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Assessment of Phthalates and Bisphenol-A in Plastic Packaged Water and Evaluation of Risk

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Abstract

Bisphenol-A and phthalates are moderately water- soluble compounds synthetic chemicals that are classified as endocrine disruptors used in the production of polyethylene bottles and other packaging materials. The present study was designed to perform a trace analysis of the two plasticizers in different brands of polyethylene-packaged water samples. Methylene dichloride was used to extract the analytes, which were then analysed for BPA and phthalate esters using high performance liquid chromatography (HPLC) and gas chromatography with mass spectrometry (GC- MS), respectively. BPA was found in several samples, however its mean concentration ranged from 554.14±44.11 to 1497.5±2.18 µg/L. The phthalates had a concentration range of 12740±1070.4 to 130±2.32 µg/L across the different water brands. Bis (2-ethylhexyl) phthalate (DEHP) and di-n-butylphthalate (DBP) noted for their embryotoxic effects were the most prevalent phthalates in the samples and they exceeded the USEPA and WHO limits in almost all the brands. While the concentrations observed in some packaged water brands were above regulatory limits, no significant health risk was observed for the regular ingestion of BPA and phthalates in the samples as the HQs for the various brands were less than one.

Keywords: Bis (2-ethylhexyl) phthalate, bottled water, BPA, di-n-butylphthalate, plasticizers, and polyethylene bottles.

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Introduction

Plastic materials are used widely in consumer goods and the food industry both for primary and secondary packaging. Depending on the container's capacity, specific polymers are used to produce bottles in the bottling industry. Each of these polymers has specific properties that affect bottle strength, storage period, dispenser type, and disposal. According to the World Packaging Organization (WPO) (2008), plastic stoppers are typically made of low-density polyethylene (LDPE), high-density polythene (HDPE), and polystyrene (PS), with major packaging usually made of high-density polyethylene (HDPE), polyethylene terephthalate (PET), and polycarbonate (PC). These plastic packaging materials are made flexible and durable by the addition of phthalates and bisphenol synthetic chemicals (Koch & Calafat, 2009). The increasing use of phthalates, BPA and nanomaterials in food packaging materials has heightened the threat to food safety concerns as these chemical contaminants leach from packaging and storage containers into food items (Brownlow, 2021). The migration of these compounds into foods is enhanced by a lower pH of the water stored, higher temperature, abrasion and use of cleaning agents for the packaging material (Lane et al., 2015). These high molecular weight compounds and their metabolites have been the sources of xenobiotics, particularly those with endocrine disrupting properties. They have been detected in the urine, and are also associated with widespread adverse health effects associated with their ability to upset the endocrine system (Romano et al., 2015; Gore et al., 2015). Other health consequences of the presence of BPA and phthalates in water include

mutagenicity, carcinogenicity, reproductive impairment, immunity and neurological effects that may result in congenital anomalies and developmental disabilities in children (Elisabet et al., 2016; Bakare, 2021). Hence, the move by the Lagos state government of Nigeria and other nations to interdict the packaging and sale of plastic bottled drinks (Solebo, 2022).

Bisphenol-A (2,2-di(p-hydroxyphenyl) propane) is a slightly water-soluble compound at ambient temperature with low volatility and a high melting point (Staples et al., 1998). It dissolves in alkaline matrices with rapid photo-oxidation and possesses moderate bio-accumulation (EPA, 2010; Heinonen et al., 2002). It has been detected and reported in several environmental matrix including wildlife and humans and packaged foods and water (Xu, Zhou, Lei, Leblanc & An, 2020; Yun, Ho, Tan & How, 2018; Fan et al., 2014; Taylor et al., 2013; Elobeid et al., 2012). By condensing phenol and acetone in the presence of a catalyst, bisphenol-A is synthesised. It has a core tetrahedral carbon atom and two methyl and benzyl hydroxyl groups on either side (Fig. 1(a)). In the area of water policy, BPA is classified as a potential significant substance that should be examined. The ester bonds in BPA-based polymers do hydrolyse and, therefore, BPA migrates into packaged items from their storage containers. Heat and acids speed up the migration process, and repeated abrasion of polycarbonate products have all been shown to increase the rate of migration of BPA (vom Saal et al., 2007).

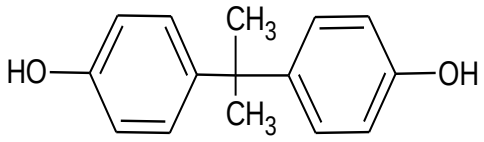

Fig. 1(a): Bisphenol-A

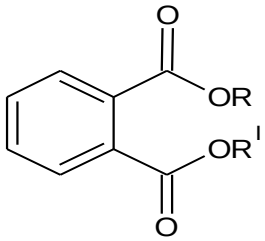

Fig. 1(b) Phthalates

Phthalates are synthetic carbon-based chemicals (Fig. 1(b)) that include di-n-butylphthalate (DBP), diethylphthalate (DEP), diiso nonylphthalate (DiNP), butylbenzylphthalate (BBzP), di-(2-ethylhexyl) phthalate (DEHP), diisododecylphthalate (DiDP) and di-n-octylphthalate (DnOP). The immense industrial success arising from the adding of DEHP to polyvinyl chlorides (PVC) to increase flexibility, led to an aggressive exponential application and usability in solvent manufacture, ink, wax, adhesive, cosmetic, pharmaceuticals, insecticide and cellulose packaged food production. They can migrate to the food in which they are in contact because they are unbound with the plastics, and have been the main source of exposure of many phthalates in human - packed foods, mainly fatty foods (Fasano et al., 2012). Phthalates are rapidly hydrolysed to the monoesters in humans, and further metabolised and excreted with urine and feces. They have, however, been detected in the breast milk, amniotic fluids, and serum (Kueseng et al., 2007).

Given the tropical nature of the country and her inadequate energy supply for food storage, the heat generated enhances the release of these endocrine disruptors into packaged foods. This study was therefore designed to assess the relative concentrations of BPA and phthalates in different brands of PET-bottled water and their associated health risks.

Materials and Methods

Reagents: The methylene dichloride was from Sigma-Aldrich (Laborchemikalien GmbH. D-30926), HPLC (Agilent 1290 infinity), rotary evaporator (Bibby sterilin Ltd. Staffordshine, ST15 0SA, UK), gas

chromatograph (Agilent 8860A) coupled with inert mass spectrometer (5977C) with ion impact source were from Agilent technology.

Sample collection: Nine brands of popular PET-bottled water were randomly purchased from popular outlets in Ilaro, Ogun State, Nigeria. The samples were stored in the refrigerator prior to solvent extraction.

Analytes extraction and clean-up: The Elobeid et al. (2012) method was modified slightly for the extraction of phthalates and bisphenol-A. 500 mL of the material was shaken in a separatory funnel with 30 mL of methylene dichloride to perform liquid-liquid extraction. The extracts were combined and reduced to 2 mL in a rotary evaporator after being extracted three times in a row. The phthalates were examined using a gas chromatograph connected to a 5977C triple-axis detector inert mass spectrometer with an electron-impact source in the SIM mode, while the BPA in the extracted samples was examined using an HPLC chromatograph. The gas chromatograph had an HP-5 capillary column and He as the carrier gas. The flow was 1.2 mL/min at an initial pressure and average velocity of 10.26 psi and 40.00 cm/sec, respectively, and sample injection was in splitless mode at 250°C. The total and pure flow to the spilt vent were 31.24 and 30.0 mL/min, respectively @ 0.35 min. The oven was set to run for 12 minutes with a 3-minute solvent delay after ramping up to 100 °C for 1 minute, then 280°C for 7 minutes. The quadrupole, ion source, and transfer line temperatures were 150, 230, and 300°C, respectively. The spectrometer was operated in the electron-impact ionisation mode at 70 eV. Using the SIM, the ions were scanned and captured between 50 and 500 amu at 2.0 s/scan. The GC-MS was calibrated with phthalates ester standards (0.3, 0.6, 1.5, and 3.00 mg/L) made from a 2000 mg/L stock. The samples were examined after calibration, and the corresponding phthalate esters were quantified.

Determination of Hazards Quotient: This is the share of the probable exposure to a contaminant and the level at which no adverse effects are expected. It is determined with

$$\mathbf{HQ} = \frac{CDI}{RfD} \qquad \textbf{Eqn. 1}$$

$$\mathbf{CDI} = \frac{C \times IR \times EF \times ED}{BW \times AT} \qquad \textbf{Eqn. 2}$$

as described by Xu et al., 2020. Where; R_fD – reference dose (0.05 and 0.4 mg/kg/day for BPA and phthalates, respectively), CDI - chronic daily intake (mg/kg/day), IR – ingestion rate (2 Litres/day), EF – exposure frequency (365 days/year), ED – exposure duration (30 years), BW - body weight (70 kg) and AT – ED*365 (10950) (EPA, 1989). When HQ < 1, adverse effects are not likely to occur, thus it can be considered to be negligible while HQ > 1 is not a statistical probability of harm occurring. Rather, it is a simple statement of whether an exposure concentration exceeds the reference dose (R_fd).

Results and Discussion

The results of this study, as summarised in Table 1, agreed with and are sometimes higher than those previously reported from other locations. The maximum concentration of BPA in the PET-bottled water was 1497.5±2.18 µg/L, and it was not detected in some samples. The highest mean concentration of the phthalates was 12740±1070.4 µg/L and the minimum mean concentration was 120 ±2.48 µg/L. The maximum level of BPA in the water reported in previous works across Africa was 251 µg/L while 224 µg/L was specifically reported for Nigeria (Rotimi et al., 2021). These concentration values are lower than those obtained in the current study because over the years Nigeria's share of total plastic consumption in Africa has increased with the increased packaging of water in PET bottles with very little regulatory oversight. The high concentration values of phthalates, particularly the DEHP and bisphenol A in this study was asserted by Buckley et al. (2019) where they expressed concern over the increased phthalates exposure and bisphenol A from ultra-processed food consumption because of packaging, thus, becoming a growing health concern because of the outcomes related to their wide-ranging adverse health effects. The most ubiquitous of the phthalates were DBP and DEHP most frequently used as plasticizers, because they can easily leach into foods and beverages from plastic containers since they are only physically bound to the polymer chains (Kueseng, Thavarungkul &

Kanatharana, 2007). Worrisome is the fact that DEHP in the current study exceeded the 8.0 and 6.0 µg/L established limits by WHO and USEPA, respectively (Xu et al., 2020). They are noted for their embryotoxic effects, and the aggregated dietary exposure to the two chemicals is 1.6–11.7 and 0.9–7.2 µg/kgbw per day for high and mean consumers, respectively (Silano et al., 2019). DEHP has been reported in curry samples from Thailand at a concentration of 0.12 – 0. µg/g, and the concentration tends to increase with storage time. Similar results were reported for different packaged foods from Italy, DEHP and BPA had the highest contamination values but still within regulatory limits (Fasano et al., 2012). Dimethyl phthalate was found in only two samples. The concentration of BPA and phthalate in water brands from PET bottles after outdoor exposure for 10 weeks at temperatures up to 30°C, by Xu et al. (2020) was 101.97 to 709.87 µg/L. Six phthalate esters, including DEP, DMP, DBP, BBP, DOP and the ubiquitous DEHP, were reported at a concentration of 0.19 to 0.98 µg/L under outdoor storage conditions. A careful examination of these results shows that they are lower than the results in our present study. The difference in results is highly connected to the nature and composition of the PET bottles and the severe temperature of our sampling location being tropical. In the same vein, the results from the current study are also higher than the phthalates and BPA of the polycarbonate bottles exposed to sunlight reported by Leena, Hassn and Abdalla, (2016). Notwithstanding the exposure to sunlight that accelerates the leaching of endocrine disruptors into the water, the concentration (4.3×10^{-3} to 0.01 µg/L) reported by the authors was lower than our current study, though some samples showed no BPA. The BPA in the current study in some bottled water samples had higher concentration values than the concentration of BPA (3.30 to 0.0113 µg/L) in bottled water samples stored at an average of 50^{o}C for three days in Malaysia notwithstanding the elevated storage temperature (Santhi et al., 2012). The 7.9×10^{-3} and 3×10^{-3} µg/L of BPA in outdoor water samples from Brazil and Spain, respectively, after several weeks of exposure to sunlight at 50°C also had lower BPA levels than what obtained in this study (Elobeid et al., 2012; Casajuana & Lacorte, 2003). The authors, however, observed that the BPA concentration in the different samples increased with storage time and temperature rise (Westerhoff et al., 2008). High temperatures and extreme weather as experienced in this part of the world increase the migration of BPA and phthalate into drinking water, this assertion was strengthened by Rotimi et al. (2020) and Xu et al. (2020) that the migration of plasticizers into packaged foods is affected by the nature of packaging material, storage conditions, source contamination during processing in plants from the usage of plastic pipes, heat treatment, and contamination during the analytical procedures arising from the use of plastic apparatuses.

Table 1: The amount of BPA and phthalate esters found in PET water samples (µg/L)

Samples	DMP	DEP	DBP	BBP	DEHP	DOP	Total phthalate	BPA
1	ND	30±2.65	260±13.23	20±3.46	20±4.36	50±5.00	380±103.6	ND
2	ND	20±4.36	180±51.96	60±10	220±62.45	200± 20	680±89.9	700.8±0.78
3	20±2.65	120±20	6040±1005.04	ND	5300±15	30 ±2.65	11510±3085.9	1497.5±2.18
4	ND	20±4.58	130±14.80	10±2.22	150±21.79	60± 23.98	370±21.53	554.14±44.11
5	ND	10±1.79	40±2.35	10±0.56	20±2.11	40 ±2.06	120 ±2.48	677.95±25.02
6	30±1.86	470±30.51	5470±1204.37	10±1.65	6730±208.62	30 ±0.85	12740±1070.4	ND
7	ND	10±1.26	70±2.43	10±0.79	430±9.03	60 ± 1.83	580±7.13	599.94±15.40
8	ND	170±1.93	1610±18.41	30±1.94	2140±23.62	40 ± 1.88	3990±36.8	ND
9	ND	10±1.15	40±1.22	10±0.88	50±1.76	20 ± 0.94	130±2.32	ND

Key: ND - Not Detected; **DMP -** dimethyl phthalate; **DEP-** diethylphthalate; **DBP -** di-n-butylphthalate; **BBP -** benzylbutylphthalate; **DEHP -** bis(2-ethylhexyl) phthalate; **DOP -** di-n-octylphthalate; **BPA -** Bisphenol A

Health Risk Assessment

The measured concentrations of BPA and phthalates in the evaluated samples were used to evaluate health assessment using a hazard quotient approach. The magnitude of chronic daily intake (CDI) was compared to the reference dose (R_fD) of BPA and phthalate at 50 and 400 µg/kg/day, respectively. In this study, the highest CDI for BPA and phthalate were 42.9 and 328.9 µg/kg/day, respectively, as shown in Table 2.

Table 2: Health risk assessment of BPA and phthalates in PET bottled water

	BPA		Phthalate	
SAMPLE	**CDI**	**HQ**	**CDI**	**HQ**
1	8.57×10^{-06}	1.71×10^{-07}	10.9	0.027
2	20	0.4	19.4	0.05
3	42.9	0.86	328.9	0.82
4	15.83	0.317	10.57	0.0264
5	19.37	0.387	3.43	8.57×10^{-03}
6	0.029	5.72×10^{-04}	364	0.91
7	17.14	0.34	16.57	0.0414
8	0.0286	5.72×10^{-04}	114	0.285
9	0.0286	5.72×10^{-04}	3.71	9.28×10^{-03}

The HQs associated with the samples under consideration were less than one, which showed no significant chronic non-carcinogenic health risk. Two of the samples, though less than unity, had the highest HQs for BPA and phthalates. Sample 3, had high BPA and phthalate HQs of 0.86 and 0.82, respectively. These values are close to unity and therefore call for regulatory attention with respect to the type of PET bottles used in the packaging and storage conditions to forestall possible negative impacts on the public. Similarly, sample 6 had the highest HQ with respect to the presence of phthalates and presented a very weak HQ for the BPA. The highest CDI in this study is below 50 and 400 µg/L for BPA and phthalate reference doses (R_fD), respectively. The chronic daily intake (CDI) values obtained in this study are similar to the 8.94×10^{-3} µg/kg/day reported for adults in the studies of Yun et al., (2018) and Fasano et al. (2012). The results of the health risk assessment of the phthalates in this study are also comparable to the results by Xu et al., (2020) for adults through the consumption of PET-bottled waters from China.

Conclusions

The various PET bottled water brands contained phthalates such as DEP, DBP, DMP, BBP, DEHP, and DOP. While DMP was detected in only two of the brands, DBP and DEHP had the highest concentration values across the different brands. Bisphenol-A was detected in five of the nine brands in varying concentrations. The concentrations of phthalates in the bottled samples were negligible in some cases while it was high in others. The CDI was below the R_fD by the US EPA standard, and no significant chronic non-carcinogenic health risk may arise due to drinking the water samples since the calculated HQ was less than

for BPA and the total phthalate**.** Additional study is needed to determine the effects of storage temperature, nature of packaging materials and production practices on phthalate and bisphenol levels in packaged water.

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