ANALYSIS OF TOXIC HEAVY METALS (ARSENIC, LEAD, AND MERCURY) IN SELECTED INFANT FORMULA MILK COMMERCIALLLY AVAILABLE IN THE PHILIPPINES BY AAS
Gian Carlo Cruz, Zaheer Din, Christian Dale Feri, Angela Mae Balaoning, Eva Marie Gonzales, Hannah Mia Navidad, Ma. Margot Flor Schlaaff, Jennifer Winter

ABSTRACT

Objective: To test for the presence of toxic heavy metals, specifically Arsenic, Lead and Mercury in formula milk available in the Philippines for infants aged 6 to 12 months through Atomic Absorption Spectrophotometry. And to compare the results gathered to an existing standard for allowable amounts of toxic heavy metals in food products as set by the World Health Organization.

Design: Experimental Study

Main Outcome Measures: The presence or absence of toxic heavy metals (Lead, Arsenic and Mercury) in selected infant formula milk and their levels within or beyond standards set by the WHO.

Results: Of the three infant formulas tested, all were negative for Arsenic and Lead, while two out of the three infant formulas tested positive for Mercury with levels of 0.6333ppm and 0.8333ppm. The levels of Mercury obtained, expressed in parts per million (ppm), from the two infant formulas tested were above the Provisional Tolerable Weekly Intake of total Mercury, which is 0.005ppm, as set by the Food and Agriculture Organization/World Health Organization Joint Expert Committee on Food Additives (JECFA, 2003).

Conclusion: Two out of three milk samples tested were positive for Mercury in concentrations beyond the allowable limits. There are numerous milk products in the market, not only for infants aged 6-12 months but for other age groups as well, but the researchers were only able to test three of these for the presence of heavy metals. It therefore cannot be generalized from this study that all of the infant formula milk available contains toxic heavy metals. However, this study can be used as a reference or a foundation for future in-depth studies. This research can also serve as an eye-opener to consumers, manufacturers, and the professionals in the health care system due to possible direct or cumulative effects it may cause.

INTRODUCTION

Numerous studies have been published recognizing the importance of breast milk for the growth, development, and long term health of infants. Republic Act No. 7600, also known as the Rooming-In and Breastfeeding Act of 1992, promotes breastfeeding since it not only strengthens the mother-infant relationship; it is also the first preventive measure that can be given to the child at birth (DOH). This act adopts rooming-in, or placing the newborn in the mother’s room after delivery, as a national policy to encourage, protect, and support the practice of breastfeeding.
(DOH). Despite the ratification of RA 7600, the proportion of babies who are exclusively fed on breast milk in their first six months dropped from 20% in 1998 to 16% in 2003 (Conde, 2007).

Mothers are consistently being reminded about the necessity to breastfeed their infants. However, some instances, like the presence of hypogalactia, inverted nipple, nipple tenderness, and other medical conditions prohibiting breastfeeding are inevitable, thus the need for infant formula milk. Infants, particularly in the 6-12 month age group, are vulnerable to infection due to their immature immune system. This is also the time when they are weaned from a pure breast milk diet to one with solid foods. Infant formula milk is one of the primary foods infants ingest during this period. Infant formula is defined as, when in liquid form, may be used either directly or diluted with water before feeding, as appropriate. In powdered form it requires water for preparation (Codex Stan 72-1981).

Heavy metals are described as those metals which, in their standard state, have a specific gravity (density) of more than about 5 g/cm³ (IOCCC, 1996). 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.

Since Arsenic, Lead, and Mercury ranked as the top three most hazardous substances in the said priority list, the researchers formulated this study to determine the presence or absence of these toxic heavy metals in selected samples of infant formula milk using Atomic Absorption Spectrophotometry (AAS). Presence of these heavy metals is to be measured in parts per million (ppm), and the obtained values are to be compared to the Provisional Tolerable Weekly Intake (PTWI) for toxic metals as set by the Food and Agriculture Organization/World Health Organization Joint Expert Committee on Food Additives (JECFA)

The PTWI (Provisional Tolerable Weekly Intake), as set by the Food and Agriculture Organization/World Health Organization Joint Expert Committee on Food Additives (JECFA), is the maximum amount of a contaminant to which a person can be exposed per week over a lifetime without an unacceptable risk of health effects. The level is provisional because it is subject to review when new information becomes available. The PTWI for toxic metals is set by the Food and Agriculture Organization/World Health Organization Joint Expert Committee on Food Additives (JECFA) levels were originally set in 1982 for infants and children, based on studies conducted with children. In 1993 the adult level was withdrawn and the infant and child level extended to all age groups.

A study entitled, “Determination of Major and Minor Elements in Milk Through ICP-AES (Inductively Coupled Plasma Atomic Emission Spectrometry)
was published by researchers in Romania and the results revealed that cow milk samples generally contain sufficient quantity of trace elements. Although toxic heavy metals were excluded from the study, their results proved that dry ashing procedure is a precise and accurate sample preparation of powdered, fresh, and pasteurized milk samples.

In another study conducted by Gundacker, et al (2002) entitled, “Lead and Mercury in Breast Milk”, it was shown that the Mercury present in breastmilk and cow milk were significantly higher than those found in infant formulas. Lead levels in breast milk are normally lower than in milk-based infant formulas (Gundacker, et al. 2002). In one of the studies cited by this journal article, it was reported that reconstitution of infant formula milk with tap water contaminated with heavy metals can result in much higher Lead concentrations. The results of the present study, however, did not reveal the presence of Lead in any of the samples tested.

Another study (Talat Zamir, et al. 2001) showed that concentrations of Lead and Cadmium in whole cream powdered milk and infant formula milk were found to be within the safe limits as recommended by WHO. The present study indicates that the infant formula milk samples tested are also contaminated with Mercury, whose concentrations have been found to exceed the allowable levels of heavy metals in food.

The studies cited above are studies used as reference and backbone to the current study. The current study is significant to the society in general. This study is to serve as a realization about the current situation of food handling and manufacture and its implication on the health of the consumers. It is to serve as an eye-opener for all members involved in the infant formula handling, including manufacturers, distributors, consumers as well as health care professionals who prescribe these products.

METHODS
Study Design
An experimental method of research was performed to assess the presence or absence of toxic heavy metals in selected infant formula milk; and the concentration of each heavy metal when present.

Research Organization and Procedure
Collection of Milk Samples
Three hospitals in Baguio City were selected randomly by drawing lots, of which three pediatricians from each of these hospitals were chosen randomly to complete a questionnaire regarding the three infant formulas they most commonly prescribe. Once the data was collated and tallied, the top 3 infant formula milk samples were bought.

Inclusion and Exclusion Criteria
Inclusion criteria for the milk samples are: (a) formula milk that are commercially available in the Philippines, but not necessarily manufactured in the Philippines; (b) formula milk recommended for infants ages 6-12 months, as stated on the label; (c) should meet the criteria of Codex Stan 72-1981; (d) must be originally contained and distributed in tin foil; and (e) must not be expired and should be
manufactured in the year 2008 regardless of the month. Exclusion criteria are: (a) formula milk that are not commercially available in the Philippines; (b) formula milk recommended for infants beyond 12 months of age, as stated on the label; (c) formulation that failed to meet the criteria of Codex Alimentarius 72-1981; and (d) formula milk that is expired and not manufactured in the year 2008, regardless of the month.

These samples were then brought to the Natural Sciences Research Unit, Saint Louis University, Baguio City by a third party in their original, unopened tin pack containers devoid of their boxes, and were subjected to analysis by the facility technician. The researchers as well as the facility technician were blinded as to what specific milk samples were labeled X, Y and Z respectively. The facility technician then labeled these samples as a third party was present to observe the entire labeling process and made sure no samples were tampered with.

**Preparation of Milk Samples for AAS**

5 grams from each milk sample was placed in different crucibles and heated in a maple furnace at 700°C for 3 hours to vaporize all other constituents and leave the heavy metals as a pure ash. The ash was cooled to room temperature before being dissolved in a 5 ml solution of nitric acid (1:6) to compound with the heavy metals, if present. The solution was subsequently heated and evaporated to half its volume using a hot plate. The resulting solution was then poured into a volumetric flask, or Erlenmeyer flask, and topped up to 25 ml with distilled water.

**Preparation of Standard of Selected Heavy Metals**

The selected heavy metals were Arsenic, Lead, and Mercury. For each of the selected metals, three standards were set for the calibration of the AAS. These are as follows: 1.0000 ppm, 1.5000 ppm, and 2.0000 ppm. The calibration curve of well prepared standards and an accurate Atomic Absorption Spectrophotometer should present as a linear curve.

**Atomic Absorption Spectrophotometric Analysis**

Analysis of the heavy metal contents in the milk samples was done with the use of the Atomic Absorption Spectrophotometer (AAS) following the 7000B Method of EPA (Environmental Protection Agency) for flame absorption spectrophotometry. The AAS not only detects the presence of heavy metals, but, if present, it is also designed to provide the concentrations in parts per million (ppm). Three trials were run on each milk sample in every replicate of the heavy metal and the averages of the concentrations were then taken and compared to Provisional Tolerable Weekly Intake as stated by the Food and Agriculture Organization/World Health Organization Joint Expert Committee on Food Additives (JECFA).

Atomic Absorption Spectrophotometry relies heavily on the Beer-Lambert Law. The electrons of the atoms in the atomizer can be promoted to higher orbitals for a short amount of time by absorbing a set quantity of energy i.e. light of a given wavelength. This amount of energy or wavelength is specific to a particular electron transition in a particular element, and in general, each wavelength corresponds to only one
element. This gives the technique its elemental selectivity. As the quantity of energy put into the flame is known, and the quantity remaining at the other side (at the detector) can be measured, it is possible, for Beer-Lambert law, to calculate how many of these transitions took place, and thus get a signal that is proportional to the concentration of the element being measured. (Wikipedia, 2009)

Data Assessment

Data was gathered from all trials and organized in tables. The concentrations, in parts per million (ppm), of milk samples that yielded positive results for the presence of heavy metals were converted into mg/kg body weight, then compared to the Provisional Tolerable Weekly Intake limit of these heavy metals as set by the Food and Agriculture Organization/World Health Organization Joint Expert Committee on Food Additives (JECFA). The results were simply assessed as values greater than or within the tolerable weekly limit of toxic heavy metals. No statistical analysis was employed.

RESULTS

Calibration curves for each heavy metal were set to ensure the accuracy of the Atomic Absorption Spectrophotometer and to establish that results of the determination proper were true and reliable. Standards with the concentration of 1.000 ppm, 1.500 ppm, and 2.000 ppm, respectively, were set for the calibration of the Atomic Absorption Spectrophotometer. The calibration curve of well prepared standards and an accurate AAS should present as a linear curve. The data on the calibration for Arsenic, Lead and Mercury are seen in Figures 1, 2 and 3 respectively.

![Figure 1: Arsenic Calibration Curve (C#:01).](image-url)
Following the proper calibration of the Atomic Absorption Spectrophotometer, as evidenced by the linear calibration curves, the formula milk samples were tested and rerun twice for the heavy metals: Arsenic, Lead and Mercury. The data on these tests are presented in Tables 1, 2, and 3 respectively. Table 4 presents a summary of the average concentrations in all toxic heavy metals for all samples. All data is expressed as parts per million (ppm).

Table 1: As (193.7) AAS Readings in ppm.

<table>
<thead>
<tr>
<th>Milk</th>
<th>Trial 1</th>
<th>Trial 2</th>
<th>Trial 3</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>-5.3217</td>
<td>-5.0892</td>
<td>-6.2985</td>
<td>-5.5775</td>
</tr>
<tr>
<td>Y</td>
<td>-2.6938</td>
<td>-2.6008</td>
<td>-2.7868</td>
<td>-2.6938</td>
</tr>
<tr>
<td>Z</td>
<td>-3.5078</td>
<td>-2.8333</td>
<td>-2.4380</td>
<td>-2.9264</td>
</tr>
</tbody>
</table>

Figure 2: Lead Calibration Curve (C#:01)

Figure 3: Mercury Calibration Curve (C#:01)
Table 2: Lead (283.3 nm) AAS Readings in ppm

<table>
<thead>
<tr>
<th>Milk</th>
<th>Trial 1</th>
<th>Trial 2</th>
<th>Trial 3</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>-0.7500</td>
<td>-0.7197</td>
<td>-0.7045</td>
<td>-0.7273</td>
</tr>
<tr>
<td>Y</td>
<td>-0.7273</td>
<td>-0.6970</td>
<td>-0.6515</td>
<td>-0.6894</td>
</tr>
<tr>
<td>Z</td>
<td>-0.6894</td>
<td>-0.7424</td>
<td>-0.6472</td>
<td>-0.7045</td>
</tr>
</tbody>
</table>

Table 3: Hg (253.7 nm) AAS Readings in ppm.

<table>
<thead>
<tr>
<th>Milk</th>
<th>Trial 1</th>
<th>Trial 2</th>
<th>Trial 3</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>0.8333</td>
<td>0.8333</td>
<td>0.2333</td>
<td>0.6333</td>
</tr>
<tr>
<td>Y</td>
<td>-2.3667</td>
<td>-2.3667</td>
<td>-2.3667</td>
<td>-2.3667</td>
</tr>
<tr>
<td>Z</td>
<td>1.8333</td>
<td>0.8333</td>
<td>-0.1667</td>
<td>0.8333</td>
</tr>
</tbody>
</table>

Table 4: Summary of AAS Readings in ppm

<table>
<thead>
<tr>
<th>Milk</th>
<th>Arsenic (As)</th>
<th>Lead (Pb)</th>
<th>Mercury (Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>-5.5775</td>
<td>-0.7273</td>
<td>0.6333</td>
</tr>
<tr>
<td>Y</td>
<td>-2.6938</td>
<td>-0.6894</td>
<td>-2.3667</td>
</tr>
<tr>
<td>Z</td>
<td>-2.9264</td>
<td>-0.7045</td>
<td>0.8333</td>
</tr>
</tbody>
</table>

After running the samples, the results revealed that milk samples X, Y, and Z are all negative for both Lead and Arsenic but both samples X and Z were positive for Mercury. Milk sample X had an average concentration of 0.6333 ppm Mercury, while milk sample Z had an average concentration of 0.8333 ppm Mercury.

The concentration of heavy metals positive in the infant formula milk samples were compared to the provisional tolerable weekly intake of toxic heavy metals as set by the Food and Agriculture Organization/World Health Organization Joint Expert Committee on Food Additives (JECFA) (Table 5). Table 6 illustrates the comparison between Mercury content in samples X and Z and the provisional tolerable weekly intake.

Table 5: Provisional Tolerable Weekly Intake of Toxic Heavy Metals (WHO, 2003)

<table>
<thead>
<tr>
<th>Heavy Metal</th>
<th>Amount in mg/kg body weight (1 mg/kg or 1 mg/L = 1 ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arsenic</td>
<td>PTWI 0.015</td>
</tr>
<tr>
<td>Lead</td>
<td>PTWI 0.025</td>
</tr>
<tr>
<td>Total Mercury</td>
<td>PTWI 0.005</td>
</tr>
</tbody>
</table>
Table 6: Comparison between Provisional Tolerable Weekly Intake of Total Mercury and Actual Concentrations from Milk Samples

<table>
<thead>
<tr>
<th>Milk sample</th>
<th>Concentration in ppm</th>
<th>Concentration in Mg/kg body weight</th>
<th>Maximum allowable limit in mg/kg body weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>0.6333</td>
<td>0.6333</td>
<td>0.005</td>
</tr>
<tr>
<td>Y</td>
<td>-2.3667</td>
<td>--------</td>
<td>0.005</td>
</tr>
<tr>
<td>Z</td>
<td>0.8333</td>
<td>0.8333</td>
<td>0.005</td>
</tr>
</tbody>
</table>

The PTWI of total Mercury is 0.005 mg/kg. However, the infant formula labeled as X had a concentration of 0.6333 mg/kg while formula Y had a concentration of 0.8333mg/kg. The concentration of Mercury in both milk samples that tested positive are above the Provisional Tolerable Weekly intake as set by WHO and JECFA.

**DISCUSSION**

The presence of heavy metals in the infant formula, Mercury at that, is an alarming finding not only because it is in a food product but because it was detected in a food product specifically for infants. Moreover, the amount of Mercury was much higher than the tolerable weekly intake limits, which makes it even more contentious.

The contamination of the infant formula milk tested has an impact on the health of the society in general. It has an implication on the manufacturers, the distributors, the consumers and all parties involved in the cycle of handling the product.

Although the study does not aim to point out the source of contamination, there are probable sources. Heavy metals accumulate in the water supply through human activity, such as industrial and consumer waste. Commercial processes like mining, agriculture, manufacturing and the discarding of wastes in landfills are common sources of heavy metal contamination. Even rainwater, with its acidic pH, can cause these compounds to leach into the surface and underground water supplies from the surrounding soil and rock. (Bock, 2005)

Mercury exposure and toxicity is far more common than most of us tend to think. Our environment has become highly contaminated with mercury as a result of the burning of fossil fuels, particularly coal, for energy. Mercury normally occurs in the Earth's crust, but is released either by burning coal, or through incineration of mercury-containing equipment such as thermometers, blood pressure cuffs, electrical switches, thermostats and computer chips. Coal-burning and medical waste incineration are the two biggest sources of mercury contamination in our environment. (Jackson, 2005) Due to the various factors that may contribute to the contamination, it is vital to find the specific source of the problem.

The findings of this research have an implication on decisions that health care professionals have to make as well. The administration of infant formula is
inevitable due to a number of reasons. Many women cannot or choose not to breastfeed even though breast milk is universally recognized as the best source of nutrition for infants and is recommended to be the exclusive food for the first 6 months of life. A baby’s health may depend on safe and nutritious alternatives to breastfeeding in a variety of circumstances such as; the baby fails to thrive because the mother's milk alone does not satisfy the baby's nutritional needs, such as, the mother has an inadequate diet or in cases of multiple births; the baby is at risk because it was born prematurely or with metabolic or other nutritional disorders; the mother’s healthcare provider advises her not to breastfeed because she suffers from an infectious disease such which could be transmitted to the baby; the mother is receiving medications that may be excreted in the breast milk; or she has been exposed to hazardous environmental agents, such as lead; the mother frequently uses addictive drugs or alcohol. (International Association of Infant Food Manufacturers, 2003)

As health care professionals are confronted with the problems stated above, the only solution would be to resort to an infant formula, which is the paramount alternative to breast milk. Health care professionals, doctors at that, who prescribe the infant formula, with the aim to uphold the health of the infant, are met with the dilemma on whether this is really the best choice. It can be added, that this is also takes into consideration that the study was done locally, on the most prescribed infant formulas by doctors.

The results of the study lead to a core dilemma which affects the most crucial member of the process – the consumer, in the person of the infants taking the infant formula. And the end point of the study is the focus on Heavy Metal Toxicity, more specifically, Mercury toxicity.

Heavy metal toxicity is frequently the result of long term low level exposure to pollutants common in our environment: air, water, food and numerous consumer products. Exposure to toxic metals is associated with many chronic diseases. Recent research has found that even low levels of lead, mercury, cadmium, aluminum and arsenic can cause a wide variety of health problems (Jackson 2006). The toxicity of heavy metals is usually cumulative in nature. In this case, infants ingest amounts of Mercury beyond the tolerable weekly intake of an adult through the infant formula.

Unfortunately, most physicians are not familiar enough with the harmful effects of mercury to consider this as a cause of many diseases. Mercury has a particular affinity to become deposited in vital organs such as the brain and nervous system, the heart, the liver, kidneys and bone marrow. Mercury is also known to cause dementia (loss of mental capacity), peripheral neuropathy (damage to nerves in the body), Parkinson's disease, and cancer. Mercury is known to present a particular hazard to children and infants. Because the developing brains of children and infants are much more susceptible to the harmful effects of mercury, both the American Academy of Pediatrics and the American College of Obstetrics and Gynecology have issued warnings about mercury exposure to children and infants. (Jackson 2006)
It can be deduced from the information collated that the health of the infant could be at stake in these circumstances. Thus it is critical that the source of contamination be determined and appropriate action be undertaken by involved parties, which include the manufacturers and distributors at most, as well as the consumers.

This study is limited to the determination of the presence or absence of only three toxic heavy metals, namely, Arsenic, Lead, and Mercury, in infant formula milk, and to compare the levels acquired, if any, to the maximum allowable limits stated by Food and Agriculture Organization/World Health Organization Joint Expert Committee on Food Additives (JECFA). It does not determine the source of the contaminants in the milk samples. The study was limited to testing only three milk samples since infant formula milk and the procedure are expensive. In addition, the three milk samples tested, which were the top three infant formula milk commonly prescribed by pediatricians, is not necessarily synonymous to the infant formula milk consumed by the patients.

In every research, sources of bias cannot be avoided. The most important of which is human error. Errors in the performance of the test may have occurred. Technical/ Systematic errors could also influence the accuracy and precision of results such as inevitable deterioration in the performance of the equipment as well as the ageing of reagents. Furthermore, the samples were not tested by the facility technician on the same day, leading to different conditions when the tests were performed.

CONCLUSION

The study aimed to test for the presence of heavy metals, specifically Arsenic, Lead and Mercury in infant formula milk for infants aged 6-12 months and to establish whether the concentrations of these heavy metals present are within standards set by the World Health Association.

Three types of infant formula milk were subjected to testing and results were collated. All of the infant formula milk samples tested were negative for both Lead and Arsenic; however, two of these milk samples were positive for Mercury (sample X = 0.6333 ppm; sample Z = 0.8333 ppm) in amounts above the maximum allowable limit, as stated by the Food and Agriculture Organization/World Health Organization Joint Expert Committee on Food Additives (JECFA).

The present research consists of a small population, in terms of the number of milk samples. There are numerous milk products in the market, not only for infants aged 6-12 months but for other age groups as well, but the researchers were only able to test three of these for the presence of heavy metals. It therefore cannot be generalized from this study that all of the infant formula milk available contains toxic heavy metals. However, this study can be used as a reference or a foundation for future in-depth studies. This research can also serve as an eye-opener to consumers, manufacturers, and the professionals in the health care system due to possible direct or cumulative effects it may cause.

Since the study revealed significant concentrations of Mercury in two of the
milk samples, it is recommended that a more specific confirmatory test be performed and more replicates be tested by future researchers. It is also recommended that further studies to find out whether the Mercuric concentrations in the infant formula milk are significant enough to cause clinical manifestations and to compare the Mercury concentration to other established limits for heavy metals, since the limits set by the Food and Agriculture Organization/World Health Organization Joint Expert Committee on Food Additives (JECFA) is the only basis of the present study for the maximum allowable limit of Mercury in food.