



Toxic Chemicals Associated With Milk Powder Consumption

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ABSTRACT

A total of 60 samples of milk powder representative as 40 canned and 20 un-canned milk powder were collected from groceries and supermarkets from different localities in Alexandria city for detection of toxic heavy metal residues including (Pb, Cd, Al and tin) and mycotoxins including (AFM1, AFM2 and Ochratoxin A); The obtained results could be summarized as Lead was detected in 6 (15%) and 7 (35%) of examined canned and un-canned milk powder with a mean values of 0.25 ± 0.06 and 0.12 ± 0.04 ppm, the samples exceeded the permissible limit 3(50%) and 4(57.14%), respectively. Cadmium residues was detected in 5 (12.5%) and 6 (30 %) of examined (canned and un-canned milk powder) with a mean values of 0.08 ± 0.02 and 0.11 ± 0.02 ppm, the samples exceeded the permissible limit 2 (40%) and 3 (50%), respectively. Aluminium was detected in 3 (7.5%) and 5 (25 %) of examined canned, and un-canned milk powder and the respective Aluminium level were mean values of 0.45 ± 0.12 and 0.37 ± 0.15 ppm, the samples exceeded the permissible limit 1(33.33%) and 4(80%), respectively. Tin was detected in 2 (5%) and 8 (40 %) of examined canned, and un-canned milk powder with a mean values of 26.8 ± 5.13 and 49.9 ± 5.50 ppm, respectively. All positive samples within the permissible limit.

AFM1 was detected in 2 (5%) and 3 (15%) in examined milk powder (canned and un-canned) samples with respective mean values of 0.033 ± 0.02 and 0.507 ± 0.21 ppb, the samples exceeded the permissible limit 0 (0%) and 3 (100%), respectively. AFM2 was detected in 1 (2.50 %) in examined milk powder (canned) samples with a mean values of 0.013 (ppb), and the positive samples within permissible limit. Ochratoxin A "OTA" was not detected in all (canned, and un-canned) milk powder samples.

1. INTRODUCTION

Milk and dairy products may contain varying amounts of different toxic contaminants (Ataro *et al.*, 2008). The level of toxic metals are an important component of safety and quality of milk and dairy products. Metals are widely released in the environment and have two major origins: human activities and geological background (Loska *et al.*, 2004) where they are present in soil through fertilizers or following atmospheric deposition and from natural weathering of the bedrock. Canned dairy products are considered source of heavy metals due to migration of metals from equipment to product through prepetition and during long storage period or damaged equipment (Oskarsson and Norrgren, 1998). Industrial and agricultural processes have resulted in an increase concentration of heavy metals in air, water, soil and subsequently,

these metals are taken by plants or animals and take their ways into food chain (Ahmad, 2002).

Exposure to toxic metals is associated with many chronic diseases. Recent research found that even low levels of lead, mercury, cadmium and aluminum can cause a wide variety of health problems (Gian *et al.*, 2009). It connected to Alzheimer's, Parkinson's, autism, lupus, amyotrophic lateral sclerosis, cardiovascular disease, depressed growth, impaired fertility, nervous and immune system disorders, increased spontaneous abortions, and elevated death rate among infants (Yuzbasi *et al.*, 2003 and Jack, 2005). Although individual metal exhibit specific signs of their toxicity, the following have been reported as general signs associated with cadmium, lead, and aluminum poisoning: gastrointestinal (GI) disorders, diarrhea, stomatitis, tremor, hemoglobinuria causing a rust-red color to stool, ataxia, paralysis, vomiting

and convulsion, depression, and pneumonia when volatile vapors and fumes are inhaled (McCluggage, 1991). The nature of effects could be toxic (acute, chronic or sub-chronic), neurotoxic, carcinogenic, mutagenic or teratogenic.

Aflatoxins are natural toxic compounds produced mainly by *Aspergillus* species of fungi and commonly found in the tropical and subtropical feeds. They are extremely toxic, immunosuppressive, carcinogenic, mutagenic and teratogenic substances and are known to induce hepatic carcinogenesis in humans. The most frequent aflatoxins are B1, B2, M1 and M2. Aflatoxin B1 (AFB1) is especially the common one and has been reported as the most powerful natural carcinogen in human and animals (Awad *et al.*, 2012).

Therefore, this study was planned to throw the light on some toxic heavy metals that may be reach milk powder and the serious health hazards that may be associated with exposure to AFM1, AFM2 and Ochratoxin A.

2. MATERIAL AND METHODS

2.1. Samples collection:

A total of 60 samples of milk powder representative as 40 canned and 20 un-canned samples were collected randomly from groceries and supermarkets at Alexandria City, Egypt. Samples were obtained as sold to the public and transferred to central laboratory of faculty of Veterinary Medicine, Banaha University to be examined.

2.2. Determination of heavy metal levels:

The collected samples were examined for detection and the levels of lead, cadmium, aluminium and tin on the basis of wet weight (mg/Kg).

2.3. Washing procedures (AOAC, 2000):

2.3.1. Digestion technique:

Accurately, 2 g of each sample were digested by 10ml of digestion mixture (60 ml of 65% Nitric acid and 40 ml of 70% perchloric acid) in screw-capped tube (Tsoumbaris and Papodoulou, 1994). The tubes were tightly closed and the contents were vigorously shaken and allowed to stand overnight at room temperature. Moreover, the tubes were heated for 4 hours in water bath starting from 60°C till reach 110 °C ensure complete digestion of the samples. The digestion tubes were vigorously shaken at 30 minutes intervals during the heating period. The tubes were then left to cool to room temperature and diluted with 1ml deionized water (30%) as well as reheated in the water bath at 70°C to ensure complete digestion of the samples. At this point, all organic matrixes have been

destroyed. Each tube was diluted with deionized water till reach 25 ml and the digest was filtered with Whitman filter paper No. 42. The filtrates were collected in Pyrex glass test tubes capped with polyethylene film and kept at room temperature until analyzed for their lead, cadmium, chromium and copper concentrations.

2.3.2. Preparation of blank and standard solutions:

Instrumental procedures for various analyses were based on those suggested in the operator manual of the Atomic Absorption Spectrophotometer. However, blank and standard solutions were prepared in the same manner as applied for wet digestion and by using the same chemicals (Shibamoto and Bjeldanes, 2000). Blank solution consisted of 10 parts of Nitric acid and 1 part of H₂O₂ then was diluted with 25 parts of deionized water and filtered. The blank was used to determine the metal contamination which may be present in the chemicals and its value was discounted from the end calculated results. Furthermore, the standard solutions using pure certified metal standards at different strengths were prepared by 10 parts of nitric acid and 1 part of H₂O₂ then was diluted with 25 parts of deionized.

2.4. Analysis:

The digest, blanks and standard solutions were aspirated by Flame Atomic Absorption Spectrophotometer (VARIAN, Australia, model AA240 FS) and analyzed for calcium concentration. The apparatus has an auto sampler, digital absorbance and concentration readout capable of operating under the following conditions recommended by the instrument instruction:

5. Quantitative determination of heavy metal residues:

Absorbency of lead, cadmium, chromium and copper was directly recorded from the digital scale of and its concentration was calculated according to the following equation:

$C = R \times (D/W)$ Where, C= Concentration of heavy metal (wet weight). R= Reading of digital scale of AAS.

D= Dilution of the prepared sample. W= Weight of the sample.

N. B. The concentration of each heavy metal in the blank solution was also calculated and subtracted from each analyzed sample.

Determination of aflatoxins: Shundo and Sabino, (2006)

Determination of Ochratoxin A: Toscan *et al.*, (2007)

Heavy metal condition	Lead	Cadmium	Aluminium	Tin
Lamp wavelength (nm)	283.3	228.8	275.6	241.4
Lamp current (m/amp)	10	4	12	10
Fuel flow rate	1.4	1.2	1.4	1.2
Used gas	Argon	Argon	A-AC **	Argon
Measurement time (seconds)	4.0	4.0	4.0	4.0
Detection limit (ppb)	8-40	0.2-0.8	0.5-1.0	5-20

N₂O/A/AC* = Nitrous oxide/Air/Acetylene A-AC** = Air/Acetylene

2. RESULTS AND DISCUSSION

Toxic metals mainly lead is the most common air pollutants and are emitted into the air as a result of various industrial activities (WHO, 2007). Various industrial environmental contamination of soil, waters, foods and plants with these metals cause their incorporation into the food chain and impose a great threat to human and animal health (Bilandzic *et al.*, 2011).

The obtained data in Table (1) showed that 6 (15%) and 7 (35%) of examined milk powder samples (canned and un-canned) were contaminated with lead and its level ranged from 0.014-0.45 and 0.012-0.29 with mean values of 0.25 ± 0.06 and 0.12 ± 0.04 ppm, respectively. Form the positive samples 3 (50%) and 4 (57.14%) of canned and un-canned milk powder exceeding the permissible limit which stated by Egyptian standards (2010) must not exceed (0.02 ppm).

It is obviously that un-canned milk powder is more contaminated than canned one. It may be due to the sachet is still opened until all the quantity is completely sold so, the probability to be contaminated is higher than canned one.

WHO, (1972) reported that the provisional tolerable intake of lead is 3 mg/person or 0.05 mg/kg body weight while for infants and children lead level is reduced to 0.025 mg/kg body weight.

US Environmental Protection Agency (2003) stated that the public health hazards of the detected metals, Pb is a potent neurotoxin for which no safety threshold has yet been found. It may cause damage to kidneys, the cardiovascular, immune, hematopoietic, central nervous and reproductive systems. Short term Exposure to high level of lead can cause gastrointestinal distress, anaemia, encephalopathy and death.

Table (1): Lead levels (ppm) in the examined milk powder samples and Comparison with Egyptian standards (2010).

Product milk powder	No. of Examined samples	Positive samples		Min-Max	Mean \pm SEM	Permissible Limit ES (2010) (ppm)	Samples exceeded permissible limit	
		No.	%				No.	%
Canned	40	6	15	0.014- 0.45	0.25 ± 0.06	0.02	3	50
Un-Canned	20	7	35	0.012- 0.29	0.12 ± 0.04	0.02	4	57.14

Table (2): Cadmium levels (ppm) in the examined milk powder samples and Comparison with Egyptian standards (1993)

Product milk powder	No. of Examined samples	Positive samples		Min--Max	Mean \pm SEM	Permissible Limit ES (1993) (ppm)	Samples exceeded permissible limit	
		No.	%				No.	%
Canned	40	5	12.5	0.01- 0.147	0.08 ± 0.02	0.05	2	40
Un-Canned	20	6	30	0.035- 0.216	0.11 ± 0.02	0.05	3	50

Table (3): Aluminum levels (ppm) in the examined milk powder samples and Comparison with standards reported by Pennington (1987).

Product milk powder	No. of Examined samples	Positive samples		Min--Max	Mean \pm SEM	Permissible Limit ES (1987) (ppm)	Samples exceeded permissible limit	
		No.	%				No.	%
Canned	40	3	7.5	0.33 - 0.73	0.45 ± 0.12	0.5	1	33.33
Un-Canned	20	5	25	0.07- 0.862	0.37 ± 0.15	0.5	4	80

Table (4): Tin levels (ppm) in the examined milk powder samples and Comparison with Egyptian standards (2010).

Product milk powder	No. of Examined samples	Positive samples		Min—Max	Mean \pm SEM	Permissible Limit ES (2010) (ppm)	Samples exceeded permissible limit	
		No.	%				No.	%
Canned	40	2	5	21.6 - 31.9	26.8 ± 5.13	50	0	0
Un-Canned	20	8	40	29.8-74.9	49.9 ± 5.50	50	0	0

Table (5): Aflatoxin M1 levels (ppb) in the examined milk powder samples and Comparison with Egyptian standards (2010).

Product Milk powder	No. of Examined samples	Positive samples		Min--Max	Mean± SEM	Permissible Limit ES (2010)	Samples Exceed permissible limit	
		No.	%				No.	%
Canned	40	2	5	0.017-.048	0.033± 0.02	0.05	0	0
Un-canned	20	3	15	0.104 - 0.821	0.507 ± 0.21	0.05	3	100

Table (6): Aflatoxin M2 levels (ppb) in the examined milk powder samples and Comparison with Egyptian standards (2010).

Product	No. of Examined samples	Positive samples		Min-- Max	Mean± SEM	Permissible Limit ES (2010)	Samples Exceed permissible limit	
		No.	%				No.	%
Canned	40	1	2.5	0.013	0.013	0.05	0	0
Un- canned	20	-	-	-	-	0.05	-	-

Cadmium is considered as one of the most toxic element to human and animal health. It has an extremely long biological half-life in man. Even low exposure level may cause in time considerable accumulation in tissues.

Results recorded in Table (2) showed that the cadmium residue was detected in the examined milk powder samples (canned and un-canned) at incidence of 5 (12.5%) and 6 (30 %) and its level ranged from 0.01-0.147 and 0.035-0.216 with mean values of 0.08 ± 0.02 and 0.11 ± 0.02 ppm, respectively.

According to Egyptian Standards (1993) which stated that cadmium level must not exceed (0.05 ppm) there are 2 (40%) and 3 (50%) of examined milk powder were exceeding the permissible limits.

Some cadmium poisoning symptoms are asthma and kidney complications. It is carcinogenic, especially in lungs and prostate tumours. The complications of cadmium pollution among pregnant women are birth defects, decreased fetal weight, abnormalities in DNA and fetal proteins. Its high infection usually leads to abortion (Abdulkhalik *et al.*, 2012).

The obtained results in Table (3) showed that Aluminium residues was detected in 3 (7.5%) and 5 (25%) of examined milk powder samples (canned and un-canned) and its level ranged from 0.33 - 0.73 and 0.07 - 0.862 with mean values of 0.45 ± 0.12 and 0.37 ± 0.15 ppm, respectively. According to Pennington, (1987) who reported that Aluminium should not exceed (0.5 ppm). One sample of canned milk powder and 4 samples of un-canned milk powder had higher level than (0.5ppm).

Large amounts of Aluminium contamination can be resulted either from the abundant and traditional use of Aluminium containers for production and storage of milk powder or by external environmental contamination during the phases of collection

and transport of milk from the farms to the dairy shops (Arafa *et al.*, 2014).

Aluminium enters the milk and milk products from a variety of sources. Milk gets contaminated before milking, from the feed and fodder fed to the dairy cows. Additionally aluminium can be introduced into the milk and milk products during the production process or by contamination from the metal processing equipment (Deeb and Gomaa, 2011). The use of aluminium utensils for processing and storage of milk may increase substantially the level of this metal in milk and milk products (Semwal *et al.*, 2006) and leaching of this metal from utensils is influenced by the quality of the containers, pH level, preparation conditions and the presence of complexing agents (Al Juhaiman, 2010).

The obtained results in Table (4) showed that Tin residues was detected in 2 (5%) and 4 (40%) of examined milk powder samples (canned, un-canned) ranged from 21.6-31.9 and 29.8-74.9 with mean values of 26.8 ± 5.13 and 49.9 ± 5.50 ppm respectively. According to Egyptian standards, (2010) which stated that tin must not exceed (200 ppm), all positive samples of milk powder (canned, un-canned) are within the permissible limit.

(Abd- El Aal, 2012) reported that the migration of Tin from the surface of the can to the products is caused not only by the length of storage but also, by the consistency of milk product. There is a low chance for contamination of foodstuffs by tin from the surroundings, and the main cause responsible for the tin concentration of foodstuffs is whether or not the food products has been in connection with metallic tin, chiefly tinplate. Therefore, great levels may be found in some processed foods owing to adding of tin-based preservatives and stabilizers, corrosion and leaching of the metal from un lacquered cans, or from tin foils in packing (Greger, 1988).

Results showed in Table (5) declared that AFM1 was detected in 2 (5%), 3 (15%) in

examined milk powder (canned and un-canned) milk powder samples with a range of 0.017-0.048, 0.104-0.821, with mean values of 0.033 ± 0.02 , 0.507 ± 0.21 $\mu\text{g/kg}$ (ppb), respectively, all positive samples were within the permissible limits of Egyptian standards (2010) which stated that AFM1 should not exceed than (0.05 ppb).

Global regulations of AFM1 contamination in milk are varied from one country to the other. It should be less than 0.05 $\mu\text{g/kg}$ in EU, Switzerland, Austria, France, China, Turkey, Japan, Mexico, Thailand, Argentina, and Honduras; less than 0.50 $\mu\text{g/kg}$ in US, Bulgaria; less than 1.0 $\mu\text{g/kg}$ and 0.0 $\mu\text{g/kg}$ in Egypt, Rumania (FAO, 1997).

Data presented in Table (6) showed that AFM2 was detected in 1 (2.5%) in examined canned milk powder with concentration of 0.013 $\mu\text{g/kg}$ (ppm), while in un-canned milk powder AFM2 was failed to be detected, The permissible limits of Egyptian Standards (2010) which stated that AFM2 must not exceed (0.05 ppm).

Ochratoxin A "OTA" was not detected in all (canned, and un-canned) milk powder samples.

Milk and other dairy products are always at risk of being contaminated with aflatoxin M1. Mycotoxin compounds are extremely stable and also dangerous in minute quantities. A few parts per billion are of concern. Once formed, they cannot be removed from the commodity. Milk, as a liquid, is a highly variable product that rapidly loses its quality and spoils if not to be treated. Since milk may be processed in numerous ways, the effects of storage and processing on stability and distribution of AFM1 are of great concern. Many researchers from different countries have carried out studies about the incidence of AFM1 in milk; many authors showed that seasonal effect influences concentration of aflatoxin M1.

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