Assessment of Organochlorine pesticides in cocoa beans from Ashanti and Brong Ahafo regions of Ghana

[*1]Boakye, S., [2]Jonfia- Essien W., [3]Voegborlo, B.R, [3]Boadi N,O

[* 1]Radford University College, Department of Applied Sciences, P. O.BOX CT 2837 Cantoments **Accra Ghana**

[2]Quality Control Company Limited (COCOBOD). P.O.BOX M 54 Accra

[3]Kwame Nkrumah University of Science and Technology. Private mail bag KNUST Kumasi Ghana

ABSTRACT

Research was conducted in the Ashanti and Brong Ahafo regions of Ghana to determine organochlorine pesticide residues in cocoa beans. The pesticides were extracted by using solvent extraction technique followed by clean up with C-18 and Envi – Carb NH_2 solid-phase extraction cartridges. Extracts were dissolved in ethyl acetate and analysis carried out by Gas Chromatograph equipped with Electron Capture Detector. Retention times were used for identification of pesticides and quantified using external calibration method. From this study, 55.5 % of cocoa beans sampled from Brong Ahafo region and 66.7 % of cocoa beans sampled from the Ashanti region had organochlorine pesticide residue concentrations above the EU permissible levels. Heptachlor had the maximum concentration of residues whilst heptachlor exo epoxide had the minimum concentration for the two regions. The results obtained reflect the enormous past use and continual usage of pesticides on cocoa beans in Ghana.

Keywords: Retention times: Pesticide residues: Solvent extraction: Cocoa beans: Gas chromatograph: External calibration: Quantified: Concentration

INTRODUCTION

Cocoa is affected by a range of pests and diseases causing a decline of global production (ICCO, 2010) and resulting in the massive use of pesticides. The use of organochlorine pesticides (OCPs) has proved to be effective in Ghana. The recommended pesticides for cocoa include Actellic/Talstar, Bifenthrin, Thiamethoxam, Promecarb and Imidacloprid.

The hazardous nature of OCPs is a result of their toxicity in combination with high degree of lipophilicity, chemical and biological stability (Biziuk, 1996). These characteristics make the OCPs prone to bioaccumulation along the food chain involving a wide range of trophic levels (Fildago Used et al., 2003). Most of OCPs have been banned because they are highly persistent, and their residues still appear as pollutants in food (Abou-Arab, 1999). Many benefits have been achieved from the use of synthetic pesticides in agriculture, but in spite of the obvious advantages, the potential adverse impact on food and consumers' health must be considered. Although pesticides are manufactured under very strict regulation processes to function with logical certainty and minimal impact on human health and the environment, serious concerns have been raised about health risks resulting from residues in food (Damalas and Eleftherohorinos, 2011; Eskenazi *et al*., 2008). Research data have shown the presence of pesticide residues in dairy products, meat, (Darko and Acquaah, 2007, 2008) fish, water, sediments (Darko *et al.*, 2008), human blood and breast milk (Ntow, 2001), fruits and vegetables (Hanson *et al*. 2007; Hussain *et al*., 2002; El-Nahhal, 2004). OCPs have a wide range of both acute and chronic health effects, including cancer, neurological damage, birth defects and endocrine disruption (Walorczik, 2008).

Recently, there have been some complaints about high levels of chemical residues in cocoa beans in general. With the signing of the Stockholm convention on Persistent Organic Pollutants and the development of global monitoring programs, there is an increased need for laboratories

to determine pesticides (Muir and Sverko, 2006). Standards and regulations for the export markets have increased over the last decade dramatically in number and stringency in response to food safety scares and a rise in concern for health and environmental aspects of food leading to the establishment of maximum residue limits (Pico, 2003). Since Ghana is one of the leading cocoa exporters worldwide, it is therefore necessary to monitor the levels of pesticide residues in cocoa beans to determine whether the cocoa beans produced in Ghana conforms to international standards.

2. METHODOLOGY

2.1 SAMPLING

Dry Cocoa beans from different districts of the Ashanti and Brong Ahafo Regions of Ghana were sampled at the Tema port using the method adopted by Quality Control Company Limited (QCC) in Ghana and were sent to the laboratory for analysis (MS, 2007).

2.2 REAGENTS AND APPARATUS

Reagents and apparatus used were acetonitrile, acetone, ethyl acetate and toluene (pesticide grade (BDH, England)); acetone, dipotassium hydrogen phosphate and potassium dihydrogenphosphate (analyte grade (BDH, England)); Sodium sulfate (pesticide grade (Aldrich-Chemie, Germany)); sodium chloride (Pesticide grade, Riedel-de Haen) and Envi-carb/LC-NH_2 (500 mg/500 mg/6 mL) from Supelco C-18 (USA).

2.3 EXPERIMENTAL CONDITIONS

All glass wares were acid washed, cleansed with distilled water and dried in the oven at 200 ^{o}C for about four hours.

2.4 ANALYSIS FOR PESTICIDES

Sample preparation, extraction, clean-up and analysis were carried out according to the procedure described in multi-residue method for agricultural chemicals (Syoku-An, 2006). Pesticide residues in cocoa beans were determined using Gas Chromatography with Electron Capture Detection. Validation of the method was achieved using spiking process at fortification levels of 2.0, 1.5 and 1.0 µg/ml. The percentage recovery ranged between 70 % to 120 %. Concentrations of the pesticide residues in each cocoa bean samples were calculated (in mg/kg).

2.4.1 PREPARATION OF ORGANOCHLORINE STANDARDS

All the pesticides standard stock solutions were prepared in ethyl acetate with the aid of an ultrasonic bath, by dissolving a weight of the pesticides which when corrected for purity will be equivalent to 1000 µg/ml. 1 ml of each of the prepared stock solutions were pipette into a 50 ml volumetric flask resulting in a mixed standard (MIX 1) of concentration 20 µg ml^{-1}. The MIX 1 was diluted to produce new concentrations of 2.0 µg ml^{-1} (MIX 2) and 0.2 µg ml^{-1}(MIX 3)

2.4.2 QUALITY CONTROL AND QUALITY ASSURANCE

Quality of pesticides was assured through the analysis of blanks and replicate samples. All reagents used during the analysis were exposed to same extraction procedures and subsequently run to check for interfering substances. In the blank for each extraction procedure, no pesticide was detected. The method was optimized and validated by fortifying the ground and homogenized cocoa beans sample with MIX 1 before analysis to evaluate the recovery of compounds. This is equivalent to a fortification level of 1 mg kg^{-1}. Extraction and clean up procedure as in the methodology were carried out before its injection into the GC. The same chromatographic conditions were used. This was repeated for fortification levels of 0.1 mg kg^{-1}

and 0.01 mg kg^{-1}. The recoveries of internal standards ranged between 70% and 120% for most of the pesticides analyzed. The percentage recovery was calculated as:

$$\% \text{ Recovery} = \frac{\text{Amount of analyte recovered}}{\text{Amount of analyte spiked}} \times 100$$

3. RESULTS AND DISCUSSION

A total of eight pesticide residues were detected in cocoa bean samples, corresponding to percentage positive of 100 %. Pesticide residues identified in the cocoa beans were HCH isomers, Dieldrin, methoxychlor, heptachlor and its metabolite. The individual retention times (Table 1) used in identifying the detected pesticides in the cocoa beans are presented on the chromatogram (Figure 1).

Table 1: Retention times of Organochlorine Pesticide standards

Standards	Retention Time (Min)
Alpha HCH	13.953
Beta HCH	13.736
Gamma HCH	13.869
Delta HCH	14.596
Methoxychlor	16.177
Heptachlor exo epoxide	18.394
Dieldrin	20.791
Heptachlor	25.292

Brong Ahafo region

Alpha and Delta HCH residues were detected in all the cocoa beans sampled. However, beta and gamma isomers were present in 80 to 90 % of the samples analysed. All samples recorded levels below the EU permissible levels for gamma HCH (Table 2). In all, 90% of the cocoa beans analysed had concentrations of lindane above Japanese MRL of 0.10 mg/kg.

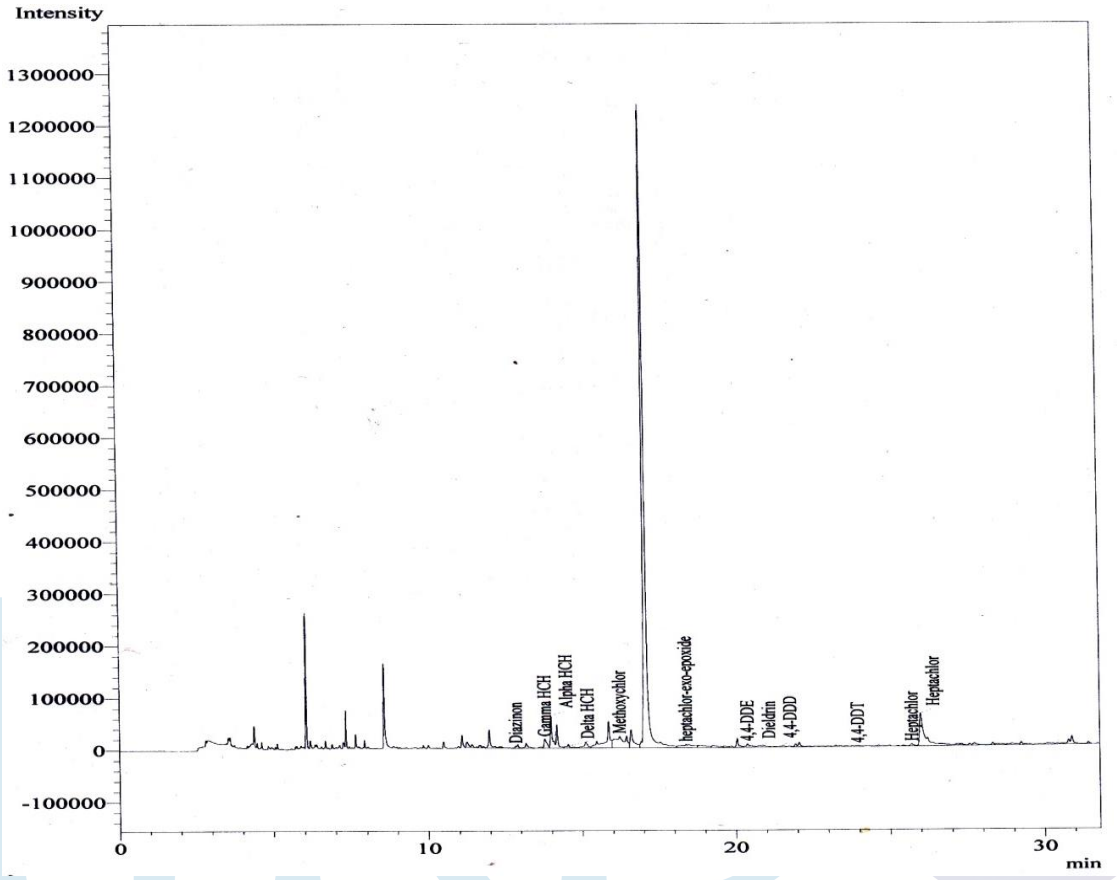

Fig.1 Chromatogram for Organochlorine pesticide standard Mix

Table 2 Concentration of OCPs in cocoa beans from Brong Ahafo regions

Pesticide	Mean Concentration (mg/kg)	EU MRL (mg/kg)	Japan MRL (mg/kg)
Alpha HCH	0.543±0.122	0.02	
Beta HCH	0.061±0.780	0.02	
Gamma HCH	0.152±0.083	1.00	0.10
Delta HCH	0.024±0.013	0.02	0.10
Dieldrin	0.050±0.000	0.02	
Heptachlor	2.300±1.208	0.02	
Heptachlor exo epoxide	0.020±0.000	0.02	
Methoxychlor	0.079±0.072	0.10	

Lynes (1978) analysed lindane residues in cocoa and found levels ranging from below detection limits to 300 µg/kg, which is higher than results obtained from this study. Levels of the alpha HCH in cocoa beans had concentrations above the MRL established by EU, Japan and Codex Alimentarius. From literature, it is not common to find high levels of alpha HCH residues in food samples especially in the tropics because the higher temperature conditions favour its photochemical degradation as compared with the temperate region (Stockholm Convention, 2007). Due to its persistence alpha-HCH can still be detected at low levels in the environment.

Ashanti region

In all, 60% of cocoa beans had concentration of delta HCH residues within Japanese allowable limits (Table 3). However, all the delta HCH residue concentrations were below the EU permissible limits of 0.02 mg/kg. This study revealed that the concentration of HCH isomers in the cocoa bean samples were higher as compared to the mean concentrations reported by Frimpong et al., (2012) and Okoffo et al., (2016). Alpha HCH had concentrations in cocoa beans higher than EU, Japanese and Codex Alimentarius (Table 3).

Table 3 Concentration of OCPs in cocoa beans from Ashanti region

Pesticide	Mean Concentration (mg/kg)	EU MRL (mg/kg)	Japan MRL (mg/kg)
Alpha HCH	0.378±0.143	0.02	
Beta HCH	0.098±0.122	0.02	
Gamma HCH	0.103±0.095	1.00	0.10
Delta HCH	0.029±0.022	0.02	0.10
Dieldrin	0.050 ±0.000	0.02	
Heptachlor	0.696±0.870	0.02	
Heptachlor exo epoxide	0.020±0.000	0.02	
Methoxychlor	0.126±0.110	0.10	

Both Brong Ahafo and Ashanti regions

Delta HCH recorded the lowest level of HCH residue while the highest concentration was recorded for alpha HCH.

Heptachlor was present in all the cocoa beans whilst 60% of heptachlor exoepoxide was detected in the number of samples from the Brong Ahafo region. Concentrations of heptachlor were higher than its breakdown oxygenated product, the epoxide. This could be due to the incomplete breakdown of heptachlor which can be attributed to unfavourable degradation conditions and persistence in the environment though it is among the list of banned pesticides by Stockholm Convention for use on food (Afful *et al*, 2010). Heptachlor epoxide was below detection limit for 50% of the cocoa beans sampled from the Brong Ahafo.

However, 90 % of cocoa beans sampled from the Ashanti region for heptachlor and its metabolite were detected in cocoa. Out of the number of samples, 60% were above the EU MRL of 0.02 mg/kg. Frimpong et al., (2012), found that heptachlor was not detected in all 20 cocoa beans samples analyzed from the Takoradi warehouse. However, mean residue concentrations of 5.0 ± 0.1 µg/kg heptachlor was recorded for Tema warehouse which were lower than results obtained in this survey.

It was observed that the concentration of heptachlor was higher than its metabolite. Generally, concentrations of heptachlor and its metabolite from the Brong Ahafo region were higher than those of the Ashanti region and there was no significant difference between heptachlor concentrations in the two regions. However, there was significant difference in concentration of heptachlor exoepoxide between Ashanti and Brong Ahafo regions.

Concentrations of dieldrin in samples were within the EU allowable level in cocoa beans. All the samples had levels below the Japanese MRL value of 0.10 mg/kg. However, the levels of

dieldrin in cocoa samples from the Brong Ahafo region were lower than the Ashanti region. There was a significant difference between cocoa beans sampled between the two regions.

Methoxychlor was present in all cocoa beans sampled from Brong Ahafo and Ashanti regions with mean concentrations of 0.079 ± 0.072 mg/kg and 0.126 ± 0.110 mg/kg respectively. There was a significant difference between the residue concentrations between the two regions. The presence of methoxychlor suggests that methoxychlor had either been used on cocoa in the past or historical use of DDT or currently illegally used, since they are banned for agricultural purposes in Ghana (WHO 1996; Bempah and Donkor 2011; Agbeve et al., 2014). The mean value of methoxychlor recorded in the study was higher than the mean value of 0.002 mg/kg reported by Frimpong et al., (2012) and 0.03 ± 0.01 mg/kg for Okoffo et al., (2016) in cocoa beans from Ghana.

4. CONCLUSION

From this study, 55.5 % of cocoa beans sampled from Brong Ahafo region had residues above EU MRL whereas 66.7 % were above EU permissible levels for the Ashanti region. Heptachlor had the maximum concentration of residues in cocoa beans whilst the minimum concentration was found to be heptachlor exo epoxide for the two regions. The results obtained reflect the continual usage and enormous past use of banned pesticides on cocoa beans in Ghana.

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