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Agro-Industrial By-Products in Animal Livers

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Abstract: The present study was carried out to analyze the liver samples for determination of their contents of agro-industrial by-products mainly pesticide residues. The study was applied on a total of 40 samples of liver which were collected on the day of slaughtering from 40 carcasses of native breeds of beef cattle. About 20 gm of each liver sample was collected directly after inspection at slaughter houses belong to EL-Gharbia governorate over the period of the four year seasons from April 2014 to January 2015. GC-ECD used for determination of the concentration of pesticide residues in the samples and confirmation using GC-MS/MS. The obtained results revealed that ppDDE was the predominant OC pesticide during Spring, Summer and Autumn with a mean of 58.276 ± 8.29 ppb, 4.183 ± 1.09 ppb and 14.32 ± 8.12 ppb, respectively. Concerning to Winter season, the mean concentrations of HCB, Heptachlor, Heptachlor epoxide, Endrin, ppDDE, ppDDT and Methoxychlor in the examined samples were 1.69 ± 0.569 ppb; 2.24 ± 0.763 ppb; 6.74 ± 2.04 ppb; 3.56 ± 1.815 ppb; 2.62 ± 1.451 ppb; 2.69 ± 1.467 ppb and 1.67 ± 0.122 ppb, respectively. ANOVA between each OC pesticide separately and the year seasons, revealed a high significant difference among the Spring, Summer, Autumn and Winter ($P< 0.05$).

Key words: Pesticide Residues · Organochlorine · Beef Liver · GC-ECD · GC-MS/MS

INTRODUCTION

The environmental pollution is a matter of great concern worldwide and consequently contaminates the food chain as well as its bad deleterious role in human health and nutrition. The rate of urbanization and industrialization is usually increased day by day in the world, so many problems are usually associated with such development and the major one is the pollution. There are numerous types of environmental pollution, which constitute a potential danger to humanity [1]. Among a large number of anthropogenic (man-made) chemicals, greater attention has been focused on semi-volatile persistent OCPs because of their high bioaccumulation, potential and harmful biological effects. OCPs present in food pose a toxicological risk that is incriminated by their persistence in the environment and their concentration in higher levels in the food chain, particularly in foods of animal origin, due to the high liposolubility of this type of pesticides [2].

Various OCPs have been produced and used for agricultural and industrial purposes for a long time and on a large scale throughout the world because of exceptional insecticidal and fungicidal properties [3]. In Egypt, all types of OCPs have been banned since late 1980s, after being used for more than 50 years for agriculture and public health reasons. However, some kind of OCPs such as DDT and γ-HCH (lindane) are still being used illegally in Egypt due to its cheap price and powerful effect on eradication of pests, coupled with a lack of law enforcement [4]. The use of pesticides in modern agriculture is necessary to guarantee the production of food in suitable quantities, as they are an effective tool in reducing losses in crop production due to attacks by plant pests [5].

Although pesticides play an important role in increasing food production, the risks they pose to humans and the environment should be considered. Pesticides are linked to various chronic health problems, such as cancer, neurologic diseases and adverse

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reproductive effects [6]. Various analytical methods including gas chromatography (GC) and liquid chromatography (LC) coupled with various detectors have been developed for the determination of pesticide residues. The most frequently used technique is gas chromatography (GC) especially coupled with electron capture detector (ECD) and mass spectrometry (MS). ECD is commonly used for the determination of OC and pyrethroid pesticides, however research using this detector for multi residues determinations show excellent results [7, 8].

The present study was carried out to determine the concentration and the incidence of some pesticide residues used in agriculture and industry within the beef liver during the different year seasons and study its dangerous public health hazards.

MATERIALS AND METHODS

Samples Collection: A total of 40 samples of liver were collected on the day of slaughtering from 40 carcasses of native beef cattle. About 20 gm of each liver sample was collected directly after inspection at slaughterhouses belong to EL-Gharbia governorate over the period of the four year seasons from April 2014 to January 2015, each sample was kept in separate sterile plastic bag in a deep freezer unit (-20°C) and then transferred to the lab in an insulated ice box for detection of their content of pesticide residues.

Apparatus and Equipments: GC/ECD [Gas Chromatography (Hewlett-Packard Model 6890) equipped with Electron Capture Detector Ni^{63}, Agilent Technologies, Inc. 2850 Centerville Road Wilmington, DE 19808-1610]; GC-MS/MS [Gas Chromatography coupled with tandem Mass Spectrometry, Trek Equipment Corp. Sausalito, CA 94965]; Rotary evaporator and Heating mantles.

Reference Standards: OC pesticides: Heptachlor; Aldrin; Heptachlor epoxide; P,P'-DDE; Endrin; P,P'-DDD; P,P'-DDT were obtained from Dr. Ehrenstorfer, Augsburg in Germany, with purities larger than 98.5%.

Extraction and Preparation of Samples: Liver tissues were pooled and weighted to produce 20 gm of liver samples. Extraction of tissue samples was conducted as described by Abd El Kader [9].

Clean up of Samples: The clean up of extracted liver samples was conducted by Mills *et al*. [10].

Preparation of Blank Solution: The same volumes of solvents (n-hexane-acetone) and sodium sulphate anhydrous which used in liver extraction were subjected to the same extraction, portioning and clean up procedures as the examined samples to detect any possible traces of the studied pesticides in the solvents or distilled water and its value was subtracted from the results.

Preparation of Stock Standard: Pesticide stock solutions were prepared according to Toyoda *et al*. [11].

Preparation of Chromatographic Working Standard: Standard solutions were prepared according to Wang *et al*. [12].

Gas Chromatographic Analysis: The extracts were concentrated and injected into Agilent GC Model 6890 equipped with an Ni^{63}–electron capture detector, a split/splitless injection inlet, capillary column capability and a 7683A autosampler. Chemistation software was used for instrument control. GC conditions: PAS-5 methyl silicone (30m x 0.32 mm i.d x 0.25 µm film thickness), nitrogen was used as a carrier gas at flow rate of 4 ml /min, injector and detector temperatures were 300°C and 320°C, respectively. The initial column temperature was initial oven temperature, 160°C for 2 min, raised at a rate of 5°C/min and then held at 230°C for 15 min.

Confirmation: Selected samples were analyzed by full-scan GC-MS to confirm the GC-ECD results. The column used was HP-5MS (Agilent, Folsom, CA) capillary column of 30 m, 0.25 mm i.d., 0.25 µm film thickness. The carrier gas was helium at a flow rate of 1 ml/min-1. Inlet temperature was 250ºC with injection volume of 1µl.

Recovery Analysis: The reliability of analytical method was examined by fortifying the tested samples with known quantities of tested pesticides following the same procedures of extraction, clean up and analysis. The percentage rate of recovery of OCPs ranged from 87 to 96%.

Statistical Analysis: Data obtained were statistical analysis using ANOVA test, Duncan Multiple Range Test, Fischer Exact Probability test, correlation and t-student test by SPSS 14 [13].

RESULTS AND DISCUSSION

It was evident from the results recorded in table 1 that ppDDE was the predominant pesticide during Spring, Summer and Autumn with incidence 90%, 100% and 100%, respectively and concentrations were ranged from 13.08 to 95.73 ppb with a total concentration of 524.49 ppb and a mean of 58.276 ±8.29 ppb during Spring, while concentrations were ranged from 1.72 to 13.47 ppb with a total concentration of 41.83 ppb and a mean of 4.183 ±1.09 ppb during Summer and from 1.07 to 80.21 ppb with a total concentration of 143.2 ppb and a mean of 14.32 ±8.12 ppb during Autumn. All examined samples during these seasons were lower than the MRL stipulated by Gracey and Collins [14] who stated that the MRL of Heptachlor, Heptachlor epoxide and Endrin should not be exceeded 200 ppb, 200 ppb and 50 ppb, respectively. So, the liver samples were fit for human consumption.

Concerning to Winter season, The results in table 2 reported that ppDDE and Heptachlor epoxide were the predominant pesticides with incidence % of 90% and concentrations were ranged from 0.05 to 13.99 ppb with a total concentration of 23.56 ppb and a mean of 2.62 ±1.451 ppb for ppDDE, while Heptachlor epoxide concentrations were ranged from 0.001 to 17.20 ppb with a total concentration of 60.65 ppb and a mean of 6.74 ±2.04 ppb. The incidence % of the other detected OCPs followed the order: HCB 70% >Heptachlor and ppDDT 60% >Methoxychlor 30% >Endrin 20% >Aldrin 10%. The concentrations of HCB were ranged from 0.07 to 3.77 ppb with a total concentration of 11.81 ppb and a mean of 1.69 ± 0.569 ppb. Heptachlor concentrations were ranged from 0.40 to 5.73 ppb with a total concentration of 13.46 ppb and a mean of 2.24 ±0.763 ppb. ppDDT concentrations were ranged from 0.001 to 8.99 ppb with a total concentration of 16.13 ppb and a mean of 2.69 ±1.467 ppb. Methoxychlor concentrations were ranged from 1.47 to 1.89 ppb with a total concentration of 5.01 ppb and a mean of 1.67 ±0.122 ppb. Endrin concentrations were ranged from 1.74 to 5.37 ppb with a total concentration of 7.11 ppb and a mean of 3.56 ±1.815 ppb, while Aldrin total concentration was 0.225 ppb.

All examined samples during Winter were lower than the MRL stipulated by Gracey and Collins [14] who stated that the MRL of HCB, Heptachlor, Aldrin, Heptachlor epoxide, Endrin and ppDDT should not be exceeded 200 ppb, 200 ppb, 200 ppb, 200 ppb, 50 ppb and 1000 ppb, respectively. So, the liver samples were fit for human consumption.

The previous mentioned concentrations nearly lower than those obtained by Kessabi *et al*. [15] who said that the incidence % of HCB was 93% and Cantoni and Balazaretti [16] determined Aldrin concentration in foods of animal origin in Italy in 1992 which was ranged from 6.7 to 8.3 ppb, while higher than those obtained by Sitarska *et al.* [17] who estimated the degree of accumulation of pp DDT and HCB in the liver, ovaries and mammary gd tissue

Table 1: OCPs concentrations (ppb) in beef liver during Spring, Summer and Autumn using GC-ECD (n=10)

Season			Spring		Summer	Autumn
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OCPs	Heptachlor epoxide	Endrin	ppDDE	Heptachlor	ppDDE	ppDDE
Incidence (%)	10	10	90	10	100	100
Mean	96.124	1.867	58.276	34.77	4.183	14.32
SE	-	-	8.29	-	1.09	8.12
Min	96.1	1.87	13.08	34.77	1.72	1.07
Max	96.12	1.87	95.73	34.77	13.47	80.21
Total	96.124	1.867	524.49	34.77	41.83	143.2
MRL*	200	50		200		

* The MRL (ppb) for organochlorines in beef liver according to Gracey and Collins

Table 2: OCPs concentrations (ppb) in beef liver during Winter using GC-ECD (n=10)

OCPs	Incidence (%)	Mean	SE	Min	Max	MRL	*Total
HCB	70.00	1.69	0.569	0.07	3.77	200	11.81
Heptachlor	60.00	2.24	0.763	0.40	5.73	200	13.46
Aldrin	10.00	0.23	0	0.23	0.23	200	0.225
Heptachlor epoxide	90.00	6.74	2.04	0.001	17.20	200	60.65
Endrin	20.00	3.56	1.815	1.74	5.37	50	7.11
ppDDE	90.00	2.62	1.451	0.05	13.99		23.56
ppDDT	60.00	2.69	1.467	0.001	8.99	1000	16.13
Methoxych-lor	30.00	1.67	0.122	1.47	1.89		5.01

Table 3: Comparison between mean of organochlorine pesticide residues in beef liver during year seasons (n =10)

OCPs	Spring	Summer	Autumn	Winter	P <
HCB				1.69 ±0.569 a	
Heptachlor		34.77 ±- b		2.24 ±0.763 a	0.001*
Aldrin				0.23 ±- b	
Heptachlor epoxide	96.124 ±- a			6.74 ±2.04 ab	0.001*
Endrin	1.867 ±- a			3.56 ±1.815 a	0.078
ppDDE	58.276 ±8.29 a	4.183 ±1.09 b	14.32 ±8.12	2.62 ±1.451 ab	0.001*
ppDDT				2.69 ±1.467 ab	
Methoxychlor				1.67 ±0.122 ab	
Total	156.267	38.95	14.32	21.44	0.001*

• Data represented as Mean ± SE.
• * Significant at $P<0.05$ using Analysis of Variance (ANOVA) test.
• a,b insignificant difference between similar litter using Duncan Multiple Range test for comparative of means at $P<0.05$.
• All other compounds of OCPs were not detected in all liver samples under examination.

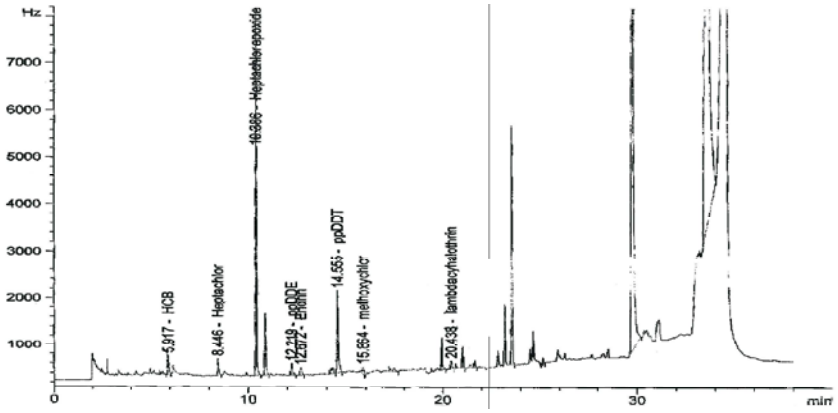

Fig. 1: Chromatogram of liver samples extracts using GC-ECD during Winter
Chromatogram of liver samples extracts using GC-ECD during Winter, carrier gas flow was nitrogen at 4ml/min and retention time 5.917 min for HCB, 8.446 min for Heptachlor, 10.386 min for Heptachlor epoxide, 12.219 min for ppDDE, 12.672 min for Endrin, 14.555 min for ppDDT and 15.864 min for Methoxychlor

of cows under natural environmental conditions and determined the presence of ppDDT in 55% and HCB in 38% of examined animals and according to Ahmed *et al.* [18] the mean concentration of Aldrin in frozen bovine meat imported from Netherds, Sudan, India and Brazil in the Egyptian market was 0.174 ± 0.004 ppb.

Table 3 revealed (ANOVA) between each pesticide (HCB, Heptachlor, Aldrin, Heptachlor epoxide, Endrin, ppDDE, ppDDT and Methoxychlor) separately and the year seasons, revealed a high significant difference among the Spring, Summer, Autumn and Winter ($P < 0.05$).

According to Alawi and Al-Hawadi [19] the concentration of OCPs were found to be high in liver and kidney samples. This is because liver and kidney are detoxification organs in animal body and tend to accumulate toxic substances. The most common health effects of OCPs include dermal toxicity, immunotoxicity, reproductive effects and teratogenicity, endocrine disruption and cancers by Mansour [20].

CONCLUSIONS

The obtained results in the present study allow to conclude that the examined liver samples exposed for sale in EL-Gharbia Governorate proved to be contaminated with pesticide residues but they did not exceed MRL. GC-ECD used for pesticide residues concentration detection and GC-MS/MS for confirmation and their accumulation in liver and adipose tissue for long run lead to higher levels than MRL causing severe public health hazards.

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