Level of Organochlorine Pesticide Residues in selected Soft drinks commonly sold in Benin City Markets

Abstract; This research studied the levels and risk assessment of pesticide residues in selected soft drinks such as sprite and coca-cola commonly sold in Benin City markets. A total of twenty-five organochlorine pesticide (OCP) residues in these samples were analyzed using with gas chromatography- electron captured detector (ECD). The estimated chronic daily intake (ECDI) of aldrin in soft drinks (sprite) was determined based on European Union (EU) and United States Agency of Toxic Substances and Disease Registry (ATSDR) standards, using two population age groups (child and adult). The results revealed the levels of organochlorine pesticide residues among the soft drinks. Aldrin, had a value of 0.0001mg/kg in soft drink (sprite), while coca-cola were below detection limit(0.00±0.00). The study, thus suggest that OCPs concentration in sprite is unsafe for human . The study therefore calls for continuous monitoring of agricultural farmlands because continuous exposure to pesticide contaminated soft drinks sold in Benin city markets (sprite) could affect the health of consumers.

Keywords; organochlorine pesticides, sprite, coca-cola., chromatogram

INTRODUCTION

Pesticides are chemical substances generally used in agriculture to increase the yield, improve the quality, and extend the storage life of agricultural produce (Fernndez-Alba and Garca-Reyes, 2008).They help society with immense benefits, such as increasing agricultural output and in the control of diseases.

There has been an alarming rate about ingestion of pesticide residues by adults and children who consume soft drinks. Consumption of soft drinks has increased over time because of its demand. Xenobiotics are a major cause for concern all over the world, given their persistence to their metabolite residues after degradation by artificial or natural means and negative effects on humans (FDA, 1999).

In 2001, about 74 percent of soft drinks sold in the United States of America (USA) were reported to contain atleast one pesticide residue greater than the acceptable level Gilden *et al*., (2010). This increase in level of pesticide residues has risen in developing countries and the fastest growing markets in Africa, Asia, South and Central America, Eastern Mediterranean Gilden *et al*., (2010).

The toxic effects of these pesticides has caused them to be banned in developed and many growing nations. Moreover, some growing countries still use them Ize-Iyamu *et al*., (2007).

In Nigeria, there have been reports of some levels of pesticide residues in soft drinks Ezemonye *et al.,* (2008) . The results have often revealed contamination by organochlorine pesticides in soft drinks sold in Benin City markets Ize-Iyamu *et al*., (2007). The organochlorine pesticides (OCPs) include aldrin, endrin, dieldrin, chlordane, heptachlor, DDT, toxaphene, endosulfan 1, and hexachlorobenzene (HCB) Papadakis *et al*., (2015). These dreaded organochlorine pesticides get into the raw materials and water for processing soft drinks via ; (lack of awareness, inappropriate use of pesticides, waste from industrial chemical production, pesticides runoff from agricultural areas, sewage and refuse dump) . Because of their potency, efficiency and low cost compared with alternative pesticides, organochlorine pesticides (OCPs) are still being used by some farmers (Daam and Van der Brink, 2010). The use of OCPs such as DDT has been outlawed since 1990 in Nigeria.

These compounds are characterized by high persistent, low polarity, low aqueous solubility and high lipid solubility (lipophilicity). They are ecotoxic, non-biodegrable and able to bioaccumulate and biomagnify in humans via consumption of contaminated soft drinks which may pose health risk to human Ezemonye *et al.,* (2008) . To estimate the risk of detected pesticides to humans (young children and adults) through drinking/ingestion of contaminated sprite, the hazard quotient (HQ) method was employed (Papadakis *et al*., 2015). Hazard quotient (HQ) = ECDI/RfD

Where ECDI is the estimated chronic daily intake

This was obtained by using the Formula ECDI (ingestion) =Cs IR(sprite) x EF xED/BW xAT where Cs is the concentration of sprite samples, IR (sprite) is the ingestion rate of sprite, EF is the exposure frequency, ED is the exposure duration, BW is the body weight, and AT is the average life Span Ezemonye *et al*., (2008)..

The aim of this study were to assess the level of organochlorine pesticide residues in soft drinks (sprite and coca-cola) sold in markets in Benin City.

MATERIALS AND METHOD

Study area

Benin City have alot of super markets located in different localities in the city where soft drinks (sprite and coca-cola) are sold for final consumption. The markets include Ekiosa, New Benin, Oba market, Ikpoba hill market and Uselu market. The soft drinks (sprite and coca-cola) are usually transported from within the state or other neigboring western, eastern, southern or northern part of the country.

Sample Collection

Random sampling of soft drinks ; sprite and coca-cola were purchased from the various markets in Benin city. However, samples were provided to the analytical laboratory for GC analysis Anastassiades *et al*., (2003)

Sample extraction

- Prepare a solvent 50:50 mix of Acetone and n-Hexane or DCM and Acetone
- Measure about 30gram aliquot of well mixed sample into a solvent rinsed beaker.
- Add 1mL of Decachlorobiphenyl, mix thoroughly using glass stir rod
- Weigh an equal amount of Sodium Sulfate and add to the sample and mix thoroughly to form a free flowing powder.
- Add 50ml of the solvent mix to the samples.
- Place sample in the Sonicator and Sonicate for about 10 – 15 minutes at about 60^0C.
- Decant the extract into a round bottom flask.
- Repeat once more with an additional 50ml of solvent mix, Sonicate and allow the beaker to settle and decant into the same round bottom flask.
- Concentrate the sample extract using Rotary Evaporator, to 1-2mL
- Add about 5mL Hexane to the extract
- Evaporate it to reduce the volume to 1-2mL.
- Make the final volume 2mL in Hexane, giving final sample weight of 15g/mL.
- The sample is ready for Sulfuric acid/Permanganate cleanup/gel permeation chromatography Anastassiades *et al*., (2003).

Sample cleanup

- Using a syringe or a volumetric pipette, transfer 1 or 2mL of the hexane extract into a 10mL vial in a fume cupboard.
- Carefully add 5mL of the 1:1 Sulfuric acid: water mixture, ensure there is no exothermic reaction.
- Cap the cap tightly and vortex for 1-2mins.
- Allow the phases to separate for at least 3mins, observe the upper hexane layer and ensure that it is not highly colored.
- If the upper layer is colored or the emulsion persist, discard the sulfuric acid layer using pipette and add fresh 5mL sulfuric acid/water mixture.
- Cap tightly and vortex as above.
- Allow the Phases to separate by allowing to stand for 2 to 3mins.
- If a the upper layer is clear, transfer the Hexane layer using a glass pipette into the 10mL vial.
- Add 1mL of n-Hexane to the sulfuric acid layer and repeat the vortex procedure.
- Allow the phases to separate, transfer the n-Hexane layer into the 10mL vial.
- Take care not to include the \acid layer into the 10mL vial as it could damage the column and instrument Anastassiades *et al*., (2003).

Permanganate cleanup

This cleanup procedure is employed when the sulfuric acid cleanup could not remove all the color from the sample extract.

- Add 5mL of 5% permanganate to the combined n-hexane extract above in the hood.
- Ensure there is no exothermic reaction, cap the vial and vortex for 1min.
- Allow the two phases to separate, if a clean separation is achieved, transfer the hexane layer into a clean 10mL vial.
- Add additional 1mL hexane to the permanganate layer
- Cap the vial and vortex for 1min
- Allow the phase to separate, and transfer the hexane to the 10mL vial using a glass pipette.
- Reduce the combined hexane extract volume to the initial 1-2mL using the appropriate method.
- Any remaining Organochlorine Pesticides could be removed using the silica gel cleanup method Anastassiades *et al*., (2003).

Silica gel cleanup

Reduce the extract volume to 2mL and the extract solvent should be hexane

- Mount the clean polypropylene cartridge on a manifold or stand.
- Block the base with sterile cotton wool or glass wool, which ever is available
- Add 2g of the activated silica gel to the cartridge and tap the wall of the cartridge for good packing.
- Add a layer of dry Na_2SO_4 to the top of the column
- Condition the column with about 15mL of n-Hexane, collect the eluate to waste.
- Do not allow the column to dry up at this stage.
- Transfer the 2mL extract to the head of the column with the help of a pipette.
- Elute the column with 15-20mL hexane, collect the fraction in a clean 50mL glass beaker. This fraction contains all the OCPs.
- Dry the sample extract using the appropriate technique.
- Make up the dry extract with 2mL n-Hexane and transfer into a GC vials. Sample extract is ready for GC analysis Anastassiades *et al*., (2003).

Calculation for sample analysis

The concentration of each analyte range in a sample was calculated directly from the Instrument using the Data Analysis Software. The final sample weight and the dilution factor can be use in the batch file and the final results would be generated by the software. OR

The use of the Response Factor Method could be employed (Renwick, 2002).

$$Cf = \frac{Area\ (p) \times Rf \times Vf \times DF \times 1000}{Wi}$$

Where:

Cf = Final Sample concentration (mg/L)

Area (p) = Measured area of peak (peaks)

Wi = Initial weight extracted (g dry weight)

Vf = Final extract volume (mL).

Df = Dilution factor of sample or extract if diluted.

RF= Response factor from the calibration standard calculation

$$RF = \frac{\text{Concentration (P)}}{\text{Area (P)}}$$

Concentration (p) = Concentration of peak or Total concentration of range

Area = Area of peak or total across range.

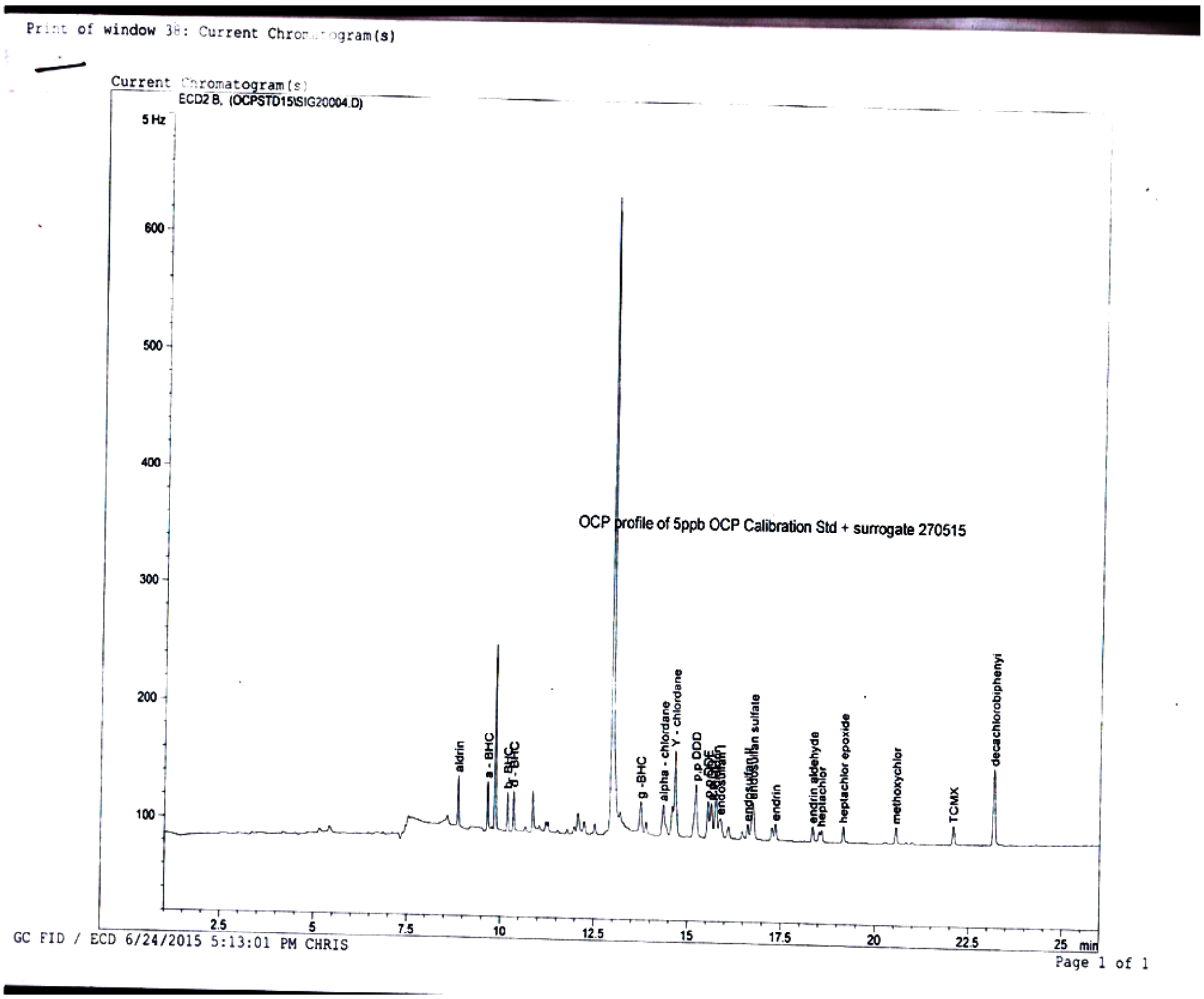


Figure 1; Standard of organochlorine pesticide used in this study

Results and Discussion

The concentration of pesticide residues in soft drinks (sprite and coca-cola) sold in Benin city, Edo state has been investigated. Figure 1, 2 and 3 shows the representative chromatogram standard and samples. No interference peaks were obtained for the blank sample chromatogram at the same retention time as the targets compounds. The mean recovery values for the spiked samples are shown in Table 1. The procedure employed in this study is reproducible, efficient and reliable for the analysis of OCPs as stipulated by EU guidelines for evaluating accuracy and precison method (European Union, 2005).

The chromatogram result in this study is in consonance with that of Ogunleye *et al*. (2010) who assessed soft drinks in Addo Ekitti, Ogun and Benin. Reports from this investigation reveal that organochlorine pesticide residues ranged from below detection limit(BL)(0.00±0.00) to 0.0001mg/kg in (Table1).

Figure 2; Chromatogram of sprite samples analysed

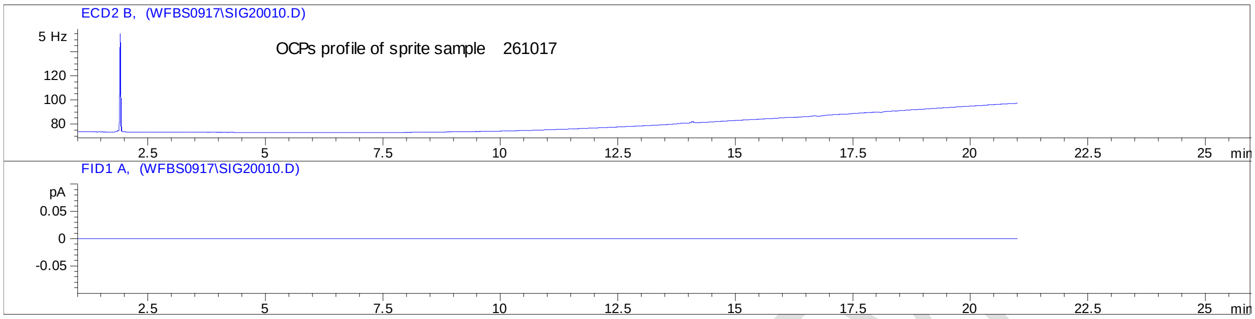
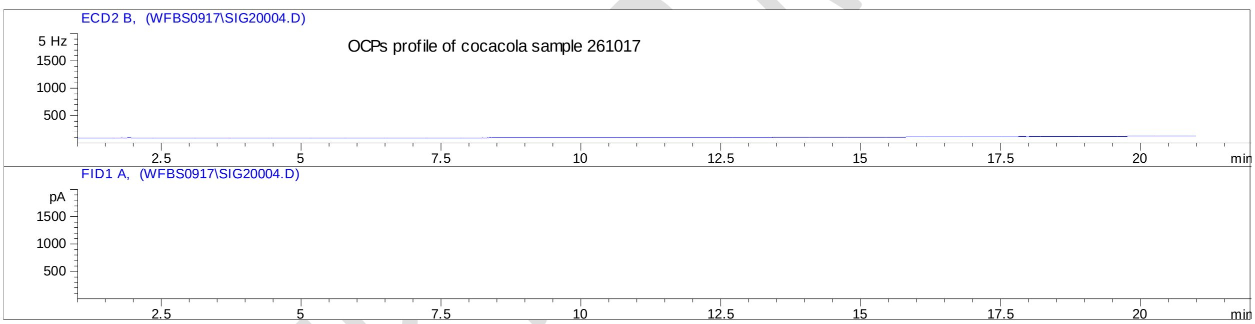


Figure 3; Chromatogram of coca-cola samples analysed



Twenty five (25) pesticides were assessed in these consumable products (Table.1) with aldrin having the highest mean concentration (0.0001mg/kg) in sprite sample.

Table 1; Mean concentration of pesticide residues in soft drinks

COMPONENT	Sample 1 sprite	Sample 2 coca-cola
ALDRIN	0.0001±0.00	0.00±0.00
a – BHC	0.00±0.00	0.00±0.00
b – BHC	0.00±0.00	0.00±0.00
d – BHC	0.00±0.00	0.00±0.00
Gamma - BHC (LINDANE)	0.00±0.00	0.00±0.00
ALPHA – CHLORDANE	0.00±0.00	0.00±0.00
GAMMA – CHLORDANE	0.00±0.00	0.00±0.00

ATRAZINE	0.00±0.00	0.00±0.00
p,p DDD	0.00±0.00	0.00±0.00
p,p DDE	0.00±0.00	0.00±0.00
p,p DDT 4, 4 DDT	0.00±0.00	0.00±0.00
DIEDRIN	0.00±0.00	0.00±0.00
ENDOSULFAN 1	0.00±0.00	0.00±0.00
ENDOSULFAN 11	0.00±0.00	0.00±0.00
ENDOSULFAN SULFATE	0.00±0.00	0.00±0.00
ENDRIN	0.00±0.00	0.00±0.00
ENDRIN ALDEHYDE	0.00±0.00	0.00±0.00
HEPTACLOR	0.00±0.00	0.00±0.00
HEPTACHOR EPOXIDE	0.00±0.00	0.00±0.00
METHOXYCHLOR	0.00±0.00	0.00±0.00
DIAZINON	0.00±0.00	0.00±0.00
PHOSPHORO METHYL GLYCINE	0.00±0.00	0.00±0.00
TCMX	0.00±0.00	0.00±0.00
CARBAMATE	0.00±0.00	0.00±0.00
DECACHLOROBIPHENYL	0.00±0.00	0.00±0.00
TOTAL OCP (mg/Kg)	**BDL**	**BDL**

KEY :OCP; Organochlorine pesticide residue, BDL;Below detection limit

The presence of these organochlorine pesticide residues in soft drink (sprite) showed that farmers still use them for the control of pest. This is because of their potency, efficiency and low cost compared with alternative pesticides (banned or not) Idowu *et al*., (2013).

Several activities (volatilization, photolysis, penetration through the plant surface, inadequate training of personnel, inappropriate use of pesticides) could be attributed to the levels of chlorinated hydrocarbon compounds in soft drink sold in Benin City markets Ezemonye *et al*., (2008). The findings in this study is in consonance with the findings of (FDA,1999) observed that organochlorine pesticide residue in sprite ranged from 0.0001 to 0.75mg/kg in Benin City, Addo Ekitti, Ogun, Lagos and Ghana metropolis.

Previous studies have demonstrated that organochlorine pesticide like gamma lindane is toxic and can affect non target organisms other than the organisms of interest, thereby causing great menace to ecosystem and to consumers Celik *et al*., (1995).

The use of organochlorine pesticides for the control of pest by farmers is worldwide . These compounds are characterized by high persistent, low polarity, low aqueous solubility and high lipid solubility (lipophilicity). They are ecotoxic, non-biodegrable and able to bioaccumulate and biomagnify in living organisms (Lars, 2000). The major problems are their toxic effects such as interfering with the reproductive systems and foetal development as well as their ability to cause cancer, cardiovascular disease, asthma and other health related diseases .

Table 2: Estimated chronic daily intake (CDI) and hazard quotient (HQ) for OCPs in sprite

Pesticide	Concentration	child		Adult		RQ
		ECDI	HQ	ECDI	HQ	
Aldrin	0.0001	1.8E-04	8.3E-06	2.4E-06	5.8E-04	
Hazard index		8.3E-06		5.8E-04		1.89E-08

However, the estimated chronic daily intake (ECDI) of (aldrin) in soft drinks (sprite) (non dietary), risk quotient (RQ), hazard quotient (HQ) and the hazard index (HI) in (Table 2) were calculated using two population groups with varying body weights (35 and 70kg) (Ezemonye *et al*., 2008). The study revealed that the risk quotient (RQ) were (1.89E-08), estimated chronic daily intake (ECDI) for both groups were (1.8E-04) and (2.4E-06) (Table 2). The hazard quotient (HQ) were (8.3E-06) and (5.8E-04). Hazard index (HI) (8.3E-06) and (5.8E-04) were also estimated for both population groups. The result in this study is in consonance with the result of Ecobichon, 2001 which was (BL -0.256mg/kg) in Ghana and Addo Ekitti.

Organochlorine pesticide residues at any given concentration are highly toxic, bioaccumulate and not readily biodegraded (FDA, 1999; Ezemonye *et al.*, 2008). Though the estimated concentrations in this study were minute, ecological risk assessment showed that there is a potential of toxic effects to soft drink (sprite) upon exposure to organochlorine pesticides. Risk projections for humans from non-dietary intake also revealed that there is potential for cancer effects. Projections showed that both children and adult were at high health risk. The result in this study is in line with the United States Agency of Toxic Substances and Disease Registry (ATSDR, 2002) and European union (EU, 2005) standard for estimation of OCPs (0.00001 to 1mg/kg) in sprite that could be considered as unsafe to human.

Conclusion

The study, thus suggest that OCPs concentration in sprite is unsafe to human. The study therefore calls for continuous sensitization to farmers from the use of these dreaded chemicals, that could contaminate the raw materials for processing and periodic investigation of soft drinks (sprite) sold in Benin city markets.

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