



## Assessment of organochlorine pesticides residues in cocoa beans from Ghana

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### ABSTRACT

Pesticide may be a necessary evil. They protect crops including cocoa from pests attack, and thus maximize productivity in agricultural businesses. However, if pesticide residues are found to be above their maximum residue limits, they pose adverse health effects to humans. Exporting nations also tend to lose foreign exchange, if produce are found to contain high residue levels. The main objective of this study was to monitor and assess residue levels of 15 organochlorine pesticides in cocoa beans from Ghana, against their various maximum residue limits. Cocoa beans were sampled at random in two cocoa storage stations located in Tema and Takoradi, cities of Ghana. The extraction method uses acetonitrile as the extracting solvent. Two solid phase extraction clean-ups were employed; bond elut C18 cartridge, followed by envi-carp/LC-NH<sub>2</sub> cartridge, using acetonitrile and a mixture of toluene/acetonitrile in the ratio 1:3 as eluting solvents, 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 method determination limit of 0.01µg/g. The widest range of organochlorine pesticides detected was from endosulfan (0.01 – 0.10 µg/g); however none of the detected organochlorine pesticides exceed their various maximum residue limits.

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### Introduction

In Ghana, cocoa is cultivated on about 1.5 million hectares of land. These are owned by some 800,000 farm families in six out of the ten regions of Ghana (COCOBOD Ghana, 2010). Since Independence, cocoa has been one of the biggest foreign exchange earner for Ghana, and the share of *cocoa* in Ghana's GDP rose from 4.9% in 2000-2004 to 8.1% in 2005/2006 (Breisinger et al., 2007).

Cocoa from Ghana continues to enjoy high premium on the World's Commodities Markets because of its unsurpassable high quality. That is, being well fermented cocoa beans of dark brown uniform colour and sizes with good cocoa flavour potential and a moisture content between 6 and 8%, and none or very little damaged beans and foreign material (Amoa-Awua et al., 2006). This status, over the years has diligently been maintained, through the effective quality control practices of the Quality Control Division (QCD), now Quality Control Company Limited of COCOBOD (COCOBOD Ghana, 2010).

However, there is limited data and studies on pesticide residues in quality cocoa beans (Botchway, 2000; Owusu-Ansah et al., 2010; Frimpong, S. K et al, 2011).

Pesticide is any substance or mixture of substances intended for preventing, destroying or controlling any pest, including vectors of human or animal disease, unwanted species of plants or animals causing harm during or otherwise interfering with the production, processing, storage, transport or marketing of food, agricultural commodities, wood and wood products (WHO/FAO, 2009). The substances can be classified into

several groups. The main pesticide groups by target pest include; fungicides - for crop diseases such as black pod in cocoa; herbicides - kill weeds; insecticides- control insect pests, but they may also be; acaricides- controlling mites; nematocides- controlling nematodes (eelworms); rodenticides - kill rats and mice (they are often such less effective against squirrels). Other pesticide types that are not used on cocoa include molluscicides and bactericides (ICCO, 2008). Among these groups of pesticides; the insecticides, specifically organochlorines have enjoyed much research publicity worldwide (C.K. Bempah et al., 2011; H. Kuranchie-Mensah et al., 2011; Dikshith, 2008; Yu et al., 2000; Soliman, 2001; How-Ran et al., 2006; Bai et al., 2006; Fontcuberta et al., 2008). Perhaps, this may be due to their peculiar characteristics: low cost, their versatility against various pests, bioaccumulative nature and potential toxic effects to wildlife and humans (C.K. Bempah et al., 2011). Also their tendency for long range transport and trans-boundary dispersion and their capacity to bioaccumulate in the food chain have also been known (Laws, 2000). Organochlorine pesticides such as aldrin/dieldrin, lindane and DDT had reportedly been used on cocoa in Ghana (Owusu-Ansah et al., 2010; H. Kuranchie-Mensah et al., 2011; Ghana's NIP, Dec 2007). Although the organochlorines are banned from importation, sales and use in Ghana, there are evidence of their presence in the ecosystem (EPA Ghana, 2009; C.K. Bempah et al., 2011; H. Kuranchie-Mensah et al., 2011). Pesticides in general, may be necessary evil. They are the sure way of protecting crops including cocoa

from pests attack, and also to maximize productivity in agriculture businesses (Baig, et al., 2009). These same pesticides pose health and economical treats, when found to contain residue levels above the maximum residue limits (Frimpong, S. K et al., 2011). Therefore, in the current study 15 organochlorine pesticides residues were monitored and assessed in cocoa beans from Ghana against two international standards for maximum residue limits (Japan and European Union; major buyers and consumers of Ghana's best cocoa).

## Material and methods

### Study Area

Tema is locally nicknamed the "Harbour City" because of its status as a seaport. The port of Tema handles 80% of the nation's import and export cargo. Most of the country's chief export, cocoa, is shipped from Tema. Figure 3.0 shows the map of Ghana from which Tema, home of one of the two main cocoa warehouses and most of the cocoa processing industries in Ghana are located. It is a city on the Atlantic coast of Ghana, coordinated 5°40'N 0°0'W. 5.667°N 0°E, lying 25 kilometres east of the Ghanaian capital city of Accra, in the region of Greater Accra. The Greenwich Meridian (00 Longitude) passes directly through the city ([www.ghanadistricts.com/Tema](http://www.ghanadistricts.com/Tema)).

It is home to an oil refinery and is an important centre of manufacturing. It is linked to the capital by railway and a highway.

Tema is one of Ghana's two deep seaports; Sekondi Takoradi is the other. The Port of Takoradi is located 2km from centre of the city and Coordinated on 4°53'5"N 1°44'26"W ([www.ghanaports.gov.gh](http://www.ghanaports.gov.gh)). Figure below shows the map of Ghana with Takoradi, a twin city to Sekondi as the capital city of the Western region of Ghana.



### Sample collection

Cocoa beans samples were collected from two main cocoa storage warehouses located in Tema in the Greater Accra region and Takoradi in Western region of Ghana. The sampling was performed randomly in each warehouse. In Tema warehouse, a total of twenty-three (23) bagged samples each weighing about a kilogram of cocoa beans was collected. While in Takoradi warehouse, a total of twenty-two (22) samples were also collected and bagged in labeled zip lock plastic bags.

In all, a total of 45 cocoa beans samples were collected within the sampling period from January 2011 to June 2011, and

were dispatched to the laboratory for sample preparation and subsequent residue analysis.

### Chemicals and Reagents

Certified pesticide 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*), with certified purity of at least 99% were obtained from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Pesticide grade *Acetonitrile, Acetone, Ethyl Acetate and Toluene*, and analytical grade *dipotassium hydrogen phosphate and Potassium dihydrogen phosphate* were supplied by BDH, England. Anhydrous sodium sulphate analytical grade were purchased from Aldrich- Chemie, Germany. *Sodium chloride and anhydrous sodium sulphate* (Pesticide grade, from 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.

### Analysis for organochlorine pesticides

Sample preparation, extraction, cleanup and analysis were carried out according to the procedure described in multi-residue method for agricultural chemicals with slight modifications (Syoku-An (2006) No. 0124001. The method was chosen because of ease of comparison and verification of results.

### Sample extraction and clean-up

The extraction procedure, as stated above, was that of a Japanese analytical method for multi-residues for agricultural chemicals (Syoku-An, 2006). Cocoa beans samples were thoroughly grinded and homogenized. 20ml of distilled water was added to approximately 10.0g of the ground sample, stirred to form a homogeneous mixture and allowed to stand for 15 minutes. 50ml acetonitrile was added and macerated for 2 min using the ultra turrax. It was then centrifuge at a speed of 3000 rpm for 3 minutes and decanted through No. 4 filter paper into labeled 100ml volumetric flask. 20ml acetonitrile was added to the residue and further homogenized for 2 min, and 5ml acetonitrile was used to rinse the dispersing element into the container. It was then centrifuged at 3000 rpm for 3 min and filtered again into each corresponding labeled 100ml volumetric flask. A further 15ml acetonitrile was used to rinse the jar and residue, filtered and all filtrates adjusted to the 100ml mark with acetonitrile. An aliquot of 20ml was pipetted into labeled 250ml separating funnel, and 10g of NaCl and 20ml of 0.5mol/L phosphate buffer (pH 7.0) were added. The separating funnel was corked and shaken for 10 min using the horizontal shaker and allowed to stand for another 10 min. The NaCl and lower aqueous layers in each separating funnel were carefully removed and the organic layer transferred into labeled 50ml beaker for further clean-up. For the clean-up, two solid phase extraction (SPE) cartridge clean-ups were employed; the first by bond elut C18 (1000mg/6ml) and then followed by an envi-carb/LC-NH<sub>2</sub> (500mg/500mg/6ml) cartridge. For the C18 cartridge, after being conditioned with 10ml acetonitrile and the extracts loaded onto it, 2 ml acetonitrile was used as the eluting solvent. The filtrates were dried over 5g anhydrous Na<sub>2</sub>SO<sub>4</sub> and concentrated to just dryness. An envi-carb/LC-NH<sub>2</sub> cartridge was conditioned with 10ml mixture of toluene/acetonitrile in the ratio 1:3. The dried extract was dissolved in 2ml of the mixture and transferred onto the cartridge. It was then eluted with 20ml of the mixture and filtrate concentrated to approximately 1 ml below 40°C on the rotary evaporator, and 10ml acetone added to the flask and further concentrated to just dryness. The extract was re-

dissolved in 1ml ethyl acetate and transferred into labeled 15ml screw capped tube, closed and placed in freezer for at least 30 min. The extract was removed afterwards and immediately centrifuged at 3000 rpm for 5 min, and the top layer carefully transferred into labeled 2ml GC standard opening vial for quantitation by gas chromatography  $^{63}\text{Ni}$  electron capture detector (GC-ECD).

#### Gas chromatographic determination

The final extracts were analyzed by Gas Chromatograph-Varian CP-3800 (Varian Association Inc. USA) equipped with combiPal autosampler and  $^{63}\text{Ni}$  electron capture detector (ECD) that allowed the detection of contaminants even at trace level concentrations (in the lower  $\mu\text{g/g}$  range) from the matrix to which other detectors do not respond. The GC conditions and the detector response were adjusted so as to match the relative retention times and response as spelt out by Japanese analytical methods for agricultural chemicals (Syoku-An, 2006). The GC conditions used for the analysis were capillary column coated with VF-5 (30 m + 10 m guard column x 0.25 mm i.d, 0.25  $\mu\text{m}$  film thickness). The injector and detector temperature were set at 270°C and 300°C respectively. The oven temperature was programmed as follows: 70°C held for 2 min, ramp at 25°Cmin<sup>-1</sup> to 180°C, held for 1 min, and finally ramp at 5°Cmin<sup>-1</sup> to 300°C. Nitrogen was used as carrier gas at a flow rate of 1.0 mLmin<sup>-1</sup> and detector make-up gas of 29mLmin<sup>-1</sup>. The injection volume of the GC was 1.0  $\mu\text{L}$ . The total run time for a sample was 31.4 min. The residues detected by the GC analysis were confirmed by the analysis of the extract on two other columns of different polarities. The columns were VF-1 (methyl polysiloxane) and ZB-17 (50% phenylmethyl polysiloxane). The conditions used for these columns were the same, as stated in analytical methods.

#### Quantification of Organochlorine Pesticide Residues

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 standards were extrapolated on their corresponding calibration curves to obtain the concentration.

#### Quality control and quality assurance

The quality of organochlorine pesticides was assured through the analysis of solvent blanks, matrix blanks and duplicate 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 organochlorine pesticide was detected. Sample of each series was analyzed in duplicates. The method was optimized and validated by fortifying the cocoa beans samples with 500  $\mu\text{L}$  of 1  $\mu\text{g mL}^{-1}$  standards mixture before analysis to evaluate the recovery of compounds. The recoveries of internal standards ranged between 70% and 110% for most of the organochlorine pesticides analyzed.

#### Results and discussion

The study involves the analysis of organochlorine pesticides in cocoa beans from Ghana. Organochlorine pesticides selected for this study are gamma-HCH (lindane), beta-HCH, delta-HCH, heptachlor, aldrin, dieldrin, endrin, gamma-chlordane, alpha-endosulfan, beta-endosulfan, endosulfan sulfate, p,p'-DDT, p,p'-DDD, p,p'-DDE and methoxychlor. However, residue concentrations of alpha-endosulfan, beta-endosulfan and endosulfan sulfate were expressed as total endosulfan, while residue concentrations of p,p'-DDE, p,p'-DDD and p,p'-DDT

were also expressed as total DDT to conform to the standard requirements of residue limits. Concentrations of the various organochlorine pesticide residues in each sample were calculated in  $\mu\text{g/g}$  sample. The results for the types and residue levels of organochlorine pesticides in the cocoa beans samples are shown in Table 1. The values in the table indicate the range, mean of residue concentrations, European Union and Japanese maximum residue limits (MRLs) for cocoa beans all in  $\mu\text{g/g}$ .

In Ghana there is no established maximum pesticide residue limits for cocoa beans. The levels of organochlorine pesticides recorded in the study were therefore compared to standard limits set by the European Union and Japan.

Among the organochlorine pesticides (Table 1), none of the mean residue concentrations were above both the EU MRLs and the Japanese MRLs. The limit of determination was 0.01 $\mu\text{g/g}$ .

#### HCHs

Even though gamma-HCH (lindane) is the only isomer of HCH that possesses insecticidal activity, appreciable amounts of residues were recorded for delta-HCH and beta-HCH; 68% and 16% residue detection of the total cocoa beans analysed, respectively. This suggests the use of technical HCH in the past (Frimpong et al., 2011). Lindane was used extensively in cocoa production in Ghana until it was officially banned in 2002 (S. Adu-Kumi et al., 2010). Out of the 45 cocoa beans samples analysed, 4 (9%) had lindane with residue concentration ranging from not detected to 0.02 $\mu\text{g/g}$ . A mean residue concentration of 0.01 $\mu\text{g/g}$  lindane was realized. This residue concentration level of lindane is by far less than both EU MRL of 1.00 $\mu\text{g/g}$  and the Japanese limit of 0.10 $\mu\text{g/g}$ . Meanwhile the mean residue concentrations of delta-HCH and beta-HCH, the other isomers of the parent compound HCH were both less than the EU MRL of 0.02 $\mu\text{g/g}$ , but were comparable to the Japanese MRL of 0.01 $\mu\text{g/g}$  (Table 1).

#### Heptachlor and Methoxychlor

Heptachlor and methoxychlor are ban pesticides in Ghana (EPA Ghana, 2009). Heptachlor and methoxychlor were not detected in all 45 cocoa beans samples analysed. This proves that heptachlor and methoxychlor had not been used on cocoa production in Ghana, or better still their presence in the environment had diminished.

#### Aldrin and Dieldrin

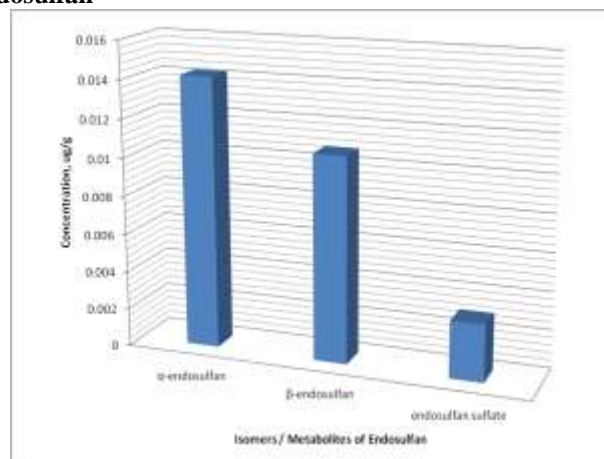
Aldrin was detected in 3 out of 45 (7%) of the cocoa beans samples analysed, with residue concentration range of not detected to 0.02 $\mu\text{g/g}$ . However, dieldrin recorded a slightly wider residue concentration range than that of aldrin; from not detected to 0.04 $\mu\text{g/g}$ . This implies a much higher rate of conversion of aldrin to dieldrin in the environment. The mean residue concentrations of aldrin and dieldrin in the cocoa beans samples analysed were 0.01 $\mu\text{g/g}$  and 0.02 $\mu\text{g/g}$ , respectively. However, both aldrin and dieldrin mean residue concentrations are below the EU MRLs of 0.05 $\mu\text{g/g}$  and the Japanese MRLs of 0.1 $\mu\text{g/g}$ .

#### Gamma-chlordane and Endrin

Only one cocoa beans sample analysed had gamma-chlordane. It ranges from not detected to 0.02 $\mu\text{g/g}$ , with a mean residue concentration of 0.01 $\mu\text{g/g}$ . On the other hand, endrin was detected in 29 out of 45 (66%) cocoa beans samples analysed, with 0.01 $\mu\text{g/g}$  as the highest residue concentration of endrin detect. The residue concentration of aldrin was 0.01 $\mu\text{g/g}$ , and it is below the EU MRL of 0.02 $\mu\text{g/g}$  but comparable to the Japanese MRL of 0.01 $\mu\text{g/g}$ . However, endrin's mean residue

concentration of 0.01 $\mu\text{g/g}$  is comparable to both the EU MRL and that of the Japanese MRL.

### Endosulfan

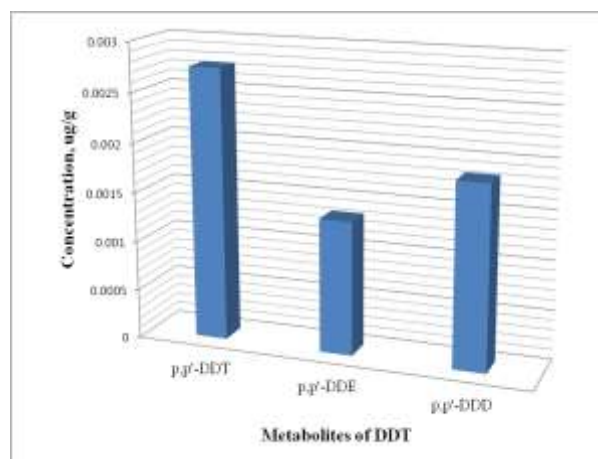


**Fig. 1: Chart of concentrations of isomers/metabolites of endosulfan**

Alpha and beta-endosulfans are isomers of the parent compound endosulfan, while endosulfan sulfate is their metabolite (ATSDR, 1993; Tomlin, 2002 and Wan *et al.*, 2005). Endosulfan was registered in Ghana for cotton and coffee production. It was also used to control ectoparasites on farm animals and pest in Ghana (Ntow, 2001; Darko and Acquah, 2008; Ntow *et al.*, 2009). 28 out of the 45 (64%) samples of cocoa beans analysed had alpha-endosulfan, while beta-endosulfan was detected in 23 out of 45 (52%) of the cocoa beans samples analysed. Meanwhile, only one cocoa bean sample had the metabolite, endosulfan sulfate. This shows the persistent nature of the parent compound endosulfan in the environment (Peterson and Batley, 1993). Generally, low residue levels of alpha-endosulfan, beta-endosulfan and endosulfan sulfate were recorded in the cocoa beans samples analysed (Fig. 1). Cumulatively, Endosulfan average residue level was 0.03 $\mu\text{g/g}$ . This residue level is below both the Japanese MRL of 0.10 $\mu\text{g/g}$  and the EU limit of 0.10 $\mu\text{g/g}$ . Endosulfan is one of the pesticides screened at the Ghana Standards Authority for shipment to Japan. With this residue level of endosulfan trade sanctions as far as cocoa beans to Japan are concerned may not exist.

### DDT and its Metabolites

In Ghana, DDT was used against cotton pests and for controlling mosquitoes (Ntow, 2001). Unfortunately the unrestrained use of DDT, especially in agriculture, led to a rapid increase in its concentration in the environment, and soon after its introduction the scientific community expressed concern about this insecticide (Doyle *et al.*, 1994). DDD, a metabolite is released to the environment as a biodegradation product of DDT (Maldonado *et al.*, 2005), whilst most of the DDT in the human body fat is present in its metabolized form DDE, which is extremely soluble in organics and persists in organism for long time (Nollet, 2000). From table 1, 15 out of the 45 (34%) of the cocoa beans samples analysed had p,p'-DDE, 32 (73%) had p,p'-DDD and 4 (9%) had p,p'-DDT. Figure 2 shows the relative residue concentrations levels of the metabolites of DDT detected in the cocoa beans samples analysed.



**Fig. 2: Chart of concentrations of residue levels of metabolites of DDT**

As indicated in Table 1 and Fig. 2, the amount of DDT and its metabolites DDE and DDD recorded could possibly imply previous use of the parent compound DDT. The average residue concentration of p,p'-DDD, p,p'-DDE and p,p'-DDT expressed as DDT was 0.01 $\mu\text{g/g}$ . This residue level is by far lower than both EU and Japanese maximum residue limits of 0.50 $\mu\text{g/g}$  and 0.05 $\mu\text{g/g}$ , respectively.

### Conclusion

The study revealed the presence of some organochlorine pesticides at varying concentrations, with endosulfan recording the widest range of residue concentration.

Generally, there were low levels of organochlorine pesticides in cocoa beans analysed; however, these were not surprising since these pesticides are banned in Ghana. This also shows that in Ghana, many chemicals are highly regulated because they are known to negatively affect human and environmental health.

Finally, in all 45 sampled cocoa beans, none of the detected organochlorine pesticides residues concentrations did exceed the European Union or Japanese Maximum Residue Limits in cocoa beans. Thus, based on the results of the current concentrations of organochlorine pesticides residues in cocoa beans from Ghana, it is safe to state, that organochlorines do not pose a serious threat to the cocoa industry in Ghana and, that organochlorine pesticides contamination should not be a limiting factor for cocoa beans uses.

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**Table 1: Summary of results of organochlorine pesticides residues in cocoa beans against their European Union and Japanese maximum residue limits (MRLs)**

| PESTICIDES   | DETECTS (%)                 | RANGE     | MEAN, * | EU(MRLs) | JAPAN(MRLs) |
|--|-----------------------------|-----------|---------|----------|-------------|
| Organochlorines:   | N = 45                      |           |         | µg/g     |             |
| Lindane  | 4 (9)                       | ND - 0.02 | 0.01    | 1.00     | 0.10        |
| Beta-HCH   | 30 (68)                     | ND - 0.01 | 0.01    | 0.02     | 0.01        |
| Delta-HCH  | 7 (16)                      | ND - 0.01 | 0.01    | 0.02     | 0.01        |
| Heptachlor   | 0 (0)                       | ND        | ND      | 0.02     | 0.01        |
| Aldrin   | 3 (7)                       | ND - 0.02 | 0.01    | 0.05     | 0.10        |
| Dieldrin   | 4 (9)                       | ND - 0.04 | 0.02    | 0.05     | 0.10        |
| Gamma-chlordane  | 1 (2)                       | ND - 0.02 | 0.01    | 0.02     | 0.01        |
| Alpha, Beta and Endosulfan sulfate expressed as Endosulfan | 28 (64)<br>23 (52) 1<br>(2) | ND - 0.10 | 0.03    | 0.10     | 0.10        |
| p,p'-DDE, p,p'-DDD and p,p'-DDT expressed as DDT           | 15 (34)<br>32 (73)<br>4 (9) | ND - 0.01 | 0.01    | 0.50     | 0.05        |
| Endrin   | 29 (66)                     | ND - 0.01 | 0.01    | 0.01     | 0.01        |
| Methoxychlor   | 0 (0)                       | ND        | ND      | 0.10     | 0.01        |

LOD = 0.01µg/g, ND = Not detected and N = Number of Samples