Pyrethroids Residues in Milk and Some Milk Products.

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Abstract

The presence of pyrethroids residues in 45 samples of raw milk, soft cheese and cream (15 of each) were collected randomly from different localities at Gharbia Governorate were investigated using High Performance Liquid Chromatography with diodea ry detector (DAD). Flumethrin residues were found in 20, 26.66 and 40 % of the examined raw milk, soft cheese and cream samples with an average concentration of 11.53±3.21, 80.82±23.18 and 107.98 ± 27.57 ppb, respectively. All the positive raw milk samples were within the Maximum Residual permissible Limit value (MRL) but those of soft cheese and cream showed levels above the MRL. While, cyhalothrin was detected in 26.66, 33.33 and 33.33% of examined samples and all of them showed levels exceeding the MRL with an average concentration of 258.25±170.31, 364.00±182.00 and 379.60±180.78 ppb, respectively. The public health importance of pyrethroids insecticides and possible sources of contamination as well as some recommendations to control such serious residues in milk and milk products were discussed.

Introduction

As the usage of many chlorinated hydrocarbons has diminished in many parts of the world, synthetic pyrethroids have often served as replacements because of their high insect potency and expected low mammalian toxicity **(7).**

Pyrethroids are broad-spectrum insecticides effective against a wide range of flying, crawling, chewing and suckling insects. They are used as household insecticides in a variety of location including residential, public and commercial buildings, animal houses, ware houses, fields and green houses. In addition, some pyrethroids are extensively used in the field of veterinary medicine as a spray, dip paths or pour-on treatment or even as ear tag applications in controlling ecto-parasites **(5 and 22)**.

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There are two types of pyrethroids, type I and type II. Further, type I pyrethroid is characterized by aggressive behavior, fine tremors, prostration and high body temperature, whereas,type II symptoms include salivation and chewing, burrowing, colonic and tonic seizures **(3)** . Flumethrin and cyhalothrin are a type II pyrethroids insecticides and accaricide that are derived from natural pyrethriins (ester of chrysanthemic and pyrethric acid extracted from chrysanthemum flowers) and used predominantly on cattle and sheep for the control of a broad range of ectoparasites **(24).**

Pyrethroids compounds may gain access to milk by various ways as its application to the lactating animals or by inhalation of toxic vapors by the animals following application of insecticides on their environment or ingestion of residues in feed and water and /or accidental contamination of milk, equipments and utensils used in processing of raw milk products during eradication of insects in dairy factories and storage rooms **(19).** All synthetic pyrethroids are potent neurotoxicants that interfere with nerve cell function and eventually causing paralysis **(3 and 20)** .Due to their lipophilic nature they are readily taken up by biological membranes and tissues so exposed organisms may exhibit symptoms of hyperexitation, tremors, and convulsions, followed by paralysis **(18).**

Thus, it is highly imperative that pyrethroids residues are kept well under recommended tolerance levels to minimize the human risk. Therefore the present work is planned out to study the prevalence of these harmful, toxic substances in milk and milk products and discussion of their public health importance as well as suggestion of some recommendations to safeguard consumers by monitoring of pyrethroids residues.

Materials and Methods

A- Collection of Samples:

To achieve the objectives of the present study, a total of forty five random samples (15 each) of raw milk, soft cheese and cream were collected from different localities in Gharbia Governorate. Milk samples

(500 ml each) were collected from street peddlers and dairy shops in clean glass containers, while soft cheese and cream (250 g of each) were collected from dairy shops and supermarkets. Each sample was labeled to identify its source, site and date of sampling, and then transferred to the laboratory with a minimal of delay.

Each sample was thoroughly mixed then, a representative 10 ml or g sample of milk or cheese and cream was subdivided in small glass containers with screw Teflon capped stoppers and kept deeply frozen at -20C till be used for pyrethroid residues analysis.

B- Pyrethroid standards:

Reference standards of synthetic pyrethroid insecticides were procured from the Central Agricultural Pesticide Laboratory, Cairo, Egypt.

C- Procedure:

1-Extraction and purification:

Ten ml of milk sample (or ten gm of cream) were transferred to an Erlenmeyer flask and acidified with 1N HCL to approximately Ph 4. Then 50 ml HPLC grade acetonitril was added to the sample and the flask was closed and shacken mechanically for 30 min. The sample was filtered in a glass funnel with a quantitative 12.5 cm diameter No. 42 Whatman paper filter (Whatman International Ltd., Kent, U.K.) and the filtrate was collected in a beaker. The residue in the paper filter was transferred to the same Erlenmeyer flask, and the procedure was repeated with 25 ml HPLC grade acetonitril for 15 min. the sample was then filtered again using the same procedure and the same filter paper. The filtered sample was also collected in the same beaker with the first filtrate.

2- Partitioning:

The filtrate was transferred to a separatory funnel, 15 ml N Hexan was added and then shaken for approximately 1 min. The procedure was repeated twice, and the hexanic phase was discarded. The acetonitril phase was taken to evaporate in a rotary apparatus.

3-Clean-up:

Clean up was performed in a chromatographic column containing 4 g silica gel previously activated in an air heater at 130 C for 5h. **(21),** then cooled and hydrated in 10% of its weight with distilled deionized water, and kept in a sealed container for 30 min. before use. The silica gel was put into the chromatographic column, and eluted with 1 ml n-hexan: diethyl ether (9:1). The dry residue was resuspended with 10 ml n-hexane and 7 ml n-hexan: diethyl ether (9:1). The filtrate was evaporated to dryness according to method recommended by **(2).**

4- Chromatography:

The dry residue was resuspended with 1 ml HPLC grade acetonitril and injected onto Liquid Chromatography and the condition of HPLC as follow : HPLC Apparatus: (Agilent 1100) equipped with diodearray detector (DAD) Column: Zorbex SBC 18 (150 mm× 4.6 mm ×0.5 µm film thickness). ; Mobile phase: acetonitril: distelled ionized water (80:20). Flow rate: 1.0 ml/min.; Detector: 266 nm ultraviolet.

5- Quantitative analysis:

The pyrethroids present in examined samples were compared with those obtained from similar injections of standard solutions. Quantitative measurements were made by the external standard technique and by measurements of the peak areas of the chromatograms.

Table (1): Recovery percent of pyrethroids pesticides as a result of fortification of examined samples was shown in the following table:

Pyrethroids	Rate of Recovery %		
	Raw milk	cheese	cream
Flumethrin	89.27	82.49	80.18
Cyhalothrin	90.01	85.19	80.30

Results

Table (1): Acceptability of the examined raw milk samples based on their content of pyrethroids residues (ppb) according to the Maximum Residual Permissible Limit (MRL)* (n=15)

Pyrethroids	positive samples				Mini.	Max.	Mean± S.E.
	No	%	Exceeding MRL				
			No.	%			
Flumethrin	3	20	0	0	6.3	17.4	11.53 ± 3.21
Cyhalothrin	4	26.66	4	26.66	54.00	695	258.25±170.31

* MRL Maximum Residual Permissible Limit for pyrethroids residues: 30 ppb **(10)**

Table (2): Acceptability of the examined soft cheese samples based on their content of pyrethroids residues (ppb) according to the Maximum Residual Permissible Limit (MRL)* (n=15).

Pyrethroids	positive samples				Mini.	Max.	Mean± S.E.
	No	%	Exceeding MRL				
			No.	%			
Flumethrin	4	26.66	4	26.66	44.80	131.40	80.82 ±23.18
Cyhalothrin	5	33.33	5	33.33	94.22	8600	364.00±182

* MRL Maximum Residual Permissible Limit for pyrethroids residues: 30 ppb **(10)**

Table (3): Acceptability of the examined cream samples based on their content of pyrethroids residues (ppb) according to the Maximum Residual Permissible Limit (MRL)* (n=15).

Pyrethroids	positive samples				Mini.	Max.	Mean± S.E.
	No	%	Exceeding MRL				
			No.	%			
Flumethrin	6	40.00	5	33.33	25.90	206.80	107.98±27.57
Cyhalothrin	5	33.33	5	33.33	83.12	905.21	379.60±180.78

* MRL Maximum Residual Permissible Limit for pyrethroids residues: 30 ppb **(10)**

Discussion

From the results recorded in table (1), it's clear that 20 and 26.66% of the examined raw milk samples were contaminated with flumethrin and cyhalothrin with a mean values of 11.53 ±3.21 and 258.25±170.31, respectively.Comparing our results with maximum permissible limit "MRL" (30 ppb) recommended by FAO/WHO (2004), we

found that all the positive examined raw milk samples not exceed such limit for flumethrin and all positive samples were exceeding the cyhalothrin limit

It is of great concern to mention that **(23)** coudn't detect any pyrethroids in examined raw milk samples. In contrast, **(24)** (found pyrethroid residues in milk of dairy cows sprayed by consecutive topical treatments. **(25)** detected residues of pyrethroid in milk until day 21 after application of cypermethrin. **(13)** mentioned that pyrethroid residues were found in milk samples over an 8- day after treatment. While, **(2)** found that the highest residues concentration for cyhathrin after its application of recommended doses of this pyrethroids with recovery percentage ranged from 78- 91%. Flumethrin and cyhalothrin exert their neurotoxicity through interference with ion-channel targets such as chloride and calcium channels **(6).**

Results in table (2) revealed that flumethrin was found in 26.66% of examined soft cheese samples with a mean value of 80.82±23.18 while cyhalothrin found in 33.33% of samples with an average of 364± 182. All positive samples for both pyrethroids were exceeding the limit (30 ppb)

Flumethrin and cyhalothrin are also modulating the release of acetylcholinesterase in the brain's hippocampus region. **(12)** and can inhibit Atpases **(16)**

Results in table (3) declared that both flumethrin and cyhalothrin residues were detected in 40 % and 33.33 % of examined cream samples with an average of 107.98 ±27.57 and 379.6 ± 180.78 respectively. Only 33.33 % of positive samples having residues of flumethrin are exceeding the permissible limit, while all positive samples for cyhalothrin are extensively beyond the MRL. Lower findings obtained by **(15)** who declared that due to the lipophilic nature, pyrethroids insecticides have a tendency to remain in milk and after processing of milk, partition into cream or cheese the residues of these insecticides gets magnify.

In addition, pyrethroids can disrupt hormone related functions (**11**). In mammals, pyrethroids decrease progesterone and estradiol production (**8**) eliciting estrogenic effects in females and antiandrogenic effects in males (**14**). Furthermore, flumethrin have been shown acytotoxic effect on both myelopiosis and erythropiosis inducing chromosomal damage in the bone marrow cells when administered to mice by intraperitonial injection (**17**). Also, flumethrin exposure diminishes hepatic enzyme levels and catalytic activities of monosxigenase systems as wellas oxidative metabolism of antipyrine in male wister rates (**1**) and have immunosuppressive effects (**9**). Additional long term effects may cause damage to respiratory surfaces and interference with renal ion regulation (**4**).

Finally, the obtained results gave an indication about the misuses and extensive uses of pyrethroids insecticides without paying attentions to their health risk assessments, considering them being safer and less harmful than other insecticides. Therefore, to prevent or reduce the presence of these toxic and harmful residues in milk and milk products, some recommendations should be kept in consideration including the fact that pyrethroids should be used according to the specification on the label / approval sheet. In addition, using of pyrethroids in dairy rooms, dairy related equipment and dairy processing planets should not be permitted as well as contaminated milk should not be used for human consumption, also treatment of ectoparasites on lactating animals or at the animal shed must only performed with approved formulation under a veterinarians supervision.

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بقايا المبيدات البيريثرودية فى اللبن الخام و بعض منتجات الالبان

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الملخص العربى

لقد زاد في الآونة الأخيرة استعمال المبيدات البيريثروديه فى مكافحة الآفات الزراعية الضارة باعتبارها اقل سمية من المبيدات الاخرى لأنها تتحلل سريعا إلى عناصرها الاوليه ولا تلحق أضرار بالبيئة لذا فهي من أهم المبيدات المستخدمه حاليا خصوصا فى زراعات القطن بالاضافة الي أنها تستخدم في إبادة الحشرات المنزليه كالناموس والذباب والحشرات الزاحفة فى المنازل والمحال التجارية ومعامل إنتاج منتجات الألبان . و علاوة علي ذلك فان لها استخدام واسع المجال في الطب البيطري كعلاج لكثير من الطفيليات الخارجية للحيوان مثل القمل ، الجرب، القراد والذباب والتي تتسبب جميعها فى خفض إنتاجيه الحيوان وأيضا تستخدم فى الحظائر لمكافحة الحشرات المتطفلة علي جسم وفراش الحيوان. لذلك تتواجد العديد من الفرص لتلوث اللبن الناتج من الحيوان بهذه المبيدات الضارة. وتهدف هذه الدراسة إلى استبيان مدى تواجد بقايا البيرثرويدات فى اللبن الخام ، الجبن الطري والقشدة والتعريف بخطورتها الصحية ان وجدت وتوضيح الأسباب التي تؤدى الى وجودها فى هذه الاغذيه مع إيجاد بعض الإرشادات للحد من هذه المشكلة وذلك للحفاظ على صحة المستهلك.

أجريت هذه الدراسة على عدد ٤٥ عينه من اللبن الخام، الجبن الطري و القشدة بواقع ١٥ عينه لكل نوع تم تجميعها من مختلف محلات الألبان والأسواق المنتشرة فى محافظه الغربية وذلك لإجراء التجارب التحليلية بواسطة جهاز الكروماتوجراف السائل عالي الأداء للكشف عن مدى تواجد بقايا البيروثريدات في العينات المجمعة وقد وجد إن تواجد مبيد الفلومثرين بنسب ٢٠ ، ٢٦,٦٦ و ٤٠ % من عينات اللبن الخام ، الجبن الطري و القشدة بمتوسط التركيزات التالية ٢١و٣±٥٣و١١ ، ١٨و٢٣ ±٨٢و٨٠ ، ٥٧و٢٧± ٩٨و ١٠٧ جزء في البليون على التوالي. أما بالنسبة إلي مبيد السيهالوثرين فقد وجد فى نفس العينات بالنسب ٢٦,٦٦ % في اللبن الخام و٣٣,٣٣ % لكل من عينات الجبن والقشدة بمتوسط تركيزات ٣١و١٧٠ ±٢٥و٢٥٨ ، ..و١٨٢± ..و٣٦٤ و ٧٨و١٨٠ ± ٦٥و٣٧٩ جزء في البليون علي التوالي .

أوضحت الدراسة ان عينات اللبن الخام ظهرت بنسب اقل تلوثا من عينات الجبن الطري والقشدة فقد كانت ٢٠ % من العينات المختبرة جاوزت الحد المسموح به بينما تواجدت بقايا الفاومثرين والسيهالوثرين بقيم جاوزت الحد المسموح بيه في عينات الجبن الطري بنسبه ٦٦و٢٦ ، ٣٣و ٣٣% علي التوالي و بنسبة ٣٣و٣٣ ، ٣٣و٣٣% فى عينات القشدة المختبرة.