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Prevalence of Some Pesticides Residues in Buffalo's Milk with Refer to Impact of Heating

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ABSTRACT

The widespread application of pesticides in agriculture, public health programs and industry as well as around the home can lead to accumulation of pesticides in the environment, however, milk could be contaminated with pesticides from fodder, soil and ingestion of insecticides. Therefore, our survey had conducted to investigate the magnitude of contamination of buffalo's milk with organochlorine (OC), organophosphorous (OP) and synthetic pyrethroids pesticides moreover to detect the effect of boiling of milk on concentration of these pesticides. The obtained results were revealed that OCPs (HCB, α-, β-, ϒ- HCH isomers, heptachlor, heptachlor epoxide, ϒ- chlordan, aldrin, endrin, methoxychlor, p,p'-DDT, p,p'-DDE, p,p'-DDD) were detected with high values exceeded the tolerance levels of FAO/WHO and EU. Concerning OP pesticides, the maximum residual levels (MRLs) of dimethoate, pirimiphos-methyl as well as its arithmetic mean were higher than MRLs set by FAO/WHO . On the other hand, cypermethrin concentration as pyrethroids exceeded the limits of FAO/WHO and EU. Application of boiling process on milk, OC pesticide residues were reduced with a different percentages while pyrethroid and OP pesticide residues were eliminated due to the effect of heat treatment. Health hazard were found to be associated with heptachlor and its metabolite, heptachlor epoxide. Accordingly, monitoring survey of pesticide residues in milk is necessary to protect consumer health.

Key words:
Organochlorine pesticide residues, synthetic pyrethroids residues, organophosphorous (OP) pesticides, buffalo's milk.

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1. INTRODUCTION

Milk is almost considered as a complete food because it is a good and relatively cheap source of protein, fat and also contains many valuable nutrients. Milk is the main constituent of the daily diet, especially for vulnerable groups such as infants, children as well as elderly people (Davies *et al*., 1986). Pesticides were used extensively in agriculture and public health programs for combating pests and vector-borne disease. Uncontrolled and random application of these pesticides may led to significant contamination of food commodities. Consumption of contaminated food has become a serious problem to human health. Milk is an ideal fluid to dissolve environmental organic pollutants such as pesticide residues since most of them have lipophilic properties (Kampire *et al*., 2011). Thus, milk is used as an indicator for determining the persistence of organic pollutants (Losada *et al*., 1996). The contamination of milk is considered as one of the main dangerous issues in the last few years. Milk can be contaminated by pesticide residues such as organochlorine (OC), organophosphorous (OP) pesticides as well as pyrethroids pesticides (Battu *et al*., 2004 and Fontcuberta *et al*., 2008). There is a number of sources where pesticide residues can reach milk, among the possible sources are (i) Foodstuffs (clover, corn, fodder, grassland, Sorghum, etc.) such crops are sprayed with OP and pyrethroids pesticides during the growing season to control crop pests. (ii) Contaminated drinking water presented to milk producing animal. (iii) Contaminated soil, which constitutes a long-term storage of pesticide residues, particularly OC pesticide residues because this group of pesticides has high affinity to adsorb to soil particles. Despite usage of this group has been banned long time ago in most countries, including Egypt, nevertheless it still exists in environmental components especially soil. (iv) Use of these pesticides directly on animal for disease vectors control and parasites (Di Muccio *et al.,* 1996). Due to lipophilic and relative stability properties of these residues may easily translate and accumulate in different animal organs (meat, fat, milk, etc.). Continuous drinking of contaminated milk may led to biomagnification of these residues in the human body, causing a chronic toxicity after long- term exposure. The chronic effects of pesticides from contaminated food on human health are not well defined, but there is increasing evidence of carcinogenicity and genotoxicity, as well as disruption of hormonal functions (Ledoux, 2011). Regarding infants, milk is considered as an essential and main diet during their development. So, drinking contaminated milk will pose a serious risk to their health, since their enzymatic and metabolic systems are not fully active (Heck *et al.,* 2007). OP and pyrethroids pesticide residues are widely used in agriculture and in animal husbandry in Egypt while, the OC pesticides have been banned a long time ago. OP and pyrethroids pesticides are less persistent than OC pesticide residues in environment. In order to ensure the safety of milk for the consumer, it is very important to know the pesticide residue levels and compared with recommended toxicological criteria such as maximum residue limit (MRL) and the acceptable daily intake (ADI). In general, studies on monitoring survey of pesticide residues in Egypt are limited on milk and its products. The under study area is Kafrelsheikh governorate which depends mainly on agriculture and livestock activities. Therefore, the present study was conducted to determine the levels of OC, OP and pyrethroids pesticide residues in buffalo's milk collected from 10 towns inside Kafrelsheikh Governorate, Egypt. On the other hand to study the effect of boiling process on the stability of the detected pesticide residues to prevent the potential adverse effect on consumer's health.

2) MATERIAL AND METHODS

2.1. Sampling

A total of 20 buffalo's milk samples (500ml of each) were collected from ten towns in Kafrelsheikh Governorate during the period from April 2014 to May 2014. Each sample was received in glass bottles and immediately stored at -20˚C until for detection of pesticides residues. All examined collected milk samples were boiled to assess the impact of boiling process on pesticide residues. Each milk sample was divided into two parts, one part of milk was analyzed as such without any treatment and the other part was subjected to boiling.

2.2. Pesticide residues analysis

All reagents and chemical used in this study were of analytical grade. Pesticide standards were used in monitoring survey contained the following OCPs: hexachlorobenzene (HCB), hexachlorocyclohexane (HCH) (α-, β-, ϒ- HCH isomers), heptachlor and its metabolite (heptachlor epoxide), aldrin, endrin, ϒ-chlordan, dichlorodiphenyltrichloroethane (p,p' -DDT) and its metabolites (p,p'-DDE and p,p'-DDD) and methoxychlor. While, OPP standard included: ethoprophos, phorate, diazinon, dimethoate, pirimiphos-methyl, chlorpyrifos, malathion, prothiophos, fenamiphos, ethion and triazophos . In addition, the synthetic pyrethroids standard was permethrin, tetramethrin, cypermethrin, deltamethrin, esfenvalerate as illustrated in Fig.1 and 2.

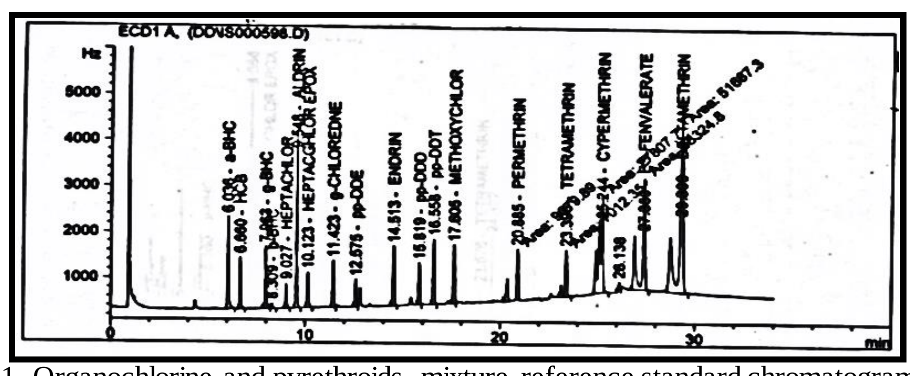

Fig.1. Organochlorine and pyrethroids mixture reference standard chromatogram

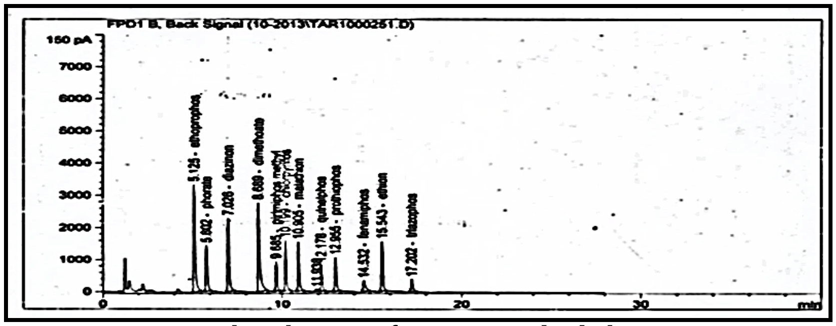

Fig.2.Organophosphorus reference standard chromatogram.

2.3. Instrument Conditions

Determination of OC and pyrethroid pesticide residues were performed on Hewlett Packared GC model 6890 N equipped with a Ni63- electron capture detector (GC -ECD). GC-ECD had a capillary column PAS-5 (30 m length x 0.32 mm internal diameter x 0.25 µm film thickness). Injector and detector temperature program were 300°C for 2 min, raised at the 3°C min-1 and then held at 260°C for 15min. The carrier gas was N2 at a flow rate of 4 ml/ min. As for OPPs the same model of gas chromatograph was used and fitted with a flame photometric detector (FPD) with phosphorus filter. It attached to a fused silica capillary column PAS-1701 (30 min length x 0.32 mm internal diameter x 0.25 µm film thickness). The Injector and detector temperature program were 240 °C and 250 °C, initial oven temperature, 200 °C for 2 min, raised at 6 °C min-1 then held at 250 °C for 15 min. The carrier gas was nitrogen at 3 ml min-1; hydrogen and air were used for combustion in 75 and 100 ml min-1, respectively.

2.4. Extraction and clean- up

Prior to extraction, all glasswares used in this study were washed with soap and water, followed by distilled water and acetone and finally heated at 220 °C in oven till use. The technique adopted to extract and clean-up multi-pesticide residues from milk samples was one used in details by **(Battu *et al.*,2004)**.

Recovery analysis was carried out on samples spiked with multi-pesticide residues standards. Extraction and clean- up were carried out on spiked samples as proposed technique. Blank analysis also has been carried out to check the interference in samples. Detected pesticide residues in all examined milk samples were calculated as ppm.

3) RESULT

The current study showed the presence of multi-pesticide residues belong to different groups of pesticides in fresh buffalo's milk samples collected from all cities that belong to Kafrelsheikh governorate. These pesticide groups in this study were of organochlorine, organophosphorus and pyrethroids. The concentrations of these pesticide residues are expressed as ppm whole milk basis. Fig. 3. Shows the frequency percentages of the detected pesticide residues in fresh milk samples.

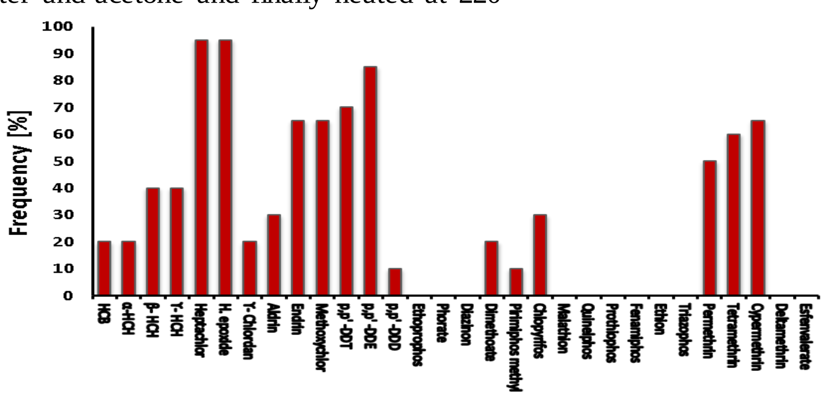

Fig. 3. Frequency distribution of pesticide residues detected in analyzed milk samples.

3.1. Organochlorine pesticide residues

Data in Table (1) represented the detected OCPs, arithmetic means ± standard errors and their minimum and maximum residue levels compared with corresponding maximum residue limits (MRLs) recommend by the Codex Alimentarius Commission (FAO/ WHO, 2013) and (European Union (EU), 2014).

Data in Table (1) and Fig.3 reflect that all analyzed raw milk samples were contaminated with OCP residues. All organochlorine pesticides in reference standards appeared in milk samples. The most frequently observed were heptachlor and its derivative, heptachlor epoxide, showing 95% frequency occurrence at mean concentrations of 0.0953 and 0.0266 ppm, respectively. The frequency of p,p'– DDT was less than its metabolite p,p'–DDE at 70 and 85 % , respectively, while, p,p'–DDD was detected only in 10 % of the samples. The mean concentrations of DDT and its metabolites were in this order p,p'–DDE (0.0847) > p,p'– DDT (0.0226) > p,p'– DDD (0.0142) ppm. As for HCH and its metabolites, α-HCH were detected in 20 % of milk samples while; the frequency percentages of β- and ϒ-HCH were two times greater than α-HCH percentage. While, their average concentrations were as this follows: β-HCH (0.8167) > α-HCH (0.267) > ϒ- HCH (0.0878) ppm. Endrin and methoxychlor were identified in 65 % of milk samples at a mean concentrations of 0.0103 and 0.0191 ppm, respectively. HCB and ϒ- chlordan were detected in 20% of milk samples, while aldrin was found in 30 % of milk samples. Their mean concentrations were 0.0231, 0.0093 and 0.0059 ppm, respectively. Statistical analytical results of OCPs concentrations found in examined milk samples were in violating levels. All levels of OCP residues detected were above their respective MRLs recommended by FAO/WHO and European Union legislation.

Table (1): Organochlorine pesticide residues (ppm whole milk basis) in examined buffalo's milk samples and respective MRLs.

No.	Detected Pesticides	Min.	Max.	Mean ±SE	MRLs	
					FAO/WHO (2013)	EU(2014)
			Organochlorine pesticides			
1	**HCB**	0.0141	0.0291	0.0231±0.004	N.E	0.01
2	**α-HCH**	0.014	1.0041	0.267±0.0246	N.E	0.004
3	**β- HCH**	0.1527	1.1284	0.8167±0.115	N.E	0.003
4	**ϒ- HCH**	0.0406	0.2758	0.0878±0.028	0.01	0.001
5	**Heptachlor**	0.0074	0.2745	0.0953±0.018	Sum = 0.006	Sum =0.004
6	**Heptachlor epoxide**	0.0031	0.0953	0.0266±0.005		
7	**ϒ- Chlordan**	0.0053	0.0183	0.0093±0.003	0.002	0.002
8	**Aldrin**	0.0018	0.0145	0.0059±0.002	0.006	0.006
9	**Endrin**	0.0022	0.0207	0.0103±0.002	N.E	0.0008
10	**Methoxychlor**	0.0123	0.0319	0.0191±0.002	N.E	0.01
11	**p,p' -DDT**	0.0043	0.0563	0.0226±0.004		
12	**p,p' -DDE**	0.0095	0.1953	0.0847±0.013	Sum =0.02	Sum =0.04
13	**p,p' -DDD**	0.0005	0.028	0.0142±0.014		

N.E: Not established

3.2. Organophosphorus pesticide residues

As for OPP residues in Table 2 and Fig. 3, only three pesticides were detected namely, dimethoate, pirimiphos methyl and chlorpyrifos. The highest incidence percentages among OPPs in milk samples were chlorpyrifos (30 %) followed by dimethoate (20 %) then pirimophos-methyl (10%) and the mean concentrations were 0.0066, 0.0388 and 0.0132 ppm, respectively. The maximum residue levels of dimethoate and pirimophos-methyl as well as its arithmetic mean were higher than MRLs set by FAO/WHO.

3.3. Pyrethroid pesticide residues

Table 3 and Fig.3, showed the presence of pyrethroid pesticide residues in collecting milk samples. Permethrin, tetramethrin and cypermethrin were monitored in milk samples while, deltamethrin and esfenvalerate were not monitored. The frequency distribution and mean residue levels of these pesticide in milk samples were in direct proportion as follows cypermethrin (13%) 0.1985 ppm, tetramethrin (12%) 0.1239 ppm and permethrin (10%) 0.0229 ppm. The cypermethrin concentrations that found in milk samples were exceeded the limits of FAO/WHO and EU. While, residue levels of permethrin were below these limits. As for tetramethrin concentrations that found in the samples we cannot decide if it stand for risk or not because no MRL has been established yet.

Table (2): Organophosphorus pesticide residues (ppm whole milk basis) in examined buffalo's milk samples and respective MRLs.

No.	Pesticides detected	Min	Max	Mean ±SE	MRLs	
					FAO/WHO (2013)	EU(2014)
			Organophosphorus pesticides			
1	**Ethoprophos**		N.D			
2	**Phorate**		N.D			
3	**Diazinon**		N.D			
4	**Dimethoate**	0.0071	0.075	0.0388±0.017	0.05	N.E
5	**Pirimiphos methyl**	0.004	0.0224	0.0132±0.009	0.01	0.05
6	**Chlopyrifos**	0.0033	0.0093	0.0066±0.001	0.02	0.01
7	**Malathion**		N.D			
8	**Quinelphos**		N.D			
9	**Prothiophos**		N.D			
10	**Fenamiphos**		N.D			
11	**Ethion**		N.D			
12	**Triazophos**		N.D			

N.D: Not detected N.E: Not established

Table (3): Pyrethroid pesticide residues (ppm whole milk basis) in examined buffalo's milk samples and respective MRLs.

No.	Pesticides detected	Min	Max	Mean ±SE	MRLs	
					FAO/WHO (2013)	EU(2014)
			Pyrethroid pesticides			
1	**Permethrin**	0.0106	0.0374	0.0229±0.003	N.E	0.05
2	**Tetramethrin**	0.019	0.6373	0.1239±0.054	N.E	N.E
3	**Cypermethrin**	0.0316	0.2969	0.1985±0.022	0.05	0.05
4	**Deltamethrin**		N.D			
5	**Esfenvalerate**		N.D			

N.E: Not established N.D: Not detected

3.4. Effect of boiling process on the examined pesticides

The effect of boiling process on the concentration of pesticide residues in buffalo's milk was studied and data was illustrated in table (4).

3.5. Risk assessment of pesticide intake in raw milk

The previous monitoring results could be used to assess the risk of pesticide intake via raw milk to egyptian consumer health. The risk assessment has been carried out by comparing calculated estimated daily intake (EDI) to toxicological criterion, acceptable daily intake (ADI), established by the Joint FAO / WHO meeting on pesticide residues (JMPRL). The EDI was calculated by multiplying the mean concentration of pesticide residue (ppm) by milk consumption rate (kg/day) and dividing by a body weight of 60 kg for an adult person. The average daily milk consumption for the egyptian people, according to food balance sheet compiled by FAO, 2011 was 64.8 kg/year. The hazard risk index (HRI) can be calculated by dividing the value of EDI by the corresponding value of ADI (WHO, 1997). Table (5) compared the values of EDI and ADI. The results of health risk assessment indicated that EDIs of ϒ- HCH, ϒ- Chlordan, Aldrin, Endrin, Methoxychlor, ∑DDT, Dimethoate, Pirimiphos methyl, Chlopyrifos, Permethrin, Tetramethrin, Cypermethrin were below the corresponding ADIs. Therefore, their HRI values ranged from 0.001 to 0.174. While, EDIs summation of heptachlor and its metabolite heptachlor epoxide exceeded corresponding ADI and RHI was greater than one. No ADIs available for HCB, α- and β –HCH so, EDIs could not be calculated.

4) DISCUSSION

Generally, the results reflected that total levels of OCPs detected were much greater than the total levels of OPPs and pyrethroid. In spite of in Egypt all types of this group have been officially banned since the late 1990 after being used for more than 50 years (El Bouraie *et al.*, 2011). This can be interpreted based on many reasons. One might be related to their properties such as their high persistence, high accumulation in organisms as well as bind strongly to soil particles, these will keep them in an environment for long time. Also, some banned pesticides are still producing and sold in developing countries such as DDT and other organochlorine pesticides (Wood Mackenize, 1994). A number of organochlorine is still used in some Nile basin countries so, it is possible to transfer these pesticide molecules by suspended particles through water river which runs to Egypt (Malhat *et al.*, 2015). The most abundant OCPs were heptachlor and its metabolite, heptachlor epoxide. Since heptachlor was used extensively in Egypt as a seed treatment to protect crops such as corn, small grains, and sorghum from pests, also, it used to control insects at home and buildings. Heptachlor can be converted inside a living organism to oxygenated metabolite, heptachlor epoxide. The mean concentration of heptachlor is more than its metabolite. Since heptachlor epoxide is more soluble in water than heptachlor consequently, the concentration of heptachlor in milk increased than its metabolite (SRC, 2007). John *et al.* (2001) found heptachlor residue in buffalo's milk samples at a level of 0.15 ppm which also exceeded the tolerance limit. Abou Donia *et al.* (2010) detected heptachlor and H. epoxide in 8.3% and 25% buffalo's milk samples with a mean values of 0.032 and 0.064 ppm fat basis respectively that is exceeded MRLs.

The technical-grade HCH is a mixture of different isomers (α, β, ϒ isomers, which detected in milk samples). Among of these isomers, β- HCH has recorded that the highest mean concentration among all pesticide residues that detected in milk samples. Because this isomer represented as the most persistent in the environment. The persistence of these three isomers in the environment is β > ϒ > α isomers and the mean concentrations found in the milk samples were in the same line of their persistence in the environment (Yamashita et al., 2000). Battu et al. (2004) could be detected ϒ-HCH(lindane) in 40.9% liquid milk samples which is nearly similar to our result but Abou Donia et al. (2010) found ϒ-HCH in 50% of buffalo's milk samples.

α-HCH could be detected in 1.4% milk samples by Nida *et al.* (2009) while, β-HCH found in 8.6% samples. The presence of ϒ-HCH(lindane) above its MRL in all contaminated samples in the present study is viewed with serious concern, as it is carcinogenic in nature, and may affect the functioning of other vital organs of the body (Vettorazzi, 1975). The frequency positive samples of HCB in our current investigation was lower than those detected by Heck *et al.* (2007) and Abou Donia *et al.* (2010) who reported the frequency positive samples were 100% and 41.7%, respectively.

Table (4): Effect of boiling process on Organochlorine, pyrethroid and Organophosphorus pesticide residue levels (ppm)in examined buffalo's milk samples

Pesticide name	Before boiling	After boiling	%Pesticide reduction After boiling
Organochlorine pesticides			
HCB	0.0219	0.0207	5.48
β-HCH	0.945	0.7136	24.49
ϒ-HCH	0.0775	0.0728	6.06
Heptachlor	0.11196	0.0869	22.38
Heptachlor epoxide	0.0039	0	100
Aldrin	0.0224	0.0123	45.1
Endrin	0.0144	0.012	16.67
Methoxychlor	0.06	0.0529	11.83
P,P-DDT	0.0317	0.0269	15.14
P,P-DDE	0.0114	0.0111	2.63
Pyrethroid pesticides			
Permethrin	0.0284	0	100
Tetramethrin	0.074	0	100
Cypermethrin	0.1208	0	100
Organophosphorus pesticides			
Phorate	0.0051	0	100
Dimethoate	0.0207	0	100
Pirimiphos methyl	0.0112	0	100
Chlopyrifos	0.0018	0	100

But the mean value of HCB was high, this is due to HCB was used frequently as fungicide to protect seeds of wheat and also it is persistent and bioaccumulative (EPA, 2011). The frequency and concentration order of DDT and its derivatives was indicated that DDE was more predominate in milk samples. Higher concentration of DDE observed in analyzed milk samples may be interpreted based on conversion of DDT to DDE as the result of UV radiation in sunlight, moreover, DDT will be metabolized in animal to DDE and DDD. So, the ratio of DDE found in milk will be higher than DDD and DDT (loganathan and Lam, 2011). Abou Donia *et al.* (2010) recorded DDT and its metabolites at lower levels than those recorded by our study. However the mean sum DDT level of 0.1215 ppm in the current study is lower than those reported for raw milk from some developing countries, for example, Uganda 3.24 ppm, Nigeria 3.83 ppm, India 6.55 ppm, Kenya 6.99 ppm, South Africa 20.10 ppm and Ethiopia 7.75 ppm (FAO, 1986).

After prohibition of DDT. Methoxychlor was used extensively with a broad spectrum insecticide on livestock as ectoparasiticide, agriculture and housholds (Ivey *et al.*, 1983). Also, aldrin, endrin and ϒ- Chlordan were used heavily as insecticides to control different pests in main crops. High level of aldrin was recorded by John *et al.* (2001) and Abou Donia *et al.* (2010) who found its residues in buffalo's milk samples at level 0.15 and 0.066 ppm respectively, which exceeded the tolerance limit. In contrast, lower levels of aldrin were determined in bovine milk samples which were 0.003 and0.002 ppm fat by IDF (1979) and Campay *et al.* (2001) respectively

Table (5): Risk assessment based on ADIs of pesticide residues in raw milk

Pesticides	ADI (ppm bw /d)	EDI (ppm bw/d)	HRI	Risk assessment[a].
HCB	N.A	0.00006	-	-
α-HCH	N.A	0.00078	-	-
β- HCH	N.A	0.00240	-	-
ϒ- HCH	0.005	0.00025	0.0518	No
∑Heptachlor	0.0001	0.00035	3.9	Yes
ϒ- Chlordan	0.0005	0.00002	0.054	No
Aldrin	0.0001	0.00001	0.174	No
Endrin	0.0002	0.00003	0.1519	No
Methoxychlor	N.A	0.00005	-	-
∑DDT	0.01	0.00035	0.035	No
Dimethoate	0.002	0.00011	0.055	No
Pirimiphos methyl	0.03	0.00003	0.001	No
Chlopyrifos	0.01	0.00001	0.001	No
Permethrin	0.05	0.00006	0.0012	No
Tetramethrin	N.A	0.00036	-	-
Cypermethrin	0.02	0.00009	0.004	No

[a]Yes: indicates to a positive health risk , No: indicated to no negative health risk

None of the liquid milk samples revealed the presence of aldrin at their detection limit of 0.01 ppm with the investigations of Waliszewski *et al.* (1997). While similar result of endrin recorded by Abou Donia *et al.* (2010) but Nida *et al.* (2009) could not detect endrin in any of the examined milk samples. OCPs are categorized as persistent organic pollutants (POPs). Organochlorine pesticides pose serious risk to human health and the environment, which may include carcinogenicity, reproductive impairment, developmental and immune system changes and endocrine disruption (IOMC, 2002). The existence of OCP residues in milk with concentrations over than their corresponding MRLs will set off the alarm bell.

Organophosphorus pesticides were used instead of organochlorine pesticides because being less stable and persistence than organochlorine. The presence of chlorpyrifos, dimethoate and pirimophos-methyl in milk samples may be explained due to their extensively used as they are of great significance in pest control in Egypt. Chlorpyrifos has the highest frequency percentage among OPPs which detected in our study because it is heavily used to combat different pests in main crops as well as forage crops that used for animal feed. Its concentrations were found below MRLs. Existence of some OPPs such as dimethoate and pirimophos-methyl in milk at concentrations over than MRLs constitute a serious risk to human health.

Currently, synthetic pyrethroids are extensively and frequently used because they are effective against different pests in agriculture, livestock and public health purposes. They have selective toxicity towards insects as well as their low toxicity to mammals and animals comparing with OCPs and OPPs (Kidd and James, 1991). Cypermethrin , is classified as restricted pesticide by EPA because its toxicity to fish, but in Egypt, it is still used to control a broad spectrum of pests in agriculture and veterinary sectors. Also, it is used to control the pests at home and buildings (Extoxnet, 1996). Cypermethrin found at concentrations that may cause adverse health impacts.

The results of pesticides in table (4) showed the efficient role of boiling of milk on degradation or elimination of pesticide residues. Abou- Arab (1991) showed that sterilization at 121˚C for 15 min. showing 83.25, 91.67 and 68.70% loss with β-BHC, lindane and P,P-DDT, respectively. The higher reduction may be due to long time of treatment. In contrary, Rachev *et al.* (1974) reported that pasteurization of raw milk at 93˚C or 100˚C for few seconds had a little effect on β-BHC and P,P-DDT. In our study, for OC pesticide residues, boiling of milk for 10 min. showed different degradation percentages in its concentrations. While boiling process of milk could eliminate residues of pyrethroid and OP pesticides completely, this may be attributed to OC pesticides are much more resistant to environment degradation than OP pesticides (Krieger, 2001). LI-Ying *et al.* (2011) revealed that the contents of the pesticides in milk subjected to heat treatment showed a decreasing trend with progressing treatment time, indicating degradation of the pesticides.

Pesticides exposure independently or in synergism with modifiable risk factors, is recognized as an important environmental risk factor associated with hemopoetic cancers, cancers of the prostate, pancreas, liver and other organs (Jaga and Dharmani, 2005).

Fat solubility of these compounds is responsible for their varied concentrations in the tissues and their accumulation in the lipoproteins of the cell membranes resulting in changing their structures and permeability (Antunes-Madeira *et al.*, 1993). OC pesticides are able to significantly decrease the ability of highly purified human natural killer (NK) cells to lyse tumor cells after exposure, ranging from 1 hour to 6 days (Beach and Whalen, 2006). Persistent OC compounds such as DDT and HCB play an important role in chronic poisoning and take part in a number of pathological processes (Lembowicz *et al.*, 1991).

In Egypt some people, especially farmers prefer to drink the milk directly after milking process without boiling. To protect those people, risk assessment of pesticide intake was carried out based on the raw milk. The data in Table (5) illustrated that ∑EDIs for heptachlor and its metabolite (H.epoxide) was about 4 times greater than its corresponding ADI recommended by FAO/WHO. So, the intake of this pesticide and this metabolite with raw milk may cause possible adverse health effects. Unfortunately this pesticide and its metabolites was detected in over than 95% of the collected samples. Our attentions were focused only on the adults because the consumption rate of milk by kids not available. It is expected that kids are at greater risk than adults because their consumption rate of the milk in this stage is higher than the adults, also their weight is much lesser than adults. Regarding the pesticides that their ADIs not available so the toxicological significance could not be evaluated.

5. CONCLUSION

It could be concluded that OC, OP and pyrethroid pesticide residues were detected in examined buffalo's milk samples. The most detected values of pesticide residues were exceeded the maximum residue limits of FAO/WHO and EU. Boiling of milk showed the efficient role in the degradation and elimination of pesticide residues in milk. The consumption of polluted raw milk with heptachlor and its metabolite will be risky. The risk of using pesticides especially OC pesticides on human health was associated with cancers and increased risk of Alzheimer disease (Singh *et al.*, 2013). So the main risk management tool is the prevention of exposure either through feed or from animal environment as well as the application of a withdrawal time (Kan and Meijer, 2007).

Monitoring the pesticide residues continuousely in milk is very essential to safeguard the consumer health and achieve the food safety.

6) REFERENCES

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