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# Toxic Metal Contamination of Banked Blood Designated for Neonatal Transfusion

**Sripriya Sundararajan[1], Allison M Blatz[2], Dorr G Dearborn[2,3,4], Arthur W Varnes[3], Cynthia F Bearer[1], and Dina El Metwally[1*]**

[1]*Department of Pediatrics, University of Maryland, Baltimore, MD 21201, USA*

[2]*Departments of Pediatrics, Case Western Reserve University, Cleveland, OH 44106, USA*

[3]*Environmental Health Sciences, Case Western Reserve University, Cleveland, OH 44106, USA*

[4]*Mary Ann Swetland Center for Environmental Health, Case Western Reserve University, Cleveland, OH 44106, USA*

[*]**Corresponding author:** Dina El Metwally, Division of Neonatology, Department of Pediatrics, University of Maryland School of Medicine, 110 S Paca St. 8th floor, Baltimore, MD 21201, Tel: 410-328-6003; Fax: 410-328-1076; E-mail: dmetwally@peds.umaryland.edu

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## Abstract

**Objective:** Very low birth weight (VLBW) infants frequently receive blood transfusions. We hypothesize that toxic metals in donor blood may pose a health risk with potential adverse neurologic effects on the developing brain of a vulnerable VLBW infant.

**Study design:** Samples from 100 donor blood units were collected from a large urban hospital. Blood was analyzed for aluminum, arsenic, beryllium, cadmium, manganese, mercury, nickel, lead and polonium. The estimated upper limit of acceptable metal concentration in donor blood was calculated assuming a transfusion volume of 20 ml/kg and using either previously published acceptable intravenous doses or oral reference doses with a conservative estimate of 10% gastrointestinal absorption. Ingested mercury was assumed to be 95% absorbed.

**Results:** Eight of the nine metals were detectable. Concentrations of arsenic, beryllium, cadmium, mercury and polonium were not of concern for any single blood transfusion. Concentrations of aluminum, manganese, nickel and lead exceeded the estimated upper limit of acceptable concentration in 5, 11, 4 and 26 units respectively. Of the 100 units, 31 had at least one toxic metal concentration high enough to pose a potential health risk.

**Conclusions:** VLBW infants are exposed to heavy metals that are toxic from blood transfusion. The number of units with concerning levels of toxic metals was higher than expected. Neonatologists should be aware of this potential exposure to toxic metals from donor blood when decision is made to administer blood transfusion. Neurodevelopmental studies of toxic metal exposed infants from blood transfusion are warranted.

**Keywords** Blood bank; Blood transfusion; VLBW; Heavy metal contamination

## Abbreviations

VLBWs: Very Low Birth Weight Infants; ELBW: Extremely Low Birth Weight Infants; CDC: Centers for Disease Control and Prevention; ETC: Estimated Tolerable Concentration; PRBCs: Packed Red Blood Cells

## Introduction

Blood transfusion is a critical part of neonatal intensive care and is life saving for neonates with severe anemia or hemorrhage [1]. During the first 2 weeks of life, approximately 50% of infants weighing less than 1000g at birth will receive their first transfusion [2] and without the use of erythropoiesis-stimulating agents, 85% to 90% of VLBW infants receive blood transfusions [3,4]. All blood products administered to VLBWs is subject to universal screening for infectious agents as directed by the Food and Drug Administration [5]. Additional procedures of blood processing may be required by the individual institutions, such as irradiation [6] and leukoreduction [7] before transfusing blood to neonates. VLBW infants are transfused with blood that has been donated by adults 17 years of age or older. These donors may have had a variety of exposures to substances including toxic heavy metals from environmental and occupational sources [8]. VLBWs are especially vulnerable to these toxic metal exposures [9-14]. The federal government has codified exposure levels acceptable to adult workers and to children exposed to environmental contaminants. These exposures occur mainly via ingestion or inhalation.

In contrast, toxic heavy metals present in transfused blood are administered intravenously. Safe levels for intravenous administration of most of these toxic metals are unknown. Lead in donor blood has previously been shown to be present at concentrations that pose a health risk for VLBW infants [12,15]. Aluminum (Al), arsenic (As), beryllium (Be), cadmium (Cd), mercury (Hg), Lead (Pb), Manganese (Mn) and Nickel (Ni) have been studied extensively due to the known serious adverse health effects associated with human exposure to these metals. According to the Agency for Toxic Substance and Disease Registry's (ATSDR) priority list of hazardous substances, the latent effects from these heavy metals include carcinogenesis, neurotoxicology and developmental deficits in humans and animals ([16-18]. Toxic metals tend to stay in the body for long periods of time

and may cause detrimental adverse effects on human growth and development [19,20]. Therefore, it is an urgent and high priority to explore and determine the burden of exposure of toxic metals from human blood to the developing VLBW infants. We hypothesized that `metals, known to have especially detrimental neurotoxic health effects, could be present in donor blood used for VLBW infants at potentially toxic concentrations. The above metals were prioritized based on carcinogenicity, neurotoxicity, teratogenicity, and prevalence in the greater Cleveland area as this was the source of the donor blood samples, and published literature suggest increased susceptibility of adverse neurological effects in children [9,12,15,21-51]. Toxic metals meeting one or more of these criteria were selected to be the focus of this study. They were Al, As, Be, Cd, Pb, Mn, Hg, Ni, and polonium (Po).

## Methods

### Blood collection

Donor blood units were selected from a convenience sample obtained from University Hospitals of Cleveland blood bank over a period of 2 weeks. Upon review by the Institutional Review Board (IRB), the study was deemed not human subjects research and thus was exempt from IRB review. Donor blood was collected by the blood bank, mixed with citrate phosphate dextrose (standard anti-coagulant) solution, and stored in standard blood bank bags which included a section of tubing divided into segments that could be reserved for any future tests. For this study, one segment was collected by cutting through the plastic connecting two of the segments. 2 mL of blood within the segment was transferred into blue-top, trace-mineral-free vacutainers (Vacutainer Brand, #369737) using Fenwal Hematype Segment Devices (Baxter, 4R5128). Blood was subsequently stored at -20°C until further analysis.

### Metal analysis

An inductively coupled plasma – mass spectroscopy method (Using Perkin-Elmer 9000 (ELAN DRC II, SCIEX, INC SHELTON, CT) was used to determine blood metal concentrations that had an ability to measure all 9 metals simultaneously. Donor blood samples that were to be analyzed were removed from the freezer and allowed to thaw at room temperature. After vigorous mixing, 500 uL of whole blood was withdrawn with an Eppendorf pipette and discarded to clean the tip. A second 500 uL aliquot was transferred to a clean, dry 23-mL Parr acid digestion bomb. The cleanliness of each Parr bomb was established before each set of sample analysis by adding 1000 uL of nitric acid (vide infra) to each bomb. If any analyte was found to be above its detection limit, the bomb was re-cleaned prior to being used to digest a blood sample.

Following addition of whole blood to the Parr bomb, 1000 uL of concentrated nitric acid (GFS Chemical double-distilled from Vycor) was then added. The bomb was then sealed and placed in an oven at 120oC for two hours. After digestion was complete, the bombs were cooled to room temperature and opened. The contents were quantitatively transferred to clean, dry 1-oz. polyethylene bottles. The sample was then diluted to 10 mL volume with deionized water and subsequently analyzed by inductively coupled plasma – mass spectroscopy (Perkin-Elmer 9000). All reagents including water and acids were high-purity grade.

To ensure quality and control of the heavy metal analysis, each batch of 10 blood samples from donors also contained 1 standard sample (NBS Standard Reference Material 1557a). For accuracy and precision, the standard sample was analyzed 10 times. The mean values and standard deviations for each metal (ug/L) in the standard sample are: Al, 2300 ±700; As, 57 ± 5; Be, 1.0 ± 0.5; Cd, 350 ± 30; Pb, 230 ± 70; Mn, 8300 ± 500; and Hg, 10 ± 7.

### Statistical analysis

Statistical analyses were performed using Excel Statistical software. Descriptive statistics was used to determine the median, range and mean of the detectable values of the various heavy metal concentrations (Al, As, Be, Cd, Pb, Mn, Hg, Ni and Po) from banked donor blood.

### Calculations of estimated tolerable acute intravenous dose

To estimate the safety of the concentrations of metals in the blood units, an estimated tolerable acute intravenous dose was calculated from available literature. If no specific literature was available pertaining to intravenous doses considered tolerable, the acute oral reference dose given by the ATSDR was used. If the acute oral reference dose was not given, the chronic oral reference dose was used. Oral reference doses were converted to intravenous doses based on a conservative assumption that only 10% of a gastrointestinal dose of heavy metal would be absorbed with the exception of Hg [52-54]. Thus, an "intravenous reference dose" would be $1/10^{th}$ of the oral reference dose. Using 20 mL/kg as the blood volume of a transfusion used in many neonatal intensive care units, the concentration of each metal in the donor blood that would yield a tolerable dose was calculated (estimated tolerable concentration (ETC), ng/mL (or µg/L)=(tolerable dose (µg/kg)/20 mL/kg).

Hg absorption was treated differently as it is known that Hg is highly absorbed and results from dietary intake of forms of organic Hg, particularly methylmercury (Centers for Disease Control and Prevention). For calculation of the ETC of Hg, the assumption was made that most Hg in blood is methylmercury, and the EPA's oral reference dose of 0.1 µg/kg/day (100 ng/kg/day) was used as the tolerable oral dose [55,56].

## Results

All units had detectable concentrations of at least one heavy metal. The descriptive statistics are given in Table 1. Po was the only metal not detected in any unit. Arsenic (As) was the only toxic metal to be detected in all 100 donor blood units. Pb was detected in 26 of the 100 units. The distributions of the concentrations of the individual metals are shown in the Figure. For 7 of the 9 metals, the majority of the units had metal concentrations below the limit of detection. In contrast, the majority of values for both As and Be were above the limit of detection.

Units with metal concentrations above the ETC are shown in Table 2. Al, Mn, Ni and Pb concentrations were high enough that in multiple units they were above their ETCs. Several units were greater than twice the ETC for Al, Ni and Pb. Thirty-one units had at least one metal above its ETC (Table 3), and 10 units had more than one metal above the ETC. Pb was present in 84% (26 units) of the 31 units with at least one metal above the ETC.

## Discussion

In this descriptive study, we show that whole blood concentration for toxic metals may exceed ETC. For one particular unit, the concentration of Al was 10 times the ETC. Our results fell within the ranges of blood metal concentrations reported by the NHANES for Pb and Cd in the 2009-2010 survey under laboratory data (1.8 to 435.2 µg/L and 0.14 to 8.67 µg/L for Pb and Cd respectively) [57]. The limit of detection (LOD) was different for each metal because multiple factors, such as the analytical sensitivity of a mass spectral line and inter-element and physical interferences are different for each metal [58]. For example, Be was detected at any concentration above 0.08 ng/ml, whereas Mn was detected at a concentration above 30 ng/ml. Thus, 97% of the samples had Be over the limit of detection, yet only 11% had Mn concentrations above the limit of detection. This difference in distribution does not necessarily represent a higher level of exposure to Be, but rather a limitation of the analytical method. Despite Po being established as a human carcinogen, environmental levels of Po are extremely low. It was reassuring to not detect Po in any of the donor blood samples, despite the fact that very low levels of Po are found naturally in the body particularly in first-hand smokers[42].

The results of this study may underestimate the dose of toxic heavy metal exposure to a VLBW infant because these infants 1) often receive multiple transfusions of packed red blood cells (pRBC) rather than whole blood as was used in this study, and 2) often receive multiple transfusions from the same donor. Because Pb binds exclusively to hemoglobin molecule in erythrocytes [59], a VLBW infant who might have been transfused with pRBC would receive nearly double the concentration of heavy metals initially present in the donor blood [15]. Several studies suggest that other metals also bind to hemoglobin [60-65]. The difference in metal concentrations between whole blood versus pRBCs may not be a trivial issue. In a recent study [66], 40 of 49 (82%) pRBC units had detectable Hg, compared to 16% of whole blood units reported here. In addition, 6.8% of the pRBC units exceeded the EPA reference dose on the day of transfusion [66]. If the concentration of Hg were doubled in pRBCs prepared from whole blood reported here, 4 units (4%) would have exceeded the reference dose for Hg on the day of transfusion. Similarly, 11 (11%) of transfusions would exceed the Mn ETC.

Another factor to consider when estimating exposure is the practice of assigning a donor unit to an infant. VLBW infants are generally assigned a dedicated unit of blood from a single donor and several transfusions can be administered from the same donor unit. This practice limits exposure of VLBW infants to multiple donor units and thereafter the risk of infection and allo-immunization to foreign antigens from various blood donors is mitigated. On the contrary, VLBW infants receiving multiple transfusions from one donor unit may be chronically exposed to the cumulative effect of toxic metals from that donor blood unit.

Any concentration higher than the calculated ETC could potentially be harmful to VLBW infants. In this study, at least 31% of the blood bank blood had metal concentrations greater than the ETC. Several strategies exist to identify such donor blood units. The first strategy will be to adopt a universal screening program of blood collected by blood banks for heavy metal concentrations. Sensitive, inexpensive assays for blood lead are readily available due to the American Academy of Pediatrics and CDC recommended blood lead screening program [53]. Screening for this one metal would identify 81% of those units with one or more metal concentrations higher than the acceptable level. The addition of screening for Al would identify 94% of unacceptable units.

The second strategy would be a targeted screening of blood units donated by members of communities known to be at high risk for elevated metal concentrations in the blood. In this study, all 100 units of banked blood had As detected above the LOD. According to the 2013 ATSDR priority list of hazardous substances, arsenic ranks at number one as the hazardous substance determined to pose the most significant potential threat to human health due to its known toxicity. For most people, diet is the predominant source of As exposure, which includes, fish, shellfish and drinking water. Other sources of inorganic As exposure include use of arsine gas in the microelectronics industry, semiconductor manufacturing units, and workers in metal smelters exposed to above-average inorganic As levels from arsine released into the air. Elevated levels of inorganic As may also be present in soil, either from natural mineral deposits or contamination from human activities, which may lead to dermal or ingestion exposure [67]. All the above listed sources and exposures could potentially lead to elevated levels of inorganic As in the human body and therefore in donor blood. As CDC continues to gather data in the Biomonitoring program, geographically relevant data will be available (Centers for Disease Control and Prevention). Such data might be used to determine which potential toxic metals, especially As, should be tested in blood donors from a given community.

A third strategy would be the development of a questionnaire to identify donors at high risk for elevated metal concentrations in their blood. An example of such a questionnaire is that developed by the CDC to identify children at risk for elevated blood Pb concentrations [23]. The results of the questionnaire would be used to target donor units for analysis of blood metals.

In conclusion, at least 31% of the donor blood in the blood bank had metal concentrations above an estimated tolerable concentration. This exposure could potentially pose a grave health risk to a vulnerable VLBW infant, especially of the developing nervous system. The number of donor blood units with concerning levels of metal(s) was higher than expected. It is suggested that neonatologists consider the potential threat of toxic metal exposure to neonates when deciding whether their patients should receive blood transfusions. Future research to determine the actual impact and burden of these toxic heavy metal exposures from blood products is imperative.

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