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Quantification of some pesticide residues in raw and heat treated milk

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Abstract

Detection of pesticides residues in milk of ruminants represents a human public health hazardous. In this study, milk was monitored using Gas chromatography (GC), for one organochlorine pesticide residue dielderin and high performance liquid chromatography(HPLC) for deltamethrin as pyrothroid pesticide residues to determine the degree of environmental pollution with both of them .The study included examination of 60 milk samples (30 fresh dairy milk which were collected from some farms at Assiut Governorate and 30 ultra- heated milk (UHT), which collected from different shops and supermarkets at Assiut Governorate.

The results of the study revealed that 30% of raw milk samples were polluted with dieldrin pesticide residue and 20% were polluted with deltamethrin pesticide residue while (13.33%) of UHT milk samples were polluted with dieldrin pesticide residue and 6.66% were polluted with deltamethrin pesticide residue.

The mean concentration of dieldrin and deltamethrin pesticide residues in raw milk were 210.1 ± 126.7ppb and 100.0 ± 83.8 ppb respectively, while in the ultra-heated milk samples, the mean concentration of dieldrin and deltamethrin pesticide residues were 98.0.25±63.0 ppb and 18.0 ±8.55 ppb respectively.

On comparison of the data recommended by the Food and Agricultural organization/ World Health Organization (FAO/WHO 2008), it was found that none of obtained values exceeded the recommended values. The results of this work indicated that in spite of the banning much of organochlorine and pyrothroid pesticides still contaminating the environment resulting in contamination of food stuffs, particularly milk. There is a potential risk of the consumption of such contaminated milk on human's health particularly infants and children.

Key words: GC, HPLC, UHT milk, organochlorine pesticide, pyrothroid pesticide.

Introduction

Pesticides are used worldwide but many persistent residues of pesticides may be resulted in environmental pollution as well as human health hazards. Pesticides are broadly used in farming for their economic benefits to fight crop pests and reduce competition from weeds, thus improving yields and protecting the quality, reliability and price of production (**Arthur *et al*., 2011**). The widespread use of these compounds may be resulted in environmental contamination (**Tariq *et al*., 2004** and **Fernandez *et***

***al*., 2008**). Egypt is one of the largest users of pesticides in order to duplicate her animal and plant production power. Several pesticides contain noxious substances that persist in environment for a long time (**Latif *et al*., 2011**). The agrochemicals particularly pesticides enter in our body via food of animal origin, especially of high lipid contents (**Shahzadi *et al*., 2014**).

Detection of pesticide residues in milk of ruminants represents a public health hazard, since milk and dairy products are widely consumed by infants, children and adults throughout the world. In view of this, many countries have enacted regulations to set the permissible limits of such pesticides residues in milk and dairy product to protect the consumers (**Tsiplakou *et al*., 2010** and **LeDoux, 2011).**

Organochlorine pesticides are substances containing chemically combined chlorine and carbon. They may be grouped into three general classes:the dichlorodiphenylethanes (DDT, DDD, dicofol, etc.), the chlorinated cyclodienes (aldrin, dieldrin, heptachlor, etc.) and the hexachlorocyclohexanes (lindane). Pyrethroid pesticides (bifenthrin, deltamethrin and lambda cyhalothrin), **(Neff *et al*., 2012**)

The present study aimed to monitor one of organochlorine pesticide residue, dielderin and one of organophosphorous pesticide (deltamethrin as pyrothroid pesticide residue) in dairy cow milk collected directly from farms and ultra-heated milk in Assuit Governorate as a trial to determine the degree of environmental pollution with both of them. Moreover, to compare the results of this study with other national and international studies.

MATERIAL AND METHODS

1. Collection of milk samples:

Sixty samples of fresh dairy cows milk and ultra-heated milk (30 of each) were collected directly from farms in some villages as a bulk milk tank samples and the ultra-heated milk (processing holds the milk at a temperature of 140 °C (284 °F) for four seconds, were milk is sterilized and not pasteurized to allows milk to be stored several months without refrigeration (**Tortora, 2010**) from different shops and supermarkets at Assiut Governorate.

2. Determination of dieldrin as organochlorine pesticide:

The procedures for estimation of dieldrin in the examined samples were applied by using Gas chromatography (GC) according to **Heck *et al*. (2007)** as follow:

2.1. Extraction:

The milk samples were homogenized and thoroughly mixed. Further, 100 ml of each sample were divided into two portions (50 ml of each). Each portion was centrifuged at 5000 rpm for 15 minutes. After centrifugation, the upper layer containing fat was removed and placed in a small clean beaker from which one g of fat was weighed out. Anhydrous sodium sulfate (25g) was mixed with the fat in a 250 ml flask. To this mixture, 100 ml petrolium ether was added and vigorously shaken for two minutes. The mixture was allowed to settle down and the petrolium ether was filtered off into another flask through a glass funnel containing anhydrous sodium sulfate to remove the traces of water. The filtered extract was evaporated to about one ml which mixed with hexane and kept in a flask for the clean-up process.

2.2. Clean up with florisil method (Darko and Acquaah, 2008):

Accurately, 50 cm long glass column of 4 mm was pulgged with glass wool and washed with hexane. It was then packed with 10g activated florisil followed by 4g anhydrous sodium sulfate. The column was lightly tapped to compact the florisil ped and then rinsed with 5 ml hexane to remove any impurities. To this column, the concentrated fat was added and the column was thed eluted with 100 ml n-hexane to extract the organochlorine (dieldrin) pesticides. The elutent was collected in a 250 ml round bottomed flask, filtered through anhydrous sodium sulfate into another round bottomed flask and then transferred into a rotary evaporator flask. Therefore, the extract was carefully concentrated to about one ml at 40°C and taken into a glass vial with hexane and evaporated until dryness by using nitrogen gas. The residue was dissolved in one ml cyclohexane for analysis by GC.

On the other side, the Stock and standard solutions of dieldrin were prepared by diluting a known volume of the solution in 100 ml hexane. Accordingly, the dieldrin residues in the examined milk samples were quantitatively determined by comparison with the standard solutions injected under identical GC conditions. Finally, the concentrations of the dieldrin residues (ppb) were calculated as follows:

Dieldrin concentration= S/ W (ppb)

Where, S= Concentration of standard solution (ppb) in corresponding to the spot of reference pesticide standard.

W=volume of unkown sample.

3-Determination of deltamethrin as pyrothroid pesticide:

3.1. Extraction and purification (AOAC, 1996):

Accurately, 10g of the sample were put in an Erlenmeyer flask and acidified with 1N HCl to approximately pH 4. Further, 50ml acitonitril were added and the flask was closed and vigorously shaked for 30 minutes. The sample was filtered in a glass funnel by Wattman filter paper NO.42. The residue in the filter paper was transferred to the same Erlenmeyer flask and the procedure was repeated with 25ml acitonitril for 15 minutes. Thus, the sample was filtered again using the same procedure and the same filter paper. The filtered sample was also collected in the same flask with the first filtrate.

3.2. Partitioning The filtrate was transferred to a separating funnel and 15ml N Hexan were added and shaken for one minute. This procedure was repeated twice and the hexanic phase was discarded. However, the acitonitril phase was applied to evaporate in a rotary apparatus.

3.3. Clean-up (Bissacot and Vassilieff, 1997):

It was performed in a chromatographic column containing 4g silica gel previously activated in an air heater at 130^{o}C for 5 hours, then cooled and 5ml distilled deionized water were added. The silica gel was put in the chromatographic column and eluted with 1ml N Hexan: Diethyl ether (9: 1). The dry residue was re-suspended with 10ml N Hexan and 7ml N Hexan: Diethyl ether (9: 1) and the filtrate was evaporated till dryness.

3.4. Chromatography:

The dry residue was re-suspended with 1ml high grade acetonitril and injected onto High Performance Liquid Chromatography (HPLC). Accordingly, the suitable conditions of HPLC were: HPLC apparatus (Agilent1100) equipped with diodearray detector (DAD); Column: Zorbex SBC 18 (150mm x 4.6mm x 0.5um film thickness); Mobile phase: acitonitril: distilled deionized water (80: 20); Flow rate: 1.0ml/ min.; Detector: 226nm ultraviolet.

The deltamethrin residues in the examined samples were compared with those obtained from similar injections of the standard solutions. Quantitative determination of these residues was obtained by the measurement of the peak areas in the chromatogram.

Results and Discussion

Statistical processing of the data included calculation of the minimum, maximum, median and mean levels of organochlorine and pyrothroid pesticide residues and the standard deviation of the mean (± SD).

For dieldrin (in raw milk samples) 9 (30%) out of 30 analyzed milk samples were contaminated with dieldrin pesticide, while 21 samples (70%) was free from contamination with dieldrin pesticide residues.

For dieldrin (in Ultra heated milk samples) 4 (13.33%) out of 30 analyzed milk samples were contaminated with dieldrin pesticide, while 26 samples (86.6%) was free from contamination with dieldrin pesticide residues.

For deltamethrin (in raw milk samples) 6 (20%) out of 30 analyzed milk samples were contaminated with deltamethrin pesticide, while 24 samples (80%) was free from contamination with deltamethrin pesticide residues.

For deltamethrin (in Ultra heated milk samples) 2 (6.66%) out of 30 analyzed milk samples were contaminated with deltamethrin pesticide, while 28 samples (93.33%) was free from contamination with deltamethrin pesticide residues.

Although many countries have banned or severely restricted the use of most dangerous organochlorines, and pyrothroid pesticides, it is thought that many of these compounds have been or continue to be used in large quantities in some developing tropical countries for agricultural and public health reasons as control of some diseases (**Latif *et al*., 2011**). Presence of organochlorine residues in soils that were heavily treated years ago is suspected to be an important present-day source (**Fiedler *et al*. 2000 and Amoah *et al*. 2006**).

In this study dieldrin resample 30 % in raw milk samples and 13.3% in ultra-heated milk samples. This could be explained by the wide spread of organochlorine pesticide contaminants in Assiut governorate. This is in agreement with (**El-Gebaly**, **2000**) who detected organochlorine residues in milk samples collected from Cairo, Giza and El-Kaliobia Governorates .Moreover, **Dogheim *et al*. (1988 and 1996**), who detected organochlorine pesticide contaminants in milk samples collected from Beni-Suef and El-Gharbia Governorates. As a result of the legal banning of organochlorine pesticides use in Egypt since 1990 (**National Resources Defense Council, 2003).**

In this study, deltamethrin resample 20% in raw milk samples and 6.66% in ultra-heated milk samples, which was very low in comparing with the result detected by **Jackson (2013)**, which was 73 % in milk samples.

In the present study, the mean concentration of dieldrin in raw milk samples was 210.1 ± 126.7ppb, which was higher than the mean concentration detected by **El-Gebaly (2000)**, which was 175 ppb in milk collected from Great Cairo and also higher than the mean concentration detected by **Abdel-Wahab *et al.* (2004)** which was 127.3±0.02 ppb. The mean concentration of deltamethrin in raw milk samples was 100 ± 83.8ppb, which was more than that recorded by **Neelam, *et al*. (2013).**

This high result of dielderin and deltamethrin residues in raw milk samples may be explained by the diverse industrial activities have been implicated to contaminate the forages with subsequent elevated organocholorine and pyrothroid compounds (**Goodarzi, *et al*. 2010**).

The mean concentration of dieldrin and deltamethrin in ultra-heated samples was 98.25±63.0 ppb, and 18.0 ±8.55 respectively which were lower than all the values detected in raw milk, this can be attributed to the fact that the highly temperature can reduce the amount or the value of the pesticide residue (**Abou-Arab *et al.* 2008).**.

The estimated pesticide residues in ultra-heated milk samples showed the efficient role of heat processing may be due to evaporation, co-distillation., thermal degradation which vary with the chemical nature of the individual pesticide (**Sharma *et al.* 2005).**

Ultra-heated milk samples contained residues were below the levels detected in raw milk. These results are in agreement with that reported by **Abou-Arab (1999)** who showed the efficient role of heat treatment on the degradation of some pesticides in milk products.

The results of this work suggested that consumption of heat-treated milk and dairy products may be safer than consumption of raw milk also indicated that some of organochlorine and pyrothroid pesticides still contaminating the environment resulting in contamination of food stuffs, particularly milk. There is a potential risk of the consumption of such contaminated milk on human's health particularly infants and children **(WHO, 2003, POP's office, 2006, FAO/WHO, 2008 and Faqir *et al*. 2012).**

Milk contamination with the pesticides residues can be controlled by preventing the contamination of feedstuffs. The findings of the study might help in extending awareness in dairy farmers and local people about pesticides and their hazardous effects on human.

Table (1): Statistical analysis of dieldrin and deltamethrin pesticide residuel levels in raw milk samples.

Pesticide residues (ppb)	No.of examined samples	Positive samples		Statistical analysis			
		No.	%	Min.	Median	Max.	Mean± SD
Dieldrin	30	9	30	34	217	380	210.1 ± 126.7
Deltamethrin	30	6	20	12	101	236	100 ± 83.8

Table (2): Statistical analysis of dieldrin and deltamethrin pesticide residuel levels in Ultra heated milk samples.

Pesticide residues (ppb)	No.of examined samples	Positive samples		Statistical analysis			
		No.	%	Min.	Median	Max.	Mean± SD
Dieldrin	30	4	13.33	41	78	196	98.2±63
Deltamethrin	30	2	6.66	10	18	26	18.0±8.5

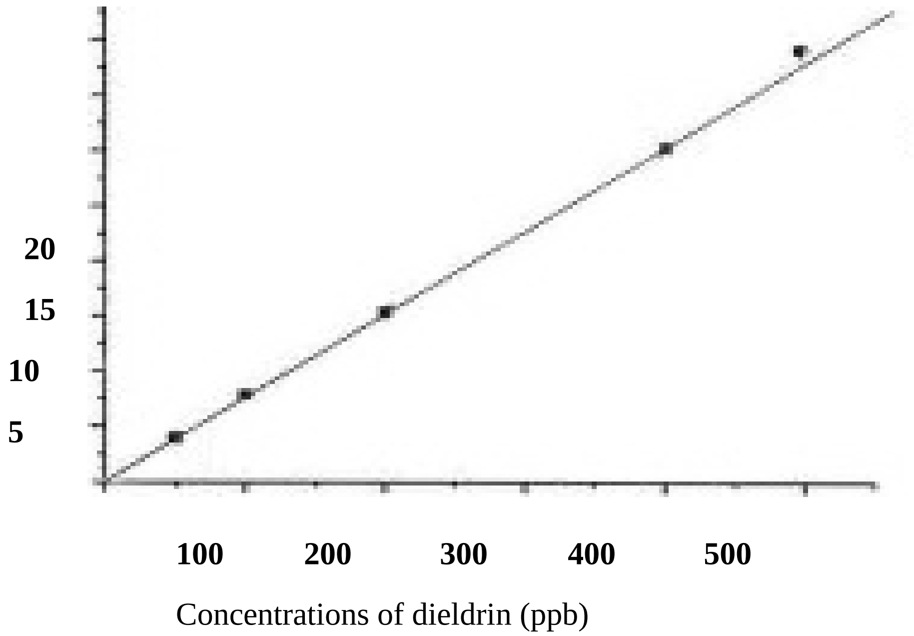

Figure (1): Calibration curve of dieldrin by using GLC in raw milk

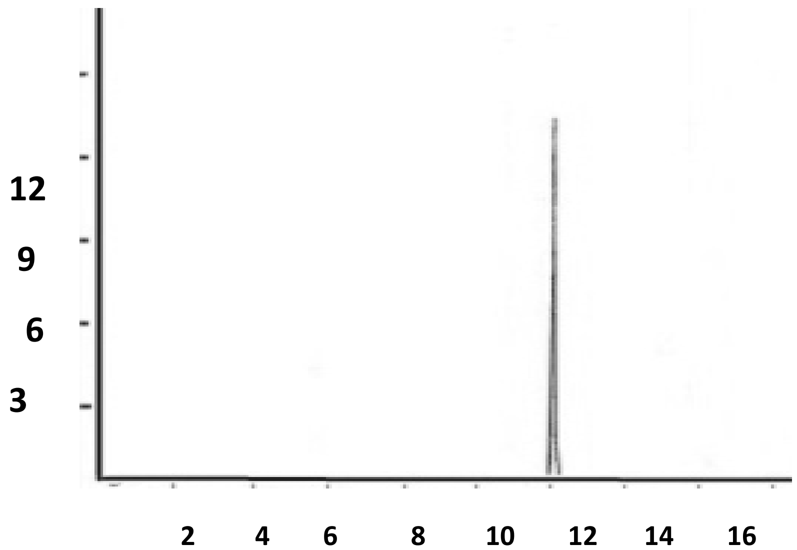

Figure (2): Chromatogram extract for dieldrin as organochlorine pesticidal residue in the examined samples of ultra -heated milk (Retention time minutes).

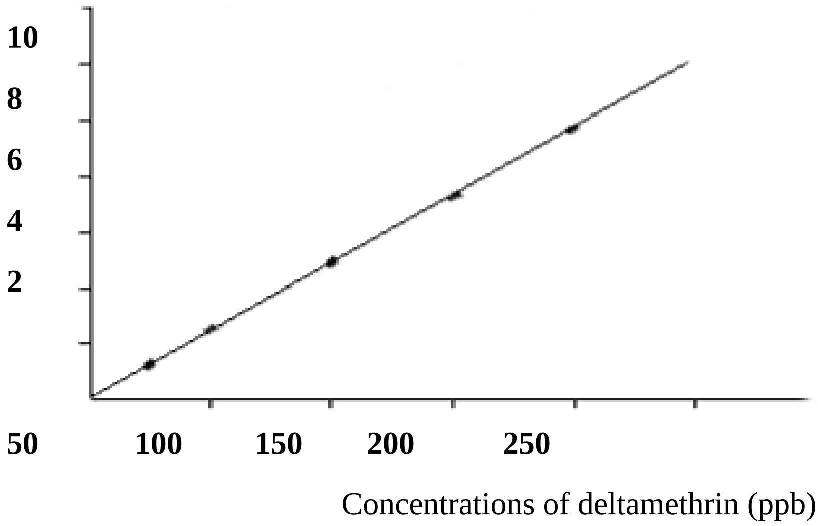

Figure (3): Calibration curve of deltamethrin by using HPLC in raw milk.

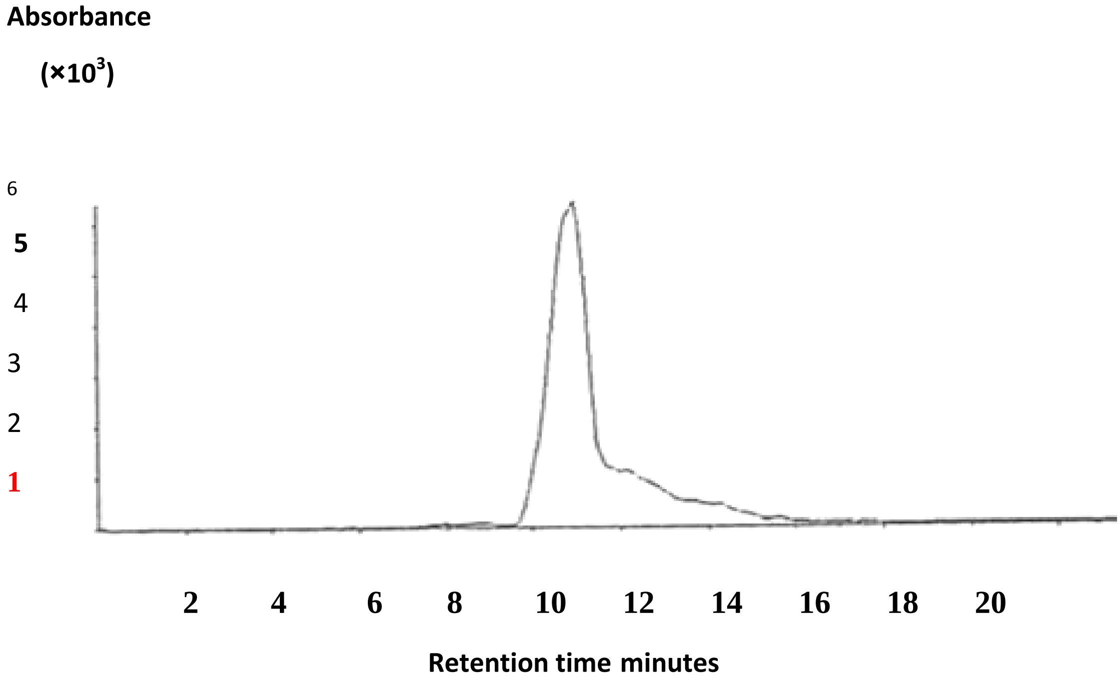

Figure (4): Chromatogram extract for deltamethrin as pyrothroid pesticidal residue in the examined samples of ultra-heated milk.

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