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Prevalence and predictors of exposure to multiple metals in preschool children from Montevideo, Uruguay

Katarzyna Kordas[a], **Elena I. Queirolo**[b,c], **Adrienne S. Ettinger**[d], **Robert O. Wright**[d], and **Rebecca J. Stoltzfus**[e]

[a] Department of Nutritional Sciences, Pennsylvania State University, University Park, PA, USA

[b] Center for Research, Catholic University of Uruguay, Montevideo, Uruguay

[c] Clinic for Environmental Contaminants, Pereira Rossell Hospital, Montevideo, Uruguay

[d] Department of Environmental Health, Harvard School of Public Health, Boston, MA, USA

[e] Division of Nutritional Sciences, Cornell University, Ithaca, NY, USA

Abstract

The extent of children's exposure to multiple toxic metals is not well described in many developing countries. We examined metals exposures in young children (6 – 37 months) from Montevideo, Uruguay and their mothers (15 – 47 y) participating in a community-based study. Hair samples collected from 180 children and their mothers were analyzed for: lead (Pb), cadmium (Cd), manganese (Mn), and arsenic (As) concentration using inductively coupled plasma-mass spectrometry (ICP-MS). Median metals levels (μg/g) were: Pb 13.69, Mn 1.45, Cd 0.17, As 0.09 for children and Pb 4.27, Mn 1.42, Cd 0.08, As 00.02 for mothers. Of the child and maternal samples, 1.7% and 2.9% were below the limit of detection (LOD) for Cd, and 21% and 38.5% were below the LOD for As, respectively. Correlations between maternal and child levels ranged 0.38–0.55 ($p<0.01$). Maternal hair metals levels were the strongest predictors of metal concentrations in children's hair. Girls had significantly lower As levels than boys ($p<0.01$) but did not differ on other metals. In addition, in bivariate logistic regressions predicting the likelihood that the child would be exposed to multiple metals, hemoglobin <10.5 g/dL (OR=2.12, $p<0.05$), blood lead (OR=1.17, $p<0.01$), and the mother being exposed to two or more metals (OR=3.34, $p<0.01$) were identified as significant predictors of increased likelihood of multiple metals exposure. Older child age (OR=0.96, $p<0.05$), higher maternal education (OR=0.35, $p<0.01$), and higher number of household possessions (OR=0.83, $p<0.01$) were significantly associated with decreased likelihood of multiple metals exposure. Preschool children in Uruguay are exposed to multiple metals at levels that in other studies have been associated with cognitive and behavioral deficits. Sources of exposure, as well as cognitive and behavioral consequences of multiple metals exposure, should be investigated in this population.

Keywords

Hair metals; manganese; lead; arsenic; cadmium; child; Uruguay

Corresponding author: Katarzyna Kordas, PhD, Department of Nutritional Sciences, Pennsylvania State University, 110 Chandlee Laboratory, University Park, PA 16802, Kxk48@psu.edu, 814-865-2170.

1. Introduction

Most environmental exposures do not occur in isolation. Thus, the strategy of studying single metal exposures may fail to adequately represent the health risks of affected populations (Cory-Slechta, 2005). Reports on exposures to multiple toxic metals in adults and children are emerging, with an increasing use of hair concentrations as the indicator of exposure. However, many of these studies focus on populations engaged in specific risk behaviors, such as smoking (Unkiewicz-Winiarczyk et al., 2009) or on specific locations with high potential for environmental contamination, such as areas located close to recycling sites (Wang et al., 2009), smelting complexes (Díaz-Barriga et al., 1993) or mines (Pereira et al., 2004). Studies on multiple exposures in children are limited, particularly when no specific source or risk behavior has been identified.

Many existing reports on hair metal concentrations are among school-aged children and have found varying levels of exposure, depending on the source (Beneš et al., 2003; Chlopicka et al., 1998; Nadal et al., 2005; Zaborowska and Wiercinski, 1997). However, there are few reports on multiple metal levels in younger children (Menezes-Filho et al., 2009). Few studies (Wilhelm et al., 1994) also include sampling from other family members in an attempt to understand risk factors for exposure.

The potential for exacerbated cognitive and behavioral deficits makes children's exposure to multiple toxic metals an important public health concern. Reports investigating the effects on cognition of at least two metals together suggest that these combinations of metals either affect different aspects of children's cognitive functioning (Thatcher et al., 1982) or result in increased toxicity (Wright et al., 2006). These findings highlight the need to assess children's exposure to multiple metals starting at an early age. Here, we report hair metal levels of 6–37 month old children living in Montevideo, Uruguay, where elevated blood lead levels have been found (Mañay et al., 2008; Queirolo et al., 2010), but where exposure to other metals has not been studied. In addition, we investigate predictors of children's exposure to multiple metals in this population.

2. Methods

The study setting, sample, and methodology have been described previously (Queirolo et al., 2010). In brief, the study was conducted between February and December 2007 in Montevideo, the capitol of Uruguay, on a sample of preschool children and their mothers. Montevideo is a city of 1.3 million inhabitants, approximately 90,000 of them being children 0–4 y of age. Participants were recruited through widely–distributed posters, leaflets, radio announcements, and letters to childcare and healthcare centers inviting 6–36 mo-old children to participate in screenings for blood lead levels and anemia. Parents brought their children to a "lead clinic" at the Center for Research, Catholic University of Uruguay, or the researchers traveled to clinics set up specifically for this study in elementary schools and religious centers. The majority of participants came from four areas of Montevideo (La Blanqueada, 15; La Teja, 84; Lezica, 14; Peñarol, 101; other, 8). The study was approved by Human Subjects Committees at Cornell University, Catholic University of Uruguay, and the Pereira Rossell Hospital for Women and Children.

After parents provided written consent, blood lead concentrations were tested in several drops of non-fasting capillary blood using the portable LeadCare Analyzer® (ESA Inc., Chelmsford, MA) following established capillary blood collection methods (Parsons et al., 1997) and as described previously (Queirolo et al., 2010). Hemoglobin was also measured in capillary blood using the HemoCue 201+ portable device (HemoCue, Lake Forest, CA).

Children with hemoglobin levels below 10.5 g/dL were referred to their pediatricians for follow-up.

Hair samples were obtained from children and their mothers using stainless steel, blunt-tip scissors. Samples were taken from the occipital region, cut close to the scalp, and deposited in clean, white envelopes. The weight required for hair sample metal analysis is 0.125 g, which corresponds to about 50 strands of hair or the thickness of a pencil. For women and girls, approximately 50 strands of hair were isolated, twisted and cut. For boys and very young children with short or very fine hair, samples were taken from several areas of the head and collected directly into the envelope. Hair was analyzed for lead (Pb), manganese (Mn), cadmium (Cd), and arsenic (As) concentrations (μg/g) at the Trace Metals Laboratory, Harvard School of Public Health (Boston, MA).

Hair samples were cleaned in 10 mL of 1% Triton X-100 solution for 15 min, followed by repeated rinsing with deionized water, and drying at 70 °C for 24 h. Subsequently, the samples were digested with 1 mL concentrated nitric acid for 24 h and diluted to 10 mL with deionized water. Acid-digested samples were analyzed using inductively coupled plasma mass spectrometry (ICP-MS) (Elan 6100, Perkin Elmer, Norwalk, CT). Analyses of Cd, Mn, and As were performed using an external calibration method, using indium as the internal standard for Cd and Mn, and tellurium as the internal standard for As. Pb was measured with thalium as the internal standard. Recovery of the analysis of quality control standard by this procedure is 90–110%, and coefficient of variation of the within-day analysis was <5%. Samples were measured in five replicates and the average of the five replicates is reported for each child. Analytical limits of detection (μg/g) were: 0.03 (Pb and Mn); 0.002 (Cd); and 0.014 (As).

During the clinic visits, parents completed a brief questionnaire asking about family size; socioeconomic status, parents' education and employment; pregnancy outcomes, breastfeeding history, child's health, use of pacifier, tendency to put fingers/toys in his/her mouth, and general diet in the previous week (Queirolo et al., 2010). The question on diet was meant to give a general understanding of the health and eating habits of the child and was not used to estimate dietary sources of exposure to metals. A "family possessions" variable was constructed to indicate socioeconomic status and was the sum of the following items (coded as 1 if present): TV, video-player, DVD-player, computer, video games, radio, sound equipment, refrigerator, washer, house phone, cellular phone, or car. Crowding in the house was calculated by dividing the number of persons living in the house by number of bedrooms. The employment question was open-ended and parents reported the jobs they held or the industry where they worked. Because a large proportion of the mothers was not employed outside the home, we collapsed mother's occupation into employed vs. stay-at-home or unemployed. In addition, we coded father's occupation according to whether he engaged in jobs with potential lead/metal exposure (coded as 1 if employed in a print shop, informal recycling, construction, mechanic, driver, manufacture of plastics and metals and 0 if employed otherwise). Parents were not specifically queried about informal work with battery, electronic waste recycling or cable burning. A question about smoking was not posed.

To conduct statistical analyses, first the distributions of child and maternal hair metal levels were explored using descriptive statistics. Because actual values were available for hair metal levels below the LOD, these values were used in statistical analyses. Hair metals concentrations were not normally distributed and were $\log_{10}$-transformed for further analyses. Spearman correlation coefficients with p-values are reported to describe the relationships between the hair metals levels. Additionally, an index of children's exposure to multiple metals was created. To be counted as exposed to multiple metals (variable coded as

1), the child had to have hair concentrations above the median of the distribution for at least two of the metals. If children had hair concentrations equal to or below the median for two or more metals, they were coded as 0 for this variable. The same procedure was used to identify mothers who were exposed to multiple metals.

To identify salient predictors of toxic metal exposure, a two-step approach was taken. First, the available child and family characteristics were tested in bivariate regression models to identify potential predictors of the hair concentrations for each metal and the likelihood of the child being exposed to at least two metals at levels above the median. The potential predictors tested included: child age (months), sex, hemoglobin <10.5 g/dL, history of anemia, capillary blood lead concentration (μg/dL), whether child puts toys/fingers in his mouth, maternal age (years), whether or not the child consumed milk products daily, maternal education (whether or not the mother completed any secondary education), number of household possessions, maternal employment, crowding in the house, father's employment in jobs with potential metal exposure, and maternal hair metal concentration (μg/g). Next, based on the results of the bivariate regressions and biological plausibility, three progressively complex models were tested for each of the metals, and for the likelihood of multiple metals exposure. In Model 1, child age, sex, hemoglobin <10.5 g/dL and capillary blood lead level were included together. Model 2 included all variables from Model 1, as well as socioeconomic characteristics of the family (maternal education and household possessions). Finally, in Model 3, we additionally included the mother's hair metal levels.

3. Results

Two hundred and forty four children, including 20 sets of siblings, were screened for blood lead and anemia. Only one child from each set of siblings was randomly selected for inclusion in the analysis, therefore the final study sample consisted of 222 children with blood lead and hemoglobin values, and their mothers. For this study, a group of 180 participating children who had hair samples available for analysis did not differ on any demographic characteristics from those children who did not have a hair sample available or had insufficient sample volume ("non-participants," N=42). The mean age of the participating children was 19.8 ± 8.1 months. This population was characterized by high prevalence (42.8%) of anemia (Hb < 10.5 g/dL) and elevated blood lead (33.9% ≥ 10 μg/dL) (Table 1).

Children's hair metals concentrations were generally higher than the mothers' levels, except for Mn (Table 1). Three (1.7%) and 37 (21.3%) children had undetectable hair Cd and As concentrations, respectively. Additionally, four (2.9%) and 52 (38.5%) of the mothers, had undetectable Cd and As levels, respectively. Children's hair metals were moderately correlated (Spearman's rho), ranging from 0.35 (Cd-As, $p<0.01$) to 0.68 (Cd-Pb, $p<0.01$). Children's lead levels, as measured in capillary blood and hair were moderately correlated (0.32, $p<0.01$). Correlations between mother and child hair metals were: 0.48 (Pb, $p<0.01$); 0.55 (Mn, $p<0.01$); 0.41 (Cd, $p<0.01$); and 0.38 (As, $p<0.01$).

Bivariate analyses revealed several child and family-related characteristics that were significant predictors of children's metal levels (Table 2). Maternal hair metal concentrations and education explained the highest proportion of variability in children's levels. For As only, maternal hair levels were significantly associated with levels among children older than 18 months of age ($\beta=0.64\pm0.10$, $p<0.001$) but not younger children ($\beta=0.12\pm0.14$, $p=0.37$). In addition, older children had lower hair Pb and Cd; girls had lower As than boys; children with low hemoglobin or elevated blood Pb had higher hair levels of all metals. Children from crowded households (>3 persons per bedroom) had higher Mn and

children with greater number of household possessions (representing higher SES) had lower Mn, As and Cd concentrations.

In multivariate linear regressions, Model 1 confirmed that older age was associated with lower hair lead and cadmium concentrations and that girls had lower hair arsenic levels than boys in covariate adjusted models (Table 3). Higher capillary blood lead was a significant predictor for all four metals measured in children's hair. On the other hand, a hemoglobin level less than 10.5 g/dL was associated with higher hair manganese and arsenic concentrations. In model 2, the addition of socioeconomic variables did not change the association between child age and hair metal levels but did attenuate the associations between sex and hair arsenic, and between hemoglobin and hair manganese and arsenic. The association between blood lead and hair metal levels also became attenuated. Finally, in Model 3, maternal hair metal concentrations were the strongest predictors of children's hair metal levels, whereas the association between socioeconomic variables and children's hair levels disappeared, suggesting a strong correlation between socioeconomic status and exposure to metals. Low hemoglobin was an important predictor of higher arsenic and lead levels in children's hair.

In bivariate logistic regressions predicting the likelihood that the child would be exposed to multiple metals, hemoglobin <10.5 g/dL (OR=2.12, $p<0.05$), blood lead (OR=1.17, $p<0.01$), and the mother being exposed to two or more metals (OR=3.34, $p<0.01$) were identified as significant predictors of increased likelihood of multiple metals exposure. Older child age (OR=0.96, $p<0.05$), higher maternal education (OR=0.35, $p<0.01$), and higher number of household possessions (OR=0.83, $p<0.01$) were significantly associated with decreased likelihood of multiple metals exposure.

A multiple logistic regression model was used to test demographic predictors of the child's exposure to more than two metals at levels above the median of the distribution for each metal. Again, models of increasing complexity were built (Table 4). In terms of child predictors (Model 1), both older age and higher blood lead were associated with higher likelihood of being exposed to multiple metals. The addition of socioeconomic factors (Model 2) did not attenuate these relationships but maternal education and household possessions were not associated with the child's likelihood of multiple metal exposures. In the final model, each 1 μg/dL increase in blood Pb was associated with a 16% higher likelihood ($p<0.01$) and maternal exposure to multiple metals was associated with a 2.6-fold higher likelihood ($p<0.01$) of the child being exposed to at least two toxic metals after adjusting for potential covariates.

4. Discussion

Our study provides much needed information on exposures to metals among preschool children and their mothers residing in Montevideo, Uruguay. Although biomonitoring studies have been carried out in the U.S. (Pirkle et al., 2005) and in European populations (Seifert et al., 2000), this is not the case for many other countries. This is particularly true in developing countries where exposures to toxic substances in the environment may be greater due to less stringent environmental and occupational controls (LaDou, 2003; Orloff and Falk, 2003). To our knowledge, this is the first study of multiple metals exposure in Uruguayan children.

Thus far, the global extent of exposure to multiple metals among children has not been well characterized. Metals are ubiquitous in the environment and exposure in children may occur through inhalation, ingestion and absorption. Major unanswered questions regarding metal exposure in children include the extent of exposure via air, soil, food and water, and the

potential synergistic effects of mixed metal exposures on health outcomes. Hair holds promise as a biomarker of metal exposure in children because it is involves a simple, noninvasive, and painless procedure for collection. Many metals are deposited in keratin, a component of hair, and the growth rate of hair by ~1 cm/month means that hair levels represent integrated exposures over several months. While external contamination is a potential issue, careful collection and analysis protocols mitigate this risk.

A wide discrepancy exists in the amount of information available on levels of different metals in children's hair and very few studies report on exposures to two or more metals together (Beneš et al., 2003; Nadal et al., 2005; Wright et al., 2006). Where data are available, distributions of hair metals are quite variable depending on timing, location and potential sources of exposure (Menezes-Filho et al., 2009; Nadal et al., 2005), and with the exception of hair mercury levels, no clear levels of concern for health effects have been established (Harkins and Susten, 2003). Thus, at this stage it is difficult to estimate how many children worldwide are exposed to levels that would result in cognitive and behavioral deficits or pose other health risks.

The preschoolers in our study had relatively high levels of hair lead, which is perhaps not surprising considering that leaded gasoline was not phased out until 2004. Their hair Pb levels exceeded those reported in children living in urban centers of Sardinia [11.8 ± 11.7 μg/g, (Sanna et al., 2003)], Poland [8.2 ± 5.6 μg/g (Chlopicka et al., 1998)], and the Czech Republic [2.05 ± 2.02 μg/g (Beneš et al., 2003)]. Arsenic levels were lower than those reported for children living in urban or smelting communities [geometric mean, 0.84 and 9.87 μg/g, respectively, (Díaz-Barriga et al., 1993)] or living in vicinity of defunct mines [0.35 ± 0.12 μg/g, (Pereira et al., 2004)]. However, even with 21% of children having undetectable As values, the mean levels in Uruguayan preschoolers were higher than among Spanish children (Nadal et al., 2005) or US children living close to a Superfund Site and in whom hair As was associated with cognitive deficits (Wright et al., 2006). For manganese and cadmium, the Montevideo preschoolers had levels higher than reported for school children in many rural and urban settings internationally (Hasan et al., 2004; Nadal et al., 2005; Pereira et al., 2004; Seifert et al., 2000; Wright et al., 2006), but not as high as urban Polish children [Cd: 0.91 ± 0.61 μg/g (Chlopicka et al., 1998)] or Spanish children living in the vicinity of a ferro-manganese plant [Mn: geometric mean, 15.2 μg/g (Menezes-Filho et al., 2009)].

Our study has several limitations. Hair may not be the preferred biomarker for all metals, such as lead, due to high variability in a given metal's deposition or the potential for environmental contamination (Harkins and Susten, 2003). However, we used strict collection and analytical techniques to decrease the latter concern, including cleaning the hair with detergent and repeated rinsing to remove external contaminants prior to laboratory analysis. In addition, lead levels in blood and hair were significantly correlated in our study. Other limitations of measuring hair metals have also been identified: individual variability in growth rates of hair, which may limit the usefulness of this biomarker as a measure of recent exposure; lack of reference values; incomplete understanding of how metals are incorporated into hair; and low (or lack) of correlation between hair metal levels and other biomarkers of exposure. Despite these limitations, however, metal biomonitoring in hair samples may be useful as a screening tool or in conjunction with other biological samples (Harkins and Susten, 2003). Considering the nature of our screening prevalence study, these results can be used to indicate that environmental metal exposure is a concern in children from Montevideo and to serve as an impetus for a more systematic assessment of exposure and its sources.

Another limitation of the study, particularly in relation to cadmium exposure, is that we did not ask questions on the smoking habits of parents or other family members. It has been shown that smoking is related to hair metal levels of the smokers themselves (Hoffmann et al., 2001; Unkiewicz-Winiarczyk et al., 2009) and their children (Özden et al., 2007). Anecdotally, a large proportion of the Uruguayan population smokes, thus future studies should include the assessment of this important risk factor for exposure to cadmium and other metals. Finally, fewer mothers than children had complete data on metal concentrations, particularly for arsenic. Nonetheless, maternal hair metal levels were the strongest predictors of children's hair concentrations, suggesting that the sources of exposure occur in their shared environment. Over 70% of the women reported not working outside of the home, so the common sources of exposure may be found in the home or the neighborhood environment, such as inhaled particles or drinking water. This study did not include environmental sampling, but the young age at which exposure is occurring in these children is a concern and indicates a clear need to examine the sources of toxic metals in Montevideo.

Age was a significant predictor of lead, manganese and cadmium levels, with older children having lower metal concentrations. This is consistent with hand-to-mouth activity being an important pathway of metal exposure because this behavior tends to subside with increasing age. We found that the behavior of "putting fingers or toys in the mouth" was a salient predictor of blood lead levels in these children (Queirolo et al., 2010). In the present study, this behavior was associated with hair Pb and Mn levels in bivariate analyses. Diets are also age specific and could represent a source of exposure. For all metals, maternal levels strongly predicted child levels. If mothers and children are exposed from a common source, their diets would likely be more similar at older ages than in infancy. As children age, they progressively transition to the typical family diet. In our study, children older than 18 months of age were more likely to eat meat 7 days/week ($p<0.05$) and eggs at least 3 days/week ($p<0.5$) than children less than 18 months of age (data not shown). For As, maternal hair levels strongly predicted children's As levels but only for children older than 18 months of age, which could result from common exposure from the diet. However, this possibility would have to be investigated more closely in future studies.

Other age-related behaviors and factors may also play a role. It is noteworthy that maternal arsenic predicted child hair levels only for children older than 18 months of age. Other researchers have found that breast milk does not contain high levels of arsenic even when environmental exposure is high (Fängström et al., 2008) and that breast milk and early infant metabolism may be protective against arsenic toxicity (Vahter, 2009). On the other hand, breast milk is an important source of lead (Ettinger et al., 2004) but perhaps not cadmium (Schoeters et al., 2006) exposure in infants. Breastfeeding in our study was prevalent (93.5% of mothers reported having ever breastfed) with the mean age at introduction of solid foods being 5.3 ± 1.8 months. Thus, the age-dependent relationship between maternal and child arsenic levels, in particular, suggests that environmental factors, perhaps related to spending time outdoors or via increased exposures through food and drinking water among older children, could be important sources/pathways of exposure in this population. The age-related findings for Pb, Mn, and Cd may also be consistent with maternal-fetal transfer of metals as a potential source of exposure. Metals cross the placental barrier with varying levels of efficiency and maternal-infant metal levels are correlated (Goyer, 1990; Krachler et al., 1999; Rossipal et al., 2000). Hair is a biomarker of cumulative exposure to metals with a longer half-life than other metal biomarkers, such as blood or urine. Young children's detoxification systems are immature (Suk et al., 2003), suggesting that a young child's body burden may be acquired, at least partially, *in utero*.

In our study, girls had lower arsenic levels than boys. This is consistent with other studies of arsenic in hair and other biological samples (Rosado et al., 2007; Wright et al., 2006) but the reason for this association is unclear. Women appear to be better at methylating arsenic than men (Hsueh et al., 1998; Lindberg et al., 2007, 2008), but this has not been demonstrated at younger ages. Methylation capacity could affect the rate of arsenic excretion and susceptibility to arsenic toxicity (Tseng, 2007). Boys also tend to be more active and may spend more time playing in contaminated environments such as outdoor playgrounds, although this is not reflected in sex differences for the other metals.

Previous work on elevated blood lead in Uruguayan children revealed that informal battery recycling and cable burning activities by families may expose children to lead (Mañay et al., 2008) and, potentially, to other metals. Activities related to electronic-waste recycling were associated with substantially elevated hair metal levels in Chinese children and adults (Wang et al., 2009). We did not find a relationship between father's occupation and children's metal levels, but it is possible that some mothers were reluctant to disclose or did not know of such recycling activities. It may be that other family members are involved in these activities, which should be queried in future studies.

Although we did not measure neurobehavioral outcomes in this study, there is evidence that the concentrations seen in this population may have toxic effects. Hair metal concentrations in our study of Montevideo preschoolers were higher than those found among school children living in the vicinity of the Tar Creek, Oklahoma Superfund site (Wright et al., 2006). Even at the low-level exposures observed in Tar Creek children, metals (As, Mn) were negatively associated with IQ, verbal learning and memory. There was also evidence of an interaction such that children with both elevated arsenic and manganese had lower IQ compared to children with exposure to only one metal (Wright et al., 2006). Twenty-two percent of the Montevideo preschoolers had manganese levels above 3 μg/g, which was the level associated with hyperactivity and oppositional behaviors in another study of school-aged children (Bouchard et al., 2007). In 1-year old Mexican children, manganese exposure showed a u-shaped association with child cognition, highlighting the element's role as both essential nutrient and potential toxicant (Claus Henn et al., 2010). Finally, in a study of Spanish adolescents (Torrente et al., 2005), low-level lead exposure, but not manganese, was associated with decreased sustained attention. This evidence suggests that the children in our study were exposed to metal levels sufficiently high to result in cognitive and behavioral deficits. Furthermore, these children were exposed to multiple metals during a period characterized by rapid brain development, raising concerns of potentially irreversible effects and magnified deficits in this young age group.

In summary, in a sample of preschool children from Montevideo, Uruguay, we found evidence of exposure to multiple metals at levels that have been associated with cognitive deficits and behavior problems in other studies. This sample may not be representative of the entire population of preschool children from Montevideo. Therefore, exposure assessment studies encompassing a wider population of children are needed. In addition, more information is needed about the sources and pathways of exposure to environmental toxicants in this population. A systematic and comprehensive study of the cognitive and behavioral consequences of early childhood exposure to multiple metals should also be designed to study the effects of metals on specific cognitive and behavioral outcomes. Better understanding of the biological interactions and toxicokinetics of multiple metals is needed for risk assessment and policy development to protect children's health, particularly in developing countries, where attention to these issues has been limited.

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Table 1

Characteristics of the study sample.

	Sample size (n)	Mean ± SD or Proportion	Range
Child characteristics			
Child age (months)	180	19.8 ± 8.1	6 – 36.6
Sex (female)	180	48.9%	
Weight (kg)	180	10.8 ± 2.1	6.4 – 16.0
Height (cm)	180	80.0 ± 8.5	60 – 105
Hemoglobin (g/dL)	180	10.6 ± 1.4	6.4 – 15.3
Anemia (< 10.5 g/dL)		42.8%	
Capillary blood lead (μg/dL)	180	9.3 ± 6.0	1.0 – 35.6
Elevated (≥ 10 μg/dL)		33.9%	
Hair lead (μg/g)	180	17.64 ± 17.43	0.44 – 161.70
Median		13.69	
Hair manganese (μg/g)	180	2.16 ± 2.25	<0.03 – 18.31
Median		1.45	
Hair cadmium (μg/g)	176[1]	0.28 ± 0.53	<0.2–6.24
Median		0.17	
Below LOD[2]		2.8%	
Hair arsenic (μg/g)	174[1]	0.13 ± 0.14	<0.014 – 1.00
Median		0.09	
Below LOD[2]		21.3%	
Family/household characteristics			
Mother's age (years)	179	27.8 ± 7.4	15 – 47
Mother's education	179		
Any primary school		45.8%	
Any secondary school		48.6%	
Any post-secondary school		5.6%	
Marital status	171		
Married or living with partner		76.6%	
Divorced or separated		23.4%	
Mother's employment (none)	179	77.1%	
Hair lead (μg/g)	142[3]	7.32 ± 9.58	0.25 – 71.90
Median		4.27	
Hair manganese (μg/g)	142[3]	2.25 ± 2.66	<0.03 – 21.18
Median		1.42	
Below LOD[2]		1.4%	
Hair cadmium (μg/g)	138[4]	0.12 ± 0.15	<0.02 – 1.35
Median		0.08	
Below LOD[2]		2.9%	
Hair arsenic (μg/g)	135[4]	0.04 ± 0.05	<0.014 – 0.38

	Sample size (n)	Mean ± SD or Proportion	Range
Median		0.02	
Below LOD[2]		38.5%	
Family possessions	171	5.9 ± 2.5	
Crowding in home (>3 persons/bedroom)	173	31.8%	

[1] Sample sizes for Cd and As levels in children are 176 and 174, respectively. This is because 4 and 6 children, respectively, had insufficient sample volume for the determination of these two metals;

[2] LOD—Limit of Detection.

[3] Number of samples for maternal hair analysis is smaller because sample was not collected or lost in shipment (n=31), child did not come to study visits with mother (n=7).

[4] Four and seven maternal samples did not have sufficient sample volume for the determination of cadmium and arsenic levels, respectively.

Table 2

Bivariate predictors of child hair metal concentrations (μg/g).

Predictor	Lead[1]	Manganese[1]	Cadmium[1]	Arsenic[1]
Child factors				
Age (months)	−0.03 ± 0.01**[2] 5.5%[2]	−0.003 ± 0.008 0.09%	−0.02 ± 0.01** 2.9%	−0.01 ± 0.01 0.2%
Sex[3]	0.03 ± 0.14 0.02%	0.07 ± 0.14 0.1%	0.08 ± 0.16 0.0%	−0.46 ± 0.16** 4.6%
Hb < 10.5 g/dL	0.29 ± 0.14* 2.4%	0.49 ± 0.13** 6.8%	0.33 ± 0.16* 2.5%	0.51 ± 0.16** 5.4%
History of anemia	0.13 ± 0.20 0.3%	0.10 ± 0.20 0.2%	0.001 ± 0.22 0.0%	0.60 ± 0.23** 4.4%
PbB (μg/dL)	0.04 ± 0.01** 7.4%	0.04 ± 0.01** 6.8%	0.03 ± 0.01* 3.6%	0.04 ± 0.01** 5.9%
Puts fingers/toys in mouth	0.17 ± 0.07* 3.3%	0.15 ± 0.07* 2.5%	0.09 ± 0.09 0.6%	0.10 ± 0.09 0.7%
Consumes milk and milk products 7d/wk	−0.83 ± 0.22# 1.8%	−0.05 ± 0.22 0.0%	−0.35 ± 0.25 1.2%	−0.04 ± 0.26 0.0%
Maternal and household factors				
Age (years)	0.01 ± 0.01 1.2%	0.005 ± 0.01 0.2%	0.02 ± 0.01 1.3%	−0.01 ± 0.01 0.9%
Education[4]	−0.48 ± 0.13** 6.9%	−0.64 ± 0.13** 12.0%	−0.39 ± 0.16* 3.5%	−0.86 ± 0.15** 15.6%
Employment[5]	−0.13 ± 0.16 0.4%	0.03 ± 0.16 0.0%	−0.25 ± 0.19 1.0%	0.14 ± 0.20 0.3%
>3 persons per bedroom in the house	0.06 ± 0.15 0.1%	0.50 ± 0.15** 6.3%	0.20 ± 0.17 0.8%	0.21 ± 0.18 0.8%
Household possessions (#)	−0.03 ± 0.03 0.6%	−0.12 ± 0.03** 10.3%	−0.06 ± 0.03# 2.2%	−0.09 ± 0.03* 4.9%
Father has a job with possible metal exposure	−0.15 ± 0.16 0.6%	0.04 ± 0.16 0.0%	0.01 ± 0.18 0.0%	0.18 ± 0.18 0.7%
Maternal hair metal level (μg/g)[1]	0.37 ± 0.07** 17.8%	0.57 ± 0.08** 28.4%	0.33 ± 0.07** 13.3%	0.41 ± 0.09** 15.0%

[1] Hair metals values log-transformed for this analysis;

[2] Values given as β ± SE and %Variability in hair metal level explained by predictor;

[3] Reference group is boys;

[4] Education level defined as having no (reference group) or any secondary education;

[5] Employment defined as working (reference group) or being unemployed/stay-at-home mother;

** p<0.01;

* p<0.05;

# p<0.1.

Table 3

Multivariate predictors for hair metal levels in Uruguayan children.

	Lead[1]	Manganese[1]	Cadmium[1]	Arsenic[1]
Model 1				
Intercept	2.54 ± 0.21**[2]	−0.12 ± 0.21[1]	−1.84 ± 0.25**[2]	−2.77 ± 0.25**[2]
Child age (months)	−0.03 ± 0.01**	0.00 ± 0.01	−0.02 ± 0.01*	0.00 ± 0.01
Sex[2]	0.09 ± 0.13	0.11 ± 0.13	0.15 ± 0.15	−0.41 ± 0.15**
Hemoglobin <10.5 g/dL	0.16 ± 0.13	0.42 ± 0.13**	0.25 ± 0.16	0.43 ± 0.16**
Blood lead (μg/dL)	0.04 ± 0.01**	0.03 ± 0.01**	0.03 ± 0.01*	0.04 ± 0.01**
Model 2				
Intercept	2.77 ± 0.31**	0.78 ± 0.30*	−1.44 ± 0.37**	−2.24 ± 0.35**
Child age (months)	−0.03 ± 0.01**	−0.01 ± 0.01	−0.02 ± 0.01*	0.00 ± 0.01
Sex[3]	0.20 ± 0.13	0.20 ± 0.13	0.19 ± 0.16	−0.28 ± 0.15#
Hemoglobin <10.5 g/dL	0.12 ± 0.14	0.33 ± 0.14*	0.23 ± 0.17	0.36 ± 0.16**
Blood lead (μg/dL)	0.03 ± 0.01**	0.01 ± 0.01	0.02 ± 0.01	0.02 ± 0.01#
Maternal education[4]	−0.45 ± 0.15**	−0.46 ± 0.15**	−0.25 ± 0.19	−0.52 ± 0.17**
Household possessions	0.02 ± 0.03	−0.07 ± 0.03*	−0.03 ± 0.03	−0.02 ± 0.03
Model 3				
Intercept	2.07 ± 0.34**	0.62 ± 0.34#	−0.76 ± 0.52	−1.83 ± 0.49**
Child age (months)	−0.02 ± 0.01*	−0.01 ± 0.01#	−0.02 ± 0.01*	0.01 ± 0.01
Sex[3]	0.19 ± 0.14	0.22 ± 0.14	0.25 ± 0.18	−0.21 ± 0.18
Hemoglobin <10.5 g/dL	0.28 ± 0.15#	0.15 ± 0.15	0.24 ± 0.20	0.51 ± 0.19**
Blood lead (μg/dL)	0.01 ± 0.01	0.00 ± 0.01	0.01 ± 0.02	0.03 ± 0.02#
Maternal education[4]	−0.16 ± 0.17	−0.25 ± 0.16	−0.28 ± 0.22	−0.11 ± 0.21
Household possessions	0.00 ± 0.03	−0.05 ± 0.03#	−0.01 ± 0.04	−0.02 ± 0.04
Maternal hair metal level (μg/g)[1]	0.30 ± 0.07**	0.49 ± 0.08**	0.32 ± 0.08**	0.30 ± 0.09**

[1] Hair metals values log-transformed for this analysis;

[2] Values given as β ± SE and %Variability in hair metal level explained by predictor;

[3] Reference group is boys;

[4] Education level defined as having no (reference group) or any secondary education;

** p<0.01;

* p<0.05;

# p<0.1.

Table 4

Predictors of the likelihood of multiple-metal exposure in children from Montevideo.

Variables	OR [95% CI][1]
Model 1	
Child age (months)	0.95 [0.92, 0.99]*
Sex[2]	0.82 [0.43, 1.59]
Hemoglobin <10.5 g/dL	1.76 [0.89, 3.45]
Blood lead (μg/dL)	1.17 [1.09, 1.27]**
Model 2	
Child age (months)	0.95 [0.91, 0.99]*
Sex[2]	0.97 [0.47, 1.98]
Hemoglobin <10.5 g/dL	1.64 [0.79, 3.41]
Blood lead (μg/dL)	1.16 [1.06, 1.26]**
Maternal education	0.59 [0.27, 1.29]
Household possessions	0.93 [0.80, 1.09]
Model 3	
Child age (months)	0.98 [0.93, 1.02]
Sex[2]	1.14 [0.50, 2.59]
Hemoglobin <10.5 g/dL	1.95 [0.83, 4.57]
Blood lead (μg/dL)	1.16 [1.06, 1.28]**
Maternal education	1.16 [0.44, 3.06]
Household possessions	0.92 [0.78, 1.10]
Mother exposed to at least 2 toxic metals[1]	2.65 [1.16, 6.03]*

[1] To be counted as exposed to multiple metals (variable coded as 1), the child/mother had to have hair concentrations above the median of the distribution for at least two of the metals. If child/mother had hair concentrations equal to or below the median for two or more metals, they were coded as 0 for this variable.

[2] Reference group is boys.

** p<0.01,

* p<0.05,

# p<0.1.