Assessment Of DDT Residues In Milk And Some Dairy Products

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ABSTRACT

A total of sixty samples of raw buffalo's milk, kareish cheese and buffalo's butter samples (20 of each) were collected from Sharkia Governorate markets for detection and determination of DDT derivatives and comparison the level with its permissible limit and the effect of heat treatment on DDT residues. The obtained results revealed that the mean values of *p, p* DDE in raw buffalo's milk and butter before boiling 2.747± 0.346 and 41.624± 4.242 ppb respectively, meanwhile; after boiling the results were 0.769± 0.103 and 10.995± 1.166 ppb respectively. The detected *p.p* DDE in both raw and boiled milk was below the permissible limit in all samples, on the other aspect *p.p* DDE exceeded the permissible limits in 16 (80%) out of 20 butter samples (before boiling), while all the boiled butter samples (ghee) within permissible limits. On the other hand, all the DDT derivatives were not detected in all the examined kareish cheese samples.

INTRODUCTION

DDT (Dichloro Diphenyl Trichloro ethan) is an organochlorine pesticide and one of the best known synthetic pesticides. It was first synthesized in 1874 by Othmer Zeiler, but its insecticidal properties were not discovered until 1939 by the Swiss scientist Paul Hermann Muller, who was awarded the 1948 Nobel Prize in physiology and Medicine for his efforts ***(1)***. In the early years of World War II, DDT was used with great effects to combat mosquitoes spreading malaria, typhus and other insect-born diseases among both military and civilian populations. Further more, malaria was eradicated from Egypt due to extensive use of DDT spraying ***(2).***

Commercial DDT is actually a mixture of several closely related compound, with DDT generally composing 77% of the formulation *o,p DDT* 15% and related compounds making up the balance. The major metabolite and breakdown product of DDT in the environment is Dichloro Diphenyl Dichloro ethylene (DDE).

DDT and other organochlorine pesticides were highly lipophilic, stable in the environment and pass through the food chain ***(3)***. Thus in 1962, Rachel Carson's book, Silent Spring was published, this book argued that organochlorine especially DDT were poisoning both wild life and the environment and were also endangering human health ***(4)***. Moreover, from the public health point of view, exposure to DDT was associated with increase neuropsychological and psychiatric symptoms, non allergic asthma ***(5)***. Further more, EPA in 1987 classified DDT as class B_2 a probable human carcinogen ***(6).***

Owing to their environmental persistence and serious risks on the public heath, DDT and other organochlorine pesticides were prohibited in several progressed countries. Although banning of this pesticide group in Egypt since 1980 ***(7),*** many recent studies recorded DDT and other organochlorine residues in food ***(8-10).***

The milk and dairy products are one of the important media for accumulation of organochlorine pesticides ***(11)***. Because of their environmental stability and lipophilic property, DDT and its metabolites are readily excreted in the milk fat ***(12).*** DDT in feed and water of the cow is converted into DDD by the action of microflora. In animal tissues DDD is further metabolized to DDE which is more lipid soluble than DDT or DDD ***(13).***

Dairy products have relatively high saturated fat content may increase breast cancer risk ***(14).*** Due to its estrogenic activity ***(15)***, *p.p* DDE comprised 84% of total DDT in

dairy product, the half-lives of DDT individuals measured in cows *(16)*, demonstrated that DDE was persistent but not DDT which was metabolized in the rumen to DDD. Virtually the most cows were exposed to DDT via feed (more than 98%). Meanwhile, 50-80% of the ingested *p.p* DDE was excreted via milk *(17)*.

The aim of this study was determination of DDT levels in milk, cheese and butter and comparing their levels with the permissible limits. Moreover, the effect of heat treatment on DDT level in the examined samples was studied.

MATERIAL AND METHODS

I- Collection of samples

A total of 60 samples of raw buffalo's milk, kareish cheese and butter (20 of each) were randomly collected during summer 2007 from Sharkia Governorate markets to determine DDT residues. Raw buffalo's milk samples (500ml each) were collected in clean glass containers; while, kareish cheese samples (250gm each) and butter samples (one kg each) were collected in clean polyethelen bags. The samples were identified and transferred to the laboratory in ice bag. Moreover the effect of heat treatment on DDT residue in raw buffalo's milk and butter was evaluated.

II- Preparation of samples

1-Milk samples: Each sample was thoroughly mixed and divided into two equal subsamples (10ml) in small glass containers with screw Teflon capped stoppers and kept deeply frozen at -20°C. The first subsample was kept for analysis before heat treatment and the second one was kept for analysis after heat treatment.

2-Kareish cheese samples: Twenty five gm of each sample was thoroughly mashed with 50ml of distilled water in a clean dry blender, then 10ml from each prepared was measured into small glass containers with screw Teflon capped stoppers and kept deeply frozen at -20°C for analysis.

3-Butter samples: Each sample was divided into two portions and each portion was kept in clean dry glass container. The first portion (250gm) was warmed to about 50°C until fat separate and decanted through dry filter paper No.10, and then 3gm were taken for analysis. The second portion (750gm) was kept in deep freezer at -20°C for heat treatment (ghee manufacture).

III- Heat treatment

Milk and butter samples which kept for heat treatment were heated with stirring to boiling point. Milk samples were cooled after 5 minutes; while, butter samples were heated until convert to ghee.

IV- Analysis

1- Extraction procedure

A- Milk and kareish cheese

Extraction of samples was carried out *(18,19)* as follows:

Ten ml of prepared samples were placed into a 50ml centrifuge tube, and 20ml n-hexane, 5ml acetonitrile and 1ml ethanol were added and mixed by vigorous shaking for 1 minute, followed by centrifugation for 2 minutes at 2000 rpm. The hexane phase was then filtered through anhydrous sodium sulphate. The sample was extracted with 2 additional 20ml portions of n-hexane. The combined extracts were concentrated extract was transferred to florisil column clean up.

B- Butter

The extraction of butter samples was conducted *(20)* as follows, 3gm of prepared butter sample were transferred to 125ml separating funnel using 12ml petroleum ether, then extracted with four additional 30ml portion of acetonitril saturated with petroleum ether. The combined acetonitril extract were drained into 1 liter separating funnel containing 650ml distilled water, 40ml saturated sodium chloride solution and 100 petroleum ether, and shaked vigorously for 1 minute. The aqueous layer was drained into a second 1 liter. Finally, the combined petroleum ether extracts were washed with two 100ml portion of distilled water. The washing

was discarded and petroleum ether layer was passed through anhydrous sodium sulphate. The extract was evaporated using rotary evaporator, then transferred to florisil column for clean up.

2-Clean up

All extracts of milk, cheese and butter were subjected to clean up procedure ***(18)*** as follows, the chromatographic column (contained 5em height florisil 60-100 mesh and topped by 2 cm anhydrous sodium sulphate) was used. The column was primary washed with about 30ml petroleum ether, then the extract of each sample was transferred to the column using about 5-10ml petroleum ether, organochlorine pesticide were eluted from the column using 200ml ethyl acetate, benzene, n-hexane mixture (1:19:180, respectively). The elutes were concentrated to about 2ml using rotary evaporator under vacuum and water bath at temperature of 35°C, then transferred quantitatively into a small glass tube. The solvent was evaporated using gentile stream of air in water bath at 35°C until dryness, then analyzed by gas liquid chromatography (GLC).

3-Preparation of stock standards

Stock standard were prepared in µg/ml concentration by dissolving 0.1gm of the standard in 100ml pesticide quality hexane using volumetric flask. The stock solution was transferred to ground glass stoppard reagent bottles and stored in refrigerator.

4-Preparation of chromatographic working standards

Standard solutions were prepared from the stock solution using microsyrings. A typical concentration (1ng/1ml) was prepared by diluting 0.1ml of stock solution to 100ml using hexane. The standard solution were transferred to ground glass stoppard bottle and stored in refrigerator.

5-Preparation of extracted samples

The extracted sample was diluted with 0.5ml of nanograde hexone. Five ml of diluted extract were injected into Electron capture gas liquid chromatography using standard microsyring.

4-Analysis on gas liquid chromatography

Gas liquid chromatography (Hewlette Packared 5890 series II) equipped with double electron capture detectors (ECD's Ni^{63}) and double columns system was used for identification of DDT residues under the conditions illustrated in Table 1 confirmation of the results were carried out by injection two different columns of gas liquid chromatography. Retention time for the tested DDT derivatives ranged between 1.27 and 1.48 min. for *pp* DDE and *pp* DDT respectively.

5-Determination of percentage rate of recovery

The reliability of analytical method was examined by fortifying the tested samples with known quantities of tested pesticide following the same procedures of extraction, clean up and analysis. The percentage rate of recovery of the tested DDT derivatives in milk ranged between 96% to 101% for o,p DDT and p,p DDE respectively, while, in butter it varied between 76% to 95% for pp DDT and pp DDE respectively. Meanwhile, the recovery rate in kariesh cheese ranged from 76% to 96% in pp DDT and pp DDE respectively.

Table 1. Conditions of gas chromatographic determination.

Columns			Temperature Program				Conditions	
	Column 1	Column 2	level	Rate °C/min	Temp. °C	Time min.		
Name	PAS-5 5% Phenyl	PAS-1701-14	1	--	90	2	Injector Temp.	255°C
	Methyl Siloxan	Cyanopropyl phenyl	2	20	150	0	Detector Temp.	300°C
		Methyl	3	6	270	25	Carrier gas	Nitrogen (2ml/min.)
Film Thickness	0.52µm	0.25 µm						
Length	25 m.	25 m.						
Column I.D.	0.32mm	0.32mm						
Phase ratio	150	320						

V- Statistical analysis

The obtained data were analyzed according to Petric and Watson *(21).*

RESULTS

The obtained results of the detected p,p-DDE in the examined milk and butter samples is outlined in Table 2, while; the other DDT derivatives (p,pDDD, o,pDDT and p,pDDT) were not detected in all the examined samples. On the other hand, all DDT derivatives could not be detected in all the examined kariesh cheese samples. The comparison between p,pDDE residues before and after boiling were showed in figures 1 and 2 in milk and butter respectively. Frequency distribution of p,pDDE residues in the examined milk and butter before and after heat treatment was outlined in Table 3. On the other aspect, Table.4 showed the comparison of acceptable daily intake (ADI) values of DDT with the calculated daily intake from raw and boiled milk.

Table 2. p,p DDE residues in the examined milk and butter samples before and after heat treatment (ppb).

Samples		*Min.*	*Max.*	*Mean ± S.E.*
Milk (n=20)	Before boiling	0.788	4.713	2.747 ± 0.3465^{a}
	After boiling	0.198	1.624	0.7693 ± 0.10314^{b}
Butter (n=20)	Before boiling	13.27	69.50	41.624 ± 4.242^{a}
	After boiling	3. 36	19.08	10.995 ± 1.1662^{b}

N.B.: The difference between letters within the same category (milk or butter) means the variation between the values of p,pDDE is significant ($P \leq 0.05$).

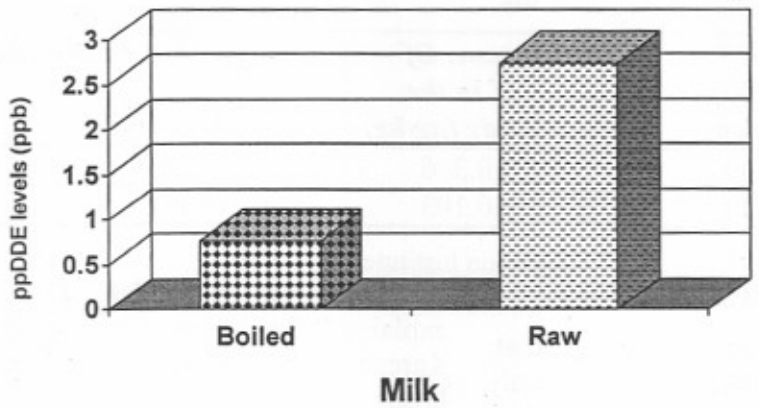

Fig. 1 . p,p DDE levels (ppb) in the examined milk samples before and after boiling

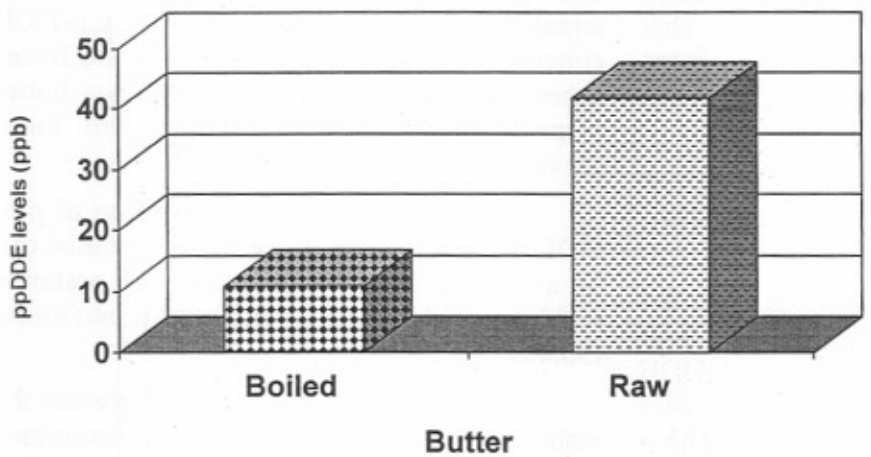

Fig. 2 .p,p DDE levels (ppb) in the examined butter samples before and after boiling

Table 3. Frequency distribution of p,pDDE residues in the examined milk and butter before and after heat treatment.

Samples		*Within P.L.**		*Over P.L.*	
		No	%	No	%
Milk (n=20)	Before boiling	20	100	0.0	0.0
	After boiling	20	100	0.0	0.0
Butter (n=20)	Before boiling	4	20	16	80
	After boiling (ghee)	20	100	0.0	0.0

P.L.: The permissible limit of DDT in milk = 20 ppb *(22)*

Table 4. Comparison of acceptable daily intake (ADI) values of DDT with calculated daily intake from raw and boiled milk.

Samples	*ADI* µg/70kg person*	*Mean conc. Of p,pDDE in the present study (µg/kg)*	*Calculated daily intake ***	
			µg/day/person	%
Before boiling	1200	2.747±0.346	0.549	0.0457
After boiling	1200	0.769±0.103	0.154	0.0128

* WHO/FAO, 1998 *(23)* ** Nutrition Institute, 1996 *(24).*

DISCUSSION

The obtained results revealed that, among four DDT derivatives residues, only p,pDDE was detected in both the examined raw buffalo's milk and butter samples. Meanwhile, the other DDT derivatives (p,pDDD, o,pDDT and p,pDDT) could not be detected in all the examined samples. This result coincide with those reported in Chinese study *(25)* which found that p,pDDE was the predominant isomer of DDT in yogurt.

On the other aspect, all the DDT derivatives were not found in all the examined kareish cheese samples.

Regarding p,pDDE residues in the examined raw milk samples before heat treatment, Table 2 showed that pp-DDE residues varied from 0.788 to 4.713 ppb with the mean value of 2.747±0.34 ppb. These levels are nearly similar with those previously recorded *(26,27).* Higher levels than our figures were detected in other investigations *(28-31).* Concerning the butter samples before processing, the obtained results revealed that p,pDDE residues ranged between 13.27 and 69.50 ppb with a mean of 41.62 ± 4.242 ppb on fat basis. Nearly similar levels were detected in Egypt *(32,33).* Also, ppDDE residues were recorded with a mean of 43 ppb in the examined butter samples in Mexico *(34).* Meanwhile, higher levels of DDT than those in the current study were cited *(35,36).* On contrary, lower p,pDDE values than our figures were recorded *(37,38)* .

Table 2 showed that the residues of p,pDDE in the examined butter (before boiling) is obviously higher than those in the examined raw milk samples, this result was attributed to the lipophilic property of organochlorine pesticides. Thus, this result explained the absence of DDT residues in Karesh cheese samples which had very low fat levels.

Regarding the effects of heat treatment on p,p-DDE residues (Table 2 and Figures 1 and 2), the statistical analysis showed significant reduction of p,p-DDE concentrations in boiled milk and boiled butter (ghee) than those in raw milk and raw butter respectively. These results agreed with those previously recorded *(32,33,35,39).*

On the other hand, the reduction of p,p-DDE residues by butter boiling-off method can be attributed to heat degradation of pesticide residue and also partial transfer of it into morta *(35,40)*

Concerning the comparison between the estimated levels of p,pDDE in the examined samples and the recommended permissible limit, Table 3 revealed that the detected p,p-DDE levels in both raw and boiled milk were below the permissible limits in all samples. On the other aspect, p,p- DDE exceeded the permissible limit in 16 (80%) out of 20 butter samples (before boiling). Meanwhile, all the boiled butter samples (ghee) contained p,p-DDE in levels within the recommended permissible limits, this result coincide with Indian study *(41)* which reported that the butter samples were comparatively more contaminated with organochlorine pesticide than ghee.

The results recorded in Table 4 indicated that the average concentrations of p,p-DDE in raw and boiled milk were 2.747±0.3465 and 0.7693±0.103 ppb respectively as mentioned previously, these concentrations gave a daily intake of about 0.549 and 0.154 µg/person for

p,p-DDE for raw and boiled milk consumers respectively (about 200 ml/person /day) and this contribute to about 0.0457% and 0.0128% respectively of acceptable daily intake (ADI) recommenced by Nutrition Institute *(24).* It could be concluded that p,p-DDE, the only detected derivative of DDT was clearly very low in comparison with the maximum tolerated daily intake of DDT from all types of food. On the other hand, the calculated daily intake of both butter and ghee in Egypt is not available.

Conclusion and Recommendations

1-Because of their low fat levels, the residues of DDT derivatives in the examined kareish cheese samples were not detected. So; regarding organochlorine residues, kareish cheese is safe for human consumption.

2-Heat treatment exhibited an important role for reduction of p,p DDE levels in the present study. Thus, heat treatment is highly recommended for raw milk before use in milk products.

3-Further studies should be enhanced to investigate the organochlorine residues in foods (especially greasy foods) to determine the relatively hazardous and safe foods.

Acknowledgment

The authors express sincere thanks to **Dr. Waiel Mohammed Salah El-Dien**, Senior Researcher of food hygiene in Zagazig provincial Lab., Animal Health Research Institute, Egypt, for his faithful guidance and advices during the course of the research.

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الملخص العربى

تقييم بقايا الـــ د.د.ت فى اللبن وبعض منتجاته

نيفين حسن إسماعيل- مها محمد سمير
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تـــم تجميع عدد (٦٠) عينة من كل من اللبن الجاموسى الخام والجبن القريش والزبد الجاموسى من أســـواق محافظـــة الـــشرقية لقياس بقايا الـــ دى دى تى ومشتقاته وقد وجد أن الـــبارا بارا دى دى تى المـــركب الوحـــيد الـــذى تم إيجاده وقياسه فى العينات وكان متوسط مستويات الـــ بارا بارا دى دى تى ٢,٧٤٧ و ٤١,٦٢٤ جزء فى البليون فى عينات اللبن الخام والزبد قبل الغليان على التوالى ولم تتواجد فى الجبن القريش فى أى من العينات المختبرة. بينما بعد الغليان كان متوسط تركيزاته فى اللبن الخام والزبد (السمن) ٠,٧٦٩ و ١٠,٩٩٥ جزء فى البليون على التوالى وكان المتوسط الإجمالى للمركب المذكور فى عيـــنات اللـــبن الخام والمغلى أقل من الحدود المسموح بها فى كل العينات. وكان متوسط التركيزات فى الـــزبد قـــبل الغلـــيان أعلى من الحدود المسموح بها حوالى ١٦ (٨٠%) من ٢٠ عينة زبد بينما متوسط المـــستويات فـــى الزبد بعد الغليان (السمن) كان فى الحدود المسموح بها. من هذه الدراسة نستخلص أن مبيدات الـــ دى دى تى تواجدت بمستويات آمنة إلى حد ما فى عينات اللبن الخام والمغلى بينما أعلى من الحـــد المسموح به فى عينات الزبد قبل الغليان. ولذلك نوصي بالمعالجة الحرارية لبعض منتجات الألبان للتقليل من مخاطر هذه المبيدات مع استمرار متابعة مستويات مبيدات ال دي دي تي في الأغذية المختلفة لمعرفة التلوث المحتمل منها.