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Monitoring of Organochlorine Pesticide Residues in Locally Produced Fruit-Based Soft Drinks in Ghana

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Abstract

The amount of some chlorinated pesticide residues in fruit-based soft drinks were monitored in Ghana. Locally produced fruit-based soft drinks were purchased from different markets and analyzed by gas chromatography equipped with electron capture detector for pesticide residues. A total of 84 fruit-based soft drink samples were analyzed for organochlorine pesticide residues (γ-HCH, pp-DDT, pp-DDE, endrin, endrin ketone, endrin aldehyde, heptachlor, aldrin, α-endosulfan and γ-chlordane). The data showed that most of the fruit-based soft drink samples analyzed contain residues of the monitored pesticides above the EU MRL level in drinking water. The results obtained also showed that 9.5 % of the fruit-based soft drink samples analyzed contained no detectable level of the monitored pesticides, 14.3 % of the samples contained 1 pesticide, 20.2 % contained 2 pesticides, 26.2 % contained 3 pesticides and 29.8 % contained 3 or more pesticide residues. The presence of these pesticides in the fruit-based soft drink samples could be attributed to the way these products are manufactured, since pesticides might be transferred from the peels to the product, probably during squeezing as these compounds are concentrated in the fruit peel. Therefore steps should be taken in order to avoid pesticide contamination in these products by changing the way they are manufactured to ensure the food safety of these products, especially considering that children are the main target and most vulnerable population subgroup.

Key words: organochlorine, pesticide residue, fruit-based soft drink, maximum residue limit (MRL), Ghana.

Introduction

Organochlorine pesticides (OCPs) are among the xenobiotics that have become constituents of the biosphere due to their great use all over the world, stability in several natural conditions and mobility in the environment (Owago et al., 2009). In Ghana, large quantities of OCPs, particularly hexachlorocyclohexane (HCH) and dichlorodiphenyltrichlorohexane (DDT) were used in agriculture and public health until early 1990s. Therefore, the occurrence of excessive OCPs residues in Ghanaian environment is believed to be serious and widespread and has over the years sustained a considerable research interest in elucidating environmental contamination status and human exposure in the country (Bempah and Donkor, 2011). The use of most OCPs is common practice in modern agriculture to control pests and diseases that damage vegetables, fruits and other crops which pose big problem in vegetable/fruit production in this case. Although these

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compounds bring unquestionable benefits in increasing agricultural production, the toxicity of pesticides makes it necessary to monitor the quality of fruits and vegetables produce to avoid possible risks to consumers. Most of organochlorine pesticides have been banned because they are highly persistent insecticides and are very resistant to hydrolysis as well as microbial degradation; hence their residues still appear as pollutants in food as well as in the environment (Zawiyah et al., 2007).

Some of these pesticides are mainly applied to growing crops particularly fruits in the final stages of production (postharvest treatment) and if the chemical is used close to harvest, contamination of the food is very likely. They may penetrate plant tissues and appear in processed products such as fruit-based soft drinks, which are widely consumed, particularly by children who are more susceptible to chemicals (Albero et al, 2003; Albero et al, 2005; Goto et al., 2005; Topuz et al, 2005).

Their high toxicity has made their use very restrictive and currently forbidden in most developed countries since the 1970s (FAO, 1985; Mansour, 2004). Because of their toxic potential, their persistence and their tendency to bioconcentrate, the widespread use of pesticides in the environment generates increasing risks to human health such as cancer and disruption of hormonal functions (Sheridan and Meola, 1999; Basheer et al., 2002). Therefore the determination of pesticide residues in food is of major importance. This has reflected in the large number of reports in the literature on this subject (Saeed et al., 2001; Mansour, 2004; Bempah et al., 2011; Bempah and Donkor, 2011).

Therefore, information on OCPs residue levels is of paramount importance to the consumers of agricultural produce. This paper presents the results of a preliminary survey carried out on the residue levels of organochlorine pesticides in fruit-based soft drinks sold on Ghanaian markets.

There has been escalation in the growth and purchase of fruit-based soft drinks in Ghana to meet domestic and international market. However, there are no strict regulations and exhaustive controls relating to pesticides residues in fruit-based soft drinks, which address them, even when there is significant consumption in vulnerable groups such as children. Despite the large quantity of these products consumed daily, no attention has been paid to enforce the safety of these products in terms of their chemical composition, even though we know they are considered representative and relevant in terms of consumption (Garcia-Reyes et al., 2008).

Therefore, low residual levels of toxic contaminants in fruit-based soft drink spell high danger to the consumers. These make information on residual levels of xenobiotics in fruit-based soft drink very important for the protection of human health particularly by vulnerable groups such as children. Fruit-based soft drinks were selected for this survey because, they are the most widely distributed food drink, which could be easily consumed daily.

Material and Methods

Sampling methodology. A total of 84 fruit-based soft drink samples of 7 different fruit types with flavors, most popular and easily available were purchased from various supermarkets in Ghana. Various commercial fruit-based soft drinks comprising of orange, mango, pineapple, mango/pineapple mix fruit drink, mango/orange mix fruit drink, pineapple/orange mix fruit drink and cocktail (mix fruit juice of apple, mango, pineapple, orange and guava). The collected samples were placed in an iced chest box. All samples were transported to pesticide residues laboratory, Ghana Atomic Energy Commission, and were refrigerated (at 5 oC). These samples were then extracted and analyzed (within 24 hrs from the time of their collection) for the presence of pesticide residues.

Extraction. The extraction procedure was according to methods described by EPA method 8081A for organochlorines with modification. Fruit drink samples were shaken well and filtered through Whatman filter paper no.1. After filtration, 500 ml of sample was taken in a one liter capacity separatory funnel and 20 ml of saturated sodium chloride solution was added. The water sample was partitioned with 100 ml of methylene chloride (thrice) by shaking the separatory funnel vigorously for 2-3 min releasing the pressure intermittently. The layers were allowed to separate. The three extracts of methylene chloride layers were combined and passed through anhydrous sodium sulfate and concentrated to about 1-2 ml using rotary vacuum evaporator. Again 10 ml methylene chloride was added for adsorption chromatography.

Clean up. The florisil column (500 mg/8 ml) cartridge was conditioned with 10 ml of ethyl acetate. A receiving flask was placed under the column to collect the eluate. 5 ml of sample extract (upper layer) was loaded into the column and elute the column with 10 ml (3, 3, 4 ml) of ethyl acetate from above. The sample extract was concentrated to dryness using the rotary evaporator with water bath aided with the water chiller. The residue was dissolved with 1 ml of ethyl acetate and then transferred quantitatively into 2 ml vial for quantification on to the gas chromatograph.

Sample Analysis. The residues were analyzed by Shimadzu gas chromatograph GC-2010 equipped with 63Ni electron capture detector that allowed the detection of contaminants even at trace level concentrations from the matrix to which other detector do not respond. The GC conditions and the detector response were adjusted so as to match the relative retention times and response. The conditions used for the analysis were: capillary column coated with ZB-5 (30 m × 0.25 mm, 0.25 μm film thickness). Carrier gas and make-up gas was nitrogen at a flow rate of 1.0 and 29 ml/min, respectively. The injector and detector temperature were set

at 280 oC and 300 oC respectively. The oven temperature was programmed as follows: 60 oC held for 1 min, ramp at 30 oC min-1 to 180 oC, held for 3 min, ramp at 3 oC min-1 to 220 oC, held for 3 min, ramp at 10 oC min-1 to 300 oC. The injection volume of the GC was 1.0 µl. The residues detected by the GC analysis were further confirmed by the analysis of the extract on two other columns of different polarities. The first column was coated with ZB-1 (methyl polysiloxane) connected to ECD and the second column was coated with ZB-17 (50% phenyl, methyl polysiloxane) and ECD was also used as detector. The conditions used for these columns were the same.
Quality control and quality assurance were incorporated in the analytical scheme. The detection limits were 0.0001 mg L-1. The samples were analyzed in triplicate. Percent recoveries in spiked samples were 95-105 %. Accordingly, the sample analysis data were corrected for these recoveries.

Results and Discussion

Organochlorine pesticides residues were found present in all the analysed samples. Generally, a wide variation between individual samples was observed. The results were examined in relation to differences in fruit types.
Incidence of Pesticide residues in fruit drinks from Accra Metropolis markets. Most popular and easily available fruit-based soft drinks of different brands purchased were investigated for the presence of organochlorine pesticides. However, due to the confidentiality reasons, detailed information about the exact brand name and the name of the manufacturing industry that produced the drink cannot be given.
From the 84 samples analysed, only 8 (9.5 %) were found to be free of the studied organochlorine pesticides. The rest of the samples were positive. Out of these, 14.3 % contained at least 1 pesticide, 20.2 % contained 2 pesticides, 26.2 % contained at least 3 pesticides and 29.8 % of the studies samples contained four or more pesticide residues.
Amongst organochlorine pesticides detected; γ− HCH (technical lindane) was detected in 81 % of the 84 samples analysed (Figure. 1). The range of concentration in the positive samples varied between 0.0001-0.0002 mg l-1 (Table 1). Minimum concentration (0.0001 mg l-1) was detected in orange-based soft dink and mango/pineapple mix fruit drink samples respectively and maximum concentration level (0.0002 mg l-1) was detected in mango/orange-based soft dink, pineapple-based soft dink and pineapple/orange mix fruit-based soft dink which is twice higher than the European Union Council Directive (80/778/EC) limit for drinking water i.e. 0.0001 mg l-1 limit for individual pesticide as per the Drinking Water Directive 80/778/EEC. Average concentration of γ-HCH detected in all the samples was 0.0002 mg l-1, which is twice higher than EU MRL for the sum of pesticides permitted.
Technical DDT (pp-DDT) along with its metabolite (DDE) was detected in 68 % and 57 % of the samples analysed respectively (Figure. 1). The range of concentration varied between 0.0002-0.002 mg l-1 for pp-DDT and 0.0001-0.0004 mg l-1 for pp-DDE (Table 1). Minimum concentration level of pp-DDT (0.0002 mg l-1) was detected in cocktail-based soft drink whilst that for pp-DDE (0.001 mg l-1) was found in pineapple based soft drink. Maximum concentration level for pp-DDT was detected in pineapple-based soft dink (0.002 mg l-1) which is 20 times the tolerated EU MRL and maximum concentration level for pp-DDE was found in cocktail-based soft drink; four times higher than the EU MRL. Average concentration of total DDT in all the samples was 0.0016 mg l-1, which is 3 times higher than the EU MRL.
Endrin along with its metabolites (endrin ketone + endrin aldehyde) was detected in 29 %, 67 %, and 45 % of all the 84 fruit-based soft drink samples analysed respectively (Figure. 1). The range of concentration in the positive samples varied between 0.0001-0.0020 mg l-1 for endrin, 0.0002-0.0004 mg l-1 for endrin ketone and 0.0003-0.0009 mg l-1 for endrin aldehyde respectively (Table 1).

Table 1. Concentration levels of the detected pesticides in the studied fruit-based soft drink samples

Pesticides	Positive samples (%)	Concentration range (mg l^{-1})	x MRL standard
γ-HCH	81	0.0001-0.0002	1.0-2.0
pp-DDT	68	0.0002 - 0.002	2.0-20.0
pp-DDE	57	0.0001-0.0004	1.0-4.0
Endrin	29	0.0001-0.002	1.0-20.0
Endrin ketone	67	0.0002-0.0004	2.0-4.0
Endrin aldehyde	45	0.0003-0.0009	3.0-9.0
Heptachlor	26	0.0001-0.0004	1.0-4.0
Aldrin	23	0.0001-0.0004	1.0-4.0
α-endosulfan	40	0.0001-0.0003	1.0-3.0
γ-chlordane	33	0.0002-0.0006	2.0-6.0

Limit of detection for all the pesticides (LOD): 0.0001 mg l^{-1}

Minimum concentration level of endrin (0.0001 mg l-1) was detected in mango/orange mix fruit-based soft drink whilst that for endrin ketone (0.0002 mg l-1) and endrin aldehyde (0.0003 mg l-1) was recorded in pineapple-based soft drink and cocktail-based soft drink. However, maximum concentration level for endrin (0.0002 mg l-1), endrin aldehyde (0.0004 mg l-1) and endrin ketone (0.0009 mg l-1) was found in cocktail, pineapple and pineapple/mango mix fruit-based soft drink samples, respectively. Average concentration of endrin (0.0004 mg l-1), endrin aldehyde (0.0002 mg l-1) and endrin ketone (0.0003 mg l-1) detected in all the samples were four, two and three times the respective EU MRL for the sum of pesticides permitted.

Heptachlor was detected in 26 % of the fruit-based soft drink samples analysed (Figure. 1). The range of the heptachlor concentration in the positive samples varied between 0.0001-0.0004 mg l-1 (from 1 - 4 times the tolerated EU MRL). Minimum concentration level was recorded in cocktail-based soft drink whilst the maximum residual concentration was recorded in pineapple-based soft drink samples (Table 1). Average concentration detected in all the samples was 0.0002 mg l-1, 2 times the EU MRL for the sum of pesticides permitted.

Likewise heptachlor, aldrin was detected in 23 % of 84 fruit-based soft drink samples analysed (Figure. 1). The residual concentration levels in the positive samples ranges between 0.0001-0.0004 mg l-1 (from 1-4 times the tolerated EU MRL). Minimum concentration level was recorded in mango/pineapple mix fruit-based soft drink and pineapple/orange mix fruit drink whilst the maximum residual concentration was recorded in pineapple-based soft drink sample (Table 1). The average residual concentration of 0.0002 mg l-1 was recorded and it was twice times higher than the EU MRL.

α-endosulfan was detected in 40 % of all the 84 fruit-based soft drink samples analysed. Its concentration levels ranges between 0.0001-0.0003 mg l-1 (Table 1). Minimum concentration level (0.0001 mg l-1) was recorded in mango, orange and mango/pineapple mix fruit-based soft drink whilst the maximum residual concentration (0.0003 mg l-1) was recorded in pineapple, mango/orange and pineapple/orange mix fruit-based soft drink samples which is 3 times the EU MRL. Average concentration level of α-endosulfan (0.0001 mg l-1) detected in all the positive samples was lower than the EU MRL (0.0005 mg l-1 for the sum of pesticides permitted).

γ-chlordane was present in 33 % of the fruit-based soft drink samples analysed (Figure. 1). The range of concentration in the positive samples varied between 0.0002-0.0006 mg l-1 (Table 1). The highest concentration level (0.0006 mg l-1) was recorded in pineapple-based soft drink, six times the EU MRL whilst the minimum concentration was recorded in pineapple/orange mix fruit drink samples (0.0002 mg l-1). Average concentration level (0.0001 mg l-1) of γ-chlordane detected in all the samples was lower than the EU MRL (0.0005 mg l-1 for the sum of pesticides permitted).

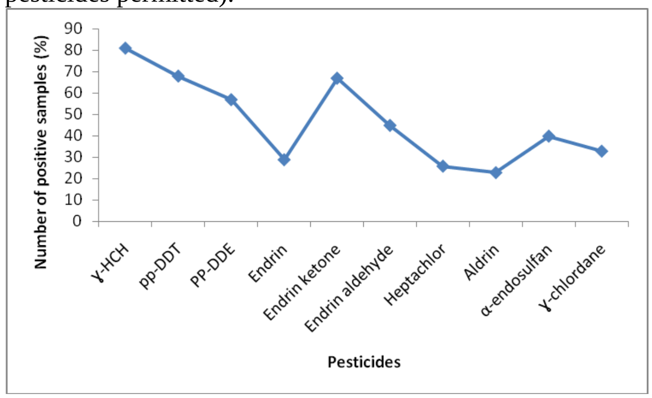

Figure1. Distribution of pesticide residues content in fruit-based soft drink

Total pesticides residues. The range of concentration of total pesticides in the 84 fruit-based soft drink samples varied between 0.0003-0.0051 mg l-1 (see Table 2). Minimum concentration of 0.0003 mg l-1 was detected in orange-based fruit drink which is 3 times the EU MRL and maximum concentration was detected in cocktail-based soft drink (0.0051 mg l-1) which is 51 times higher than the EU MRL. Average concentration of total pesticide residues was 0.0019 mg l-1 which is 4 times much higher than the EU MRL for the sum of pesticides permitted in all the positive samples. The overall concentration values detected in fruit-based soft drinks exceed the EU MRL level in drinking water.

Table 2 Average residues in different fruit-based soft drink samples

S. No.	Fruit-based soft drink types	Total pesticides Mg l^{-1}	EU MRL for total pesticides -0.0005 mg l^{-1}	Deviation from EU MRL (No. of times)
1	Cocktail	0.0051	0.0005	10
2	Mango	0.0007	0.0005	14
3	Orange	0.0003	0.0005	1
4	Pineapple	0.0018	0.0005	4
5	Pineapple/mango	0.0021	0.0005	4
6	Pineapple/orange	0.0016	0.0005	3
7	Mango/orange	0.0018	0.0005	4

Considering the individual pesticides, total DDT registered the highest concentration level, followed by total endrin (+ endrin ketone and endrin aldehyde), then to γ-HCH, heptachlor and aldrin, having the same residual concentration level of 0.0002 mg l-1(Figure 2). Lowest concentration value was α-endosulfan and γ-chlordane (0.0001 mg l-1 each).

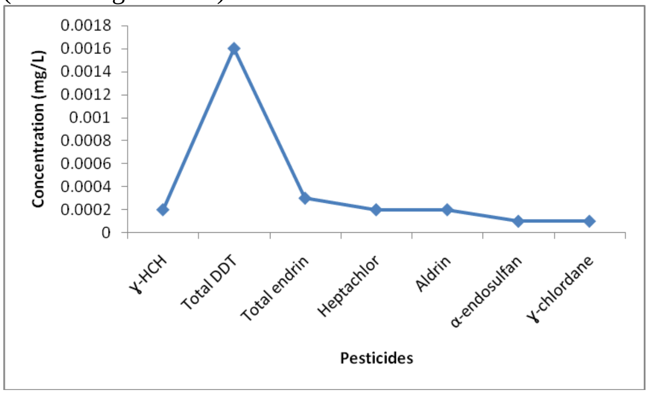

Figure 2 Concentration level of individual pesticide residues

There were cases where more than six different pesticide residues found in the same sample at relevant concentrations. The presence of more than one chemical can enhance the toxic effect of others. The combine effect of a cocktail comprised of various pesticides can be more harmful than the sum of the individual effects from each of the alone (Garcia-Reyes et al., 2008).

The source of contamination could be attributed to bad practices when manufacturing the product and the ingredients (raw materials including water) used in production. However, in a similar study conducted in carbonated soft drinks in India, the pesticides detected were found to be present in the raw water used to prepare the soft drinks. The Center of Science and Environment in India reported that the origin of pesticides in soft drink came from the raw water used, which represented 90 % of its composition. They found that the samples of groundwater taken from inside the factory were contaminated with the same pesticides as were found in the finished product. Therefore, the origin of this source was the contamination of raw water used to prepare the products, which might be contaminated due to decades of pesticides use in agriculture (CSE Report, 2006).

Unlike the Indian Scenario where the presence of pesticides caused by environmental contamination is practically unavoidable unless a dedicated treatment is applied to raw water, the source of contamination in this case might be related to the way these products are manufactured, since pesticides might be transferred from the peels to the product, probably during squeezing.

Conclusion and Recommendation

It can be concluded that pesticide residues were found in all the fruit-based soft drinks analysed. In all cocktail fruit-based soft drink contained the highest pesticide residues (0.0051 mg l-1), followed by pineapple/mango mix fruit-based soft drink (0.0021 mg l-1), pineapple soft drink (0.0018 mg l-1), mango/orange mix fruit-based soft drink (0.0018 mg l-1), pineapple/orange (0.0016 mg l-1), mango-based soft drink (0.0007 mg l-1) and orange-based soft drink (0.0003 mg l-1) in that order.

Considering the distribution of individual pesticide residues in the various fruit-based soft drink samples, γ-HCH was found to be present in 81 % of the fruit-based soft dink, followed by pp-DDT (68 %), endrin ketone (67 %), pp-DDE (57 %), endrin aldehyde (45 %), α-endosulfan (40 %), γ-chlordane (33 %), endrin (29 %), heptachlor (26 %), and aldrin (23 %) in that order. In all, total-DDT registered the highest residual concentration level, followed by total endrin (+endrin aldehyde and endrin ketone), technical lindane (γ-HCH), heptachlor and aldrin all registering the same residual concentration level of 0.0002 mg l-1. The lowest concentration value recorded in all the fruit-based soft dink was α-endosulfan and γ-chlordane (0.0001mg l-1 each).

The results obtained also showed that 9.5 % of the fruit-based soft drink samples analyzed contained no detectable level of the monitored pesticides, 14.3 % of the samples contained 1 pesticide, 20.2 % contained 2 pesticides, 26.2 % contained 3 pesticides and 29.8 % contained 3 or more pesticide residues.

The overall concentration values detected in fruit-based soft drinks exceed the EU MRL level in drinking water. The presence of these pesticides in the fruit-based soft drink samples could be attributed to the way these products are manufactured, since pesticides might be transferred from the peels to the product, probably during squeezing. Therefore steps should be taken in order to avoid pesticide contamination in these products by changing the way they are manufactured to ensure the food safety of these products, especially considering that children are the main target and consumers.

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