Journal of Agricultural Chemistry and Environment, 2019, 8, 58-67
http://www.scirp.org/journal/jacen
ISSN Online: 2325-744X
ISSN Print: 2325-7458

Occurrence and Levels of Chlorinated Pesticides Residues in Cow Milk: A Human Health Risk Assessment

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How to cite this paper: Ibigbami, O.A., Aiyesanmi, A.F., Adesina, A.J. and Popoola, O.K. (2019) Occurrence and Levels of Chlorinated Pesticides Residues in Cow Milk: A Human Health Risk Assessment. *Journal of Agricultural Chemistry and Environment*, **8**, 58-67.
https://doi.org/10.4236/jacen.2019.81005

Received: January 2, 2019
Accepted: February 24, 2019
Published: February 27, 2019

Abstract

Background: The study determined the presence and concentration of persistent organochlorine pesticides (OCPs) residues in cow milk from Ekiti State University Agricultural farm in Ado-Ekiti, Nigeria. The study was investigated in order to monitor consumer's exposure to these chemicals pesticides. **Methods:** Qualitative identification and quantification evaluation of the extracted pesticides after clean-up on silica gel were done with a Gas Chromatography coupled with an Electron Capture Detector (GC-ECD). **Results:** The results revealed the presence of 11 OCPs residues in the milk samples, with concentration range of 0.001 - 0.189 mg/l, while α-BHC, endrin, endrin aldehyde, endosulfan II, endosulfan sulphate and methoxychlor were not detected. The analysis of variance revealed no significant variation in the levels of all the analysed pesticides except dieldrin. **Conclusion:** The hazard indices (HIs) were significantly lower than 1 with the range of 0.00063 - 0.107, indicating no potential health risk.

Keywords

Cow Milk, Organochlorine Pesticides, Gas Chromatography, Risk Assessment

1. Introduction

Livestock plays a very important role in Nigeria agriculture, contributing about 12.7% of the agricultural GDP [1]. According to National Agricultural Sample Survey, Nigeria is endowed with an estimated cattle population of 19.5 million [2]. The most population of these cattle is in the hands of pastoral Fulani. The

Fulani control at least 95% of the cattle population. In Nigeria, pastoral communities produce the bulk of milk consumed in the rural areas of Nigeria. In 1992, milk consumption rate for Nigeria was 18 g per person per day, it was said to have increased to 22 g per person per day in 2007 (22 g per person per day).

Milk has usually been studied as an indicator of the bioconcentration process of environmentally persistent organic pollutants, such as organochlorine pesticides [3] [4]. Due to their lipophilic properties [5], pesticides are primarily stored in fat-rich tissues and subsequently translocated and excreted through milk fat [6] [7]. Persistent organic pollutants including organochlorine pesticides (OCPs), are of global concern because of their toxicity, resistance to degradation, potential for long-term transport and their tendency to accumulate in fatty tissues (lipophilicity) [8]. Based on reports of the toxicity and adverse harmful effects of OCPs to the environment and humans, many OCPs have been banned or restricted internationally [9]. Despite the benefits of pesticides for agriculture production and public health, the increased higher application of pesticides has resulted in food contamination. Contamination of food results in exposure to toxic pesticide residues for the resident populations leading to harmful health effects. Bio-concentration and bioaccumulation of pesticides in animal tissues or system are capable of reaching toxic levels even when the exposure is low. Due to the general prevalence of pesticides, it is important to detect and determine the concentrations levels of these pesticides in environmental samples, especially food [10]. The toxicity of pesticides to target and non-target organisms generally depends on the amount present in the environment, the proportion available and ultimately in the amount actually encountered and adsorbed by the organism [11]. This study determines the presence and extent of contamination of OCPs in consume milk cows from Ekiti State University Agricultural farm in Ado-Ekiti, Nigeria, so as to monitor consumer's exposure to pesticides.

2. Materials and Methods

2.1. Sampling and Sample Preparation

Raw cow milk samples (500 ml each) were collected from cows in Ekiti State University Agricultural farm in Ado-Ekiti, Nigeria in clean glass containers. Six samples were randomly collected and immediately stored in ice chest with dried ice at −4˚C. The samples were stored at −20˚C (temperature at which all microbial actions in biological samples are ceased [12]) in a freezer prior to analysis. Samples were collected in the month of April 2017.

2.2. Pesticides Extraction and Clean-Up Procedure

The extraction procedure was carried out by the method [13]. The cow milk samples frozen at −20˚C were allowed to thaw and then stirred thoroughly. Ten mililitres of the milk samples were homogenized with 40 ml of 1:1 acetone/n-hexane mixture by macerating the mixture with aid of an ultra-Turrax T25 basic at a speed of 9500 rpm at about 60˚C for 2 min to enhance extraction.

The homogenate were then centrifuged at 2500 rpm for 2 min. After centrifuging, the organic layer was collected into an already weighed round bottom flask. The milk phase was re-extracted twice with two separate aliquots of 30 ml of n-hexane and acetone. The combined organic phase collected was evaporated to dryness by the rotary evaporator at 40˚C. The extract was re-dissolved in 5 ml n-hexane and later concentrated to 2 ml in a rotary evaporator.

A column of about 15 cm (length) × 1 cm (internal diameter) was packed with glass wool and later with 2 g of activated silica gel (Silica gel 60 F_{254}). About 1 g anhydrous Na_2SO_4 was placed at the top of the column to absorb water. Pre-elution was done with 15 ml n-hexane prior to the clean up. The extract was run through the column and eluted with 20 ml n-hexane and diethyl ether (1:1 v/v). The eluate was concentrated to dryness on the rotary evaporator and then recovered into 2 ml n-hexane. The final extract was later transferred into GC vials for GC analysis.

2.3. Gas Chromatographic Condition

The gas chromatography conditions for the analysis were as follows: GC model: Hewlett Packard 7890A series II coupled with electron capture detector (GC-ECD); injector and detector temperature were 250˚C and 290˚C, the purge activation time was 30 s; inlet mode: splitless with flow rate of 2 mL/min; carrier gas: helium; make-up gas: nitrogen; inlet temperature: 250˚C; column type: DB-17 fused silica capillary column; column dimension: 30 m × 250 μm × 0.25 μm film thickness; oven condition: initial temperature at 150˚C and increase to 280˚C at 6˚C/min. The total run time was 21.667 min.

2.4. Quality Assurance and Quality Control

For the set of samples, a procedural blank and spike samples consisting of all reagents was run to check for interference and cross contamination. The limits of detection (LOD) of the pesticides were calculated as three times the standard deviation of the pesticides level in procedural blanks. A strict regime of quality control was employed before the onset of the sampling and analysis program. Multi level calibration curves were created for quantification and good linearity ($r^2 > 0.999$) was achieved for tested intervals that included the whole concentration range found in sample. Peak area ratios were plotted against the concentration ratios.

2.5. Health Risks Assessment

The estimated daily intake and hazard indices of the pesticides in the milk samples were calculated to estimate the potential health risks to consumers. The available daily intake (ADI) is a measure for the toxicity of substances by long term and repeated ingestion. The estimated daily intake (EDI) was calculated using international guidelines [14] equation EDI = C × M/W, where C = mean concentration of individual pesticides (mg/l), M is the milk consumption rate

per person (22 g per person per day) for Nigeria, while W is the average body weight of an adult (70 kg). The hazard index was calculated by dividing the estimated daily intake (EDI) by their corresponding acceptable daily intake (ADI).

2.6. Statistical Analysis

Data generated in the study were subjected to statistical analysis to test for spatial variations with analysis of variance (ANOVA) using SPSS 15.0 package. One level of confidence limit (p = 0.05) was considered in the interpretation of the statistical results. In order to estimate the degree of association among OCPs compounds, Pearson Correlation (two-tailed) was also employed.

3. Results and Discussion

Table 1 depicted the concentration of OCPs in the raw cow milk from the selected samples. The OCPs concentrations ranged from ND to 0.114 mg/l with mean value of 0.003 (*p,p*'-DEE) to 0.051 (β-BHC) mg/l and coefficient of variation (CV%) of 62.5 (heptachlor-epoxide) to 143 (endosulfan 1) which reflected high spread value of the OCPs concentration. The percentage occurrence of the pesticides in the samples ranged from 33.3% - 100%. In the pesticides concentrations, β-BHC and dieldrin were the highest concentrated, while *p,p*'-DDE showed the least with concentration trend of β-BHC > dieldrin > heptachlor > aldrin > Υ-BHC > δ-BHC > heptachlor-epoxide > endosulfan 1 > *p,p*'-DDD > *p,p*'-DDT > *p,p*'-DDE. In the real figure values we have percentage levels of these pesticides over the total organochlorine pesticide levels as follows: β-BHC (26.5%), dieldrin (17.4%), heptachlor (15.4%), aldrin (14.9%), Υ-BHC (6.67%), δ-BHC (5.13%), heptachlor-epoxide (4.10%), endosulfan 1 (3.59%), *p,p*'-DDD (3.08%), *p,p*'-DDT (2.05%), *p,p*'-DDE (1.54%). The percentage occurrence of the pesticides obtained in this study were lower than what [15] [16] reported for breast and davish milk. In general, α-BHC, endrin, endrin aldehyde, endosulfan II, endosulfan sulphate and methoxychlor showed not detected in all the samples.

The concentration of the benzenehexachloride (BHC) ranged from ND - 0.102 mg/l, while the mean concentration ranged from 0.010 ± 0.008 (δ-BHC) to 0.051 ± 0.042 (β-BHC) mg/l. A wide spatial variation in the concentrations of all BHCs was also noticed as revealed by the CV which ranged between 88.9% (δ-BHC) and 92.3% (Υ-BHC). The percentage occurrence (%) of β-BHC, Υ-BHC and δ-BHC was 66.7%, 83.3% and 100% respectively. The high percentage occurrence of the BHCs in the samples could be due to high persistent nature of the pesticides. All the mean BHCs except β-BHC were below the Codex Alimentarius MRL [17] in food. The BHCs level of 0.298 - 0.686 mg/l and ND - 1.08 mg/l (Table 2) reported by [18] [19] were comparatively higher, while those reported by [20] showed similar ranges in most cases to the values reported in this study.

The concentration of heptachlor and heptachlor-epoxide ranged from ND -

0.081 and ND - 0.017 mg/l respectively. The sum of heptachlor concentration ranged from 0.001 - 0.098 mg/l. The concentration of heptachlor reported in this study were similar to the levels reported [21] [22] for cow and bovine milk, while those reported [18] in breast milk were higher than those reported in this study. Mathur *et al.* [23] reported 0.0006 mg/l (**Table 2**) in human blood, a concentration lower than the present study. The level of heptachlors in this study were below maximum residue limits (MRLs) set by European Union in foods.

The dichlorodiphenyltrichloroethane concentrations ranged from ND - 0.013 mg/l with mean concentration of 0.003 ± 0.004 (*p,p*'-DDE) to 0.006 ± 0.006 (*p,p*'-DDD) mg/l and percentage occurrence of 50% - 66.7%. The DDT level were similar in some cases to what [22] [23] reported, while [18] [19] [20] [24] were higher than the present study. None of the samples exceeded the EU MRL of 0.50 mg/kg for DDT in food.

Table 1. Concentration (mg/l) of organochlorine pesticides residues in the milk samples.

	Range	Mean ± SD	CV%	% Occurrence
α-BHC	-	-	-	-
β-BHC	ND - 0.102	0.051 ± 0.042	89.4	66.7
ϒ-BHC	ND - 0.035	0.013 ± 0.012	92.3	83.3
δ-BHC	0.01 - 0.024	0.010 ± 0.008	88.9	100
ΣBHC	0.004 - 0.144	0.070 ± 0.058	82.8	100
Heptachlor	ND - 0.081	0.030 ± 0.036	117	83.3
Heptachlor-epoxide	ND - 0.017	0.008 ± 0.006	62.5	83.3
Σheptachlor	0.01 - 0.098	0.038 ± 0.039	103	100
p,p'-DDE	ND - 0.009	0.003 ± 0.004	133	50
p,p'-DDD	ND - 0.013	0.006 ± 0.006	100	66.7
p,p'-DDT	ND - 0.008	0.004 ± 0.003	75	66.7
ΣDDT	0.004 - 0.26	0.013 ± 0.008	61.5	100
Aldrin	ND - 0.075	0.029 ± 0.026	89.6	83.3
Dieldrin	ND - 0.114	0.034 ± 0.048	141	33.3
Aldrin + dieldrin	ND - 0.189	0.063 ± 0.073	116	83.3
Endrin	ND	-	-	-
Endrin aldehyde	ND	-	-	-
ΣEndrin	ND	-	-	-
Endosulfan 1	ND - 0.025	0.007 ± 0.010	143	33.3
Endosulfan II	ND	-	-	-
Endosulfan sulphate	ND	-	-	-
ΣEndosulfan	ND - 0.025	0.007 ± 0.010	143	33.3
Methoxychlor	ND	-	-	-
TOCP	0.029	0.288 ± 0.204	70.8	100

TOCP = Total organochlorine pesticides; ND = Not detected; SD = Standard deviation; CV = Coefficient of variation.

Table 2. Comparison of OCPs in the present study and other studies.

	α-BHC	*β*-BHC	*Υ*-BHC	*δ*-BHC	Hepta-Chlor	Hepta-chlor-epoxide	*p,p*'-DDE	*p,p*'-DDD	*p,p*'-DDT	Aldrin	Dieldrin	Endrin	Endrin Aldehyde	Endo-sulfan I	Endosulfan II	Endosulfan sulphate	Methoxychlor	Reference
Buffalo milk	ND-0.212	ND-0.482	0.001-1.08	ND	-	-	ND-0.97	ND-0.094	ND-0.13	-	-	-	-	ND-0.021	ND-0.022	ND-0.002	-	[19]
Human breast milk	ND-0.060	-	-	ND-0.024	-	-	ND-0.112	-	ND-0.068	-	-	-	-	-	-	-	-	[20]
Human breast milk	-	-	0.298 (80.9%)	0.686 (95.2%)	0.514 (76.2%)	-	1.124 (100%)	-	0.371 (76.2%)	0.156 (85.7%)	0.115 (71.4%)	0.125 (81%)	0.224 (42.9%)	0.211 (81%)	-	4.907 (85.7%)	0.716 (42.9%)	[18]
Cow milk	-	-	ND-0.042 (80%)	-	-	-	ND-0.086 (60%)	ND-0.092 (60%)	ND-0.23 (54%)	ND-0.406 (51%)	ND-0.095 (63%)	-	-	-	-	-	-	[25]
Cow milk	-	-	0.15-0.196 (20%)	-	0.038-0.082 (30%)	0.044-0.072 (20%)	-	-	0.064-0.128 (35%)	-	0.096-0.162 (15%)	0.036-0.082 (25%)	-	-	-	-	-	[21]
Human breast milk	ND	ND	ND	-	-	ND	ND-0.02	ND	ND-0.043	ND	ND	ND	-	-	-	-	-	[27]
Breast milk	-	-	-	-	(57.4%)	(57.4%)	-	-	(31.7%)	(31.7%)	(31.7%)	(18.8%)	-	(9.9%)	-	-	-	[15]
Human breast milk	-	0.20-2.10	-	-	-	-	7.80-734	0.20-7.30	2.5-647	-	-	-	-	-	-	-	-	[24]
Bovine Milk	0.45-2.34 (3%)	ND	0.45-0.64 (5%)	-	1.22-3.05	1.23-3.25 (7%)	6.67-6.67 (1%)	2.32-2.32 (1%)	-	0.10-1.86 (44%)	0.98-14.7 (50%)	3.91-3.91 (1%)	-	1.00-4.03 (12%)	0.13-12.2 (24%)	-	ND	[22]
Davish milk	-	-	(93%)	-	-	-	(82%)	-	-	-	(30%)	-	-	-	-	-	-	[16]
Human blood	-	-	ND-0.105 (100%)	-	0.0006 (5%)	-	ND-0.255 (95%)	0.001 (55%)	0.01 (50%)	ND-0.016 (80%)	ND	-	-	0.004 (25%)	0.0002 (5%)	-	-	[23]
Present study	-	**0.051**	**0.013**	**0.010**	**0.03**	**0.008**	**0.003**	**0.006**	**0.004**	**0.029**	**0.034**	-	-	**0.0077**	-	-	-	

(%) = Percentage occurrence; ND = Not detected.

Aldrin concentrations ranged from ND - 0.075 mg/l with the mean concentration of 0.029 ± 0.026, while dieldrin ranged from ND - 0.114 mg/l with average concentration of 0.034 ± 0.048 mg/l. This level is comparably lower in breast and cow milk (0.156 and ND - 0.406 mg/l) as reported [18] [25], while [23] (ND - 0.016 mg/l) reported similar range for human blood. The mean concentration level of aldrin was below EU (0.05 mg/l) MRL, while dieldrin reported in this study exceeded the EU MRL of 0.02 mg/l in food.

For endosulfans, only endosulfan 1 were detected with concentration range of ND - 0.025 mg/l, while endosulfan II and endosulfan sulphate showed not detected in all the samples. Comparatively, high concentration of endosulfan 1 was reported in human breast milk [18] compared to the present study, but similar to what was reported [19] in buffalo milk; while those reported [22] [23] for bovine milk and human blood were lower. The endosulfan 1 level in this study was lower than the EU and Codex Alimentarius MRL for endosulfan 1 in food.

To determine the potential human health risk of the OCPs residues in the milk, intakes of the pesticides from the milk consumption were estimated in **Table 3**. The estimated daily intakes (EDI) for all the OCPs were within the available daily intake for each pesticide. The hazard indices (HIs) were significantly lower than 1with the range of 0.00063 - 0.107, indicating no potential human health hazard. It may be concluded that human population consuming milk

Table 3. Estimated dose values and hazard indices of the OCPs in the milk samples.

	WHO/FAO ADI (mg/kg/day)	EDI (mg/kg/day)	Hazard index
α-BHC	0.005	-	-
β-BHC	0.005	1.60×10^{-5}	0.0032
Y-BHC	0.005	4.08×10^{-6}	0.00082
δ-BHC	0.005	3.14×10^{-6}	0.00063
Heptachlor	0.0001	3.03×10^{-6}	0.0943
Heptachlor-epoxide	0.0001	2.74×10^{-6}	0.0025
p,p'-DDE	0.0005	2.94×10^{-6}	0.0021
p,p'-DDD	0.0005	7.63×10^{-6}	0.0038
p,p'-DDT	0.0005	7.14×10^{-6}	0.0025
Aldrin	0.0001	2.54×10^{-6}	0.0911
Dieldrin	0.0001	6.75×10^{-5}	0.107
Endrin	0.0002	-	-
Endrin aldehyde	0.0002	-	-
Endosulfan 1	0.0005	2.20×10^{-6}	0.0044
Endosulfan II	0.0005	-	-
Endosulfan sulphate	0.0005	-	-
Methoxychlor	0.1	-	-

Table 4. Correlation matrix of the OCPs in the milk samples.

	β-BHC	*Υ*-BHC	*δ*-BHC	Heptachlor	Hept-epoxide	*p,p'*-DDE	*p,p'*-DDD	*p,p'*-DDT	Aldrin	Dieldrin	Endosulfan 1
β-BHC	1										
Υ-BHC	0.780	1									
δ-BHC	0.706	0.487	1								
Heptachlor	0.814*	0.330	0.633	1							
Hep-epox.	0.873*	0.623	0.694	0.620	1						
p,p'-DDE	0.801	0.533	0.678	0.553	0.936	1					
p,p'-DDD	0.997	0.775	0.675	0.833*	0.841*	0.759	1				
p,p'-DDT	−0.599	−0.576	−0.016	−0.503	−0.383	−0.128	−0.643	1			
Aldrin	0.481	−0.098	0.584	0.654	0.634	0.738	0.451	0.160	1		
Dieldrin	0.599	0.124	0.566	0.590	0.776	0.896*	0.561	0.079	0.946	1	
Endosulfan 1	0.684	0.104	0.628	0.840*	0.719	0.776	0.670	−0.112	0.953	0.920	1

* Correlation is significant at the 0.05 level (2-tailed).

from Ekiti State University Agricultural Farm was not at risk due to relatively low hazard indices. It has been reported that if the hazard index (HI) is greater than I, the chemical has exceeded the maximum acceptable level and may cause harm to human [26]. Therefore, the milk may be considered to be at safe levels of exposure.

Analysis of variance revealed no significant variation ($p > 0.05$) in the levels of all the analysed pesticides except dieldrin. The matrix of correlation coefficients of the pesticides in the milk samples at 0.05 confidence levels are shown in **Table 4**. It was observed that heptachlor and heptachlor-epoxide showed significant positive correlation with *β*-BHC and also *p,p'*-DDD; heptachlor positively correlated with endosulfan I; and *p,p'*-DDE was significantly correlated with dieldrin at 0.05 confident level. The pesticides with significant positive correlations likely shared common sources and were probably affected by related factors in the cow's system.

4. Conclusion

Residues of *β*-BHC, *δ*-BHC, *Υ*-BHC, aldrin, dieldrin, *p,p'*-DDD, *p,p'*-DDE, *p,p'*-DDT, heptachlor, heptachlor-epoxide and endosulfan I were detected at varying concentration in the examined milk samples with wide spatial variation in most pesticides. The mean concentrations of the OCPs except dieldrin were below EU MRL in food. The study indicates no potential health risk to human population consuming the milk as revealed by the calculated hazard indices. The detectable levels of the pesticides make it inevitable to conduct regular monitoring so as to ensure that the residual levels remain below prescribed limits by national and international standards.

Acknowledgements

The authors wish to acknowledge the technical assistance rendered by the Chemical Laboratory of the Nigerian Institute of Oceanography and Marine Research, Victoria Island, Lagos, Nigeria.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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