

Dietary Intake of Pesticides Based on Import Animal Liver Consumption: A Case Study, Cairo, Egypt

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ABSTRACT: A total of 18 pesticides (12 organochlorines, and 6 pyrethroids) in 32 different imported animal liver samples collected from local markets in Cairo governorate in Egypt in different seasons, were detected the contamination of organochlorines and pyrethroids pesticides using Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) method. The presence of organochlorines and pyrethroids pesticides residues were determined by gas chromatography with electron capture detector (GC-ECD). The results indicated that, the pesticides residues were found in all samples and 2 samples above the maximum residue levels (MRLs). Organochlorine residues had the highest percentage of contamination and violation (i.e. 100 and 6.25%, respectively) in imported animal liver samples, whereas synthetic pyrethroid residues had the lowest percentage of contamination (i.e. 6.25%) while their percentage of violation were 0%. However, the most frequently found pesticides were P,P'-DDE and heptachlor-epoxide while the lowest frequently found pesticides were aldrin, cypermethrin and deltamethrin. Furthermore, the health risk index for heptachlor-epoxide was the greatest which may be due to its physiochemical properties. A potential regular pesticides residues monitoring program in imported animal liver should be conducted to protect the consumers' health.

KEYWORDS: Monitoring, dietary intake, animal liver, organochlorine, synthetic pyrethroids, QuEChERS, Egypt.

1 INTRODUCTION

When polluted feed consumed by animals, aflatoxin, heavy metal and pesticide intakes may reach up to levels, treating human health by passing into end products such as meat and milk. To prevent becoming animal food hazardous, first of all, feed utilized should be kept under control. Although, throughout the world some study have been carried out on the raw materials, no serious work has been undertaken covering aflatoxin, heavy metal and pesticide content of compound feeds which is the final product taken into the animal body. (DAĞAŞAN, Ö., & ÖZEN, N. 2011). QuEChERS is a (quick, easy, cheap, effective, rugged and safe) method which has been mainly applied for the extraction of different classes of pesticides. The QuEChERS method is a simple, rapid, and inexpensive procedure requiring little labor and few materials, space, and solvents. This method achieved the status of Official Method of AOAC International (Lehotay 2007; Abdel. Rahman et al., 2015). Governments and international organizations are regulating the use of pesticides and are setting the acceptable MRL. When these compounds are applied according to good agricultural practices, MRL are not exceeded, but there in correct application may leave harmful residues, which involve possible health risk and environmental pollution. Teratogenic, carcinogenic and toxic properties of these compounds have been reported by (Abdellseid and Abdel. Rahman, 2014). Analysis of pesticide residues in food is a key tool for monitoring the levels of human exposure to pesticide residues. Pesticide residues in food are usually monitored with reference to Maximum Residue Limits (MRLs) and acceptable Daily Intakes (ADIs). The MRL is an index that represents the highest concentration (expressed in mg kg⁻¹) of pesticide residue that is legally permitted or accepted in a food or animal feed after the use of pesticides. A consumer exposure is of concern if the estimated dietary exposure to a pesticide exceeds the ADI.

The ADI is the estimate amount of a chemical in food ($\text{mg kg}^{-1}\text{body weight day}^{-1}$) that can be ingested daily over a life time without appreciable health risk to the consumer (FAO2002).

The current study monitors the levels of pesticide residues in some different imported animal liver samples collected from Egyptian local markets and compare the detected levels with the international established permissible limits MRL's. Although this gives a good indication, it lacks the information necessary for a proper interpretation and in terms of food safety. To evaluate the safety of consumers regarding pesticide residues, the exposure needs to be assessed and compared to health safety limits or toxicological endpoint values such as the ADI (acceptable daily intake) or the ARfD (acute reference dose). This work also provides estimation of human health risk through estimated average daily intakes (EADIs) as compared with ADIs set by (FAO/WHO 2010).

2 MATERIALS AND METHODS

2.1 SAMPLES COLLECTION

A total of 32 samples of imported animal liver were collected on the day of local markets in Cairo governorate in Egypt. About 20 gm of each liver sample was collected directly after inspection at local markets in Cairo governorate in Egypt 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.

2.2 SOLVENTS

All organic solvents were of HPLC grade and supplied by Merck, USA. Primary and secondary amine (PSA, 40 μm Bondesil) was purchased from Supelco (Supelco, Bellefonte, USA). Anhydrous magnesium sulfate was of analytical grade, purchased from Merck, USA, and was activated by heating at 250°C for 4 h in the oven before use and kept in desiccators. A stock standard solution ($100 \text{ } \mu\text{g ml}^{-1}$) was prepared with methanol and stored at -20°C . The standard working solutions were prepared from stock solution by serial dilution with methanol at 0.01, 0.05, 0.1, 0.2, 0.5, 1.0 and $2.0 \text{ } \mu\text{g ml}^{-1}$ and were stored at 4°C before use

2.3 PESTICIDES DETECTED

18 pesticides were studied for identification and quantification, the detected residues organochlorine pesticides included: alpha-HCH, beta-HCH, gamma-HCH, heptachlor, heptachlor-epoxide, aldrin, dieldrin, p,p-DDE, endrin, o,p-DDT, p,p-DDD, p,p-DDT. Synthetic Pyrethroids included fenpropathrin, permethrin, lambda-Cyhalothrin, cypermethrin, fenvalerate and deltamethrin

2.4 ANALYSIS OF TESTED AND MONITORED PESTICIDES

The samples were comminuted (10 g) of each was then placed into 50 mL polyethylene tube. Samples were extracted and cleaned up immediately after sampling using QuEChERS methodology (Anastassiades *et al.*, 2003). 15 mL of acetonitrile was added into each tube. The samples were well shaken using a vortex mixer at maximum speed. Afterwards, 6 g of anhydrous magnesium sulfate and 1.5 g of sodium chloride were added, then extract by shaking vigorously on vortex for 5 min and centrifuged for 10 min at 4,000 rpm. An aliquot of 4 mL was transferred from the supernatant to a new clean 15 mL centrifuge tube containing 100 mg PSA and 600 mg anhydrous magnesium sulfate. The samples were again vortexed for 3 min and then centrifuged for 10 min at 4,000 rpm.

2.5 GAS CHROMATOGRAPHIC ANALYSIS

The pesticides were analyzed on Hewlett Packard (HP) serial 6890, gas chromatograph, equipped with electron capture detector (GC-ECD). GC analysis was conducted on a HP-5 MS capillary column of 30 m, 0.25 mm id., 0.25 μm film thicknesses. The oven temperature was programmed from an initial temperature 80°C for 1 min, then increasing at $30^{\circ}\text{C min}^{-1}$ up to 160°C (2 min hold) then increasing to 260°C at a rate of $3^{\circ}\text{C min}^{-1}$ and was maintained at 260°C for 12 min. Injector and detector temperature were maintained at 300°C and 320°C , respectively. Nitrogen was used as a carrier at flow rate of 3 ml min^{-1} . With each set of samples to be analyzed, a solvent blank, a standard mixture and a procedural blank were run in sequence to check for contamination, peak

2.6 METHOD VALIDATION

The validation of the proposed analytical method (GC-ECD) was carried out according to the (SANCO document 10684/2009). Linearity was evaluated by constructing matrix matched calibration curves in the range of 0.1–20 µg /l for GC-ECD. Method sensitivity and recovery were determined by using samples spiked with the tested pesticides at three different levels (0.05, 0.01 and 0.001mg/kg). Fortified samples were extracted as described earlier and the average recovery percentages for fortified samples were determined. Limits of detection (LOD) and quantification (LOQ) were evaluated as the pesticide concentration that produces a peak signal-to-noise ratio of 3:1 and 10:1, respectively. The previous procedures were presented in Table. 1.

Table 1. The group, pesticide, LOD, LOQ, r², RSD%, and average recoveries percentage of OC and PY pesticides in animal livers samples using GC-ECD.

Group	Pesticides	RT	LOD (mg/kg)	LOQ (mg/kg)	r ²	RSD%	Average recoveries %
organochlorine pesticides	Alpha-HCH	10.44	0.005	0.015	0.995	12	75.72
	Beta-HCH	11.96	0.005	0.015	0.997	10	88.53
	Gamma-HCH	13.1	0.001	0.004	0.996	11	90.51
	Heptachlor	14.46	0.01	0.03	0.995	9	87.43
	Aldrin	17.07	0.01	0.03	0.997	9	87.47
	Heptachlor-Epoxyde	17.31	0.001	0.003	0.996	18	82.21
	Dieldrin	17.72	0.001	0.003	0.991	13	89.95
	P,P-DDE	18.98	0.001	0.003	0.988	7	88.52
	Endrin	19.15	0.001	0.003	0.997	16	85.64
	O.P-DDT	20.07	0.002	0.006	0.992	16	91.57
	P,P-DDD	20.5	0.002	0.006	0.991	13	94.56
P,P-DDT	21.09	0.002	0.006	0.992	15	90.86	
synthetic pyrethroids pesticides	Fenpropathrin	21.98	0.001	0.003	0.997	12	94.82
	Lambda-Cyhalothrin	25.02	0.001	0.003	0.995	17	92.92
	Permethrin	26.17	0.001	0.004	0.994	15	89.72
	Cypermethrin	28.75	0.001	0.003	0.998	12	83.11
	Fenvalerate	34.58	0.005	0.016	0.992	13	99.43
	Deltamethrin	40.96	0.001	0.003	0.996	14	84.11

LOD = limits of detection

LOQ = limits of quantification

RSD% = relative standard deviation percent

2.7 HUMAN RISK ASSESSMENT

From a potential health perspective, it is certainly important to compare exposure estimates to established toxicological criteria such as ADI. Actually EDI is a realistic estimation of pesticide residues exposure that was calculated in the agreement with the international guidelines. EDI of pesticide residues for each combination of pesticide and commodity was calculated by multiplying the mean residual pesticide concentration (mg kg⁻¹) in the food of interest and the food consumption rate (kg d⁻¹) and divided by body weight (Darko and Akoto, 2008) as shown in the equation:

$$\text{Exposure} = (\text{Concentration of pesticide residue} \times \text{Food consumed}) / \text{body weight}$$

The food consumption figures used were based on the consumption data issued by WHO/Global Environment Monitoring System–Food Contamination, The health risk indices were obtained by dividing the EDI by their corresponding values of ADI (FAO/WHO, 2010); assuming average adult's body weight of 60 kg. Estimated daily intakes (EDIs) of a pesticide residue and food consumption assumption were used to determine long term health risks to consumers.

When the health risk index >1; the food involved is considered a risk to the consumers. When the index <1, the food involved is considered acceptable (Hamilton and Crossley, 2004 and Darko and Akoto, 2008). Then HRI of the residues was computed using the equation, HRI = EDI/ADI, (EFSA2013). Cumulative risk obtained for the detected pesticides belonging to the same chemical group organochloride, and pyrethroids by summing up HRI for the individual pesticides (ΣHRI's).

3 RESULTS AND DISCUSSION

3.1 METHOD VALIDATION

The method was evaluated according to the guidance "Method Validation and Quality Control Procedures for Pesticide Residue Analysis in Food and Feed" (SANCO/10684/2009) for its repeatability, linearity, recovery, limit of detection and quantification. Linearity was evaluated by the calculation of a five-point linear plot with three replicates (Table 1), based on linear regression and squared correlation coefficient, r^2 , which should be >0.988 . Average recovery and the highest RSD were obtained in repeatability studies from samples of spiked animal livers at three different concentration levels (LOQ, 2 X LOQ and 5 X LOQ); Table 1 shows the results for recoveries studies. For the analysis of pesticides at sub $\mu\text{g/L}$ levels recovery values between of 70% and 120%, are considered as acceptable. The accuracy of the presented method was acceptable for all pesticides tested being in the range of 75.72–99.43 %, which fulfill the recommendation of SANCO guidelines (SANCO/10684/2009).

The RSD values were less than 20% for all the concentration levels tested. Limits of detection (LOD) values were in the range of 0.001-0.01 mg/kg. The experiments showed that there were no interference peaks from the animal livers matrix on the elution region of the specific pesticides. All results obtained for all compounds confirm the efficacy of the present method for the determination of multi-residue pollutants in poultry feeds sample.

3.2 MONITORED ORGANOCHLORINES AND SYNTHETIC PYRETHROIDS PESTICIDE RESIDUES IN IMPORTED ANIMAL LIVER SAMPLES

The organochlorines and synthetic pyrethroids pesticide residues found in imported animal liver samples results are shown in Table 2. It is shown that, a total of 32 imported animal liver samples were analyzed. All samples were contaminated with organochlorines pesticide, while 3 samples were contaminated with synthetic pyrethroids pesticide residues and Total contamination % was 100 % in all samples, 6.25% from these samples were exceeded MRL limits. From these pesticides heptachlor and endrin were detected in imported animal liver samples were more than MRL value. The other pesticides found were less than MRL value. All collected imported animal liver samples were free from any detectable residues of alpha-HCH, beta-HCH, dieldrin, , p,p-DDD o.p-DDT, p,p-DDT, permethrin and fenvalerat pesticides. The most frequently found pesticides were P,P'-DDE (in 31 samples) and heptachlor-epoxide (in 8 samples) while the lowest frequently found pesticides were aldrin, cypermethrin and deltamethrin (in one sample).

The results of imported animal liver samples Tables 2.indicated that, two pesticides (heptachlor-epoxide and P,P'-DDE) found in samples collected in winter season, their residues means were (9.9 and 42.75 ng/kg) respectively. in summer season heptachlor and P,P'-DDE found in samples and their residues means recorded (13and 137.125 ng/kg) respectively. the residues mean of P,P'-DDE was 80.87 ng/kg in imported animal liver samples collected during Autumn season .while 10 pesticides (gama-HCH , heptachlor , aldrin , heptachlor-epoxide, p,p-DDE , endrin , cypermethrin, fenprothrin, lambda-Cyhalothrin and deltamethrin) found in imported animal liver samples which collected during spring season , their residues means were (6.94,89.5,89,116.33,60.85,59,64.1,56.5,133.75 and 20 ng/kg) respectively.

Data in Table 2. showed that, the highest contamination of pesticides residues were found in imported animal liver samples which collected during spring season while the lowest contamination of pesticides residues were found in imported animal liver samples which collected during Autumn season.

3.3 RISK ASSESSMENT

When assessing chronic exposure, the level of pesticide exposure over a lifetime and the likely effects on health of such exposure is considered. This assessment method is well developed and considers the mean levels of exposure in relation to the Acceptable Daily Intake (ADI) values established for individual pesticides. In the case of consumers exposed to residues of chronically toxic pesticides, their health would only be at risk if their dietary intake exceeded the ADI every day for an extended period of time. The calculation of the chronic exposure assessment in Table 3. is based on the assumption that food with levels of pesticides found is consumed on a daily basis over a lifetime. Therefore, it is regarded as an overestimate of the real exposure to pesticides.

The exposure to pesticide residues was calculated on a total of 10 residues. The detected residues higher than LOQ are involved in calculation of exposure to avoid over estimation of EDI. The total exposure to a given pesticide residue, was obtained by summing exposures from all residue pesticide/food combinations.

Table 3. showed the estimated average daily intake (EDI ug kg ⁻¹bw ⁻¹) and the hazard index (HI) for each pesticide residues (the ratio of EDI to ADI) in samples of imported animal liver analysed .The data showed that, the highest intake and the hazard index (HI) of pesticides group through the imported animal liver in descending order were, organochlorines pesticide followed by pyrethroids with values of (1.80926E-05, and 3.34292E-06) (ug kg ⁻¹bw ⁻¹ day⁻¹) and (0.062327484 and 0.001023014) respectively.

The data in **table 3.** also showed that, none of individual HI of pesticides detected in imported animal liver samples exceeded one indicates no risk associated with consumption of such animal liver.

Table 2. Minimum, maximum, mean, frequency, contamination and violation of pesticides residues monitored in 32 samples of imported animal livers collected from local markets in Egypt during January 2015 to December 215

Season	Total no. of sample	Pesticides Found	Frequency		Range: Minium-maximum (mean) (ng/kg)	Contaminated Samples		MRLs (ng/kg)	Violated Samples	
			No.	%		No.	%		No.	%
Winter	8	Heptachlor-Epoxide	1	12.5	9.9-9.9 (9.9)	8	100	200	0	0
		P,P-DDE	8	100	17-100 (42.75)			1000		
Summer	8	Heptachlor	1	12.5	13-13 (13)	8	100	200	0	0
		P,P-DDE	8	100	69-278 (137.125)			1000		
Autumn	8	P,P-DDE	8	100	49-137 (80.870)	8	100	1000	0	0
Spring	8	Gamma-HCH	5	62.5	4.8-14.6 (6.94)	8	100	20	0	0
		Heptachlor	5	62.5	15-208 (89.5)			200	1	12.5
		Aldrin	1	12.5	89-89 (89)			200	0	0
		Heptachlor-Epoxide	6	75	30-186 (116.33)			200	0	0
		P,P-DDE	7	87.5	18-120 (60.85)			1000	0	0
		Endrin	2	25	28-90 (59)			50	1	12.5
		Cypermethrin	1	12.5	64.1-64.1 (64.1)			200	0	0
		Fenpropathrin	2	25	43-70 (56.5)			500	0	0
		Lambda-Cyhalothrin	4	50	40-230 (133.75)			500	0	0
		Deltamethrin	1	12.5	20-20 (20)			30	0	0
Total	32				32	100		2	6.25	

Table 3. Acceptable daily intake (ADI), estimated daily intake (EDI), and health risk index (HRI) for pesticide residues found in imported animal livers samples studied.

Pesticide found	ADI (mg/kg body weight/day) (source; year)	EDI (mg/kg body weight/day)	HRI (EDI/ADI)	Health risk
Gamma-HCH	0.005 JMPR 1994	1.58447E-07	0.000158447	No
Heptachlor	0.0001 JMPR 1994	2.10274E-06	0.021027397	No
Aldrin	0.0001 JMPR 1994	4.06393E-07	0.004063927	No
Heptachlor-Epoxide	0.0001 JMPR 1994	3.23233E-06	0.032323288	No
P,P-DDE	0.01 JMPR 2000	1.14712E-05	0.001147119	No
Endrin	0.0002 JMPR 1994	7.21461E-07	0.003607306	No
Σ O.C		1.80926E-05	0.062327484	
Cypermethrin	0.02 Dir 04/58	2.92694E-07	1.95129E-05	No
Fenpropathrin	0.03 JMPR 1993	5.15982E-07	1.71994E-05	No
Lambda-Cyhalothrin	0.02 Reg. (EU) 2016/146	2.44292E-06	0.000977169	No
Deltamethrin	0.01 JMPR 2000	9.13242E-08	9.13242E-06	No
Σ PY		3.34292E-06	0.001023014	

3.4 CARCINOGENICITY OF PESTICIDES DETECTED IN THE PRESENT STUDY

As described by the EPA's Classification System for Carcinogens, when assessing possible cancer risk posed by a pesticide, EPA considers how strongly carcinogenic the chemical is (its potency) and the potential for human exposure. The pesticides are evaluated not only to determine if they cause cancer in laboratory animals, but also as to their potential to cause human cancer. In this issue, **Table 4** shows the carcinogenicity of pesticides detected in the present study as described by the EPA's classification system for carcinogens. As seen in **Table 4**, Heptachlor, aldrin, heptachlor-epoxide and P,P-DDE were classified as Group B2—Probable Human Carcinogen, while cypermethrin was classified as Group C (possible human carcinogen). This group is used for agents with limited evidence of carcinogenicity in animals in the absence of human data (**U.S. EPA. 1989**). However, endrin and lambda-cyhalothrin were classified as Group D—Not classifiable as to human carcinogenicity. This group is generally used for agents with inadequate human and animal evidence of carcinogenicity or for which no data are available.

This descriptor is used when the evidence from human or animal data is suggestive of carcinogenicity, which raises a concern for carcinogenic effects but is judged not sufficient for a conclusion as to human carcinogenic potential. Two pesticides were classified as Group E—evidence of non carcinogenicity for humans and this group is used for agents that show no evidence for carcinogenicity in at least two adequate animal tests in different species or in both adequate epidemiologic and animal studies (**U.S. EPA. 1989**). In addition, two pesticides (fenpropathrin and deltamethrin) were described unlikely to be carcinogenic to human, and this means that, the available data on these compounds are considered robust for deciding that there is no basis for human hazard concern (**U.S. EPA. 2006**).

On the other hand, Gamma-HCH pesticide was classified as suggestive evidence of carcinogenicity but not sufficient to assess human carcinogenic potential.

The obtained findings are in agreement with those obtained by Khalid Ibrahim (**Sallam and Alaa Eldin 2008**) they determined organochlorine pesticide residues in a total of 270 meat samples; comprising the muscle, liver, and kidney collected from 90 carcasses (30 each of camel, cattle and sheep) slaughtered in Sharkia Province, Egypt. All samples were

analyzed for their residual contents of DDT compounds (DDTs), hexachlorocyclohexane isomers (HCHs), lindane (c-HCH), aldrin, dieldrin, endrin, hexachlorobenzene (HCB), toxaphene, and chlordane compounds.

The residual contents of the organochlorines detected in all of the contaminated samples analyzed from the three different species were well below the respective maximal permissible limits set by local or international organizations.

(Alawi and Al-Hawadi2008) they monitoring fifteen organochlorine pesticides in 30 samples of liver, kidney and adipose tissues from sheep (male and female) gathered from slaughter houses in Amman/Jordan. Three groups of the organochlorine pesticides in addition to hexachlorobenzene (HCB) namely: DDT, HCH, and Cyclodiene groups were determined. The results show that almost all samples are contaminated with HCB, HCH’s heptachlor and aldrin at relatively high concentrations and almost all studied organochlorine pesticides were found in the studied sample.

(Abd Elhafez et al., 2015), they 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. 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.

Table (4):- Carcinogenicity of pesticides as described by EPA’s classification system for carcinogens. Supplementary Data.

Pesticides found	Carcinogenicity
Gamma-HCH	Suggestive Evidence of Carcinogenicity, but Not Sufficient to Assess Human Carcinogenic Potential
Heptachlor	Group B2–Probable Human Carcinogen
Aldrin	Group B2--Probable Human Carcinogen
Heptachlor-Epoxide	Group B2–Probable Human Carcinogen
P,P-DDE	Group B2--Probable Human Carcinogen
Endrin	Group D–Not classifiable as to human carcinogenicity. This group is generally used for agents with inadequate human and animal evidence of carcinogenicity or for which no data are available.
Cypermethrin	Group C–Possible human carcinogen, this group is used for agents with limited evidence of carcinogenicity in animals in the absence of human data.
Fenpropathrin	Group E -Not likely to be carcinogenic to humans, this descriptor is used when the available data are considered robust for deciding that there is no basis for human hazard concern
Lambda-Cyhalothrin	Group D–Not classifiable as to human carcinogenicity. This group is generally used for agents with inadequate human and animal evidence of carcinogenicity or for which no data are available.
Deltamethrin	Group E -Not Likely to Be Carcinogenic to Humans, this descriptor is used when the available data are considered robust for deciding that there is no basis for human hazard concern

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