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CHEMICAL COMPOSITION, METALS CONTENT AND PESTICIDE RESIDUES IN RAW, PASTEURIZED AND UHT MILK AND THEIR DIETARY INTAKE

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ABSTRACT

Chemical composition, metal contents and pesticide residues were determined in raw, pasteurized and UHT (Ultra High Temperature) milk samples collected from local markets in Cairo Governorate, Egypt. Fat, T.P. (Total Protein), ash, lactose and T.S. (Total Solids) contents were (3.50, 3.20, 0.71, 4.10 and 11.40 %), (3.00, 3.50, 0.80, 4.65 and 11.50 %), and (3.00, 3.40, 0.80, 4.44 and 11.50 %) in raw, pasteurized and UHT milk, respectively. Levels of Zn, Fe, Cu, Mn, Cr, Ni, Co, Pb, Cd and Sn were (3.59, 0.62, 0.17, 0.06, 0.03, 0.036, 0.032, 0.032, 0.025 and 0.002 mg/kg), (3.11, 0.55, 0.15, 0.041, 0.030, 0.030, 0.030, 0.021, 0.022 and 0.002 mg/kg) and (3.11, 0.60, 0.180, 0.050, 0.020, 0.030, 0.030, 0.020, 0.020 and 0.002 mg/kg) in raw, pasteurized and UHT milk, respectively. Organochlorine pesticides (HCB, lindane, aldrin, heptachlor, heptachlor epoxide, chlordane, endrin and DDT metabolites) were detected in raw milk at levels higher than the tolerance limits. However, these chemicals were detected in pasteurized milk at slightly higher levels and in UHT milk at levels lower than the tolerance limits. On the other hand, non of raw, pasteurized and UHT milk revealed the presence of organophosphorus pesticides (malathion, profenofos, pirmiphos-methyl and dimethoate). To assess any possible risks to consumers of milk, dietary intakes have been estimated using the mean concentration of various metals and pesticide residues in this monitoring. Data showed that, daily intake of the investigated metals and pesticides in milk were lower than the recommended levels except that Pb and Cd are higher than it.

Keywords: Chemical composition, Metals, Pesticides, Milk, Raw, Pasteurized, UHT, Dietary Intake, Risk.

INTRODUCTION

Milk is one of the basic foods which is liable to be contaminated by toxic substances, such as heavy metals and pesticides. The rationalization of production and identification of quality markers for milk is of great importance.

Heavy metals are a quasi-scientific term, used to describe group of toxic metallic elements and their compounds, of which a few are known to travel long distances through the atmosphere via the grasshopper effect. Some metals such as Cu, Zn, Fe and Mn are essential at very low concentrations and have a variety of biochemical function for the survival of all forms of life. While, they can be toxic when taken in excess, both toxicity and necessity vary from element to element (Tripathi *et al.,* 1999). On the other side, there are different types of metals that called potentially toxic elements such as lead and cadmium. These elements in milk are of particular concern because milk is largely consumed by infants and children (Tripathi *et al.,* 2005). Minerals and trace element concentrations in milk are not constant but mainly vary according to many kinds of factors, those related with secretion from the mammary gland extrinsic factors and environment. In this respect, several studies have been carried out to assess mineral content of milk from different areas (Lante *et al.,* 2004; Hermansen *et al.,* 2005; Sola-Larranaga and Navarno-Blasco, 2008). The high levels of metals in milk in most cases may be due to the high contamination of animal feed by such pollutants and also may be reached to the milk through handling process. In fact, animal nutrition affects the hygienic quality of milk, but it is not easy to control and to evaluate contamination coming from industrial processes, agricultural practices and atmospheric depositions (Caggiano *et al.,* 2005).

Milk can be also contaminated by residues of pesticides. Pesticides are extensively used to increase agricultural products by preventing losses due to pests. Among the major groups of pesticides, organochlorines (OC) are more potent due to their persistence and stability and are either banned or severely restricted from use in most of the developed countries in the world. The major source of OC pesticide residues is from fodder and soil, while contamination of organophosphorous (OP) pesticides is mainly associated with ingestion (through licking) of insecticide used for parasite control on the animal (Waliszewski *et al*., 1997). OC pesticides play an important role in chronic poisoning and take part in a number of pathological processes (Sitarska *et al.,* 1991). Fat solubility of these compounds is responsible for their varied concentrations in the tissues and their accumulation in the lipoproteins of the cell membranes, thus changing their structure and permeability (Antunes-Madeira *et al.,* 1993). OC pesticides are able to significantly decrease the ability of the highly purified human natural killer (NK) autolyse tumor cells after exposures, ranging from 1 hr to 6 days (Beach and Whalen, 2006). Pesticide exposure independently or in synergism with modifiable risk factor associated with hemopoetic cancers, cancers of prostate, pancreas, liver and other body system. (Jaga and Dharmani, 2005). As a direct result of using the pesticides for controlling plant pests and diseases and due to the lipophilic nature of these compounds, their residues are transferred to milk and other fat-rich substances.

In Egypyt, there is a growing interest in documenting levels and presence of chlorinated pesticides in different environmental samples. Although their use has been restricted, many studies have shown their presence in milk (Dogheim *et al.,* 1990; Abou-Arab, 1997; 1999).

The aim of this study is to compare chemical composition of different types of milk and assess any possible risks to consumers of milk in Cairo governorate (Egypt) by detecting and determining pesticide residues and heavy metals in raw, pasteurized and UHT milk. Also, calculating the dietary intake of these contaminants for adults and children due to milk consumption.

MATERIALS AND METHODS

Materials:

1. Milk:

Ninety samples of milk (about 1 kg each) representing 30 samples of pasteurized, UHT (Ultra High Temperature), and raw milk, were obtained randomly from local markets in Cairo Governorate, Egypt during (2007/2008).

2. Heavy metals standards:

Standard solutions of heavy metals, i.e., zinc (Zn), iron (Fe), copper (Cu), manganese (Mn), chromium (Cr), nickel (Ni), cobalt (Co), lead (Pb), cadmium (Cd) and tin (Sn) were provided, in 1000 ppm solutions for each one, by Merck (Merck, Darmastadt, Germany).

3. Pesticides standards:

Standards of organochlorine (OC) pesticides, i.e., (Hexachlorobenzene (HCB); lindane; aldrin; heptachlor; hept.epoxide; chlordane; endrin; 1,1,1-trichloro-2,2,bis(p-chlorophenyl) ethane (p,p'-DDT); 1-(o-chlorophenyl) -1- (p-chlorophenyl)-2,2,2-trichloroethane (o,p'-DDT) ; 1, 1- dichloro-2,2-bis (p.chlorophenyl) ethylene (p,p'-DDE); 1-(o-chlorophenyl)-1-(p-chlorophenyl) -2,2-di-chloroethane (o,p'-DDE); 1-chloro-2,2-bis(p-chlorophenyl)ethane (p,p'-DDD);1- (o-chlorophenyl)-1-(p-chlorophenyl) -2,2-dichloroethane(o,p'-DDD) and organophosphorus (OP) pesticide standards as (malathion; dimethoate; profenofos and pirmiphos-methyl) were purchased from Chem. Service, Inc. (West Chester, PA).

4. Chemicals:

All solvents and chemicals used were reagent grades and purchased from Merck, (Merck, Darmastadt, Germany).

Methods of Analysis

1. Chemical analysis:

Titratable acidity, Fat, total protein (T.P), lactose, total solids (T.S) and ash were determined according to AOAC (1995). The pH value was measured by using Knick-Digital pH meter model 646.

2. Metals content:

Metals were extracted from the raw milk and heat treated milk according to AOAC (1995) by dry ashing (about 15 g of milk sample in crucible) in a muffle at 500-550° C for 8 hours, then dissolved by 1 ml of concentrated HCl and diluted to a definite volume by distilled water. The metals concentration were measured by Atomic Absorption Spectrophotometer (AAS) (Perkin Elmer 2380). Maximum absorbance was obtained by adjusting the Cathode lamps at specific slit and wave lengths. Fe was measured at (248.3 nm), Cu (324.8 nm), Mn (279.5 nm), Zn (319.9 nm), Pb (217.0 nm), Cd (228.8 nm), Cr (357.9 nm), Ni (232.0 nm), Co (240.7 nm) and Sn (286.3 nm). The limit of detection (sensitivity) was 10 µg/kg for Pb, Cd, Ni, Co and Sn as well as 1.0 µg/kg for the other metals. The recovery of metals was studied by adding known amounts of standard solution to milk samples. The recoveries in different samples were 96-103% for Cu, Mn and Zn and were 91-98% for Pb, Cd, Cr, Ni, Co and Sn. All of the obtained results were corrected according to the recovery percentages. Concentrations of heavy metals in different analyzed milk samples were calculated as mg/kg whole milk.

3. Pesticide residues:

Pesticide residues were extracted by petroleum ether (40/60), acetonitrile, methanol and diethyl ether, from different samples under investigation by AOAC (1995), and Pesticide Analytical Manual (1991). Aliquots of 1-2 µl of extracts were injected into a Hewlett Packard GC Model 5890 equipped with Ni^{63} Electron Capture Detector (ECD) and Flame Ionization Detector (FID), and fitted with HP- 101 capillary column (Cross linked methyl silicon Gum), 30 m length, 0.25 mm diameter, and 0.25 µm film thickness. The oven temperature was programmed to start by 160ºC and raised to 220ºC with rate of 5ºC/min and was held for 30 min. Injection and detector temperatures were 220ºC and 300ºC, respectively. The flow rate of carrier gas (nitrogen) was obtained by adjusting it at the pressure of 10 psi. Concentrations of pesticide residues in different analyzed milk samples were calculated as mg/kg fat.

To determine method's accuracy, a recovery study was performed on clean milk samples that were fortified by the investigated pesticides. After extraction, the samples were analyzed according to the proposed method. The recovery values were calculated from calibration curves constructed from the concentrations and peak areas of the obtained chromatograms with standards of OC and OP pesticide. Blank analysis was performed in order to check interference from the sample. Mean recoveries ranged from 90 to 94% with S.D < 6 indicating excellent repeatability and the limit of detection (LOD) was 0.001 mg/kg for OC pesticides and 0.005 mg/kg for OP pesticides. .

RESULTS AND DISCUSSION

Chemical composition of different types of milk samples:

Chemical composition of the collected milk samples (raw, pasteurized and UHT) from local markets of Cairo were determined (Table 1). Results showed that mean levels of fat, T.P, ash, lactose and T.S in raw milk were 3.50, 3.20, 0.71, 4.10 and 11.4 %, respectively. These levels were far lower than those levels determined by Kholif *et al.,* (1994) and Abou-Arab (1996) who reported that fat, T.P., ash, lactose and T.S contents were (5.9-6.0), (3.23-3.41), (0.84-0.86), (4.66-4.71) and (14.98-15.25)% in buffalo's milk. In cow's milk the results were (4.33), (3.02), (0.67), (3.88) and (12.34) %, in the same order (Kholif *et al.*, 1994). Also, gross composition in the present study was lower than those reported by Castagnetti *et al.,* (1996) who showed that fat, protein and lactose were 8.54, 4.5 and 5.12%, respectively. These variations may be due to genetics, physical and environmental factors (Kholif *et al.,* 1994; Abou-Arab, 1996). Chemical composition of heat treated milk (pasteurized or UHT) were varied. T.P; ash; lactose and T.S in pasteurized and UHT milk were detected at levels higher than those detected in raw milk. On the other hand, fat content in raw milk was higher than those found in the other two types. Titratable acidity and pH values in raw milk were higher than the others, due to keeping factors.

Heavy metal contents in raw, pasteurized and UHT milk:

Concentrations of essential elements and potentially toxic elements in different milk samples (raw, pasteurized and UHT) collected from Cairo Governorate are presented in (Table 2). The obtained results show considerable differences among the heavy metals content in different milk samples. The data proved that, the levels of the essential metals (Zn, Fe, Cu, Mn, Cr, Ni and Co) were much higher in the different samples than those of the non-essential metals (Pb, Cd and Sn).

The heavy metal zinc in raw, pasteurized and UHT milk samples was detected at the highest mean levels, which recorded 3.59, 3.11 and 3.11 mg/kg, respectively, followed by Fe. The corresponding mean values for Fe were 0.62, 0.55 and 0.60 mg/kg. However, the other essential elements, i.e. Cu, Mn, Cr, Ni and Co were detected at lower levels (Table 2). Regarding to toxic metals, i.e., Pb, Cd and Sn, they were detected in raw, pasteurized and UHT milk at mean levels of (0.032, 0.025 and 0.002 mg/kg); (0.021, 0.022 and 0.002 mg/kg) and (0.020, 0.020 and 0.002 mg/kg), respectively. These results indicate losses of some metals content in heat treated milk (pasteurized and UHT) due to processing methods, but these losses were not high. Comparing our results with the accepted upper limit (Harding, 1995), iron was detected at mean level higher than the legally accepted upper limit (0.5 mg/kg). Also, mean value of lead was below the general statutory limit of 1 mg/kg set by The Lead in Food Regulations (1979) as mentioned, but above the proposed European maximum limit of 20 micrograms/kg (European Commission, 1997). It is worthy to mention that cadmium as a toxic metal was detected at lower levels than the permissible limits due to Cd accumulates mainly in liver and kidney (Prankel *et al.,* 2004) and carry-over to milk is very low or absent (Bluthgen, 2000). Generally, the levels of various metals were mostly found below the safe limits adopted from international food standards.

Mean concentrations of heavy metals in our study were compared to the mean concentrations found in raw milk samples taken from Cairo governorate during 1988 to 1990 by (Abou-Arab,

1991). Our results were far below the earlier results. The author reported that Zn, Fe, Cu, Mn, Pb and Cd were detected at mean values, 4.25, 0.95, 0.22, 0.06, 0.05 and 0.348 mg/kg, respectively.

In this respect, several studies have been carried out to assess metal contents of milk from different areas. Tripathi, *et al.,* (1999) reported that Zn, Cu, Pb and Cd were detected in cow's and buffalo's milk samples at levels ranged from (3.177 to 3.697 mg/l), (0.043 to 0.195 mg/l), (1.70 to 3.35 µg/l) and (0.07 to 0.10 µg/l), respectively. However, Onianwa, *et al.*, (1999) found Zn, Cu, Fe, Cr, Pb, Cd, Co and Ni at levels ranged from (0.39 to 2.75), (0.07 to 0.67), (1.68 to 15.1), (0.005 to 0.030), (0.03 to 0.18), (0.004 to 0.009), (0.03 to 0.12) and (0.04 to 0.09 mg/l), respectively. Also, the mean values of Zn, Cu, Fe, Mn, Cr, Pb and Cd were 4.631 mg/l, 0.0518 mg/l, 0.290 mg/l, 0.0291 mg/l, 0.004 mg/l, 5.23 µg/l and 0.40 µg/l, respectively according to Lante *et al.,* (2004). On the other side, Caggiano *et al.,* (2005) found mean levels of 0.13, 0.20, 0.20 and 0.06 mg/kg for Mn, Cr, Pb and Cd, respectively. Also, Tajkarimi *et al.,* (2008) determined Pb in raw milk samples collected from milk tankers and found it at a mean of 7.9 ng/ml, with a range of 1 to 46 ng/ml.

The concentration ranges of certain health-related elements are not constant but mainly vary according to two factors, those related with secretion from the mammary gland, such as the lactation state, animal species and health status and extrinsic factors, such as season, dairy cattle ration (nutritional status of cow), environment (nature of soil and locality of the farm) (Lante *et al.,* 2004; Hermansen *et al.* 2005; Sola-Larranaga and Navarno-Blasco, 2008). The metals Zn, Fe, Cu and Mn are essential micro nutrients and have a variety of biochemical functions in all living organisms. While, they can be toxic when taken in excess, both toxicity and necessity vary from element to element (Tripathi *et al.,* 1999). Lead residues in milk are of particular concern because milk is largely consumed by infants and children (Tripathi *et al.,* 2005), and the determination of Pb level in milk is particularly attended by international organizations (Codex, 2003). The high levels of Pb in milk may result from industrial air pollution. One of the probable reasons for the increase in other countries may be the wide use of leaded gasoline during recent decades (Rizov, 2001). One of the most important sources of Pb contamination in food and feed is water, especially in more contaminated areas (Codex, 2003). The high levels of heavy metals in some milk samples in this investigation may attribute to the high contamination of animal feed by such pollutants and also may be reached to the milk through handling procedures.

Table (1): Chemical composition of different milk samples collected from local markets, Cairo Governorate, Egypt.

Nutrient	Raw milk		Pasteurized milk		UHT milk	
	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range
Fat	3.50±0.88	3.10-3.80	3.00±0.15	2.90-3.10	3.00±0.12	2.90-3.10
T.P	3.20±0.46	3.10-3.50	3.50±0.08	3.40-3.70	3.40±0.08	3.45-3.60
Ash	0.71±0.48	0.66-0.85	0.80±0.06	0.70-0.95	0.80±0.08	0.70-0.90
Lactose	4.10±0.65	3.90-4.40	4.65±0.12	4.45-4.85	4.44±0.10	4.40-4.50
T.S	11.4±0.84	11.20-12.40	11.50±0.15	11.25-11.85	11.50±0.12	11.40-11.60
Acidity	0.18±0.16	0.16-0.19	0.16±0.08	0.15-0.17	0.16±0.06	0.15-0.17
pH value	6.55±0.92	6.40-6.6	6.65±0.08	6.60-6.70	6.70±0.08	6.60-6.80

Table (2): Heavy metal contents (mg/kg whole milk) in different milk types collected from local markets in Cairo governorate, Egypt.

Metals	Raw milk		Pasteurized milk		UHT milk	
	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range
Zn	3.59±0.96	2.96-4.06	3.11±0.66	2.96-3.20	3.11±0.56	2.84-3.42
Fe	0.62±0.40	0.36-1.04	0.55±0.30	0.33-0.85	0.60±0.40	0.48-0.83
Cu	0.17±0.08	0.08-0.22	0.15±0.08	0.12-0.16	0.18±0.20	0.16-0.26
Mn	0.06±0.03	0.05-0.08	0.041±0.04	0.03-0.07	0.05±0.03	0.03-0.08
Cr	0.03±0.02	0.01-0.08	0.030±0.02	0.01-0.07	0.02±0.02	0.01-0.03
Ni	0.036±0.02	0.022-0.07	0.030±0.02	0.02-0.06	0.03±0.02	0.02-0.05
Co	0.032±0.02	0.028-0.046	0.030±0.03	0.020-0.04	0.03±0.02	0.01-0.05
Pb	0.032±0.02	0.030-0.067	0.021±0.02	0.016-0.036	0.02±0.02	0.01-0.04
Cd	0.025±0.03	0..016-0.04	0.022±0.02	0.012-0.036	0.02±0.02	0.01-0.05
Sn	0.002±0.00	0.0-0.004	0.002±0.0	0.0-0.004	0.002±0.0	0.0-0.004

Pesticide residues in milk (raw, pasteurized and UHT):

Milk samples (raw, pasteurized and UHT) were tested for pesticide residues. Data presented in (Tables 3&4) showed that, about 67 and 40% of the residues were p,p'-DDE and o,p'- DDE, respectively in the collected raw milk samples. DDT is very persistent and remains in 27% as p,p'-DDT and 20% as o,p'-DDT in the same samples. About 13 and 17% of the raw milk samples contained p,p'-DDD and o,p'-DDD, respectively. Regarding to the total DDT and its derivatives concentrations in raw milk (0.112 mg/kg fat), data showed that the mean levels were exceeded the tolerance limits (0.02 mg/kg) reported by FAO/WHO (2008). DDT values in raw milk samples in the present study was similar to the levels recorded by Waliszewski *et al.,* (1997) and higher than those detected by Heck *et al.,* (2007); Darko and Acquaah (2008). However, these levels were lower than those detected in some developing countries, such as Uganda (3.24 mg/kg), Nigeria (3.83 mg/kg), India (6.55 mg/kg), Kenya (6.99 mg/kg), South Africa (20.10 mg/kg) and Ethiopia (7.75 mg/kg) (FAO,1986). The tested raw milk in the present study proved that 20 and 60% of the samples contained heptachlor and heptachlor epoxide, respectively at mean levels of 0.015 and 0.045 mg/kg fat. The sum of these values exceeded the tolerance levels (0.006 mg/kg) prescribed by FAO/WHO (2008). However, these levels were lower than those detected by John *et al.,* (2001) as 0.15 mg/kg. On the other hand, heptachlor was not detected above the detection limit of 0.002 mg/kg in raw milk samples analyzed by Waliszewski *et al.,* (1997) who found heptachlor epoxide in 5% of the collected samples at mean level of 0.007 mg/kg. Chlordane detected in 53% of raw milk samples at mean level of 0.022 mg/kg exceeding the tolerance levels (0.002 mg/kg) prescribed by FAO/WHO (1993). Another chlorinated hydrocarbon insecticides, HCB was detected in 33% of the collected raw milk samples at mean level of 0.155 mg/kg. This level exceeded the maximum residue limits (MRL) reported by FAO/WHO (2008). The % positive samples of HCB in the present study was lower than those detected by Campay *et al.,* (2001); Heck *et al.*, (2007), who reported that the frequency positive samples were 94-100 and 100% (mean value 0.003 mg/kg fat), respectively. On the other hand, 27% of the collected raw milk samples were contaminated by lindane, aldrin and endrin at mean levels as 0.032, 0.048 and 0.016 mg/kg, respectively. These values exceeded the tolerance levels recorded by FAO/WHO (1993), which were 0.01, 0.006 and 0.0008 mg/kg with lindane, aldrin and endrin, respectively. These compounds in the present study were higher than those detected by Waliszewski *et al* (1997); Heck *et al.*, (2007); Darko and Acquaah (2008). However, the levels in our study were lower than those reported by John *et al.*, (2001).

The estimated pesticide residues in pasteurized and UHT milk samples presented in (Table 4) showed the efficient role of heat treatments on the degradation or elimination of the residues of pesticides. The mean concentrations of pesticides detected in pasteurized milk were lower than those detected in raw milk. On the other hand, UHT milk contained residues below the levels detected in both raw and pasteurized milk and also lower than the tolerance levels. These results are in agreement with that reported by Rachev *et al.*, (1974) and Abou-Arab *et al.,* (1999), who showed the efficient role of heat treatment on the degradation of some pesticides in milk products. Heck et al., (2007) found that o,p'-DDD and t-DDT levels were significantly higher in raw milk than in pasteurized and UHT milk, while, p,p'-DDD was significantly higher in UHT milk. On the other hand, p,p'-DDD was significantly higher in UHT milk than in raw milk. Costabeber *et al.,* (2000) evaluated organochlorine pesticides in UHT milk from Santa Maria (Brazil) and they found lindane (0.01 mg/kg fat) and HCB (0.001 mg/kg fat). In other study, pasteurized milk was analyzed in Argentina to determine organochlorine pesticides (Maitre *et al.*, 1994). Mean concentrations (mg/kg fat) were 0.0068, 0.0421, 0.0549, 0.0388, 0.0232 and 0.990 for HCB, γ-HCH, the sum of heptachlor and heptachlor epoxide, aldrin, chlordane and DDT (sum of isomers), respectively. Martinez *et al.*, (1997) reported that p,p'-DDE ranged between 0.003 to 0.538 ppm in the collected pasteurized samples. They added that 45.36% of samples contained this metabolite at a mean concentration of 0.045 ppm. It was also detected by Garrido *et al.,* 1994, at a mean concentration of 0.024 ppm. Pozo *et al.*, (1977) had also previously observed that p,p'-DDE was the metabolite that most frequently contaminated Spanish milk. Aldrin was detected in only 1.03% of pasteurized milk; however, dieldrin was detected in 72.16% with a mean level of 0.028 ppm (Frank *et al.,* 1985) in samples from Ontario.

Organochlorine pesticides and their residues are highly lipophilic nature and are hardly metabolized (Falandysz *et al.*, 2004). Hence it may easily concentrate in milk fat. Although, OC pesticides were banned from agricultural use in the early 1970s in most countries, their residues still appear. The levels of OC pesticides detected in raw milk samples were higher than the tolerance levels. However, these residues decreased in pasteurized milk, but their levels still slightly higher than the MRL. On the other hand, UHT milk contained the lowest levels of these

contaminants and in most cases not detected because of the efficient role of heat treatment. So, the presence of OC pesticides in raw milk above its maximum residue limits is viewed with serious concern. According to these results, the consumption of UHT milk may be safer than the raw milk followed by pasteurized milk.

Interestingly, non of the raw, pasteurized or UHT milk revealed the presence of any organophosphorus pesticides (malathion, profenofos, pirmiphos-methyl and dimethoate). These results could be attributed to conversion of these compounds to other compounds, or due to the relative high water solubility of these compounds. Therefore, animals secrete most of these chemicals in the urine and feces (IDF, 1979).

Estimated daily intake of metals and pesticides in milk:

The contribution of various foods to the intake of contaminant depends on the quantity of food consumed and the contaminant concentration in that food. The average consumption of liquid milk for an adult is 250 ml/day (Gopalan and Rao, 1980; Anonymous, 1998) and 300 ml for child/day (Gopalan and Rao, 1980). To assess any possible risks to consumers of milk and to provide an indication of any risks to consumers, dietary intakes have been estimated using the mean concentration of various metals and pesticide residues shown in Tables 2 and 4.

Table (3): % of positive milk samples for pesticide residues.

Pesticides	Raw milk samples		Pasteurized milk samples		UHT milk samples	
	No. of (+)	%	No. of (+)	%	No. of (+)	%
HCB	10	33	6	20	5	17
Lindane	8	27	3	10	2	7
Aldrin	8	27	3	10	2	7
Heptachlor	6	20	2	7	-	-
H. epoxide	18	60	6	20	-	-
Chlordane	16	53	7	23	-	-
Endrin	8	27	3	10	-	-
p,p'-DDT	8	27	4	13	-	-
o,p'-DDT	6	20	3	10	2	7
p,p'-DDE	20	67	12	40	2	7
o,p'-DDE	12	40	8	27	2	7
p,p'-DDD	4	13	2	7	-	-
o,p'-DDD	5	17	2	7	-	-

(-): Not detected

Table (4): Pesticide residues (mg/kg fat basis) in some milk types collected from local markets in Cairo Governorate, Egypt.

Pesticides	Raw milk		Pasteurized milk		UHT milk	
	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range
HCB	0.155±0.11	0.008-0.182	0.066±0.05	0.042-0.089	0.009±0.01	0.002-0.016
Lindane	0.032±0.02	0.026-0.054	0.015±0.02	0.009-0.022	0.004±0.01	0.002-0.008
Aldrin	0.048±0.04	0.041-0.066	0.033±0.04	0.008-0.042	0.002±0.01	0.001-0.003
Heptachlor	0.015±0.02	0.009-0.030	0.009±0.01	0.004-0.011	ND	ND
H.epoxide	0.045±0.02	0.022-0.082	0.025±0.02	0.006-0.042	ND	ND
Chlordane	0.022±0.01	0.011-0.040	0.016±0.03	0.005-0.022	ND	ND
Endrin	0.016±0.01	0.010-0.030	0.007±0.02	0.002-0.009	ND	ND
p,p'-DDT	0.024±0.03	0.016-0.046	0.006±0.02	0.002-0.011	ND	ND
o,p'-DDT	0.003±0.01	0.001-0.008	0.002±0.01	0.001-0.004	0.001±0.00	0.001-0.001
p,p'-DDE	0.012±0.01	0.010-0.020	0.004±0.01	0.002-0.006	0.001±0.00	0.001-0.001
o,p'-DDE	0.055±0.03	0.022-0.080	0.006±0.02	0.002-0.008	0.001±0.00	0.001-0.001
p,pDDD	0.006±0.01	0.002-0.096	0.003±0.01	0.002-0.004	ND	ND
o,p'-DDD	0.012±0.01	0.009-0.026	0.008±0.01	0.004-0.010	ND	ND

ND: Not detected

1. Daily intake of metals in milk:

The daily intake of Zn, Fe, Cu and Mn by adults ranged between (0.778-0.898), (0.138-0.155), (0.038-0.045) and (0.010-0.015 mg/person/day), respectively (Table 5). These levels were lower than those recorded by United States of Food and Drug Administration Daily Value Criteria (Anonymous, 1998). Regarding to daily intake of Pb, Cd and Sn, data showed that their levels were far below than the recommended levels recorded by WHO (1989 and 1993), the daily intake of Cr, Ni and Co was also below (0.005 to 0.008 mg/person/day).

The daily intake of Zn, Fe, Cu, Mn, Cr, Ni, Co, Pb, Cd and Sn by infants and children through raw, pasteurized and UHT milk marketed in Cairo Governorate has also been estimated (Table 6). The daily intake of Zn (0.934-1.078) and Cu (0.045-0.054 mg/kg body wt) are well below the recommended tolerable levels of (3-5 mg/kg body wt infant; 10 mg children) and (0.5-1.0 mg/kg body wt infant, 2.0 mg children) for Zn and Cu, respectively (Ostapczuk *et al.*, 1987; Vijaya, 1993). However, Pb (6.0-10.0 µg/kg) and Cd (6.0-8.0 µg/kg body wt) were higher than the permissible levels (3.57 µg/kg for Pb; 0.8-1.0 µg/kg for Cd). It is well established that Pb and Cd are toxic, and children are more sensitive to these metals than adult.

Table (5): Estimated daily intake of metals in raw, pasteurized and UHT milk for adults.

Metals	Estimated daily intake (mg/person/day)			Daily Intake requirements, (mg/person/day)
	Raw Milk	Pasteurized Milk	UHT Milk	
Zn	0.898	0.778	0.778	*15.0
Fe	0.155	0.138	0.150	*18.0
Cu	0.043	0.038	0.045	*2.0
Mn	0.015	0.010	0.013	*2.0
Cr	0.088	0.008	0.005	ND
Ni	0.009	0.008	0.008	ND
Co	0.008	0.008	0.008	ND
Pb	0.008	0.005	0.005	**0.214
Cd	0.006	0.006	0.005	**0.006
Sn	0.001	0.001	0.001	**0.120

ND: Not Detected
*: Anonymous, 1998
**: WHO, 1989 and 1993

In this respect, in China, the lead contamination level in milk- based products increased during 10 years (Wang, 2003). However, levels of Pb in milk seem to have risen slightly in other countries over the past 20-30 years, but there is no evidence of any real increase in dietary lead during recent decades. Recent comparisons of lead in food in North America with those obtained over 20 years earlier also do not suggest any increase in lead intake by man from this source (WHO Food Additive Series, 1972). Many reports calculated the daily intake of some metals in milk. Knowles *et al.*, (2006) reported daily intake of Fe (0.07 mg), Zn (0.9 mg), Cu (8.0 µg) and Mn (13.0 µg) lower than those calculated in our study. Also, Pb (2.9 µg) and Cd (0.09 µg) in the study of Tripathi *et al.*, (1999) were lower than the levels recorded in the present study. However, their levels of Zn (4.125 mg) and Cu (128.7 µg) were higher than those recorded in our study. The daily intake of these metals through different types of milk is computed for infants between 6-12 months assuming the daily consumption rates of 150 ml/kg and an average body weight of 6.5 kg (Guptes, 1978; Nelson, 1996). This suggests that the intake of essential elements through whole milk is lower by factors of 1.5 and 3.0 for Zn and Cu, respectively.

Table (6): Estimated daily intake of metals in raw, pasteurized and UHT milk for infants and children.

Metals	Estimated daily intake (mg/person/day)			*Daily intake requirements for infants&children (mg/kg body wt/day)
	Raw Milk	Pasteurized Milk	UHT Milk	
Zn	1.078	0.934	0.934	3-5mg infants, 10mg children
Fe	0.186	0.165	0.18	-
Cu	0.051	0.045	0.054	0.5-1.0mg infants, 2.0mg children
Mn	0.018	0.012	0.015	-
Cr	0.009	0.009	0.006	-
Ni	0.011	0.009	0.009	-
Co	0.01	0.009	0.009	-
Pb	0.01	0.006	0.006	3.57 µg
Cd	0.008	0.007	0.006	0.8-1.0 µg
Sn	0.001	0.001	0.001	-

*: (Ostapczuk *et al.,* 1987 and Vijaya, 1993.
(-): Not Detected.

2. Daily intake of pesticide residues in milk:

Daily intake of pesticide residues detected in raw, pasteurized and UHT milk in the present study were estimated for adult and children (Tables 7&8). According to the Codex Alimentarius Commission, the acceptable daily intake (ADI) of lindane, aldrin, heptachlor, chlordane, and DDT are prescribed as 8.0, 0.1, 0.1, 0.5 and 20.0 (µg /kg body wt/day), respectively (FAO / WHO, 1993). An estimate of

this value of ADI were calculated for an average adult and child weighting 60 and 10 kg, respectively with average consumption of liquid milk for an adult (250 ml) and children (300 ml). On the basis of mean residue levels observed for pesticides in the collected samples, the estimated intake was within the international ADI. Hence, there is no indications of health risks associated with milk consumption (raw, pasteurized or UHT) in Cairo governorate, Egypt, but what should be taken into account is its long-term action because of its accumulation in human fat tissues. In this respect, Martinez *et al.*, (1997) estimated daily intake of pesticide residues in pasteurized milk. Their calculation was based on the daily consumption of milk in Spain, 343 ml (Anonymous, 1986), and the mean concentration of each pesticide as well as the average weight per person, estimated at 70 kg. The author reported that the estimated dose of chlordane was 1.526×10^{-2} ppm. The estimated daily intake of pesticides through milk by elementary school children of Santa Maria (Brazil) was determined (Hech *et al.,* 2007). They reported that aldrin and dieldrin exceeded the ADI recommended by FAO/WHO. Mallatou *et al.*, (1997) determined the ADI of lindane from milk in Greece at 0.025 µg/day and for α-HCH at 0.110 µg/day.

From previous presentation, it could be concluded that this survey confirmed that milk can be contaminated by some metals and organochlorine pesticides owing to their use in sanitary actions. Iron and lead were present in raw milk at concentrations higher than the tolerance levels and declined in pasteurized and UHT milk. Some of OC pesticides were determined in raw milk at concentrations higher than the MRL. However, these compounds detected in pasteurized milk at slightly higher than the tolerance levels. On the other hand, these contaminants occurred in UHT milk at very low concentrations and in some cases these residues disappeared by heat treatments. Data showed that daily intake of metals and OC pesticide residues in raw, pasteurized and UHT milk were far below than the recommended levels. Although these residues occurred at very low concentrations in the analyzed samples, they may accumulate to higher levels in human beings who consume these types of milk. This investigation showed that we need further monitoring studies in order to follow up and to confirm food safety since these compounds represent a potential risk to human health.

Table (7): Estimated daily intake (µg/person/day) of pesticide residues in raw, pasteurized and UHT milk for adults.

Pesticides	Estimated daily intake (µg/person/day)			*ADI (µg/kg body weight/day)	**ADI (µg/person/day)
	Raw Milk	Pasteurized Milk	UHT Milk		
HCB	1.356	0.495	0.068	-	-
Lindane	0.280	0.113	0.030	8.0	0.480
Aldrin	0.420	0.248	0.015	0.1	0.006
Heptachlor	0.131	0.068	-	0.1	0.006
H.epoxide	0.394	0.188	-		
Chlordane	0.193	0.120	-	0.5	0.030
Endrin	0.140	0.053	-	-	-
p,p'-DDT	0.210	0.045	-	20.0	1.200
o,p'-DDT	0.026	0.015	0.008		
p,p'-DDE	0.105	0.030	0.008		
o,p'-DDE	0.481	0.045	0.008		
p,p'-DDD	0.053	0.023	-		
o,p'-DDD	0.105	0.060	-		

*: **FAO/WHO, 1993.** ** **ADI* x 60. (-): Below limit of detection (LOD)**

Table (8): Estimated daily intake (µg/person/day) of pesticide residues in raw, pasteurized and UHT milk for children.

Pesticides	Estimated daily intake (µg/person/day)			*ADI (µg/kg body weight/day)	**ADI (µg/person/day)
	Raw milk	Pasteurized Milk	UHT Milk		
HCB	1.628	0.594	0.081	-	-
Lindane	0.336	0.135	0.036	8.0	80.0
Aldrin	0.506	0.297	0.018	0.1	1.0
Heptachlor	0.158	0.081	-	0.1	1.0
H.epoxide	0.475	0.225	-		
Chlordane	0.232	0.144	-	0.5	5.0
Endrin	0.169	0.063	-	-	-
p,p'-DDT	0.253	0.054	-	20.0	200.0
o,p'-DDT	0.032	0.018	0.009		
p,p'-DDE	0.127	0.036	0.009		
o,p'-DDE	0.580	0.054	0.009		
p,p'-DDD	0.063	0.027	-		
o,p'-DDD	0.127	0.072	-		

*: FAO/WHO, 1993. **: ADI*x10 (-): Below limit of detection (LOD)

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