Concentrations of Toxic Metals and Trace Elements in the Meconium of Newborns from an Industrial City

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Introduction

Meconium is the dark viscous stool that is passed by newborns in the first few days of life. This material may provide an index of mineral accumulation by the fetus, since stool is normally not excreted in utero [1]. Nutritional elements and toxic substances are transported from mother to fetus via blood circulation, and excessive levels of toxins and certain toxic metals harm the fetus. The levels of trace elements and toxic metals in fetal blood may not reflect the degree of fetal exposure to toxic metals or actual degrees of excess or deficiency of trace metals and trace elements. Also there were positive correlations between the levels of Zn, Fe, and parental occupations, and between the level of Fe and location of residence of the parents (proximity to the petroleum refinery or the dye industries).

Conclusion: All the meconium samples were positive for toxic metals, and thus may reflect environmental pollution in the city. The occupation environments and the location of the family residence are linked with levels of trace elements in meconium.

Key Words
Cadmium · Lead · Toxic metal · Trace elements · Meconium

Abstract
Objective: To investigate fetal exposure to toxic metals [lead (Pb), cadmium (Cd)] and fetal levels of trace elements [zinc (Zn), copper (Cu), and iron (Fe)] in newborns from an industrial city. Relationships between meconium mineral contents and parental occupation and location of residence were also tested. Method: The meconium mineral contents of 117 healthy newborn infants were measured by flame atomic absorption spectrophotometer. Results: The median concentrations (interquartile range) of toxic metals and trace elements in the meconium were as follows: Pb: 46.5 (1,399) μg/g dry weight (wt), Cd: 2.3 (55.6) μg/g dry wt; Zn: 234 (3,049) μg/g dry wt; Cu: 11.8 (818.7) μg/g dry wt, and Fe 105 (2,980) μg/g dry wt. All the meconium samples contained both toxic metals and trace elements. The proportions of trace elements in the meconium samples with concentration higher than 100 μg/g dry wt of the substances tested were Zn 90%, Cu 64%, and Fe 53%. There were significantly positive correlations between the concentrations of toxic metals and trace elements. Also there were positive correlations between the levels of Zn, Fe, and parental occupations, and between the level of Fe and location of residence of the parents (proximity to the petroleum refinery or the dye industries). Conclusion: All the meconium samples were positive for toxic metals, and thus may reflect environmental pollution in the city. The occupation environments and the location of the family residence are linked with levels of trace elements in meconium.
elements. Many substances processed by the fetus accumulate in the fetal intestine, thus meconium analysis may also be used to assess levels of fetal exposure to toxic metals and mineral content [1–8]. It has been suggested that, in dry meconium, concentrations of trace elements higher than 100 µg/g (0.01%) can indicate toxicity [4]. Replacing trace element deficiencies or detoxifying after toxic metal exposure pose significant challenges for the neonatologist in the first few weeks of an infant’s life [6].

Several reports discuss the detection of environmental toxic metal [lead (Pb), cadmium (Cd), mercury, and aluminum] in cord blood, placental tissue, antenatal maternal blood, and breast milk. No data has been published about mineral contents from meconium samples obtained in our country. Kocaeli is the city that is located on the highway, autobahn, and railroad connecting Europe to Asia and takes place between Black and Marmara Sea. About 2 million people live in Kocaeli. The city of Kocaeli is mainly an industrial center with numerous industrial establishments surrounding the Gulf of Izmit. Seventy-five of 500 important industrial establishments in Turkey are located in Kocaeli. The most important of these are petroleum refinery and fuel additives, PVC plastics, crystal glass production, dye, chemistry, drug, detergent, rubber, and automobile industry of Turkey. Lead-free oil is seldom used by people in Turkey. The aim of this study was to measure concentrations of toxic metals (Pb and Cd) and trace elements [zinc (Zn), copper (Cu), and iron (Fe)] in the meconium of newborns from the industrial city of Kocaeli. The parents of all infants had lived within a 20-km radius of a petroleum refinery or dye industry for at least 5 years. Relationships between meconium contents and parental occupation and location of residence were also tested.

Materials and Methods

Sample Collection

Meconium specimens were collected from 117 healthy newborn infants (60 females, 57 males) born in Kocaeli in June and July of 2001 whenever available. The investigation was conducted at the Central Public Hospital and the University Hospital. The local pediatric research ethics committee approved the study, and parental consent was obtained. For each case, we recorded certain characteristics of the mothers (smoking habit, alcohol or drug abuse, blood pressure); type of employment held by the mother and father; and the baby’s sex, gestational age, birth weight, head circumference at birth, Apgar score at birth, and any systemic abnormalities or malformations noted at birth. At the time of meconium collection, none of the newborns was receiving intravenous fluids, electrolyte support, total parenteral nutrition, or any orally administered essential minerals or vitamins.

We categorized the babies according to type of job held by the mother, type of job held by the father, and location of residence (dye factory region versus petroleum refinery region).

Measurement of Heavy Metals and Trace Elements in Meconium

Each meconium sample was analyzed for levels of Fe, Cu, Zn, Cd, and Pb. The meconium from each diaper was weighed, transferred to a metal-free glass tube in ice, and then frozen immediately and kept at ~20°C until testing was done. For analysis, all specimens were dried in a drying oven at 120°C for 1 h, after which dry weights were recorded. Small amounts (approximately 0.8–1 g) of each sample were transferred to a series of test tubes. Three milliliters of concentrated nitric acid was added to each tube, and the mixture was allowed to digest in the oven at 100°C for 1 h. After cooling, 3 ml of 60% perchloric acid was added to each tube. Next, the mixtures were heated at 120°C and reduced to half volume. The digested material was then diluted to 10 ml with deionized water, centrifuged at 500 g, and filtered to remove insoluble material. Each final dilution was mixed in a shaker for 15 min just before measurements were made [9, 10].

Meconium levels of Pb, Cd, Zn, Cu, and Fe were determined in a flame atomic absorption spectrophotometer (Shimadzu AA-680), based on comparison with external standards. The standards were freshly prepared from standard stock metal solutions (Titrisol 1000 ± 0.002 mg/l, Merck) just prior to analysis, and were used for initial calibration for each substance. These solutions were also used as internal quality standards Hollow Cathode Lamp and Background Correction (with Deuterium Lamp) modes were selected to element analyses. Each result was corrected for the appropriate reagent used and matrix blanks. For the accuracy of trace element concentrations, each sample was measured twice for each element analysis and the differences <5% between two measurements were accepted. The result for each substance was expressed as micrograms per gram of dry meconium (µg/g dry wt) [1, 9, 10]. Deionized water was used to clean the chamber and zero control for each analysis. The stock standard metal solutions for every metal (Merck, 1,000 mg/l) were used for positive control and tested for every 25 samples, for the reliability of measurement. Minimum detectable limits for Pb, Cd, Zn, Cu, and Fe were 0.045, 0.009, 0.037, 0.080, and 0.014 µg/ml, respectively. The recovery of trace elements (Zn and Cu) spiked in meconium ranged between 94.1% (92.4–96.7%) and 93–101% and percent variance ranged around 2.6% (2.2–3.1%) [11]. The recovery of heavy metals (Pb and Cd) spiked in meconium ranged between 100 and 102%. Interassay coefficient of variability ranged between 0.34 and 8.9%; intra-assay coefficient of variability ranged between 0.44 and 8.44% [12]. The precision, as RSD, was between 3.9 and 6.7% for Cd [13].

Statistical Analysis

A standard statistics program (SPSS for Windows, release 11.5) was used to determine the median and interquartile range for each parameter investigated. For quantitative variables that were not normally distributed, the Mann-Whitney U test and the Kruskal-Wallis test were used to compare results, as seemed appropriate. Spearman’s rho correlation test was used for correlation analysis. Using the data from the entire sample we examined relationships...
between the toxic metals and trace elements by applying multiple linear regression analysis and taking each of three parameters (Pb, Cd, and Zn) as the dependent variable.

### Results

None of the mothers smoked or abused alcohol or drugs. In addition, none of these women had diabetes mellitus, and none had suffered preeclampsia. Seventy-five percent of the mothers worked at home and the rest were categorized as ‘office workers’ (defined as secretaries, teachers, sales assistants). Thirty-eight percent of the fathers were factory workers and the rest were office workers (see definition above) or farmers. The mean gestational age of the infants was 38.4 ± 2.5 weeks and the mean birth weight was 3,085 ± 754 g. The mean head circumference was 34.6 ± 1.5 cm. The birth weights and head circumferences were between the 10th and 95th percentiles. The Apgar scores at 1 and 5 min ranged from 8 to 10. None of the newborns developed sepsis, jaundice, asphyxia, seizures, tremor, or hypoglycemia in the first 24 h after birth, and there were no congenital malformations.

The median (interquartile range) concentrations of toxic metals and trace elements in the 117 meconium specimens are shown in Table 1. All the samples contained both toxic metals and trace elements. The proportions of the samples with concentrations of trace elements considered to be toxic (levels >100 µg/g dry wt) were as follows: Zn 90%, Cu 64%, and Fe 53%.

Neither birth weight nor head circumference was significantly correlated with meconium levels of Pb, Cd, Zn, Cu, or Fe (p > 0.05 for all). Meconium Pb level was correlated with meconium Zn, Cu, and Fe levels (r = 0.858, p < 0.0001; r = 0.227, p < 0.013; and r = 0.389, p < 0.0001, respectively). Meconium Cd concentration was correlated with meconium Zn and Fe concentrations (r = 0.675, p < 0.0001; r = 0.378, p < 0.0001, respectively). Meconium Zn level was correlated with meconium Fe and Cu levels (r = 0.326, p < 0.0001; r = 0.516, p < 0.0001, respectively).

Multiple linear regression analysis was performed using the data from all 117 meconium specimens. After potential confounding factors (birth weight, head circumference, gestational age, mothers’ employment, fathers’ employment, place of residence) were controlled for, the results showed that Pb and Zn level were both significantly and independently associated with all the other parameters investigated (R^2: 0.735, p < 0.0001, 95% CI: 103.7–192 for Pb; p = 0.99 for Cd).

Neither the mothers’ nor the fathers’ employment was correlated with meconium levels of Pb, Cd, or Cu (p > 0.05 for all); however, the results for Zn and Fe were noteworthy (tables 2, 3). The median Zn level in the meconium of infants whose fathers were factory workers was significantly higher than the median Zn for the group whose fathers were office workers or farmers (p < 0.05). When the data from the factory-worker category were further divided according to type of industry, the babies whose fathers worked in the dye industry had the highest meconium Zn levels of all the father-employment categories. However, there was only a small number of samples in the dye-industry group, so the p value for comparison with other father-employment groupings was not statistically significant (p > 0.05). The median Zn level in the meconium of infants whose mothers worked in the factory industry was significantly lower than the median Zn in the meconium of infants whose fathers worked in the dye industry (p < 0.05).

### Table 1. Levels of toxic metals and trace elements in meconium: comparison of findings in the current study and previous studies, mean ± SD

<table>
<thead>
<tr>
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<th>Toxic metals, µg/g dry weight</th>
<th>Trace elements, µg/g dry weight</th>
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<tr>
<td></td>
<td>Pb</td>
<td>Cd</td>
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<tr>
<td>Current study median (interquartile range)</td>
<td>46.5 (1,399)</td>
<td>2.3 (55.6)</td>
</tr>
<tr>
<td>Haram-Mourabet et al. [6]</td>
<td>Not studied</td>
<td>Not studied</td>
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<tr>
<td>Gonzales de Dios et al. [8]</td>
<td>28.9 ± 27.1</td>
<td>Not studied</td>
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<td>Ostrea et al. [15] (Manila, 1997) (min–max)</td>
<td>63.9 ± 74.5 (4.1–301.5)</td>
<td>7.02 ± 3.51 (1.1–3.7)</td>
</tr>
<tr>
<td>Ostrea et al. [16] (Manila, 1998)</td>
<td>109.7 ± 79.2</td>
<td>7.1 ± 3.6</td>
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<tr>
<td>Ostrea et al. [17] (Manila, 2000)</td>
<td>12.0 ± 14.9</td>
<td>3.0 ± 7.44</td>
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<td>(% of samples with detectable levels)</td>
<td>(26.5%)</td>
<td>(8.5%)</td>
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Table 1. Levels of toxic metals and trace elements in meconium: comparison of findings in the current study and previous studies, mean ± SD
Toxic and Trace Elements in Meconium

**Discussion**

We found that concentrations of toxic metals and trace elements investigated were higher than levels noted in previous reports (table 1) [6–8, 12, 14–17]. Especially, the levels of trace elements were found higher than defined normative data on meconium mineral concentration in term newborns that was documented by Haram-Mourobet et al. [6]. Minerals account for no more than 4% of...
wet weight of the meconium [18], or approximately 16% of its dry weight. The concentration of minerals in the meconium may reflect: (1) the rates of placental transport of minerals and maternal sufficiency of the same; (2) the equilibrium between the nutrients transferred to the fetus, its utilization, and distribution in fetal tissues; (3) its excretion capability, and (4) pharmacological use of inorganic salts, e.g., magnesium, or mineral supplementation during gestation, and especially during antepartum period. In consequence, the composition of these first postnatal excreta can be considered to partially reflect the nutritional ‘history’ of the fetus, since all inorganic elements are received from the mother. A premature infant, other than the understandable developmental immaturity signified by its untimely birth, has not accrued the full complement of the inorganic macro- and micronutrients with which the full-term infant is endowed [6]. The transport of mineral elements across the placenta has been studied to a far lesser extent than other placental transport processes. The recent availability of stable (non-radioactive) isotopes has opened greater possibilities [6, 19]. However, the inherent invasiveness of the approach has discouraged the extension of animal studies to humans. The growth milestones of the fetus in utero can be fairly accurately monitored by ultrasonography [20]. Nevertheless, size, estimated weight, and head circumference do not necessarily reflect possible intrauterine nutritional deficiencies. One of the potential causes for subclinical mineral insufficiency is the different type of transport mechanisms operating for macro- and microelements. Characteristically, certain minerals, such as Zn, are known to be transported across the placenta against a concentration gradient [21]. Therefore the observation of higher Zn, Fe, and Cu in the full-term infants, as compared to previous reports, refer to interference with the rates of placental transport of trace element.

All the specimens contained detectable levels of Pb and Cd, and the mean concentrations for both these elements were higher than those documented by Gonzales de Dios et al. [8] in meconium of newborns in Spain (table 1). In contrast to our observed rates of 100% fetal exposure to Pb and Cd, Ostrea et al. [12] found lower exposure rates (26.9 and 8.5%, respectively) but higher mean concentrations of these two elements in meconium of newborns in Manila, Philippines. They did not find Pb and Cd in any of the meconium samples in newborns in Michigan and Townsville [14, 15]. Ramirez et al. [22] studied mercury levels in meconium from newborns in a village (Tagum) located in Philippines. Mercury was used to extract the gold in Tagum. Vahter et al. [23] studied Pb and Cd levels in the feces of nonsmoking women. Whitehall et al. [14] measured levels of pollutants (pesticides, pentachlorophenol, chlorpyrifos, chlordane) and toxic metals in meconium from newborns in Townsville. This city, located in Australia, is ethnically Aboriginal and Islander, prone to low birth weight and sudden infant death syndrome. Pesticides and smoking are common, but no toxic metals were detected in these samples [14]. Meconium has been used as a means to monitor coccaine, heroin, and cannabinoid use by the mother, proving to be a more reliable indicator of prenatal exposure to xenobiotics than the urine of the newborn [24]. Regarding toxic metal exposure of the newborn, such as Pb and Cd, stool analysis in populations at risk for inorganic contaminants has been shown to be an effective way of estimating oral intake of these elements [14]. There appears to be no precedent for the systematic examination of fecal material of infants or children to determine intrauterine exposure to toxic metals, although Pb and aluminum have been quantitated in meconium [8]. Also there are differences in methodology in different studies including unit of heavy metals, standard deviation with mean, and different atomic absorption spectrophotometers (not flame).

It was found the high Pb and Cd levels in the meconium of newborns were correlated with high levels of Cu, Zn, and Fe. In addition, high Zn was positively correlated with Fe and Cu. Previously, Tscuchiya and Iwao [25] in adult feces, and Nowak and Chmielnicka [26] in adult teeth, hair, and nails revealed that high Zn was positively correlated with both Cu and Fe. In the adult body, excess Zn appears to interfere with Fe and Cu metabolism by altering utilization of these elements at the cellular level, by causing blood levels of Fe and Cu to rise, and by altering their excretion [26]. Therefore, it may be speculated that the high Zn levels appear to interfere with Fe and Cu metabolism by altering their gastrointestinal excretion. Also previous studies have investigated correlations among Pb, Cd, and trace elements in hair, teeth, and nails, but ours is the first to have assessed such associations in meconium. High Pb concentration both decreased Fe concentration and altered Fe/Cu and Fe/Zn ratios [27]. Other research has demonstrated a significant negative correlation between Cd and Zn in blood, but a significant positive correlation between Cd and Zn in seminal plasma [28]. A study done on children’s blood showed associations between elevated Pb concentration and elevated levels of Zn in whole blood, erythrocytes, and plasma [29]. Skoczynska et al. [30] showed that Cu and Zn concentrations in rat tissues rose when the ani-
mals were exposed to Pb and Cd. The results of the above-mentioned studies and this study may suggest that Pb and Cd status might influence the kinetics of Zn, Cu, and Fe in meconium as well [11, 25–30].

In this study we have extended previously incomplete observations on the mineral composition of meconium, which did not precisely discriminate the proximity of the family residence to industrial sites, parental occupation, and relationship between toxic metal and trace elements. Paternal lifestyles and working conditions influence exposure to toxic metals in utero, and may increase the risk of neural tube defects, stillbirth, preterm delivery, and small-for-gestational age status [30–35]. Iarushkin [31] assessed various samples from individuals living near a metal manufacturing industry that used Zn, and found high levels of Pb, Cd, and Zn in maternal blood, newborn blood, placenta, meconium, and breast milk. In the current study, the newborns whose mothers worked in the home and whose fathers worked in dye factories had higher levels of Zn as did the study reported by Iarushkin; their families lived near the industry area. Pannett et al. [32] found that high Zn level in infant blood was associated with both maternal and paternal job type, because dye contents Zn and Fe, especially in the form of ZnO(*), and dye industry may have emitted these compounds to the near environment [36].

There were some limitations in this study. First, we did not analyze the mercury level of meconium samples because of lack of the hollow-cathode lamp for mercury in our system. Second, the analysis of more meconium samples were also costly and we have limited source. The number of meconium samples was insufficient according to the size of population of Kocaeli. The associations between the occupation environments, the location of family residence and levels of trace elements and heavy metals in meconium were observed but statistical analysis was limited due to the small size of the population. Third, we did not study meconium samples of newborns where there was no air pollution, as control. Although these observations were interesting, further investigations will be required, with the use of larger population size and adequately powered study.

Our findings suggested that maternal and paternal working environments are linked with levels of toxic metals and trace elements in meconium. We also found that meconium from babies whose families resided in the petroleum refinery region had lower levels of Fe than specimens from babies whose families resided in the dye industry region. This difference is likely due to the different toxins people are exposed to in these industrial zones.

In conclusion, this study shows that fetuses in the industrial center of Kocaeli are at high risk for exposure to toxic metals and high levels of trace elements. All the meconium samples were positive for toxic metals, and thus may reflect environmental pollution in the city. The working environments and location of residence of parents are linked with levels of toxic metals and trace elements in meconium. It may suggest that Pb and Cd status might influence the kinetics of Zn and Fe in meconium. The results of this study may potentially serve to investigate the association between developmental abnormalities and alteration in the mineral composition of meconium. It is conceivable that analysis of meconium may be used as noninvasive approach to further clarify unanswered questions regarding the effect of environmental pollution to fetus. In addition, by investigating meconium in multiple births and looking for possible relationship between concentration of trace elements and toxic metals in meconium and cord blood, nutrient supply processes of the fetus during the latter part of gestation could be further clarified.

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