ABSTRACT

Background: Infants are the population group most vulnerable to the toxic effects of heavy metals. Exposure to heavy metals during growth and development can result in long-term effects on the health of children. Infant foods are the main source of heavy metals intake by this population, primarily due to contamination of raw materials used and rarely by food processing itself. Monitoring of these elements in infant formula is of considerable health importance in a bid to protect the infants from their acute and chronic toxicity on infants.

Objective: To determine the concentration of Lead and Cadmium in infant milk formulas selected from Alexandria, Egypt. Materials and Methods: Fifteen infant milk formulas were collected from three different types sold in pharmacies of Alexandria, Egypt (ordinary, lactose free, and extra care formulas). Atomic Absorption Spectroscopy AA-800 with atomizer flame (AAS), flame conducted by air/acetylene for the furnace was used to detect lead and cadmium in infant milk formulas and their concentrations (ppm).

Results: The mean lead contents (ppm) of infant milk formulas ranged from 0.134 ppm in Extra care formulas to 0.332 ppm in Ordinary formulas (brand 3), while cadmium contents ranged from 0.0010 to 0.0038 ppm in ordinary formulas (brand 1) and Extra care formulas; respectively with significant differences in lead and cadmium contents among different types of milk formulas, P < 0.05.

Conclusion: There were significant variations in lead and cadmium contents among different types of infant milk formulas and not all samples complied with acceptable levels lead specified in the Egyptian Standard although cadmium levels complied with EC Regulation by American public Health Association and Indian Standards. No data on lead and cadmium contents was declared on the label of the formulas to compare with their actual contents. Further studies are required to evaluate the heavy metal contents of infant formulas on a greater number of samples and a wider diversity of brands with a need to investigate the sources of their contaminant.

Keywords: Infant Milk Formula, Heavy Metals, Lead, Cadmium

INTRODUCTION

Infants are the population group most vulnerable to the toxic effects of heavy metals due to the higher absorption of metals by the gastrointestinal tract, faster metabolic processes, an incompletely developed detoxification system, and higher food consumption in relation to body weight (1,2). Additionally, the undeveloped blood-brain barrier allows elements noxious to infant health (primarily lead and mercury compounds) to accumulate in the brain, causing dysfunction of the central nervous system (3). Exposure to heavy metals during growth and development can result in long-term effects on the health of children (4). Infant foods are the main source of heavy metals intake by this population, primarily due to contamination of raw materials used and rarely by food processing itself (5,6). Metal pollution as a result of increasing industrialization has penetrated into all sectors of the food industry and as such pose fears for infant milk formulas (7). Labelling on the package, however, does not indicate the minimal levels of the elements present. As a matter of fact, elements and ions may find route in foods as a result of processing, packaging, farming activities and industrial emission (8).

Special attention is required in the case of toxic metals, including lead and cadmium, which are regarded as particularly harmful to the organism. Both lead and cadmium are characterized by a high accumulation factor in living organisms. The circulation of heavy metals in the environment is linked to the food chain: soil-plant-animal-man. When metals pass to a higher link, their content accumulates increasing. It should be noted that a positive correlation has been observed between the concentration of toxic metals in children's organisms and the incidence of autism (9). Heavy metals are persistent as contaminants in the environment and come to the fore front of dangerous substances causing health hazards in human. Lead, Cadmium and Aluminum are among the most important of these elements (10). Exposure to toxic metals is associated with many chronic diseases can cause a wide variety of health problems (11).

Infants, particularly those born prematurely, have reduced renal function and their developing kidneys are more susceptible to damage caused by excessive Cd and Pb in their diet (12). Lead is toxic to the blood and the nervous, urinary, gastric and genital...
systems. Furthermore, it is implicated in causing carcinogenesis, mutagenesis and teratogenesis in experimental animals (13). Babies with anemia caused by iron deficiency had a higher cadmium concentration than healthy infants. The level of lead and ferritin in the blood were conversely correlated (14). Measurement of heavy metal contents, comprising lead (Pb) and cadmium (Cd) is helpful in assessment of quality of milk during its manufacturing and production (15). Cadmium is considered to be one of the most toxic metals. In addition, it is implicated in high blood pressure (16), prostate cancer, mutations and fetal (embryonic) death (17). It has a carcinogenic effect and can lead to kidney dysfunction (18). Cd has estrogenic properties and causes an increased incidence of cancer in mice (19).

The presence of heavy metals in dairy products may be attributed to contamination of the original cow’s milk, which may be due to exposure of lactating cow to environmental pollution or consumption of feeding stuffs and water (20). Moreover, raw milk may be exposed to contamination during its manufacture (21). Many elements are present in foods naturally or through human activities, such as processing, storage, farming activities, industrial emission, use of poor quality water for formula preparation, improper handling of formula by mothers or through intentional addition (22). Presence of toxic elements in foods or food products is as a result of possible contamination during industrial food processing, metal contaminated raw materials, contaminated food preservative or leakage of metals from packaging material (23,24). Monitoring of these elements in infant formula is of considerable health importance in a bid to protect the infants from their acute and chronic toxicity on infants (25).

Heavy metal pollution is a result of increasing industrialization throughout the world, which has penetrated into all sectors of the food industry. Because of that, the World Health Organization (WHO) classifies heavy metals as one of the risks people are exposed to through food. In cooperation with the U.S. Environmental Protection Agency, the Agency for Toxic Substances and Disease Registry (ATSDR) has compiled a priority list in 2001 called the “Top 20 Hazardous Substances”. The heavy metals Arsenic, Lead, Mercury, and Cadmium ranked 1st, 2nd, 3rd, and 4th in the list, respectively (11). So, the
aim of the present study was to determine the concentration of Lead and Cadmium in infant milk formulas selected from Alexandria, Egypt using Atomic Absorption Spectrophotometry (AAS)

MATERIALS AND METHODS

A total of fifteen infant milk formulas were collected from three different types sold in pharmacies of Alexandria, Egypt (ordinary, lactose free, and extra care formulas). The ordinary formulas were collected from three different commercial brands. Three samples were collected from the lactose free formulas, extra care formulas, and from each of three brands of the ordinary formulas. Samples were either powdered or cow milk based formulas packaged in tin cans.

Samples Selection. Commercially available in Alexandria/Egypt, but not necessarily manufactured there; Recommended for infants ages 0-6 months, as stated on the label; Manufactured in the same year of the study regardless of the month and not expired.

Preparation of Infant Milk Formulas Samples. Five grams of each sample were placed in crucibles and heated in a muffle furnace at 700°C for 3 hours to remove water and the organic matter and leave heavy metals as pure ash. The ash was cooled to room temperature before being dissolved in a 10 ml solution of 10% HCL. The resulting solution was then poured into an Erlenmeyer flask, and topped up to 50 ml with distilled water.

Preparation of Heavy Metals Standards. Preparation of Heavy Metals Standards and Atomic Absorption Spectroscopy Analysis were carried out according to AOAC, 1980 and Fiorino et al.,1973 (26 ,27). For each of the selected metals (lead and cadmium), three standards with the concentrations of 10 000 ppm, 15 000 ppm, and 20 000 ppm were set for the calibration of the AAS. A linear calibration curve indicated well prepared standards and an accurate Atomic Absorption Spectroscopy.
**Atomic Absorption Spectroscopy Analysis.** Atomic Absorption Spectroscopy AA-800 with atomizer flame (AAS), flame conducted by air/acetylene for the furnace was used to detect lead and cadmium in infant milk formulas and their concentrations (ppm). Three trials were run on each sample and the average was calculated.

**RESULTS AND DISCUSSION**

Table (1) and figures (1,2) show that the mean lead contents (ppm) of infant milk formulas ranged from 0.134 ppm in Extra care formulas to 0.332 ppm in Ordinary formulas (brand 3), while cadmium contents ranged from 0.0010 to 0.0038 ppm in ordinary formulas (brand 1) and Extra care formulas; respectively. There were significant differences in the lead and cadmium contents among samples collected from different types of milk formulas, $P < 0.05$. It is worth mentioning that there were no data on lead and cadmium contents declared on the label of the formulas to compare the actual contents with those declared on its label.

Recent studies have reported contamination of infant milk formulas by various substances such as nitrates, nitrites, aluminum, cadmium, mercury, nickel, lead and melamine (28). A study to determine the concentration of selected trace and toxic elements in breast milk and infant milk formulas reported concentrations to be tenfold higher than in breast milk; thus confirming infant milk formulas as an exposure route of toxic elements to infants (29). Many reports indicate the presence of heavy metals in milk, and often it is needed to assess the levels of heavy metals in food. Lead, cadmium and mercury residues in milk are of particular concern because milk is largely consumed by infants and children (30,31). The determination of these heavy metals levels in milk is particularly attended by international organizations (32).

The present study revealed that the mean level of lead in infant milk formulas collected from Alexandria, Egypt ranged from 0.134 mg/l in extra care formulas to 0.332 mg/l in brand 3 with significant variations among different types of milk formulas, $P \leq 0.05$. It should be noted that food is one of the factors which most frequently put infants and little
children at risk of contact with lead. Numerous studies demonstrated that food addressed to this particular group of consumers is not safe as far as the content of this toxic element is concerned (33, 34). Other studies reported lower mean lead concentrations, 0.00016 mg/l in powdered milk in Romania (16), 0.003 mg/l in different Spanish infant formulas (35), 0.018 ppm in different branded infant formulae and baby foods from different area and pharmacies of Riyadh region; Saudi Arabia (8). A Nigerian study reported that lead was not detected in any formulae collected from developing countries while it was detected by a level of 0.400 mg/l in formulae from developed countries (36).

According to the Indian Standard, IS/2006, stipulating that maximum lead contents in milk formulas shall not exceed 0.2 mg/l, all samples of the present study comply with this standard except for ordinary formula brand 3 (0.332) and lactose free formulas (0.314), Pb particles may travel very long distances and may be mixed with the soil and water through rainfall. In districts close to waste yards, air, drinking water, and food may be contaminated with Pb (37, 38). The present study revealed that the average lead levels exceeded this acceptable level in all infant milk formulas moreover, the levels were three times higher in Ordinary formula "brand 3" and lactose free formulas. The Egyptian Standard (ES: 7136 / 2010) states that the maximum level for lead must be 0.02 (mg/kg wet weight) (39). Our study revealed that the average lead levels not exceeded this acceptable level in all tested infant milk formulas except in Ordinary formula "brand 3" and lactose free formula.

Cadmium is ubiquitous environmental contaminant arising primarily from electroplating, plastics manufacturing, mining, paint pigments, alloy preparation, and batteries. Food is the most important source of cadmium in the non-smoking, non-occupationally exposed population (40). Cadmium causes tissue damage in humans and animals and many toxicological studies have found the functional and structural changes in the kidneys, liver, lungs, bones, ovaries and fetal effects (41). The present study revealed that the mean cadmium level in infant milk formulas ranged from 0.0010 mg/l in brand 1 ordinary formulas to 0.0038 mg/l in extra care formulas (0.0038) mg/l with significant
variations, $P < 0.05$, among different types of milk formulas collected from Alexandria, Egypt. These levels are lower than mean level reported by a Saudi Arabian study (0.007 mg/l) (8) but higher that reported by a Spanish study (0.00071 mg/l) (35). The mean levels of cadmium in infant milk reported in the present study comply with Cadmium levels stated in both the Indian Standard (shall not exceed 0.1 mg/kg) (37) and the EC Regulation by American public Health Association (shall not exceed 0.005 mg/l) (42). Our study revealed that the acceptable limit of cadmium was exceeded for all formulas except for ordinary formula (brand 1), whereas in all the remaining formulas the content of lead was significantly higher than the norms.

**CONCLUSION**

There were significant variations in lead and cadmium contents among different types of infant milk formulas and not all samples complied with acceptable levels lead specified in the Egyptian Standard although cadmium levels complied with EC Regulation by American public Health Association and Indian Standards. No data on lead and cadmium contents was declared on the label of the formulas to compare with their actual contents. Further studies are required to evaluate the heavy metal contents of infant formulas on a greater number of samples and a wider diversity of brands with a need to investigate the sources of their contaminant.

**Conflicts of Interest**

The authors indicated no potential or actual conflict of interest pertaining to this study.

**Authors' Contributions**

All authors made full contribution to data acquisition, interpretation of results, drafting and revising the final manuscript. All authors read and approved the final manuscript.

**Study Limitations**
Limited number of infant formula samples were collected and analyzed due to financial constraints.

**Competing interest**

Authors declare that they have no competing interests.

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Table 1: Lead and cadmium contents (ppm) of infant milk formulas collected from Alexandria, Egypt

<table>
<thead>
<tr>
<th>Heavy Metal Content</th>
<th>Infant Milk Formulas</th>
<th>Ordinary #</th>
<th>Lactose free</th>
<th>Extra care</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Lead</td>
<td>Minimum</td>
<td>0.147</td>
<td>0.123</td>
<td>0.33</td>
</tr>
<tr>
<td></td>
<td>Maximum</td>
<td>0.153</td>
<td>0.153</td>
<td>0.336</td>
</tr>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>0.150±0.003</td>
<td>0.142±0.016</td>
<td>0.332±0.003</td>
</tr>
<tr>
<td></td>
<td><em>P value</em></td>
<td><em>P ≤ 0.05</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cadmium</td>
<td>Minimum</td>
<td>0.0007</td>
<td>0.0013</td>
<td>0.0007</td>
</tr>
<tr>
<td></td>
<td>Maximum</td>
<td>0.0013</td>
<td>0.0027</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td>Mean ± SD</td>
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<td>0.002±0.0007</td>
<td>0.0018±0.011</td>
</tr>
<tr>
<td></td>
<td><em>P value</em></td>
<td><em>P ≤ 0.05</em></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*# From three commercial brands (1, 2 and 3)*
Figure 1: Mean lead contents (part per million, ppm) of infant milk formulas collected from Alexandria, Egypt
Figure 2: Mean cadmium contents (part per million, ppm) of infant milk formulas collected from Alexandria, Egypt