



## Organochlorine pesticides levels in fermented dried cocoa beans produced in Ghana

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### ABSTRACT

Organochlorine pesticides have been used on cocoa. These pesticides have been banned in Ghana, however due to their persistent nature and slow degradation rate, organochlorine pesticides can remain in the environment long after application and in organism long after exposure. The main objective of this study was to determine the levels of organochlorine pesticide residues in fermented dried cocoa beans produced in Ghana, using gas chromatography mass spectrometry. Fermented dried cocoa beans were sampled at random in the two main cocoa storage warehouses located in Tema and Takoradi. The extraction method used consists of addition of distilled water to the pulverized cocoa beans samples, and allowed to stand for 15 minutes followed by maceration with acetonitrile. Extract clean-up then follows by solvent partitioning with aqueous phosphate buffer solution followed by two solid phase extractions; bond Elut C18 and Envi-carp superclean cartridges clean-up respectively. The investigated pesticides were beta-HCH, lindane, delta-HCH, aldrin, dieldrin, endrin, heptachlor, gamma-chlordane, alpha-endosulfan, beta-endosulfan, endosulfan sulfate, p,p'-DDT, p,p'-DDD, p,p'-DDE and methoxychlor. The percentage recoveries ranged from 70-110 percent, with instrumental detection limits of 0.15µg/kg. The widest range of organochlorine pesticides detected was from endosulfan (1.0 – 103.0 µg/kg), which was previously registered for cotton production in Ghana.

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### Introduction

Organochlorine pesticides are synthetic organic insecticides that contain carbon, chlorine, hydrogen and sometimes oxygen. They may also be named “chlorinated hydrocarbons” (P. O. Yeboah, 2009). Organochlorines break down slowly and can remain in the environment long after application and in organisms long after exposure (Dikshith, 2008).

Examples of organochlorine pesticides include aldrin, chlordane, DDT, dieldrin, endrin, hexachlorobenzene, Endosulfan, methoxychlor, lindane and heptachlor. The contamination of the environment and food by these chlorinated organic pesticides has become an issue of considerable concern in many parts of the world (Benson et al, 2011). Due to this concern, many researchers have and are investigating their occurrence, distribution and concentrations in fruits, vegetables, milk and water (Yu et al., 2000; Soliman, 2001; How-Ran et al., 2006; Bai et al., 2006; Fontcuberta et al., 2008).

In Ghana, pesticides have been used on cocoa for more than 50 years, with notable early research carried out independently in the former West African Cocoa Research Institute (now the Cocoa Research institute of Ghana). (Entwistle et al (1959) gave an excellent description of the early development of mirid control measures in cocoa production. Insecticide application techniques on cocoa remain essentially based on experiments that were carried out in the 1960s when the organochlorine

gamma-HCH (also called BHC and lindane) was the active ingredient of choice. The active ingredient aldrin was also extensively used on cocoa in Ghana, and was marketed under the trade name Aldrex 40 (Kuranchie-Mensah, H., et al, 2011 and Nollet, 2000). DDT was officially used in agriculture as the main insecticide against cocoa capsids and for malaria and filariasis control programmes by the Ghana Cocoa Board and Ministry of Health respectively. It was officially banned in 1985 (Ghana's NIP, Dec 2007). Many of these pesticides, such as lindane have some fumigant and insecticidal actions but have been banned in Ghana.

Contamination of cocoa beans can occur directly by treating the crop with pesticides before harvest, storage and distribution. It can occur indirectly by uptake from the soil of residual pesticides by the subsequent cocoa farming, from the atmosphere or drifting from neighbouring fields, or from a storage space pretreated with pesticides (Belitz *et al.*, 2004).

Survey conducted by the Cocoa, Chocolate and Confectionary Alliance on pesticide residues in cocoa beans and products indicated lindane residues in unroasted cocoa beans, roasted cocoa beans, cocoa mass and cocoa butter from Ghana. The residue levels of the results ranges from not detected to 300 ug/kg, with unroasted cocoa beans having the highest range and roasted beans recording the least residue amount (Lynes, 1978). However, in 2010, an assessment of lindane residue in Cocoa

beans in the Twifo Praso district of Ghana, revealed no pesticide residues in all five selected communities samples analysed (Owusu-Ansah et al, 2010). This might be due to degradation of lindane in the environment following the pesticide's withdrawal from the pesticide register of Ghana (EPA, Ghana, 1990).

In the current study, levels of fifteen organochlorine pesticides were assessed in fermented dried cocoa beans produced in Ghana. There is however still a paucity of data existing for the current levels of organochlorine pesticides in cocoa beans from Ghana. Thus, there is the need for surveillance and monitoring of organochlorine pesticide residues in the Cocoa Industry of Ghana, since these chemical stay in the environment for a long time and breaks down slowly.

## Material and Methods

### Sampling

Fermented dried cocoa beans ready for export were sampled at random from the two main cocoa storage warehouses located in Tema and Takoradi respectively. At Tema, a total of twenty-four (24) bagged samples, each weighing about a kilogram of fermented dried cocoa beans were collected in November, December 2010 and January 2011. These were identified from the six cocoa growing regions of Ghana. The same sample identification was carried out at Takoradi storage house, with a total of twenty (20) samples collected in November and December 2010, and were bagged in labeled zip lock plastic bags.

A total of forty-four (44) fermented dried cocoa beans samples, ready for export were sampled, labeled accordingly and were transported to the laboratory.

### Chemicals and Reagents

*Acetonitrile, Acetone, Ethyl Acetate and Toluene* were pesticide grade and obtained from BDH, England. *Acetone, dipotassium hydrogen phosphate and Potassium dihydrogen phosphate* were analytical grade and obtained from BDH, England. *Sodium sulfate* was pesticide grade obtained from Aldrich- Chemie, Germany), *Sodium chloride* (Pesticide grade, Riedel-de Haen), *Envi-carb/LC-NH<sub>2</sub>* (500mg/500mg/6mL) from Supelco and *Strata C18-E* (55um, 70A, 1000mg/6mL) from Phenomenex.

*Individual certified reference standards; lindane, beta-HCH, delta-HCH, aldrin, heptachlor, gamma-chlordane, alpha-endosulfan, p,p'-DDE, dieldrin, endrin, beta-endosulfan, p,p'-DDT, p,p'-DDD, endosulfan sulfate and methoxychlor* used for the identification and quantification were obtained from Dr. Ehrenstorfer GmbH (Augsburg, Germany).

### Sample Processing and Preparation

Foreign objects found in each sample were removed by hand picking. Using the hammer mill, each labeled fermented dried cocoa beans sampled was ground into fine powder and collected into new sample plastic bags and re-labeled accordingly to form each individual analytical samples, each 500 grams weight. After each sample ground, the mill was thoroughly cleaned with a brush. And to avoid cross-contamination, few grams of the next sample to be prepared were ground and discarded before the analytical sample was collected into a new labeled sample plastic bag.

### Sample Extraction

Extraction of fermented dried cocoa beans samples were carried out according to procedures described by a slightly modified multi-residue method for Agricultural chemicals by GC/MS (Department of Food Safety, Ministry of Health, Labour and Welfare, Japan (2006). Prepared fermented dried cocoa

beans were weighed in triplicate sub samples into batches of twenty samples including reagent blanks and fortified samples. Within each batch of twenty samples, 10 g analytical portions were weighed into 250 ml nalgene jars and labeled accordingly. And 20 ml of distilled water was added to each jar in the batch, stirred to form a homogeneous mixture and allowed to stand for 15 minutes. A 50 ml acetonitrile was added and homogenized using the ultra Turrax for 2 minute. Four samples were centrifuged at a time, with a speed of 3000 rpm for 3 minutes and decanted through filter paper into labeled 100 ml volumetric flasks. To the residue, 20 ml acetonitrile was added and further homogenized for 2 minutes, and 5 ml acetonitrile was used to rinse the dispersing element into the jar. The four were centrifuged at 3000 rpm for 3 minutes and filtered again into each corresponding labeled 100 ml volumetric flask. A further 15ml acetonitrile was used to rinse the jar and residue, filtered and all filtrates adjusted to the 100 ml mark with acetonitrile. From each filtrate, 20 ml was pipetted into labeled 250 ml separating funnel, and 10g of NaCl and 20 ml of 0.5mol/L phosphate buffer (pH 7.0) were added to each. The separating funnels were corked and shaken for 10 minutes using the horizontal shaker and allowed to stand for another 10 minutes. The NaCl and lower aqueous layers in each separating funnel were carefully removed and the organic layers transferred into labeled 50 ml beakers for further clean-up.

### Sample Extracts Clean-up

#### First Clean up using Bond Elute C-18 Cartridge

Bond elutes C-18 (1000mg/6ml) cartridges were conditioned using 10 ml each of acetonitrile. Labeled 30 ml flasks were placed under the columns to collect elutes. Sample extracts from the extraction stage were loaded onto each corresponding columns, and 2 ml acetonitrile was used to elute each column.

Anhydrous Na<sub>2</sub>SO<sub>4</sub>, 5 g were placed on filter paper in funnels and the extracts dried over them. The receiving flask was rinsed with acetonitrile and passed over the Na<sub>2</sub>SO<sub>4</sub>. Each filtrate was collected into labeled 50 ml round bottom flask and was concentrated below 40°C to dryness using the rotary evaporator. The residue was re-dissolved in 2 ml of a mixture of toluene/acetonitrile in a ratio of 1:3 prior to the second clean-up step.

#### Second Clean up Using Envi-Carb/LC-NH<sub>2</sub> Cartridge

ENVI-Carb/LC-NH<sub>2</sub> (500mg/500mg/6ml) cartridges were conditioned using 10 ml of 1:3 toluene:acetonitrile mixture. Labeled 50 ml pear shape flasks were placed under the columns, and the extracts from the previous clean-up step loaded onto the corresponding cartridges. The extracts were allowed to filter and the cartridges eluted each with 20 ml of the toluene/ acetonitrile mixture in four portions with intermittent vacuum use. All filtrates were concentrated below 40°C to approximately 1 ml on the rotary evaporator, and 10 ml of acetone added to each flask and further concentrated just to dryness. The extracts were re-dissolved in 1ml ethyl acetate and transferred into labeled 15ml screw capped tube, closed and placed in freezer for about 20 minutes. They were removed and immediately centrifuged at 3000rpm for 5 minutes, and the top layer carefully transferred into labeled 2ml GC standard opening vial prior to running on GC-MS and quantification by GC-ECD. Extracts were kept frozen until quantification was achieved.

### Instrumental Analysis

A Varian CP-3800 Gas Chromatograph (Varian Associates Inc. USA) equipped with 1177 type injector, Saturn 2200 Mass

Spectrometer (MS) as detector and 8400 Varian autosampler was used for GC analysis. Sample extract of 2  $\mu\text{L}$  aliquots was injected and the separation was performed on a fused silica gel capillary column (VF- 5ms, 30 m + 10 m column guard x 0.25 mm id., 0.25  $\mu\text{m}$  film thickness). The carrier gas was ultra pure helium at flow rate of 1.2  $\text{mLmin}^{-1}$ . The temperature of the injector operating in splitless mode was 270°C and the MS detector with an Ion trap analyzer was set to scan mass range of 40  $\text{m/z}$  – 450  $\text{m/z}$  at auto EI. The column oven temperature was programmed as follows; 70°C for 1 min, then at 30°C  $\text{min}^{-1}$  up to 240°C and finally at 5°C  $\text{min}^{-1}$  to 300°C held for 2.3 min. The total run time for a sample was 30 min. Additionally, Gas Chromatograph-Varian CP-3800 (Varian Association Inc. USA) equipped with 1177 type injector,  $^{63}\text{Ni}$  electron capture detector (ECD) and a combiPAL autosampler was used to quantify the detected organochlorine pesticides. Sample extract of 1  $\mu\text{L}$  aliquots was injected and the separation was performed on a fused silica gel capillary column (VF- 5ms, 30 m + 10 m column guard x 0.25 mm id., 0.25  $\mu\text{m}$  film thickness). The carrier gas was nitrogen at a flow rate of 1.0  $\text{ml/min}$ . The detector make-up gas was also nitrogen at a flow rate of 29  $\text{mlmin}^{-1}$ . The injector and detector temperatures were 270°C and 300°C respectively. The column oven temperature was programmed as follows; 70°C for 2 min, then at 25°C  $\text{min}^{-1}$  up to 180°C held for 1 min and finally at 5°C  $\text{min}^{-1}$  to 300°C. The total run time for a sample was 31.4 min. The residue levels of organochlorine pesticides were quantitatively determined by the external standard method using peak area. Measurement was carried out within the linear range of the detector. The peak areas whose retention times coincided with the reference standards were extrapolated on their corresponding calibration curves to obtain their concentration.

#### Quality Control

All reagents used during the analysis were exposed to same extraction procedures and solvents used were run to verify for any interfering substances within the runtime. In all batches of organochlorine pesticide residues analysis, reagent blanks, procedural matrix blanks and triplicate samples were included. For the reagent blanks in each extraction procedure, no organochlorine pesticides were detected. All extracts were kept frozen until quantification was achieved.

Recalibration curves were run with each batch of samples to check that the correlation coefficient was kept around  $r^2=0.99$

The method used was an international method, optimized and validated using various agricultural products (Chung et al, 2010). A fortification level of 0.05  $\text{mg/kg}$  of standard mixtures was chosen before analysis to evaluate the recovery of compounds in the cocoa beans samples analysed. Fortified samples were determined with good recoveries. The recoveries of organochlorine pesticide residues ranged between 70% and 110% for most of the organochlorine pesticides analyzed.

Recovery (%) =  $\frac{\text{Concentration of Pesticide recovered from fortified sample}}{\text{Concentration of organochlorine pesticide added to sample}} \times 100$

Concentration of organochlorine pesticide added to sample

#### Limit of Detection

The limit of detection of the organochlorine pesticides determined was based on the extract of the fortified samples that were serially diluted by factor of two to give different concentrations. One out of each concentration that gave a response three times the standard deviation of the least fortified sample was noted. And this was used to estimate the statistical significance of differences between low level analyte responses

and the combined uncertainties in both the analyte and the background measurement (G. Wells et al, 2011).

#### Data Analysis

The mean of samples, maximum values, corresponding standard deviation and statistical significant test were determined using XLSTAT 2011 software and SPSS version 16 software for windows. All other calculations were performed using Microsoft excel. Statistical analyses incorporated in the work include mean of samples, minimum and maximum values, corresponding standard deviation and statistical significant test. All test were regarded as statistically significant when  $p < 0.05$ . Ranges were compiled from minimum and maximum values for levels detected in each individual organochlorine pesticide residues detected in the study.

#### Results and Discussion

The study involves the analysis of organochlorine pesticides in fermented dried cocoa beans produced in Ghana. Organochlorine pesticides selected for this study are Gamma-HCH (Lindane), Beta-HCH, Delta-HCH, Heptachlor, Aldrin, Gamma-chlordane, Alpha-endosulfan, p,p'-DDE, Dieldrin, Endrin, Beta-endosulfan, p,p'-DDT, p,p'-DDD, Endosulfan sulfate and Methoxychlor. Concentrations of the various organochlorine pesticide residues in each sample were calculated in  $\mu\text{g/kg}$  sample. Raw data calculations of percentage organochlorine pesticide residues detected are shown in Table 1 of this work.

#### Variation of Organochlorine Pesticides in Fermented Dried Cocoa Beans

The results for the types and levels of organochlorine pesticides in fermented dried cocoa beans are shown in Table 1. The values in the figure indicate mean, standard deviation and statistical significance of concentration of cocoa beans sampled. Significantly, varying levels of organochlorine pesticides were observed in cocoa beans samples between the two warehouses (Tema and Takoradi).

Lindane was detected in 4 out of 24 (17 %) of the cocoa beans samples analysed from Tema warehouse. The mean residue concentration of Lindane in cocoa beans from Tema warehouse was  $9.3 \pm 4.2 \mu\text{g/kg}$ , and fell within the range of 6.0  $\mu\text{g/kg}$  and 15  $\mu\text{g/kg}$  (Table 1). This range is quite smaller than that recorded by Lynes (1978) from not detected to 300  $\mu\text{g/kg}$ . Meanwhile, Lindane was not detected in all 20 cocoa beans samples analysed from Takoradi warehouse. This gives a good indication that the application and residue levels of lindane on cocoa had reduced. Similar result was reported by Owusu-Ansah et al., (2010) who recorded no lindane residues in cocoa beans in all five communities selected in Twifo Praso district.

Even though gamma-HCH, lindane is the only isomer of HCH that had pesticidal activity, beta-HCH and delta-HCH were also detected in cocoa beans from both warehouses. These suggest the use of technical HCH in cocoa production, either now or in previous times. Beta-HCH occurred in 17 out of 24 (71%) of the samples from Tema warehouse with mean residue value of  $16.5 \pm 9.7 \mu\text{g/kg}$ . As indicated in Table 1, from Takoradi warehouse, beta-HCH occurred in 13 out of 20 (65%) of the samples with mean residue concentration of  $21.5 \pm 13.0 \mu\text{g/kg}$  (Table 1). The difference in mean residue concentration between Tema and Takoradi warehouses however, was not statistically significant ( $p=0.236$ ) (Table 1).

Higher mean residue concentration of Delta-HCH was recorded for samples from Takoradi warehouse than from Tema warehouse. The mean residue concentrations were  $12.0 \pm 2.8$

$\mu\text{g}/\text{kg}$  and  $2.4 \pm 0.6 \mu\text{g}/\text{kg}$  respectively, and were significantly different from each other statistically ( $p=0.008$ ).

Heptachlor, Aldrin and Gamma-chlordane were not detected in all 20 cocoa beans samples analysed from the Takoradi warehouse. However, mean residue concentrations of  $5.0 \pm 0.1 \mu\text{g}/\text{kg}$  Heptachlor,  $8.7 \pm 2.0 \mu\text{g}/\text{kg}$  Aldrin and  $20.3 \pm 0.1 \mu\text{g}/\text{kg}$  Gamma-chlordane were recorded for cocoa beans samples analysed from Tema warehouse. This suggests different origins of the fermented dried cocoa beans sampled.

Dieldrin mean residue concentration from cocoa beans samples from Tema was  $1.0 \pm 0.1 \mu\text{g}/\text{kg}$ . And this value was realised from 1 out of 24 (4%) samples analysed. From Takoradi, Dieldrin was detected in 3 out of 20 (15%) of the cocoa beans samples analysed, with mean residue concentration of  $22.7 \pm 4.7 \mu\text{g}/\text{kg}$ . This suggests a higher rate of conversion of Aldrin to Dieldrin for Takoradi samples. The difference in mean residue values between samples from Tema and Takoradi warehouses, however was significantly different from each other ( $p=0.015$ ).

Endrin occurred in 15 out of 24 (63%) of the samples from Tema warehouse with mean residue concentration of  $5.4 \pm 4.3 \mu\text{g}/\text{kg}$ . Whereas out of 20 samples analysed from the Takoradi warehouse, 14 (70%) of the cocoa beans had Endrin with mean residue concentration of  $14.5 \pm 5.9 \mu\text{g}/\text{kg}$ . These gave statistically different means with a  $p$ -value of 0.041.

Alpha-endosulfan, an isomer of the parent chemical Endosulfan, was detected in 13 out of 24 (54 %) of the samples from the Tema warehouse, and in 15 out of 20 (75%) of the samples from the Takoradi warehouse. The mean Alpha-endosulfan residue concentration in cocoa beans from Tema warehouse was  $16.7 \pm 6.8 \mu\text{g}/\text{kg}$ . The highest level of Alpha-endosulfan residue detected was  $103.0 \mu\text{g}/\text{kg}$ . The mean Alpha-endosulfan residue concentration in cocoa beans from Takoradi warehouse was  $11.7 \pm 5.8 \mu\text{g}/\text{kg}$ . However, the mean residue concentrations of Alpha-endosulfan from the two warehouses were not significantly different from each other ( $p=0.490$ ). The highest residue level of Beta-endosulfan, the other isomer of endosulfan was  $30.0 \mu\text{g}/\text{kg}$  (Table 1). Beta-endosulfan was detected in 9 out of 24 (38%) of cocoa beans samples analysed from Tema warehouse with mean concentration of  $9.4 \pm 4.8 \mu\text{g}/\text{kg}$ , while 14 out of 20 (70%) of the beans analysed from Takoradi warehouse had Beta-endosulfan residues with mean concentration of  $12.1 \pm 5.8 \mu\text{g}/\text{kg}$ . The difference in mean however was not statistically significant ( $p=0.257$ ). Endosulfan sulfate, metabolite of parent compound Endosulfan (Peterson and Batley, 1993), was not detected in all 20 cocoa beans samples analysed from Takoradi warehouse, however 1 out of the 24 (4%) of the beans samples analysed from Tema warehouse had Endosulfan sulfate with mean residue concentration of  $3.0 \pm 0.1 \mu\text{g}/\text{kg}$ . This indicates the degree of persistence of endosulfan in the environment (Peterson and Batley, 1993) and also suggests current use of the parent compound endosulfan, with a very low degradation rate.

$p,p'$ -DDT, an isomer of the parent pesticide DDT was detected in cocoa beans samples analysed from both warehouses. The mean residue concentration from Takoradi was  $3.5 \pm 2.1 \mu\text{g}/\text{kg}$ , quite higher than that of Tema with mean concentration of  $2.0 \pm 1.0 \mu\text{g}/\text{kg}$ , although the difference was not statistically significant ( $p=0.346$ ).  $p,p'$ -DDE occurred in 8 out of 24 (33%) of the samples from Tema warehouse with mean residue concentration of  $1.1 \pm 0.4 \mu\text{g}/\text{kg}$ . For samples from the Takoradi warehouse,  $p,p'$ -DDE mean residue concentration of

$1.6 \pm 0.8 \mu\text{g}/\text{kg}$  was recorded from 7 out of 20 (35%) cocoa beans samples analysed. The difference in mean was not statistically significant ( $p=0.170$ ).  $p,p'$ -DDD, a metabolite of DDT was detected in 18 out of 24 (75%) cocoa beans samples analysed from Tema warehouse. And was also detected in 14 out of 20 (70%) of the cocoa beans samples analysed from Takoradi warehouse. Its mean residue concentration in cocoa beans from Tema warehouse was  $1.9 \pm 0.7 \mu\text{g}/\text{kg}$  which was not statistically significantly different from the mean residue concentration from Takoradi ( $1.8 \pm 0.7 \mu\text{g}/\text{kg}$ ). It was observed from both warehouses that the levels of DDD were higher than DDE, which suggest a high rate of degradation of the parent compound DDT in the selected samples. The low levels of DDT detected from both warehouses, less than  $4.0 \mu\text{g}/\text{kg}$  (Table 1) also suggest previous usage of the parent chemical DDT only.

The mean residue concentrations of methoxychlor were  $1.9 \pm 1.6 \mu\text{g}/\text{kg}$  and  $1.6 \pm 1.1 \mu\text{g}/\text{kg}$  for samples from Tema and Takoradi warehouses respectively. These mean residue concentrations however were statistically not significant in difference.

The differences in residue concentrations of Tema and Takoradi warehouses could be attributed to several factors. Among these factors include differences in farming and crop storage practices. It may also be ascribed to the different sources from where the cocoa beans samples were cultivated (Brong Ahafo, Ashanti, Western, Eastern, Central and Volta regions of Ghana).

#### Conclusion

Organochlorine residue levels of pesticides in cocoa beans produced in Ghana as determined by Gas chromatography coupled with Electron Capture Detector and Mass Spectrometry shows significantly varying results.

There were appreciable residue amounts of Lindane ( $6.0 - 15.0 \mu\text{g}/\text{kg}$ ), Beta-HCH ( $4.0 - 34.0 \mu\text{g}/\text{kg}$ ) and Delta-HCH ( $1.0 - 14.0 \mu\text{g}/\text{kg}$ ). These confirm the fact that Lindane and technical HCH had been used extensively in cocoa production in Ghana. The widest range of organochlorine pesticides detected was from endosulfan ( $1.0 - 103.0 \mu\text{g}/\text{kg}$ ), which was previously registered for cotton production in Ghana. This notwithstanding; considering the residue levels of most of the organochlorine pesticides detected (aldrin, dieldrin, endrin, heptachlor, chlordane, DDT and its metabolites DDD, and DDE, and methoxychlor); it can be concluded that, these pesticides are not being used in the cocoa industry of Ghana today.

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**Table 1: Summary of analysis of variance results for Organochlorine Pesticide Residue ( $\mu\text{g}/\text{kg}$ ) in Fermented Dried Cocoa Beans from Tema and Takoradi Warehouses.**

Organochlorine Pesticides	TEMA (n=24)			TAKORADI (n=20)			Df	p-value
	Range	Detects (%)	$\bar{x} \pm \text{sd}$	Range	Detects (%)	$\bar{x} \pm \text{sd}$		
Lindane	6.0 - 15.0	4 (17)	$9.3 \pm 4.2$	ND	0 (0)	ND	NA	NA
Beta-HCH	4.0 - 34.0	17 (71)	$16.5 \pm 9.7$	8.0 - 51.0	13 (65)	$21.5 \pm 13.0$	28	0.236
Delta-HCH	1.0 - 7.0	5 (21)	$2.4 \pm 0.6$	10.0 - 14.0	2 (10)	$12.0 \pm 2.8$	5	0.008
Heptachlor	ND - 9.0	1 (4)	$5.0 \pm 0.1$	ND	0 (0)	ND	NA	NA
Aldrin	1.0 - 17.0	3 (13)	$8.7 \pm 2.0$	ND	0 (0)	ND	NA	NA
Dieldrin	ND - 2.0	1 (4)	$1.0 \pm 0.1$	19.0 - 28.0	3 (15)	$22.7 \pm 4.7$	3	0.015
Endrin	1.0 - 14.0	15 (63)	$5.4 \pm 4.3$	2.0 - 59.0	14 (70)	$14.5 \pm 5.9$	27	0.041
Gamma-chlordane	ND - 38.0	1 (4)	$20.3 \pm 0.1$	ND	0 (0)	ND	NA	NA
Alpha-endosulfan	1.0 - 103.0	13 (54)	$16.7 \pm 6.8$	6.0 - 30.0	15 (75)	$11.7 \pm 5.8$	26	0.49
Beta-Endosulfan	3.0 - 20.0	9 (38)	$9.4 \pm 4.8$	10.0 - 30.0	14 (70)	$12.1 \pm 5.8$	21	0.257
Endosulfan sulfate	ND - 7.0	1 (4)	$3.0 \pm 0.1$	ND	0 (0)	ND	NA	NA
p,p'-DDT	1.0 - 3.0	3 (13)	$2.0 \pm 1.0$	2.0 - 5.0	2 (10)	$3.5 \pm 2.1$	3	0.346
p,p'-DDE	1.0 - 2.0	8 (33)	$1.1 \pm 0.4$	1.0 - 3.0	7 (35)	$1.6 \pm 0.8$	13	0.17
p,p'-DDD	1.0 - 8.0	18 (75)	$1.9 \pm 0.7$	1.0 - 3.0	14 (70)	$1.8 \pm 0.7$	30	0.745
Methoxychlor	1.0 - 5.0	7 (29)	$1.9 \pm 1.6$	1.0 - 4.0	7 (35)	$1.6 \pm 1.1$	12	0.704

ND=Not Detected, NA=Not Applicable,  $\bar{x}$ =Mean, sd=Standard Deviation, LOD = 1.0  $\mu\text{g}/\text{kg}$ , Df=Degree of freedom  
P=Probability.