

Monitoring of pesticide residues in bovine and buffalo milk samples collected from Kafr El-Sheikh and El-Gharbia Governorates, Egypt

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ABSTRACT

The widespread use of pesticides in agriculture, public health and industry leads to the accumulation of pesticides in the environment. Therefore, monitoring has been conducted to investigate the magnitude of contamination of bovine and buffalo milk with organochlorines, organophosphates and carbamates pesticide residues from Kafr El-Sheikh and El-Gharbia Governorates, Egypt. Milk samples from bovine and buffalo were collected monthly for a year period and determined by gas liquid chromatography with an electron capture detector (ECD) for organochlorine and nitrogen phosphorus detector (NPD) for organophosphate and carbamate pesticides. The results indicated that all milk samples (bovine and buffalo) were contaminated with organochlorine pesticides. The residue levels of organochlorine pesticides (OCPs) were much lower than maximum residue limits (MRLs) for pesticides in milk, which reflects a degree of health safety for human. Residues of organophosphate and carbamate pesticides were below the detection limits for all samples. However, lindane and heptachlor were detected with higher concentrations than other detected compounds either in bovine or buffalo milk in all samples. The concentration levels of detected pesticides were clearly higher in buffalo milk than bovine milk. Comparison among the mean concentrations of detected pesticides between the two Governorates showed that the mean concentrations of detected pesticides in El-Gharbia were higher than in Kafr El-Sheikh Governorate.

INTRODUCTION

Pesticide residues and their metabolites represent one of the most harmful issues to human health. As known milk and its products were represent an essential source of human daily food, especially for infants and children all over the world. Dairy products in particular have been shown to

have a high incidence of contamination with residues of persistent organochlorine insecticides (Kalra *et al.*, 1983; Kalra and Chawla, 1985; Kannan *et al.*, 1992; Mukherjee and Gopal, 1993; Gupta *et al.*, 1997; John *et al.*, 2001 and Battu *et al.*, 2004).

Feed and fodder offered to animals are often contaminated with pesticide residues (Sandhu, 1980; Raikwar and Nag, 2003) and after feeding; these residues pass through the body systems (Prasad and Chhabra, 2001). The continuous intake of pesticide residues in ruminants is particularly a serious problem in the case of the organochlorines, which are highly liposoluble and deposited in adipose tissues, body fats and remained *in situ* for a long time. Contamination of milk in both animals and humans by DDT [1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane], hexachloro cyclohexane (HCH, commonly known as BHC), aldrin, dieldrin and heptachlor has been reported by researchers in different countries over the last few decades, and the use of most of these chemicals have been banned in certain countries (Williams and Mills, 1964; Kapoor and Kalra, 1988 and 1997; Singhal and Mudgal, 1990; Surendra *et al.*, 1998).

Since about 25 years, the use of DDT and many other organochlorine pesticides in Egyptian agriculture has been banned (Sameeh, 2004). However, these long persistent compounds are still detectable in many different types of environmental samples e.g., water, fish, sediment, vegetables, fruits, milk and foodstuffs (Sandhu, 1980 and Raikwar and Nag, 2003).

The present study was conducted to assess the current status of milk contamination (bovine and buffalo) with pesticide residues in Kafr El-Sheikh and El-Gharbia Governorates, particularly after the imposition of the bans on the use of organochlorine pesticides (OCPs) as well as the currently used pesticides, organophosphates and carbamates.

MATERIALS AND METHODS

Chemicals

Organochlorine pesticide standards were DDT (dichloro-diphenyl-trichloroethane), DDE (1, 1-(dichloro 2,2 bis (4-chlorophenyl) ethane), aldrin, heptachlor and lindane (γ -HCH). Standard solutions were prepared in hexane. Organophosphate and carbamate pesticide standards were

dimethoate, malathion, chlorpyrifos, diazinon, monocrotophos, aidicarb and carbofuran. Standard solutions were prepared in ethyl acetate. The standards were obtained from the Environmental Protection Agency (EPA), USA.

The organic solvents used were acetone, acetonitrile, methanol, ethylacetate and *n*-hexane, analytical grade, anhydrous sodium sulfate and Florisil-PR grade, (60-100 mesh) for column chromatography, obtained from Merck Co., Germany.

Sample Preparation

Bovine and buffalo milk samples (10 kg from each place) were collected monthly from three regions in Kafr El-Sheikh Governorate (Kafr El-Sheikh, Seedy Salem and Abo-Zyada) and two regions in El-Gharbia Governorate (Tanta and El-Gemmeza) from one whole year. After collection, all the samples were stored at 4°C until the extraction was done.

Extraction procedures

Extraction of the organochlorine pesticide residues was done within 2 days of collection. Prior to the extraction, all the glassware were properly washed with soap and water followed by distilled water, and finally rinsed with acetone and dried in an oven at 200-220°C to avoid any contamination with pesticides. Extraction and cleanup of the collected samples were done using the method of Takie *et al.*, (1983) with some modification. The first extraction was made with the mixture of acetone (4 ml), acetonitrile (2 ml), and hexane (15 ml). Second extraction was done with acetone (2 ml), acetonitrile (2 ml), and hexane (10 ml). The solvent layer was washed with acetonitrile (3 ml), 2% Na₂SO₄ (3 ml), and hexane (3 ml). Cleanup was made using a glass column packed with florisil (5 g) and Na₂SO₄ anhydrous (5 g). The column was eluted with hexane (15 ml) and 1% methanol in hexane (10 ml).

After cleanup, the final extracts were evaporated to dryness using rotatory vacuum evaporator. The dried material was dissolved in 2 ml ethylacetate for gas chromatographic (GC) analysis. One-microliter aliquot was injected into GC.

The Organophosphate and carbamate insecticides were extracted and cleaned up according to Hill *et al.*, (1967) and Rangaswamy *et al.*, (1976), respectively.

Gas chromatograph (HRGC-ECD and NPD)

Residues of the monitored pesticides were analyzed by using a gas chromatograph (GC) model HP-5890 equipped with a tritium electron capture detector (^3H -ECD) for detection of chlorinated pesticides. A nitrogen phosphorus detector (NPD) was employed to determine the organophosphorus and carbamate pesticides. Megabore column Hp- 608 part No. 190955-023; (30m x 0.53 mm id, 0.25 μm film thickness) was used. Split injection (0.8 min. hold) was applied with temperature at 220°C. For ^3H -ECD the carrier gas used was helium at a flow rate of 2.5 ml/min, the make up gas was nitrogen at 35 ml/min., anode purge, nitrogen at 4 ml/min., temperature was 280 C. The initial oven temperature was 80°C (1 min) $\rightarrow$ (30°C/min) 170°C $\rightarrow$ (10°C/min) 300°C hold 10 min.

For the NPD, the carrier gas used was hydrogen at a flow rate of 4 ml/min. and the make up gas was helium at 30 ml/min with temperature at 225 C. Initial oven temperature was 80°C (1 min) $\rightarrow$ (25°C/min) 190°C $\rightarrow$ (2°C/min) 225°C $\rightarrow$ (5°C/min) 280 hold 20 min.

Calibration, qualitative and quantitative analysis were carried out in addition to recovery experiments at concentration level of 1 $\mu\text{g/L}$ by fortification. The percentage recovery of organochlorine ,organophosphate and carbamate pesticides used were >90%, 83% and 84%, respectively for all milk samples.

RESULTS AND DISCUSSION

Milk samples analyzed for pesticide residues revealed that all the milk samples were contaminated with organochlorine pesticides, DDE (1,1-(Dichloro 2,2 bis (4-chlorophenyl) ethane) aldrin, heptachlor and lindane (γ -HCH). On the other hand, dimethoate, malathion, chlorpyrifos, diazinon, monocrotophos, aldicarb and carbofuran were below the detectable level in all samples.

The concentrations of the detected pesticide residues from Kafr El-Sheikh and El-Gharbia Governorates in bovine and buffalo milk during sampling time are listed in Tables (1-4). The mean concentrations of the detected organochlorine compounds in Kafr El-Sheikh and El-Gharbia Governorates are illustrated in Figures 1 and 2, respectively.

The sequence of organochlorine compounds, depending on their concentration level in both milk samples from El-Gharbia and Kafr El-Sheikh Governorates was heptachlor > lindane > aldrin > DDE.

The concentration of the detected pesticides in bovine milk ranged from 13.33 to 0.14 ng/kg (Table 1) and in buffalo milk from 31.12 to 0.05 ng/kg (Table 2) in Kafr El-Sheikh Governorate. While in El-Gharbia Governorate, the concentrations level ranged from 26.57 to 0.27 ng/kg in bovine milk (Table 3) and in buffalo milk ranged from 32.45 to 0.13 ng/kg (Table 4).

The results in Fig. 1 and 2 showed that, the heptachlor and lindane had higher mean levels than the other detected pesticides in all sampling areas for both bovine and buffalo milk. However, these concentrations were below the FAO maximum residue level (FAO 1993). The detected levels were below the MRL, in both milk types due to the long time banning from the pest control programs in Egypt. This result agreed with the previous findings of many investigators (Waliszewski, *et al.*, 1997; Hanafy and Bayoumi, 2001 and Battu *et al.*, 2004).

The detection frequency of pesticides during the study period in all sampling areas in Kafr El-Sheikh and El-Gharbia Governorates from bovine and buffalo milk were illustrated in Figs (3 and 4). These figures revealed that, the detection frequency of pesticides was higher in buffalo than bovine milk in all sampling areas except of Gemmezia and Abo-Zayada village.

The results in Tables (1-4) showed that, heptachlor and lindane were the most detected compounds in all sampling areas in both milk samples. Moreover aldrin and DDE were the lowest detected compounds during the study period in both milk types at all sampling areas.

From the previous results, it was clearly obvious that, the detectable concentrations of the pesticides were higher in buffalo milk, due to higher fat content, than bovine milk. Also, El-Gharbia Governorate gave higher residues than Kafr El-Sheikh Governorate for both milk samples (Tables 1-4). This is due to El-Gharbia Governorate is located near one of the biggest pesticide factories in Egypt. In the light of Tables (1-4), the concentrations of pesticide detected are below the maximum residues limits which clearly safe on human health.

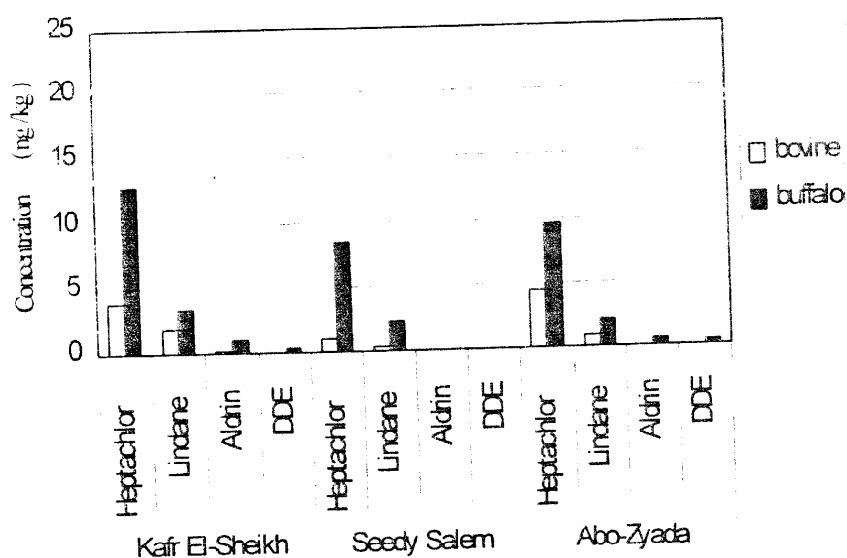


Fig. 1. Mean concentration of pesticide residues in bovine and buffalo milk during different months in Kafr El-Sheikh Governorate.

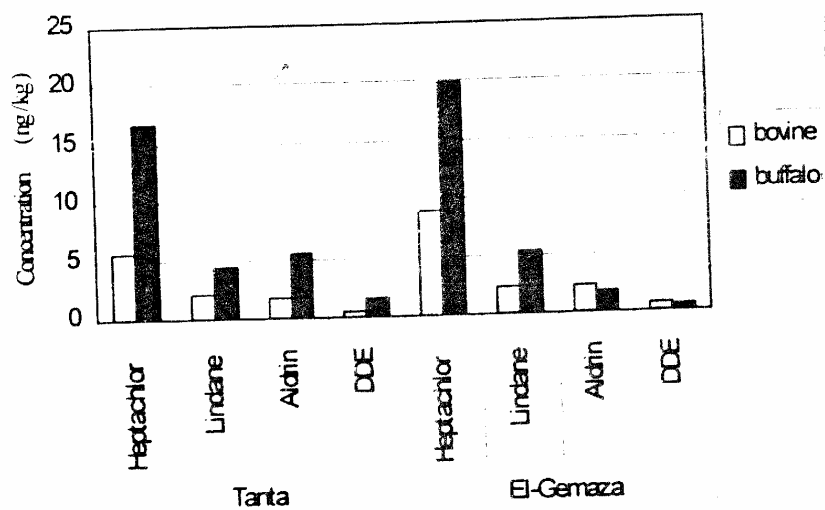


Fig. 2. Mean concentration of pesticide residues in bovine and buffalo milk during different months in El-Gharbia Governorate.

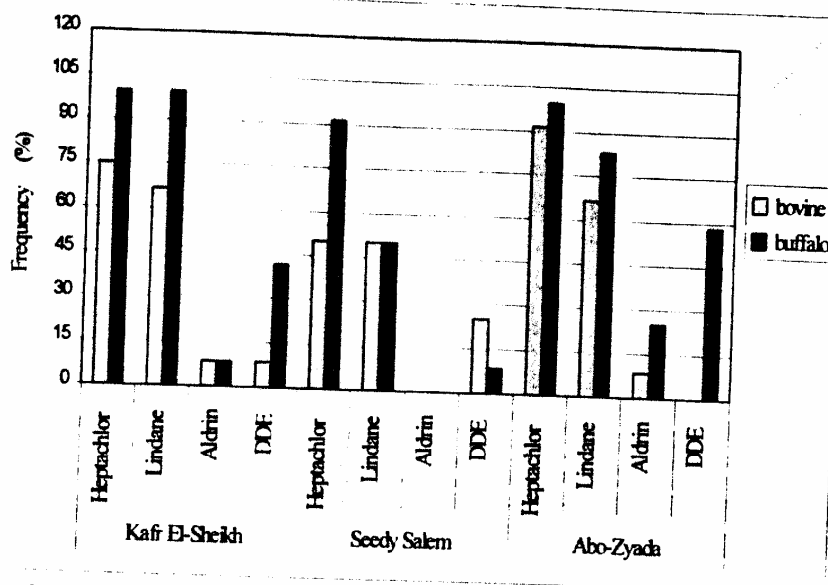


Fig. 3. Percent of frequency from pesticide residues in bovine and buffalo milk in Kafr El-Sheikh Governorate.

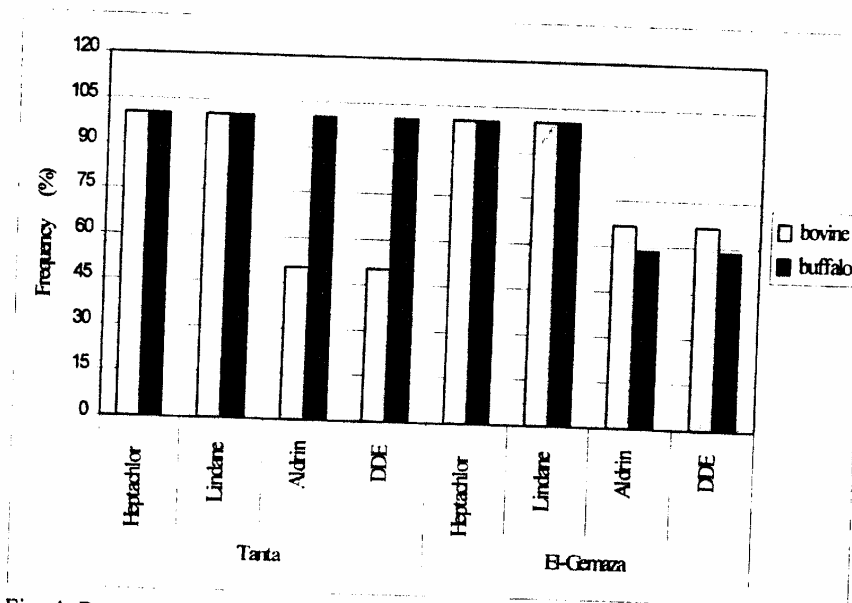


Fig. 4. Percent of frequency from pesticide residues in bovine and buffalo milk in El-Gharbia Governorate.

Being a fat-rich food, milk is an important source of organochlorine pesticides (OCPs) accumulation. All the milk samples analyzed were found to be contaminated with residues of organochlorines and these agree with (Kaphalia *et al.*, 1985; Agnihotri, 1999 and John *et al.*, 2001). DDT was not detected in all analyzed samples while the detection of DDE was a result of its metabolism (Dhaliwal and Kiara 1978).

According to the Codex Alimentarius Commission, the acceptable daily intake (ADI) of heptachlor, lindane and aldrin are given as 0.0005, 0.008 and 0.0001 mg/kg body wt/ day, respectively (FDA, 1993 and Commonwealth of Australia 2005). Gopalan and Rao (1980) have reported an average consumption of liquid milk for an adult and child to be 250 and 300 g, respectively. An estimate of this ADI value for an average adult weighing 60 kg and an average child weighing 10 kg were calculated to be 30 and 5 µg to heptachlor, 480 and 80 µg to lindane, 6 and 1 µg aldrin, respectively. Depending on the mean concentration level of detected pesticides in both milk type and average consumption of milk compared with the estimated intake for pesticide residues, it was realized that, it was remained within the safe limits during this study period. Interestingly, none of the samples analyzed showed the presence of commonly used organophosphate or carbamate compounds.

The present study showed, the contamination of both bovine and buffalo milk with organochlorine pesticides. The levels of pesticide residues in milk samples were much lower than their maximum residue levels which may reflect such kind of safety on human health. Since the obtained results are alarming, as many of these compounds are reported to be carcinogenic, mutagenic, and teratogenic, it becomes essential to check the pesticide pollution problem by educating through the mass media and adopting Integrated Pest Management (IPM) by utilizing alternate methods like mechanical, cultural, biological, and use of botanical pesticides for the control of pests and disease vectors.

Table (1): Mean average of some pesticides residues in bovine milk (ng/kg) in three regims of Kafr El-Sheikh Governorate

Time of sampling	Kafr El-Sheikh				Seedy Salem				Abo-Zyada			
	Heptachlor	Lindane	Aldrin	DDE	Heptachlor	Lindane	Aldrin	DDE	Heptachlor	Lindane	Aldrin	DDE
January	N.D	4.54	N.D	N.D	0.62	0.14	N.D	0.23	1.13	1.66	N.D	N.D
February	1.04	N.D	N.D	N.D	0.64	0.45	N.D	0.217	13.19	0.65	N.D	N.D
March	N.D	0.276	N.D	N.D	N.D	0.276	N.D	N.D	N.D	0.167	N.D	N.D
April	1.01	2.402	N.D	N.D	N.D	0.26	N.D	N.D	7.31	0.52	N.D	N.D
May	4.31	4.03	N.D	N.D	0.55	0.43	N.D	0.29	6.21	0.44	N.D	N.D
June	4.7	N.D	N.D	N.D	2.73	N.D	N.D	N.D	2.07	N.D	N.D	N.D
July	3.39	N.D	N.D	N.D	2.49	N.D	N.D	N.D	1.80	N.D	N.D	N.D
August	6.01	N.D	N.D	N.D	N.D	N.D	N.D	N.D	2.07	N.D	N.D	N.D
September	13.33	1.52	2.25	N.D	N.D	N.D	N.D	N.D	9.93	1.24	N.D	N.D
October	8.67	1.54	N.D	N.D	N.D	N.D	N.D	N.D	4.63	4.15	N.D	N.D
November	N.D	2.79	N.D	0.309	5.43	2.172	N.D	N.D	2.35	N.D	0.61	N.D
December	5.566	4.90	N.D	N.D	N.D	N.D	N.D	N.D	2.25	1.82	N.D	N.D
Total Conc.	48.03	22.00	2.25	0.31	12.46	3.73	--	0.74	52.94	10.65	0.61	--
Mean Conc.	4.00	1.83	0.19	0.03	1.04	0.31	--	0.06	4.41	0.89	0.05	--
MRL(mg/kg)	0.006	0.01	0.006	0.02	0.006	0.01	0.006	0.02	0.006	0.01	0.006	0.02

N.D = Not detected, organophosphate and carbamate pesticides were below the detectable level

Table (2): Mean average of some pesticides residues in buffalo milk (ng/kg) in three regions of Kafr El-Sheikh Governorate

Time of sampling	Kafr El-Sheikh				Seedy Salem				Abo-Zyada			
	Heptachlor	Lindane	Aldrin	DDE	Heptachlor	Lindane	Aldrin	DDE	Heptachlor	Lindane	Aldrin	DDE
January	5.57	2.38	N.D	N.D	1.90	N.D	N.D	N.D	2.41	1.70	1.47	0.32
February	10.94	1.93	N.D	0.204	1.89	N.D	N.D	N.D	18.67	1.41	N.D	0.101
March	5.956	8.521	N.D	0.10	6.02	8.63	N.D	0.11	1.34	0.358	N.D	N.D
April	8.26	2.16	N.D	0.198	1.42	N.D	N.D	N.D	9.35	0.78	N.D	0.05
May	5.11	1.25	N.D	0.122	1.52	N.D	N.D	N.D	8.17	0.67	N.D	0.05
June	14.7	8.74	N.D	N.D	5.64	1.15	N.D	N.D	18.05	1.21	N.D	0.29
July	19.23	4.36	N.D	N.D	3.42	N.D	N.D	N.D	11.47	N.D	N.D	N.D
August	11.08	2.35	N.D	N.D	N.D	N.D	N.D	N.D	5.89	N.D	N.D	N.D
September	18.48	2.38	12.41	N.D	30.11	5.94	N.D	N.D	12.69	7.65	2.30	3.18
October	47.16	2.189	N.D	2.57	31.12	6.83	N.D	N.D	12.45	1.89	N.D	N.D
November	2.101	2.362	N.D	N.D	12.31	2.66	N.D	N.D	9.21	5.17	N.D	N.D
December	5.43	2.38	N.D	N.D	5.88	2.43	N.D	N.D	5.36	3.4	1.52	0.63
Total Conc.	154.02	41.00	12.41	3.19	101.23	27.64	--	0.11	115.06	24.24	5.29	4.62
Mean Conc.	12.83	3.42	1.03	0.27	8.44	2.30	--	0.01	9.59	2.02	0.44	0.39
MRL(mg/kg)	0.006	0.01	0.006	0.02	0.006	0.01	0.006	0.02	0.006	0.01	0.006	0.02

N.D = Not detected, organophosphate and carbamate pesticides were below the detectable level

Table (3): Mean average of some pesticides residues in bovine milk (ng/kg) in two regions of El-Gharbia Governorate

Time of sampling	Tanta				El-Gemeza			
	Heptachlor	Lindane	Aldrin	DDE	Heptachlor	Lindane	Aldrin	DDE
January	0.98	0.86	N.D	N.D	26.57	0.65	N.D	N.D
February	1.12	0.66	N.D	N.D	15.12	1.43	6.12	N.D
March	10.51	1.61	4.98	0.76	16.11	1.65	5.65	0.76
April	1.22	0.47	N.D	N.D	1.30	0.55	N.D	N.D
May	0.99	0.74	N.D	N.D	1.20	0.66	N.D	N.D
June	0.472	0.49	2.6	1.8	2.2	2.51	0.88	1.05
July	0.351	0.27	0.8	1.31	2.01	2.42	1.21	1.09
August	9.03	3.68	1.85	0.32	5.23	0.27	0.75	0.27
September	16.15	9.82	5.74	0.74	14.58	0.97	7.99	0.97
October	16.11	1.62	5.83	0.88	4.60	1.02	N.D	2.21
November	8.120	4.96	N.D	N.D	9.25	5.72	2.2	0.98
December	1.66	0.72	N.D	N.D	8.89	8.64	3.65	0.87
Total Conc.	66.71	25.90	21.80	5.81	107.06	26.49	28.45	8.20
Mean Conc.	5.56	2.16	1.82	0.48	8.92	2.21	2.37	0.68
MRL(mg/kg)	0.006	0.01	0.006	0.02	0.006	0.01	0.006	0.02

N.D = Not detected, organophosphate and carbamate pesticides were below the detectable level.

Table (4): Mean average of some pesticides residues in buffalo milk (ng/kg) in two regions of El-Gharbia Governorate

Time of sampling	Tanta				El-Gemeza			
	Heptachlor	Lindane	Aldrin	DDE	Heptachlor	Lindane	Aldrin	DDE
January	20.11	3.90	2.91	0.45	24.12	5.10	N.D	N.D
February	15.31	6.70	3.10	0.35	19.32	4.80	N.D	N.D
March	12.12	1.21	6.14	0.56	12.2	0.86	6.48	0.75
April	19.41	5.11	6.24	2.92	19.20	8.97	2.24	0.66
May	10.11	6.25	7.11	3.10	22.14	6.78	2.91	0.76
June	9.03	3.76	4.65	1.80	10.21	5.89	1.97	0.253
July	7.73	2.69	5.11	0.49	11.58	2.70	0.76	0.165
August	13.14	2.32	4.52	2.35	11.36	2.33	0.91	0.13
September	16.92	3.77	7.90	3.61	17.06	3.97	5.71	3.72
October	33.20	5.94	8.11	0.46	32.45	8.11	N.D	N.D
November	21.00	4.80	7.11	3.08	30.11	7.98	N.D	N.D
December	22.56	6.61	2.41	0.86	29.11	4.90	N.D	N.D
Total Conc.	200.64	53.06	65.31	20.03	238.86	62.39	20.98	6.44
Mean Conc.	16.72	4.42	5.44	1.67	19.91	5.20	1.75	0.54
MRL(mg/kg)	0.006	0.01	0.006	0.02	0.006	0.01	0.006	0.02

N.D = Not detected, organophosphate and carbamate pesticides were below the detectable level

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تتبع متبقيات المبيدات فى عينات الالبان التى تم تجميعها من محافظتى كفر الشيخ والغربية
عثمان شكرى بيومى ،محمد على عشرين ،أحمد عبد الحميد ابوزيد إسماعيل،
على سليمان درباله، اسماعيل ابراهيم الفخرانى

قسم المبيدات — كلية الزراعة — بكفر الشيخ — جامعة طنطا
هذه الدراسة اجريت للتعرف وتتبع متبقيات المبيدات الكلورينية والفوسفورية العضوية
والكارباماتية فى اللبن الجاموسى والبقرى فى ثلاث مواقع فى محافظة كفر الشيخ (كفر الشيخ-سيدى
سالم-ابوزيادة) وموقعين فى محافظة الغربية (طنطا-الجميزة) فى جمهورية مصر العربية. عينات
اللبن تم جمعها شهريا ولمدة سنة كاملة وتحليلها بجهاز الـ GC باستخدام كشاف الـ ECD للمركبات
الكلورينية والـ NPD للمركبات الفوسفورية والكارباماتية. ولقد اوضحت النتائج ان جميع عينات
اللبن البقرى والجاموسى سواء فى محافظة كفر الشيخ او الغربية كانت ملوثة بالمركبات الكلورينية
العضوية. وكان متوسط تركيز المبيدات الكلورينية المكتشفة فى كلا المحافظتين اقل بكثير من اقصى
متبقى للمبيد موصى به فى اللبن مما يعكس درجة الامان العالية على الصحة العامة للانسان. فى
حين انه لم يتم اكتشاف اى من المركبات الفوسفورية العضوية والكارباماتية. وكان لكل من اللذين
والهبتاكلور اعلى متوسط تركيز بين المركبات المكتشفة سواء فى اللبن البقرى او الجاموسى فى كل
مواقع جمع العينات. كما اوضحت النتائج ايضا ان مستوى متبقيات المبيدات فى اللبن الجاموسى كان
اعلى منه فى اللبن البقرى وبمقارنة مستوى تركيز المبيدات فى المحافظتين محل الدراسة اوضحت
النتائج ان متوسط التركيز فى محافظة الغربية كان اعلى منه فى محافظة كفر الشيخ.