of babies in both Butte, MT, and Columbia, SC, using the protocols developed, and if b) concentrations of total metals found in babies residing near the Butte Area Superfund Site were greater than or similar to those of babies residing in or near Columbia, SC (non-Superfund area). Samples at both locations were analyzed using appropriate and nearly identical techniques and QA/QC procedures, which we have previously published (Hong et al., 2016; Hailer et al., 2017). Logistical constraints for this pilot study meant that samples were not shared between sites and measured in duplicate.

A *t*-test was used to determine if there was a significant difference between the means of the two groups. The Wilcoxon signed-rank test is a non-parametric statistical test that was used to compare the two samples to assess whether their mean ranks differed (i.e. it is a paired difference test).

### 3. Results

The high concentrations of metals, shown in Table 1, for the 15 newborns in Butte are cause for immediate concern. The magnitude of the differences in concentrations in Butte compared to Columbia is 1792-fold higher for Cu, 1650-fold higher for Mn and 1883-fold higher for Zn. Both the *t*-test and the Wilcoxon rank sum test showed that the differences between the values from Butte and Columbia were statistically significantly different ( $p \leq 0.0001$ ). The measurement of As was not sensitive enough to detect the low actual values in the meconium collected in Columbia. The analyses showed that the values in Columbia were no higher than 1.4  $\mu\text{g kg}^{-1}$ , which was the minimum limit of detection, but the values could have been substantially lower, i.e. 0.01–005  $\mu\text{g kg}^{-1}$ .

### 4. Discussion

The relatively low concentrations in the 17 newborns from Columbia are similar to levels reported in other studies of meconium and in the low  $\mu\text{g kg}^{-1}$  range (or less) (Arbuckle et al., 2016; Ettinger et al., 2017; Aziz et al., 2017; Turker et al., 2013). It is noteworthy that the primary metal being monitored by the EPA in Butte, MT on an ongoing basis is blood Pb levels. Our meconium results showed that Pb was below the limit of detection for all the infants in Columbia and Butte, with the exception of one infant from Butte (the single specimen with a value above the limit of detection was 5.0  $\mu\text{g kg}^{-1}$ ). The other metal

that the EPA has episodically monitored is urinary As levels (*The Butte-Silver Bow County Environmental Health Lead Study, Final Report, 1992*), and in that case the Butte median was 23-fold higher, when compared to Columbia.

Heavy metal exposure can damage human health in numerous ways, giving rise to potential hazards and risks. As and Pb induce oxidative cell stress (Karri et al., 2016); Arsenic can injure DNA through induction of apoptosis of cerebral neurons by activating protein kinase and altering glucose metabolism (Arbuckle et al., 2016; Karri et al., 2016; Lucchini et al., 2017); and Pb can alter calcium metabolism (Karri et al., 2016; Lucchini et al., 2017). Cu, Zn, and Mn have also been widely distributed throughout Butte, and these metals have been implicated in the literature as potential neurotoxins, especially when exposure is chronic (Turker et al., 2013; Karri et al., 2016; Lucchini et al., 2017). A study from Turkey compared meconium levels of Pb, Cd, Zn, Fe and Cu in preterm surviving and non-surviving fetuses/stillbirths, and reported statistically significant higher metal levels in the non-survivors (Turker et al., 2013). The concentrations in the live births of that study were comparable to the Columbia data and the stillbirths had levels of metals that were actually lower than the measurements made in Butte. Clearly, this preliminary study warrants further work in integrating data on environmental exposure, other exposures and potential hazards and mechanisms of action, to: i) understand the processes causing potential risk, and ii) inform a full risk assessment (RA). Fuller risk assessment studies are again self-evidently required.

## 5. Conclusion

We identified an approach that provided straightforward evidence of elevated exposure to metals in a mining-exposed population. The approach was inexpensive, thorough, and required no advanced statistical analysis. We believe that there is an urgent need for further research to understand the mechanisms and the human consequences of this potential public health emergency.

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## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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