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EXPOSURE TO POLYCYCLIC AROMATIC HYDROCARBONS THROUGH CONSUMPTION OF SOME POPULAR SOFT DRINK PRODUCTS IN NIGERIA

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ABSTRACT

In this study, levels and types of polycyclic aromatic hydrocarbons (PAHs) were determined in the commonly consumed soft drink products in Nigeria. This was done to assess the possible human health risks associated with the long-term regular consumption of the soft drink products. Two batches of twelve samples of differently packaged soft drink products were obtained from Ile-Ife, Osun Sate, Nigeria. The PAHs were extracted by Liquid-Liquid Extraction (LLE) method using n-hexane as the extracting solvent, while the cleaned-up samples were analysed for their PAHs content using Gas Chromatography coupled with Flame Ionization Detector (GC-FID). The results obtained from the study indicated that seventeen PAHs could be detected from the soft drink samples at levels (μg/mL) that varied diversely among the soft drink samples. Pyrene had the highest level of prevalence while 2-methyl naphthalene had the lowest level of prevalence; it was present in only one sample. Levels of ΣPAHs were lowest in sample B while the least levels of ΣPAHs were found in sample F. The result indicated that B had a higher potential for carcinogenic risks from consumption than the other soft drink samples. The observed values of estimated Dietary Daily Intake (DDI) for the total PAHs and carcinogenic toxic equivalents (TEQ) in sample B were the highest indicating that there could be a higher risk of exposure and carcinogenic human health risk from regular consumption of B in preference to the other soft drink products.

Key words: Soft drinks, Consumption, Health risks, Polycyclic aromatic hydrocarbons, Nigeria

INTRODUCTION

Soft drinks usually refer to a wide range of carbonated or non carbonated non-alcoholic or mildly alcoholic drinks stored in cans, cartons, plastics or bottles (ranging from small bottles to large multi-liter containers) with constituents put together in a way that conforms to healthy diet specifications (AICR, 2007). As a way of making the taste more appealing to the consumers, soft drinks may contain fruit juices, natural or artificial sweetening agents, herbal mixtures, natural or artificial flavors, milk derivatives, artificial colourings, caffeine, edible acids, and other preservatives (AICR, 2007; Vaux, 2011).

Soft drinks are widely consumed for three major reasons. One, in their various forms soft drinks are consumed so as to rehydrate the body because virtually all soft drinks contain a large quantity of water which serves as the major rehydration agent in soft drinks. Two, where the purification integrity of available water is in doubt people would prefer to drink soft drinks to avoid waterborne diseases, such as cholera, dysentery and other health compromising micro-organisms (Prato and Parent, 1993; Clancy *et al.*, 1998; Olanrewaju *et al.*, 2017). Also, several people are attracted to soft drinks because they are heavily promoted through media advertising, sponsorships in sports or concerts, movies and TV programmes, pleasantly emblazoned street bill boards.

These factors have made the consumption of soft drinks to keep increasing across virtually all age grades. By the close of the nineteenth century, several carbonated drinks, such as soda water, ginger beer, ginger ale, lemonade, citrus drinks, quinine tonic water, bitter lemon, colas, sarsaparilla, root beer, cream soda and so on were already on sale (Riley, 1958;Prato and Parent, 1993). The 2004 global consumption of soft drinks was estimated at 480 billion litres of which cola and other carbonated drinks accounted for

40% with current sales in Asia increasing at around 3.5% each year (AICR, 2007).

The ingredients and processes used in the formulation of carbonated soft drinks vary widely depending on the bottling company and targeted consumers. In all cases, three conditions are expected to be met: food and drink products must present no health risk to the consumers; correct procedures must be adopted; and ingredients are selected to meet legal requirements of purity and conform to the legislative controls that apply (Binnie *et al*., 2002; Ashurst, 2004). Such ingredients include water, carbondioxide, different sweetners, acidulants, flavourings, colours and preservatives (Mitchell, 1990; Gleick, 1996; EC, 1999; Saltmarsh, 2000; Binnie*et al.,* 2002). Ideally, these ingredients and raw materials should be added according to specification and workable limits for microbial activity so that there is little chance of excessive contamination in the finished beverage product (Hofman*et al*., 2001). However, the ingredients used in the formulation of carbonated soft drinks, by their diverse nature and sources, could be the source of a wide range of contaminants that may be detected in such food items. Some of the contaminants speculated to be present are trace metals and trace organics, such as polycyclic aromatic hydrocarbons (PAHs).

Polycyclic aromatic hydrocarbons (PAHs) consist of hydrocarbons with two or more fused benzene rings in various structural configurations with no hetero atoms or substituents (Yang*et al*.,2003). Polycyclic hydrocarbons containing up to four rings are refer to as light PAHs and those that contain more than four rings are classified as heavy PAHs (Kuppusamy*et al*., 2016). Polycyclic aromatic hydrocarbons were ranked as the ninth most threatening compounds to human health (King *et al*., 2002; Simko, 2002). In view of their higher genotoxic potentials, high molecular or heavy PAHs are more stable, have relatively low solubility in waterbut are highly lipophilic and more toxic than the light PAHs (Yang *et al*., 1988; Yamada *et al*., 2013). Most of the PAHs with low vapour pressure can readily be detected in the air adsorbed on particles and can undergo photodecomposition when exposed to ultraviolet light from solar radiation.

Many of these compounds, namely: benzo[a]pyrene, benzo[a]anthracene, dibenzo[a,h]anthracene and chrysene, have been reported to possess carcinogenic and genotoxic properties (IARC, 1973). Thus, as food contaminants, PAHs are detrimental to humans when they exceed certain threshold limits. Globally, the role of PAHs in the environment is increasingly becoming an issue of serious concern because of their toxic nature to living organisms at certain threshold levels within the various environmental compartments that are considered as very important ecological crossroads in the environment(Abdel-Shafy and Hussein, 2016).The worldwide distribution of PAHs in the environment can be traced to several sources, such as coal and wood burning, petrol and diesel oil combustion, high temperature industrial processes, forest fires, incineration of biomass matters, automobile exhausts, volcanoes, refining and hydrothermal processes (Guillen *et al*., 1997; Chrysikou *et al*., 2008; Baxter *et al*., 2014; Ortuno *et al*., 2014; Luo *et al*., 2016).

Polycyclic aromatic hydrocarbons are widely distributed environmental contaminants that have detrimental biological effects, toxicity, mutagenicity and carcinogenicity. Eye irritation, nausea, skin inflammation and irritation in form of allergic reactions in skin in animals and humans, vomiting, diarrhea and confusion are some of the symptoms associated with exposures to elevated levels of pollutants containing PAHs (such as Anthracene, benzo[a]pyrene and naphthalene) and other substances (Burchiel and Luster, 2001; Unwin *et al*., 2006; IPCS, 2010). More than their toxic effects, the ability of the reactive metabolites of PAHs, such as epoxides and dihydrodiols, to cause biochemical disruption of the cellular proteins and DNA leading to mutations, developmental malformations, tumors, and cancer has remained a major concern (Armstrong *et al*.,2004; Zhou and Zhao, 2012). There are strong enough evidences coming from laboratory experiments and occupational exposures to prove that mixtures of PAHs are partly responsible for an increased risk of skin, lung, bladder and gastrointestinal cancers in humans (USEPA, 2008).

The present study involved the determination of

the levels of PAHs in six different types of carbonated soft drink products known to be massively consumed in Nigeria. The choice of the carbonated soft drinks was based on the frequency of consumption at parties, homes, schools, offices and social gatherings. The aim of the study was to determine the regular and long-term consumption safety of each of the carbonated soft drinks with respect to the levels of their PAHs content.

MATERIALS AND METHODS

Reagents and Chemicals Used

The analytical grade anhydrous sodium sulphate, silica gel, n-hexane and ethanol were purchased from Sigma-Aldrich sales representative outlets in Nigeria. Doubly distilled water was used for the washing and rinsing of apparatus used in the preliminary extraction stages.

Pre-treatment and Sterilization of Apparatus

All standard laboratory glassware (beakers, sample bottles and vials, measuring cylinder, separating funnel, volumetric flask, conical flasks, funnel, glass columns) were cleaned by soaking for 48 hours in a detergent solution in a wash basin. Each glassware was scrubbed clean with a nylon brush, rinsed with hot water, followed by cold water, doubly distilled water and finally with acetone to preclude any trace organic matters that can cause cross-contamination of the analyte of interest. All cleaned and dried glassware were wrapped with clean aluminum foil and stored in a cupboard to prevent cross contamination by fallout from laboratory air**.** The glass wool was soaked in acetone overnight and later dried and wrapped in aluminium foil pending further use.

Sampling

Two categories of carbonated soft drinks were purchased from sales outlets in Ile-Ife, Osun State, Nigeria. The first category was made up of six different commonly consumed soft drinks contained in plastic bottles (coded: A, B, C, D, E and F), while the second category was made up of brands of the first category stored in glass bottles (coded: A', B', C', D', E' and F'). The different samples were stored in a cool place at room temperature prior to analysis.

Extraction of PAHs from the Soft Drink Samples

For the extraction of PAHs, 300 mL of each sample was carefully measured and poured into a 1000 mL separating funnel and 30 mL of analytical grade n-hexane was added to the sample in the flask. The mixture was shaken vigorously for about 20 minutes with occasional venting of the trapped gas or volatilized n-hexane. The mixture was then allowed to settle for about 1 hour to properly separate into two immiscible layers. The denser layer, which is the sample, was eluted into a beaker while the lighter layer, which contains the PAHs, was eluted into a labeled amber coloured vial. This procedure was carried out in triplicate for each sample. All the eluted extracts for a give sample were added together into a vial which was securely covered and then stored in a refrigerator at a temperature of about 4°C in readiness for clean-up.

Clean-up Procedure

The clean-up employed the principle of chromatography which involves a stationary phase (silica gel) and a mobile phase comprising n-hexane and ethanol. The ethanol was used to solubilize the extract that appeared viscous. The packed column was first washed with n-hexane to prevent any interference from extraneous trace organics. The clean-up process was performed to remove all other forms of impurities which might be present in the eluate, or reduce them to the barest minimum (Oyekunle *et al.,* 2011). The recovered eluate after clean-up was collected in amber-coloured vials, evaporated to dryness and then reconstituted with 1 mL of n-hexane and stored in at 4°C prior to instrumental analysis.

Instrumental Analysis

The qualitative identification and quantification of the PAHs were carried out using a Gas Chromatograph coupled with Flame Ionization Detector (GC-FID) available at the Nigerian Institute of Oceanography and Marine Research, Victoria Island, Lagos, Nigeria. The identification of the PAHs was based on the comparison of the retention times of the peaks with those obtained from serially diluted mixture of PAHs standards (supplied by instrument manufacturer). Quantification was based on external calibration curves prepared from the standard of each of the PAHs.

Validation of Procedure

Recovery Analysis

No standard reference material was available to the researchers in the course of this study. Hence, percentage recovery (%R) was carried out to evaluate the efficiency of the analytical procedures adopted for the analysis. Also, fluorene, anthracene, phenanthrene and chrysene were the only pure forms of PAHs available. Thus, a mixture of 10 μg/mL of the available PAHs was prepared and added into a known volume (X) of the carbonated soft drink sample, shook vigorously, left in a corked container overnight before extraction was done. An equal volume (Y) of the carbonated soft drink was left unspiked. Both samples were stored under the same conditions and taken through the extraction and clean-up protocols. The extracts were analyzed for their PAHs content. The %R was evaluated from the relationship:

$$\%R = \quad x\,100 \qquad [1]$$

Human Health Risk Assessment

The Dietary Daily Intake (DDI) of PAHs

The D$\frac{x-y}{10}$ry Daily Intake (DDI) of PAHs in the carbonated soft drinks was evaluated using Equation 2 (Halek *et al.*, 2007):

$$DDI = Ci \times IR \qquad [2]$$

where C*i* is the concentration of PAHs and IR is the ingestion rate of the soft drinks averagely based on the content of the bottle (350 mL ≈ 350 g).

Evaluation of Dietary Daily Intake (DDI) was calculated for individual PAHs, the sum of the 17 PAHs analyzed (Total PAHs) and also for the sum of those PAHs considered possible human carcinogens (Total Carcinogenic PAHs).

Carcinogenic Risk Assessment of PAHs in Carbonated Soft Drinks

Cancer risk due to dietary exposure to PAHs in carbonated soft drinks was calculated using the individual PAH carcinogenic potencies and the carcinogenic toxic equivalents (TEQs). The Carcinogenic potencies, B(a)P TEQs, of individual PAHs was evaluated by multiplying the PAH concentration in the sample by the individual toxicity equivalency factor (TEF) (Nisbet and LaGoy, 1992). The TEF is an estimate of the relative toxicity of individual PAH fraction compared to benzo(a)pyrene.

Carcinogenic potencies of individual PAHs,

$$(BaP\ TEQs) = Ci \times TEFi \qquad [3]$$

The carcinogenic toxic equivalents (TEQs) were then obtained by summing the carcinogenic potencies of individual PAHs (BaP TEQs) (Ding *et al.*, 2012).

Health Risk Index of PAHs

The risk index is defined as a quotient between the estimated exposure to daily PAHs intake (DPI) and reference dose oral (RfD) for each PAH (USEPA, 2002). An index more than 1 is considered as not safe for human health (USEPA, 2002). Daily intake was calculated by the following equation:

$$\text{Daily intake of PAHs (DIP)} = Ci \times \frac{D}{B} \qquad [4]$$

where Ci, D and B represent the PAHs concentrations in soft drink samples (μg/g), daily intake of soft drinks and average body weight (45 kg) respectively.

Statistical Analysis of Data

Basically, Pearson correlation coefficient was used in this study to predict the relative association and, possibly, the sources of the PAHs detected in the samples.

RESULTS AND DISCUSSION

Validation of Analytical Procedures

The reliability of the analytical procedure adopted in this study was tested in terms of percentage recovery of the available PAHs standards. The percentage recoveries ranged from 80.7% Anthracene to 99.0% Chrysene for glass bottled soft drinks and from 89.9% Chrysene to 108.3% Anthracene for plastic bottled soft drinks (Table 1). These values were within the 70 – 110% recovery range stipulated by the EU (1999) as the acceptable limit within which the analytical procedure is adjudged to be reliable.

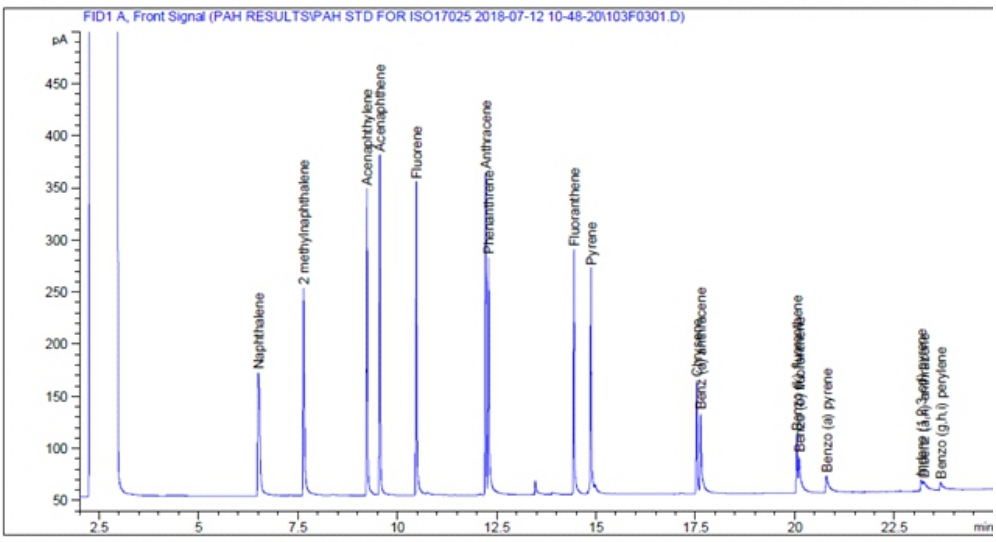
Table 1: Percentage Recovery (%R) of PAHs in Soft Drink Samples

	Glass bottled soft drink			Plastic bottled soft drink		
PAHs	X	Y	%R	X	Y	%R
Fluorene	56.91	48.55	83.6	57.09	47.65	94.4
Phenanthrene	93.36	84.89	84.7	94.63	85.15	94.8
Anthracene	34.51	26.44	80.7	32.46	21.63	108.3
Chrysene	20.91	11.01	99.0	15.35	6.36	89.9

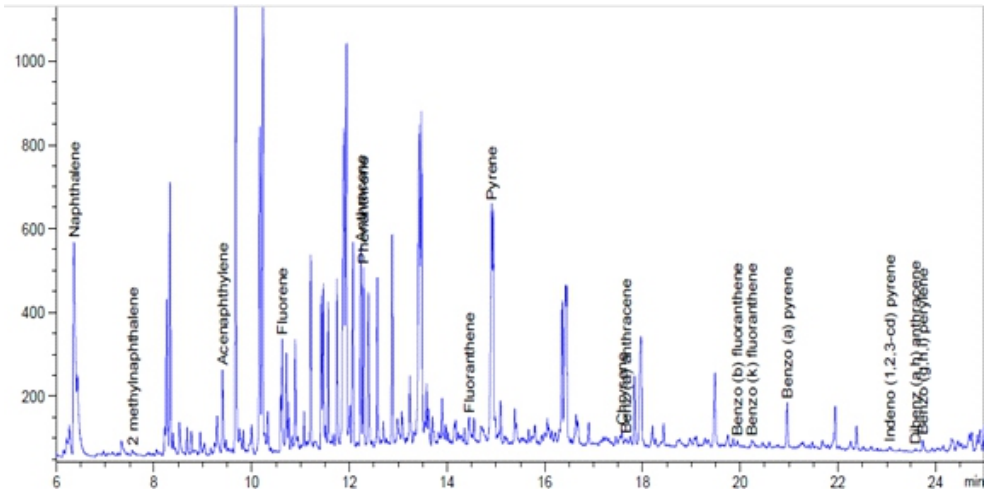
Levels of Polycyclic Aromatic Hydrocarbons in Soft Drink Samples

Seventeen (17) different types of PAHs were detected at various levels in the soft drinks (Fig.1). A summary of the types and concentrations of the various PAHs present in different soft drink samples studied is presented in Table 2.

The results obtained from the study indicated that the levels of naphthalene in plastic bottled soft drinks ranged from not detected (ND) to 0.88 µg/mL, while the range was between ND and 0.31 µg/mL in the glass bottled soft drinks. Respective levels of prevalence (LoP) of naphthalene were 0.90 and 0.34 µg/mL in plastic and glass bottled soft drinks. Basically, naphthalene was detected only in four samples at 0.88, 0.02 µg/mL (in B and D), 0.03 and 0.31 µg/mL (in B' and C'). Based on a full 350 mL content per bottle, a consumer who drinks a whole bottle of B or D or B' or C' would have consumed not less than 7.0 and up to 308.0 µg of naphthalene. Ingestion of a large amount of naphthalene damages some of the red blood cells, thus leading to hemolytic anemia (ATSDR, 2005) especially in people with an underlying G6PD (glucose-6-phosphate dehydrogenase) deficiency and may cause confusion, nausea, vomiting, diarrhea, blood in the urine, and jaundice (Santucci*et al.*, 2000). It is on record that children have developed this problem after eating naphthalene-containing mothballs or deodorant blocks (ATSDR, 2005). In pregnant women, naphthalene can move from the mother's blood to the baby's blood and in lactating mothers, naphthalene may also be transferred from the mother to the baby through the breast milk. The International Agency for Research on Cancer (IARC) classifies naphthalene as possibly carcinogenic to humans and animals (Group C) and that acute exposure causes cataracts in humans, rats, rabbits, and mice, and that hemolytic anemia can occur in children and infants after oral or inhalation exposure or after maternal exposure during pregnancy (ATSDR, 2005; IARC, 2010). The National Institute for Occupational Safety and Health (NIOSH, 1997) has set a recommended exposure limit of 0.01- 0.02 µg/mL.



(a): Chromatogram of standard PAHs



(b): A representative chromatogram of PAHs in soft drinks
Figure 1: Representative chromatograms of PAHs in soft drink samples

17Occurrence of 2-methyl naphthalene was only in sample B at 0.03 µg/mL. If present at relatively higher quantities and with regular contact, the gas-exchange part of the lungs can become filled with an abnormal material leading to a type of lung injury called pulmonary alveolar proteinosis (ATSDR, 1995; ATSDR, 2005). From the results of the present study, 2-methyl naphthalene occurred as the least contaminant of soft drink products.

Acenaphthylene levels in plastic bottled soft drinks ranged from 0.01 µg/mL in E, F and D' to 0.19 µg/mL in B. These values translated to a range of 3.5 to 66.5 µg per plastic bottle of 350 mL content. In glass bottled soft drinks, the range was from 0.01 µg/mL in D' to 0.09 µg/mL in B'. These values translated to a range of 3.5 to 31.5 µg per glass bottle of 350 mL content. The International Agency for Research on Cancer (IARC) has not classified Acenaphthylene as a carcinogen, but the recommended USEPA daily oral exposure to acenaphthylene is 0.06 mg/kg (or 0.06 µg/mL). The concentrations of acenaphthylene in A (0.04 µg/mL), C (0.02 µg/mL), E and F (0.01 µg/mL) were lower than the recommended amount, but equal to or higher than the recommended level in B (0.19 µg/mL) and D (0.06 µg/mL). In the same vein, levels of acenaphthylene in glass bottled soft drinks were equal to or higher than the 0.06 µg/mL in B', C' and E' but lower in the others. Thus, levels of acenaphthylene in B, D, B', C' and E' could lead to disturbance of respiratory system, cough, wheezing, shortness of breath, bronchitis, vomiting, kidney and liver damage (IARC, 1973) upon regular and prolonged consumption.

In the case of acenaphthene, values obtained ranged from 0.01 µg/mL in C, D, E and F to 0.95 µg/mL in B for plastic bottle soft drinks and 0.01 µg/mL in D' and E' to 0.66 µg/mL in B' for the glass bottled soft drinks. These values translated to a minimum of 3.50 µg/mL to a maximum of 332.50 µg/mL in the soft drink samples. Acenaphthene is not classified as carcinogenic as listed by OSHA. However, exposure to high doses of acenaphthene in a short period can cause damage to the skin, cause headaches, nausea, loss of appetite, inflammation or swelling of the stomach and intestines (Spacie*et al.*, 1983). The daily oral exposure likely to be without an appreciable risk of deleterious effects during a lifetime for acenaphthene is 0.3 mg/kg/day (i.e. 0.3 µg/mL/day) as recommended by the USEPA. The concentrations of acenaphthene in all the samples were below this limit except for B which had 0.95 µg/mL and B' which had 0.66 µg/mL.

Fluorene levels in glass bottled soft drinks ranged from 0.02 to 0.26 µg/mL, while its concentrations ranged between from 0.01 and 0.29 µg/mL in plastic bottled soft drinks. By implication, anyone who consumes a whole bottle of 350 mL of any of the soft drinks may consume between 3.5 and 101.5 µg fluorene. Samples B and D' had fluorene concentrations higher than the recommended limits of 0.06 µg/mL implying that caution should be exercised in consuming large amounts of soft

drinks B and D' on regular basis. Understandably, fluorene has not been classified as a cancer causing agent, but effects of its short term exposure to higher concentrations include irritation and burning of eyes and skin (EFSA, 2008).

The USEPA (1993) daily oral exposure limit of anthracene is 0.3 mg/kg/day which translates to 0.3 μg/mL. Exposure to high doses of anthracene at a short time can damage the skin, cause headaches, nausea, loss of appetite, inflammation or swelling of the stomach and intestines (Warshawsky*et al.*, 1993). In the present study, anthracene levels ranged from 0.05 to 0.46 μg/mL in plastic bottled soft drinks and 0.15 to 0.46 μg/mL in D'. Irrespective of the brand consumed, a minimum of 17.5 μg of anthracene would have been ingested each time a 350-mL content of a soft drink is consumed. Regular consumers of high-level anthracene containing soft drinks may suffer skin damage, headaches, nausea and loss of appetite, among others.

In plastic bottled soft drinks, levels of phenanthrene fell within the range 0.04 μg/mL in C and 0.35 μg/mL in B, while the levels ranged from 0.11 μg/mL A' to 0.35 μg/mL in D'. Based on a 350 mL content bottle, these values translated to 14.00 μg in C to 122.50 μg in B and D'. There is no sufficient data to derive an oral reference dose or inhalation reference concentration for phenanthrene (USEPA, 1993), but based on no human data and inadequate data from animal bioassays, USEPA (1993) has placed phenanthrene in weight-of-evidence, not classifiable as to human carcinogenicity. Phenanthrene is not classified as a carcinogen to humans by the International Agency for Research on Cancer, but as an established irritant, can cause photosensitization of skin in the presence of intense light (IARC, 1973).

The levels of fluoranthene ranged from 0.01 μg/mL to 0.12 μg/mL in soft drinks available in plastic bottles, while the range was from 0.02 to 0.08 μg/mL in glass bottled soft drinks. This means that the least amount of fluoranthene one could consume is 3.5 μg while the amount could be up to 42.00 μg per 350 mL of soft drink consumed depending on the brand. Concentrations of fluoranthene in samples B, D, B', D', and F' exceeded the USEPA (2008) daily recommended limit of 0.04 mg/kg/day although fluoranthene is not classified as genotoxic (Cavalieri, 1988).

Pyrene is a skin irritant, a carcinogenic agent, a suspected mutagen, and an unequivocal tumour-causing agent that can be absorbed by oral ingestion and through the skin. Workers exposed to 3-5 mg/kg of pyrene would exhibit some teratogenic effects (USEPA, 2002). The levels of pyrene in the present study ranged from 0.12 μg/mL to 0.88 μg/mL in plastic bottled soft drinks and 0.02 to 0.95 μg/mL in glass bottled samples. Although the concentrations of pyrene in all the samples were below the recommended limit, and hence would not cause immediate health challenges. It nonetheless had the highest level of prevalence across all the soft drink samples. The source of pyrene in the soft drinks cannot be linked with the nature of packaging material because the difference was generally not significant at $p \leqslant 0.05$.

United States EPA has classified chrysene in the category of weight-of-evidence Group B2, a probable human carcinogen group that has been established to cause cancer in laboratory animals (USEPA, 1993). The levels of chrysene fell within the range 0.01 and 0.17 μg/mL in plastic bottled soft drinks, while in glass bottled samples, the range was between 0.04 to 0.36μg/mL. These values translated to a range of 3.5 to 126.0 μg/350 mL liquid content of the soft drinks. Undoubtedly, the samples had chrysene concentrations far above the recommended limit of 0.007 μg/g in food items. As such, reckless and regular consumption of these soft drinks should be discouraged among consumers.

Benzo[a]anthracene was detected in all samples within the range 0.01 and 0.09 μg/mL, which translated to consuming a minimum of 3.5 μg mL or maximum of 31.5 μg whenever a 350 mL content is consumed. Benzo[a]anthracene can cause irritation of eyes, nose, throat and skin. With the USEPA recommended daily oral exposure likely to be without an appreciable risk of deleterious effects during a lifetime for benzo[a]anthracene being 0.3 mg/kg/day (USEPA, 1993), it could be inferred that the

concentrations of benzo[a]anthracene were not high enough to cause serious health infractions as their levels in the samples were below the recommended limit.

The position of the National Institute for Occupational Safety and Health Administration is that oral exposure to carcinogens be limited to the lowest feasible concentration. In other words, it is commendable if items meant for consumption could only contain tolerable levels or even less. For benzo[k]fluoranthene, a carcinogenic substance, exposure limit is 0.1 mg/kg (or 0.1 μg/mL) (NIOSH, 1997). In the samples chosen for this study, benzo[k]fluoranthene was detected in all the samples at levels ranging from 0.01 to 0.37 μg/mL. By implication, between 3.5 μg to 129.5 μg could be orally added to the human system whenever 350 mL of any of the soft drinks is consumed. About 50% of the samples under investigation had benzo[k]fluoranthene concentrations higher than the recommended level.

For benzo[b]fluoranthene, the National Institute for Occupational Safety and Health Administration (NIOSH, 1997) has set a recommended exposure limit of 0.1 mg/kg (i. e. 0.1 μg/mL). In the present study, levels of benzo[b]fluoranthenein the soft drink samples ranged from 0.01 to 0.1 μg/mL. Based on a 350 mL content, the values translated to a range of 3.50 μg to 35.00 μg. Benzo[b]fluoranthene has been classified to be genotoxic andcarcinogenic by the United States Environmental Protection Agency (USEPA, 2008). Apart from sample B in which the benzo[b]fluoranthene concentration was the same as the recommended 0.1 μg/mL limit, all other samples contained lower levels of benzo[b]fluoranthene, and hence, these values indicated a low concern for consumer health as the dietary exposures were only minimal.Benzo[a]pyrene, BaP, the most common cancer causing PAH in animals, is notable for being the first chemical carcinogen to be discovered (USEPA, 2008). At a threshold level, BaP has been implicated as being responsible for genetic damage of lung cells in a way similar to the DNA damage usually observed in most malignant lung tumours (Cavalieri*et al.*, 1988; Butler *et al.*,1993). The recommended oral exposure limit of BaP is 0.0003 mg/kg/day (i.e. 0.0003 μg/mL/day) (USEPA, 1993). The levels ofbenzo[a]pyrene in the samples ranged from 0.03 to 0.87 μg/mL.These values translatedto a range of 10.5 μg to 304.5 μg per 350 mL bottle content. Benzo(a)pyrene was present at significantly higher levels than the recommended limit across all the samples. Thus, the dietary exposure from regular drinking of soft drinks could lead to BaP related serious health concerns.

Indeno[1,2,3-cd]pyrene, a carcinogen, occurred in 100% of the plastic bottled soft drinks at levels that ranged from 0.01 to 0.43 μg/mL, while it occurred in only 50% of glass bottled soft drinks at 0.17 to 0.30μg/mL range. Indeno[1,2,3-cd] was present in all six soft drink samples. Indeno[1,2,3-cd] is a carcinogen. Apparently, plastic containers contributed more Indeno[1,2,3-cd]pyrene to the soft drink contents than glass bottles.

Dibenzo(a,h)anthracene was detected in 83% of plastic bottled soft drinks within the range 0.01 to 0.15 μg/mL, and at 0.09μg/mL in 17% of the glass bottled soft drinks. To some extent, plastic bottles appeared to contribute to the level of Dibenzo(a,h)anthracene recorded in the soft drink samples. A dose-related increase of dibenzo[a,h]anthracene has been inferred to be responsible for skin carcinoma formation, as well as decreased survival time and tumor latency period (ATSDR, 2005).

The levels of Benzo[g,h,i]perylene in the samples ranged from 0.01 to 0.59 μg/mL, with occurrence in 83% of the plastic bottled samples and 100% of the glass bottled samples. Regular consumption of 350 mL content per bottle of any of these soft drinks could lead to an addition of between 3.5 to 206.5 μg of benzo[g,h,i]perylene in which case reproductive problems, damage to skin, body fluids alteration and compromise of the immune system may be experienced as have been demonstrated in laboratory animals (Devault *et al.*, 1990). Generally, PAH-rich mixtures have been identified as having high carcinogenic risk to humans (Burchiel and Luster, 2001).

Coefficient of variation (CV) helps to have an overview of degree of diverse distribution pattern of the measured contaminants in a sample or an

environment. Widely varying CV values (Table 4) with a range of 78 – 167 for plastic bottled soft drinks and 100 – 157 for glass bottled soft drinks is an indication that the amount of PAHs contributed by each of the ingredients used in the formulation of the soft drinks varied widely in proportion. A deeper understanding of how much of PAHs is contributed by each ingredient could lead to methods that will ensure their reduction and enhance safer content of soft drinks produced in future.

Pearson correlation matrix for PAHs

The correlation coefficients of the PAHs in Table 3 indicated that out of 136 possible pairs of different congeners, 75 pairs (55.1%) were very strongly positively correlated in the range of 82 and above, while 14 pairs (10.3%) had fairly strong positive correlations between 70 and 81. There was no negative correlation among the 136 pairs. It could therefore be concluded that the PAHs in the soft drinks were contributed by the same factors that might basically include the ingredients used, the processing method of the ingredients and the bottling procedure with the containers used for packaging contributing little or no PAHs.

Health Risk Assessment of Polycyclic Aromatic Hydrocarbons in Soft Drinks

Health risk of PAHs in the soft drinks (Table 4) was evaluated in terms of dietary daily intake (DDI) and carcinogenic potencies [B(a)Pteq]. These were done because of the high rate of consumption of soft drink products all over the world. The United States Environmental Protection Agency (USEPA, 2002) stipulates that a dietary daily intake (DDI) value of PAHs greater than 1 is considered as not safe for human health. In the analyzed soft drink samples, all the DDI values were below the stipulated value, thus no immediate health infarctions arising from the PAHs content of the soft drinks is envisaged. Values of carcinogenic potencies, BaP_{teq}, were several folds higher in all the plastic bottled soft drink samples than in the glass bottled soft drink samples. This observation is suggestive of the fact that there is a higher tendency of those regularly consuming plastic bottled soft drinks to be more prone to cancer cases in the future.

Table 2: Levels of PAHs in the Soft Drink Samples

	Plastic Bottled Soft Drinks							Glass Bottled Soft Drinks						
PAH	A	B	C	D	E	F	*LoP	A'	B'	C'	D'	E'	F'	*LoP'
Naphthalene	ND	0.88	ND	0.02	ND	ND	0.90	ND	ND	ND	0.03	ND	0.31	0.34
2-methyl naphthalene	ND	0.03	ND	ND	ND	ND	0.03	ND	ND	ND	ND	ND	ND	ND
Acenaphthylene	0.04	0.19	0.02	0.06	0.01	0.01	0.33	0.01	0.04	0.02	0.09	0.06	0.08	0.30
Acenaphthene	0.33	0.95	0.01	0.01	0.01	0.01	1.32	0.01	0.05	0.02	0.66	0.01	0.21	0.96
Fluorene	0.04	0.29	0.01	0.03	0.05	0.01	0.43	0.02	0.14	0.03	0.26	0.03	0.05	0.53
Anthracene	0.22	0.46	0.05	0.20	0.12	0.07	1.12	0.15	0.25	0.16	0.46	0.16	0.31	1.49
Phenanthrene	0.17	0.35	0.04	0.15	0.09	0.06	0.86	0.11	0.19	0.12	0.35	0.12	0.24	1.13
Fluoranthene	0.03	0.12	0.02	0.06	0.03	0.01	0.27	0.02	0.04	0.02	0.08	0.02	0.07	0.25
Pyrene	0.24	0.88	0.20	0.74	0.26	0.12	2.44	0.36	0.46	0.02	0.95	0.31	0.59	2.69
Chrysene	0.07	0.17	0.04	0.15	0.08	0.01	0.52	0.05	0.06	0.36	0.11	0.04	0.07	0.69
Benz(a)anthracene	0.05	0.08	0.02	0.09	0.05	0.01	0.30	0.02	0.02	0.02	0.05	0.01	0.03	0.15
Benzo(k)fluoranthene	0.11	0.16	0.04	0.37	0.11	0.01	0.80	0.05	0.08	0.02	0.19	0.05	0.11	0.50
Benzo(b)fluoranthene	0.03	0.10	0.01	0.08	0.04	0.01	0.27	0.02	0.02	0.02	0.07	0.02	0.03	0.18
Benzo(a)pyrene	0.24	0.76	0.06	0.56	0.15	0.03	1.80	0.25	0.30	0.25	0.87	0.20	0.46	2.33
Indeno(1,2,3-cd) pyrene	0.14	0.24	0.03	0.43	0.05	0.01	0.90	ND	0.17	ND	0.30	ND	0.23	0.70
Dibenz(a,h)anthracene	0.04	0.05	0.01`	0.15	0.01	ND	0.26	ND	ND	ND	0.09	ND	ND	0.09
Benzo(g,h,i)perylene	0.01	0.59	0.01	0.19	0.01	ND	0.81	0.09	0.12	0.11	0.54	0.08	0.21	1.15
Total load of PAHs (ΣPAHs)	1.76	6.30	0.57	2.67	1.07	0.37	13.36	1.16	1.94	1.17	5.10	1.11	3.00	13.48
Range	ND-0.33	0.03-0.88	ND-0.20	ND-0.74	ND-0.26	ND-0.12	0.03-2.44	ND-0.36	ND-0.46	ND-0.36	ND-.95	ND-0.31	ND-0.59	ND-2.69
Mean ± s. d.	0.10	0.37	0.03	0.19	0.06	0.02	0.79	0.07	0.11	0.07	0.29	0.06	0.17	0.77
	±0.10	±0.32	±0.05	±0.21	±0.07	±0.03	±0.62	±0.10	±0.13	±0.11	±0.30	±0.09	±0.17	±0.79
Coef. of Variation (CV)	100	86	167	111	117	150	78	143	118	157	103	150	100	103

*LoP = Level of prevalence

Table 3: Pearson correlation matrix for PAHs

	NAP	2MNAP	ACPLC	ACP	FLR	ANT	PHR	FNT	PYR	CHR	BaA	BbA	BkF	BaP	IP	DahA	BP
NAP	1.00																
2MNAP	1.00**	1.00															
ACPLC	0.97**	0.96**	1.00														
ACP	0.94**	0.94**	0.93**	1.00													
FLR	0.99**	0.99**	0.96**	0.95**	1.00												
ANT	0.90*	0.89*	0.96**	0.94**	0.93**	1.00											
PHR	0.90*	0.90*	0.96**	0.94**	0.93**	1.00**	1.00										
FNT	0.92**	0.91*	0.97**	0.86*	0.93**	0.95**	0.94**	1.00									
PYR	0.74	0.73	0.86*	0.65	0.74	0.83*	0.82*	0.94**	1.00								
CHR	0.67	0.66	0.80	0.62	0.71	0.83*	0.82*	0.91*	0.97**	1.00							
BaA	0.48	0.47	0.65	0.47	0.54	0.74	0.72	0.78	0.90*	0.97**	1.00						
BbA	0.12	0.10	0.34	0.07	0.15	0.40	0.38	0.50	0.75	0.79	0.89*	1.00					
BkF	0.74	0.72	0.85*	0.66	0.77	0.87*	0.85*	0.94**	0.98**	0.98**	0.93**	0.75	1.00				
BaP	0.78	0.76	0.90*	0.74	0.79	0.91*	0.90*	0.96**	0.98**	0.97**	0.90*	0.71	0.98**	1.00			
IP	0.29	0.27	0.52	0.27	0.30	0.56	0.54	0.63	0.84*	0.84*	0.90*	0.97**	0.81*	0.82*	1.00		
DahA	0.08	0.06	0.32	0.05	0.09	0.36	0.34	0.44	0.71	0.72	0.82*	0.98**	0.67	0.68	0.98**	1.00	
BP	0.96**	0.95**	0..99**	0.87*	0.94**	0.92**	0.92**	0.98**	0.90*	0.83*	0.67	0.40	0.88*	0.91*	0.55	0.36	1.00

**Correlation is significant at the 0.01 level (2-tailed); *Correlation is significant at the 0.05 level (2-tailed).

NAP	=	Naphthalene	CHR	=	Chrysene
2MNAP	=	2-Methylnaphthalene	BaA	=	Benzo[a]anthracene
ACNAP	=	Acenaphthylene	BbF	=	Benzo[b]fluoranthene
ACP	=	Acenaphthene	BkF	=	Benzo[k]fluoranthene
FLR	=	Fluorene	BaP	=	Benzo[a]pyrene
ANT	=	Anthracene	IP	=	Indeno[1,2,3-cd]pyrene
PHE	=	Phenathrene	DahA	=	Dibenz[a,h]anthracene
FLT	=	Fluoranthene	BP	=	Benzo[g,h,i]perylene
PYR	=	Pyrene			

Table 4: Health Risk Assessment of PAHs in the Soft Drinks

PAHs	TEF	Plastic Bottled Soft Drinks											
		A		B		C		D		E		F	
		DDI (mg/day)	#B(a)P_{teq} (µg/mL)	DDI (mg/day)	#B(a)P_{teq} (µg/mL)	DDI (mg/day)	#B(a)P_{teq} (µg/mL)	DDI (mg/day)	#B(a)P_{teq} (µg/mL)	DDI (mg/day)	#B(a)P_{teq} (µg/mL)	DDI (mg/day)	#B(a)P_{teq} (µg/mL)
NAP*	0.001	-	-	0.308	880	-	-	0.007	20	-	-	-	-
2MNAP*	0.001	-	-	0.011	30	-	-	-	-	-	-	-	-
ACPL*	0.001	0.014	40	0.067	190	0.007	20	0.021	60	0.004	10	0.004	10
ACP*	0.001	0.116	330	0.333	950	0.004	10	0.004	10	0.004	10	0.004	10
FLR*	0.001	0.014	40	0.102	290	0.004	10	0.011	30	0.018	50	0.004	10
ANT*	0.010	0.077	2200	0.161	46000	0.018	500	0.007	2000	0.042	1200	0.025	700
PHR*	0.001	0.060	170	0.123	350	0.014	40	0.053	150	0.032	90	0.021	60
FLT*	0.001	0.011	30	0.042	120	0.007	20	0.021	60	0.011	30	0.004	10
PYR*	0.001	0.084	240	0.308	880	0.070	200	0.259	740	0.091	260	0.042	120
CHR**	0.010	0.025	700	0.060	1700	0.014	400	0.053	1500	0.028	800	0.004	100
BaA**	0.100	0.018	5000	0.028	8000	0.007	2000	0.032	9000	0.018	5000	0.004	1000
BkF**	0.100	0.039	11000	0.056	16000	0.014	4000	0.130	37000	0.039	11000	0.004	1000
BbF**	1.000	0.011	3000	0.035	1000	0.004	1000	0.028	8000	0.014	4000	0.004	1000
BaP**	0.100	0.084	240000	0.266	76000	0.021	60000	0.196	560000	0.053	150000	0.011	30000
IP**	0.100	0.049	14000	0.084	24000	0.011	3000	0.151	43000	0.018	5000	0.004	1000
DahA**	5.000	0.014	200000	0.018	250000	0.004	50000	0.053	750000	0.004	50000	-	-
BP**	0.010	0.004	100	0.207	5900	0.004	100	0.067	1900	0.004	100	-	-
Glass Bottled Soft Drink													
PAHs	TEF	A'		B'		C'		D'		E'		F'	
		DDI (mg/day)	#B(a)P_{teq} (µg/mL)	DDI (mg/day)	#B(a)P_{teq} (µg/mL)	DDI (mg/day)	#B(a)P_{teq} (µg/mL)	DDI (mg/day)	#B(a)P_{teq} (µg/mL)	DDI (mg/day)	#B(a)P_{teq} (µg/mL)	DDI (mg/day)	#B(a)P_{teq} (µg/mL)
NAP*	0.001	-	-	-	-	-	-	0.011	0.003	-	-	0.109	0.031
2-MNAP*	0.001	-	-	-	-	-	-	-	-	-	-	-	-
ACPL*	0.001	0.004	0.001	0.014	0.004	0.007	0.002	0.032	0.009	0.021	0.006	0.028	0.008
ACP*	0.001	0.004	0.001	0.018	0.005	0.007	0.002	0.231	0.066	0.004	0.001	0.074	0.021
FLR*	0.001	0.007	0.002	0.049	0.014	0.011	0.003	0.091	0.026	0.011	0.003	0.018	0.005
ANT*	0.010	0.053	0.150	0.088	0.250	0.056	0.160	0.161	0.460	0.056	0.160	0.109	0.310
PHR*	0.001	0.039	0.011	0.067	0.019	0.042	0.012	0.123	0.035	0.042	0.012	0.084	0.024
FLT*	0.001	0.007	0.002	0.014	0.004	0.007	0.002	0.028	0.008	0.007	0.002	0.025	0.007
PYR*	0.001	0.126	0.036	0.161	0.046	0.007	0.002	0.333	0.095	0.109	0.031	0.207	0.059
CHR**	0.010	0.018	0.020	0.021	0.600	0.126	0.360	0.039	0.110	0.014	0.040	0.025	0.070
BaA**	0.100	0.007	0.500	0.007	0.020	0.007	0.200	0.018	0.050	0.004	0.100	0.011	0.300
BkF**	0.100	0.018	0.500	0.028	0.800	0.007	0.200	0.067	0.019	0.018	0.500	0.039	0.011
BbF**	1.000	0.007	0.200	0.007	0.200	0.007	0.200	0.025	0.070	0.007	0.200	0.011	0.030
BaP**	0.100	0.088	2.500	0.105	0.030	0.088	25.00	0.305	0.087	0.070	0.020	0.161	0.046
IP**	0.100	-	-	0.060	0.017	-	-	0.105	0.030	-	-	0.081	0.023
DahA**	5.000	-	-	-	-	-	-	0.032	4.500	-	-	-	-
BP**	0.010	0.032	0.090	0.042	0.120	0.039	0.110	0.189	0.540	0.028	0.080	0.074	0.210

TEF values for the PAHs were adopted from Nisbet and LaGoy (1992).

* Non-Carcinogenic PAHs; ** Carcinogenic PAHs (EPA, 2008)

#Values are x 10^{-2}

Carcinogenic Toxic Equivalents of PAHs in the Soft Drink Samples

The carcinogenic toxic equivalents (TEQ) approach was implemented to further gain an insight into level of the carcinogenicity of the combined PAHs contamination of the soft drink samples. The TEQ values in Table 5 indicated that D (1.00) > B (0.43) > A (0.31) > E (0.14) > C (0.08) > F (0.02) in the plastic bottled samples, while in the glass bottled samples, the order was D' (0.06) > A' (0.04) ≈ C' (0.04) > B' (0.02) > E' (0.01) ≈ F' (0.27). The results of TEQ clearly indicated that regular consumption of D could be accompanied with highest potential for carcinogenic risks than when other soft drinks are consumed over a period of time. Also, TEQ values indicated that consumers of plastic bottled soft drinks could be at a higher risk of PAHs inflicted health hazards over a prolonged regular consumption.

Table 5: Estimated Carcinogenic Risk Indices of PAHs in the Soft Drink Samples

	Plastic bottled soft dr inks					
Carcinogenic Risk Index	A	B	C	D	E	F
ΣDDI (mg/day)	0.62	2.21	0.20	1.09	0.38	0.13
ΣDDI for carcinogenic PAHs (mg/day)	0.24	0.75	0.08	0.71	0.18	0.03
TEQ (μg/mL)	0.31	0.43	0.08	1.00	0.14	0.02
	Glass bottled soft drinks					
Carcinogenic Risk Index	A'	B'	C'	D'	E'	F'
ΣDDI (mg/day)	0.45	0.69	0.43	1.80	0.35	1.01
ΣDDI for carcinogenic PAHs (mg/day)	0.19	0.28	9.96	0.80	0.09	0.39
TEQ (μg/mL)	0.04	0.02	0.04	0.06	0.01	0.01

CONCLUSION

Polycyclic aromatic hydrocarbons in plastic and glass bottled soft drinks were evaluated in this study. Varying concentrations of PAHs were observed in the soft drink samples with the highest concentrations of PAHs in sample B. Generally, the concentrations of pyrene and benzo(a)pyrene in the six soft drink samples were significantly higher than the other congeners. The observed values of estimated Dietary Daily Intake (DDI) for the total PAHs and carcinogenic toxic equivalents (TEQ) in the soft drink samples indicated that there might be no immediate PAHs associated health challenges for those who consume the drinks occasionally. However, regular and prolonged consumption could translate to higher risk of exposure and subsequently result to carcinogenic effects in humans.

REFERENCES

Hussein, I. A. and Mona, S. M. M. 2015. A review on polycyclic aromatic hydrocarbons: Source, environmental impact, effect on human health and remediation, *Egyptian Journal of Petroleum*, 25(1): 107 - 123.

Agency for Toxic Substances and Disease Registry (ATSDR). 1995. Public Health Statement for Naphthalene, 1-Methylnaphthalene, and 2-Methylnaphthalene. US Department of Health and Human Services, Public Health Service, Atlanta, GA.

Agency for Toxic Substances and Disease Registry (ATSDR). 2005. Toxicology Profile for Polycyclic Aromatic Hydrocarbons, US Department of Health and Human Services, Public Health Service, Atlanta, GA.

Armstrong, B., Hutchinson, E., Unwin, J. and Fletcher, T. 2004. Lung cancer risk after exposure to polycyclic aromatic hydrocarbons: a review and meta-analysis, *Environmental Health Perspectives*, 112: 970 - 978.

Ashurst, P. R. 2004. Chemistry and Technology of Soft Drinks and Fruit Juices, 2nd Edn, Blackwell Publishing, Oxford.

Baxter, C. S., Hoffman, J. D., Knipp, M. J., Reponen, T. and Haynes, E. N. 2014. Exposure of firefighters to particulates and polycyclic aromatic hydrocarbons, *Journal of Occupational and Environmental Hygiene*, 11: D85 - D91.

Binnie, C., Kimber, M. and Smethurst, G. 2002. Basic Water Treatment, 3rd edition, Royal Society of Chemistry, London.

Burchiel, S. W. and Luster, M. I. 2001. Signaling by environmental polycyclic aromatic hydrocarbons in human lymphocytes, *Clinical Immunology*, 98: 2 - 10.

Butler, J. P., Post, G. B., Lioy, P. J., Waldman, J. M. and Greenberg, A. 1993. Assessment of carcinogenic risk from personal exposure to benzo(a)pyrene in the total human environmental exposure study, *J. Air Waste Manage. Association*, 43: 970 - 977.

Cavalieri, E. L., Rogan, E. and Cremonesi, P. 1988. Tumorigenicity of 6-halogenated derivatives of benzo[a]pyrene in mouse skin and rat mammary gland, *J. Cancer Res. Clin.Oncol.* 114: 10 - 15.

Chrysikou, L., Gemenetzis, P., Kouras, A., Manoli, E., Terzi, E. and Samara, C. 2008. Distribution of persistent organic pollutants, polycyclic aromatic hydrocarbons and trace elements in soil and vegetation following a large scale landfill fire in Northern Greece, *Environment International*, 34: 210 - 225.

Clancy, J. L., Marshall, M. M. and Dyksen, J. E. 1998. UV light inactivation of cryptosporidium oocysts, *Journal of the American Water Works Association*, 90(9): 92 - 102.

Ding, C., Ni, H. and Zeng, H. 2012. Parent and halogenated polycyclic aromatic hydrocarbons in rice and implications for human health in China, *Environmental Pollution*, 168: 80 - 86.

European Food Safety Authority (EFSA). 2008. Polycyclic aromatic hydrocarbons in food: Scientific opinion of the Panel on Contaminants in the food chain. European Food Safety Authority, (EFSA-Q-2007-136), 724: 1 - 114

European Commission (EC).1999. Official Journal of the European Communities. L84,Vol. 42. Register of Flavouring Substances, Regulation (EC) No. 2232/96.

European Union. (1999). European Union Directive 1999/74/EC. Laying down minimum standards for the protection.

Gleick, P. H. 1996. Water resources. In: Schneider, S. H. (Ed.) Encyclopaedia of Climate and Weather, 2: 817 - 823. Oxford University Press, New York.

Guillen, M. D., Sopelana, P. and Partearroyo, M. A. 1997. Food as a source of polycyclic aromatic carcinogens, *Rev. Environ. Health*, 12: 133 - 146.

Halek, F., Nabi, G. and Kavousi, A. 2007. Polycyclic aromatic hydrocarbons study and toxic equivalency factor (TEFs) in Tehran, Iran, *Environmental Monitoring and Assessment*, 143: 303 - 311.

Hofman. T., Rothe, M. and Schieberle, P. 2001. State-of-the-art in flavour chemistry and biology, *Proceedings of the 7th Wartburg Symposium,* 139 - 145.

International Agency for Research on Cancer (IARC). 1973. Certain polycyclic aromatic hydrocarbons and heterocyclic compounds. Monograph on the Evaluation of Carcinogenic Risks of the Chemical to Man, 3: 54 - 78.

International Agency of Research on Cancer (IARC). 2010. Some non-heterocyclic polycyclic aromatic hydrocarbons and some related exposures. IARC Monographs on the Evaluation of Carcinogenic Risks to Human, 92: 1 - 853.

International Programme on Chemical Safety (IPCS). 2010. WHO Human Risk Assessment Toolkit: Chemical Hazards, pp. 88.

King, S., Meyer, J. S. and Andrews, A. R. J. 2002. Polycyclic aromatic hydrocarbons in soil using hollow fibermembrane solvent microextraction, *J. Chromatogram. A*, 982: 201-208.

Kuppusamy, S., Thavamani, P., Megharaj, M. and Naidu, R. 2016. Biodegradation of polycyclic aromatic hydrocarbons (PAHs) by novel bacterial consortia tolerant to diverse physical settings - Assessments in liquid- and slurry phase systems, *International Biodeterioration and Biodegradation*, 108: 149 - 157.

Luo, P., Bao, L. J., Li, S. M. and Zeng, E. Y. 2016. Size-dependent distribution and inhalation cancer risk of particle-bound polycyclic aromatic hydrocarbons at a typical e-waste recycling and an urban site, *Environmental Pollution*, 200: 10 - 15.

Mitchell, A. J. 1990. Formulation and Production of Carbonated Soft Drinks.Blackie, Glasgow and London.

National Institute for Occupational Safety and Health (NIOSH). 1997. Elements of Ergonomics Programs, CDC Pub. No. 97 - 117.

Nisbet, I. C. T. and Lagoy, P. K. 1992. Toxic equivalency factors (TEFs) for polycyclic aromatic hydrocarbons (PAHs), *Regulatory Toxicology and Pharmacology*, 16: 290 - 300.

Olanrewaju, A. N., Ajani, E. K. and Kareem, O.K. (2017). Physico-Chemical Status of Eleyele Reservoir, Ibadan, Nigeria, *J. Aquac. Res. Development*, 8: 512. doi: 10.4172/2155 -9546.1000512 [accessed Oct. 18, 2019].

Ortuno, N., Conesa, J. A., Molto, J. and Font, R. 2014. Pollutant emissions during pyrolysis and combustion of waste printed circuit boards, before and after metal removal, *Science of the Total Environment*, 499: 27 - 35.

Oyekunle, J. A. O., Ogunfowokan, A. O., Torto, N. and Akanni, M. S. 2011. Determination of organochlorine pesticides in the agricultural soil of Oke-Osun farm settlement, Osogbo, Nigeria, *Environ. Monit. Assess.* 177: 51 - 61.

Prato, T. A. and Parent, R. G. 1993. Nitrate and nitrite removal from municipal water supplies with electro-dialysis, Proceedings of the American Water Works Association Membrane Conference.

Riley, J. J. 1958. A History of the American Soft Drink Industry – Bottled Carbonated Beverages. American Bottlers of Carbonated Beverages, Washington DC.

Saltmarsh, M. 2000. Essential Guide to Food Additives.Leatherhead Publishing, Surrey, UK.

Santucci, K. and Shah, B. 2000. Association of naphthalene with acute hemolytic anemia, *Academic Emergency Medicine*, 7(1): 42 - 47.

Simko, P. 2002. Determination of polycyclic aromatic hydrocarbons in smocked meat products and smoke flavouring food additives, *J. Chromatogram. B*, 770: 3 - 18.

Spacie, A., Landrum, P. F. and Laversee, G. J. 1983. Uptake, depuration and biotransformation of anthracene and benzo[a]pyrene in bluegill sunfish, *Ecotoxicol. Environ. Saf.*, 7(3): 330 - 341.

U. S. Environmental Protection Agency (EPA). 1993. A case review of ecological assessment case studies from a risk assessment perspective, Washington, DC: Risk Assessment Forum, EPA/630/R-92/005.

U. S. Environmental Protection Agency (USEPA). 2002. Region 9, preliminary remediation goals.

U. S. Environmental Protection Agency (EPA). 2008. EPA's 2008 Report on the Environment. National Center for Environmental Assessment, Washington, DC; EPA/600/R-07/045F.

Unwin, J., Cocker, J., Scobbie, E. and Chamber, H. 2006. An Assessment of Occupational Exposure to Polycyclic Aromatic Hydrocarbons in the UK, *The Annals of Occupational Hygiene*, 50(4): 395 - 403.

Vaux, B. 2011. What is your generic term for a sweetened carbonated beverage? Harvard Dialect Survey, Accessed: 26/10/2013.

Warshawsky, D., Barkley, W. and Bingham, E. 1993. Factors affecting carcinogenic potential of mixtures, *Fundam. Appl. Toxicol.*, 20: 376 - 382.

American Institute for Cancer Research (AICR). 2007. World Cancer Research Fund and American Institute for Cancer Research Food, Nutrition, Physical Activity and the Prevention of Cancer: A Global Perspective. Washington, DC

Yamada, J., Wang, L., Fu, P. P. and Yu, H. 2013. Photomutagenicity of 16 Polycyclic aromatic hydrocarbons from the USEPA priority pollutant list, *Mutation Research*, 557: 99 - 108.

Yang, S. K. and Silverman, B. D. 1988. Polycyclic aromatic hydrocarbon carcinogenesis: Structure activity relationships. CRC Press, Boca Raton, FL.

Yang, M., Kim, S., Lee, E., Cheong, H. K., Chang, S. S., Kang, D., Choi, Y., Lee, S. M. and Janh, J. Y. 2003. Sources of polycyclic aromatic hydrocarbons exposure in non-occupationally exposed Koreans, *Environ. Mol. Mutagen.*, 42: 250 - 257.

Zhou, B. and Zhao, B. 2012. Population inhalation exposure to polycyclic aromatic hydrocarbons and associated lung cancer risk in Beijing region: Contributions of indoor and outdoor sources and exposures, *Atmospheric Environment*, 62: 472 - 480.