ORIGINAL RESEARCH PAPER
Volume - 10 | Issue - 10 | October - 2021 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

ESTIMATION OF PHTHALATES IN BOTTLED DRINKING WATER, MANUFACTURED IN INDIA, USING LIQUID CHROMATOGRAPHY MASS SPECTROMETRY (LC-MS/MS)

Environmental Science

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ABSTRACT

Phthalate easters are known endocrine disrupter and possible carcinogen. Studies have carried out in different countries to investigate possible migration of phthalate easters into packaged drinking water and beverages and resultant toxic effect on human health. This study aimed to determine the level of phthalate migration into bottled drinking water, manufactured commercially in India and to identify a possible relationship between the amount and type of phthalate migration. Eight phthalate easters were investigated. The analysis included 375 samples (75 sets of 5 bottles each from 5 manufacturers, having same batch numbers and manufacturing dates) of drinking water packed in 1-Litre bottles made from polyethylene terephthalate (PET). The samples were incubated and analyzed at the Centre of Mass Spectrometry (Analytics Department) of the CSIR-Indian Institute of Chemical Technology, Hyderabad on Agilent 6420 QQQ MS/MS system coupled to Agilent 1290 UPLC pump and Thermo TSQ Altis coupled to Thermo RSLC 3000 system at room temperature (27^{0}C) and two temperatures of extreme conditions representing refrigeration temperature (4^{0}C) and summer outdoor temperature (45^{0}C) at the interval of 0, 30, 60, 120 and 180 days, 180 days (6 months) being the projected self-life for bottled drinking water in India. Of eight investigated phthalate esters, Di-butyl Phthalate (DBP) was detected in 94% and Di-isobutyl phthalate (DiBP) in 80% of samples analyzed. The highest migration of 0.0027 mg/l was recorded from PET bottles to drinking water for DBP, followed by 0.0024 mg/l for DiBP. DEHP (Bis(2-ethylhexyl) phthalate) was detected in 40% of sample sets with maximum concentration of 0.0006 mg/l. DPP (Di-pentyl phthalate) was detected in the least number of samples (21.3%) and its maximum concentration observed was 0.0004 mg/l. Migration of all eight investigated esters were detected in drinking water samples stored for 180 days at the three temperature conditions. In other temperature and storage conditions, frequency of detection varied between 0-66%. This study did not account for the factors like source of raw water, manufacturing process, PET types (virgin or recycled), and composition, etcetera. This is probably reflected in widely varied standard deviation. The phthalate levels measured in these samples pose no risk for human health considering reference dose determined by USEPA, EU and FSSAI, for daily oral exposure to the human population. Nevertheless, the accumulation of small individual quantity taken with time may increase the lifelong phthalate exposure and eventually threaten the exposed person's life. Further studies with larger sample size and variants may be desirable. Also, drinking water quality standards needs to be revisited to include all significant phthalate esters.

KEYWORDS

Bottled drinking water in India, Phthalate esters migration

1. INTRODUCTION

The global bottled water market size was valued at USD 217.66 billion in 2020 and is expected to expand at a compound annual growth rate (CAGR) of 11.1% from 2021 to 2028 to reach USD 505.19 billion by 2028 ***[1].*** The global bottled water consumption has been estimated to have surpassed 111 billion gallons in 2019. India is the third in Asia and the fifth overall leading country in the world with increase of consumption of bottled water from 4553.3 million gallons in 2014 to 6492.7 million gallons in 2019 with 7.9% growth of compound annual growth rates [CAGR] over the period 2014/2019 ***[2].***

The increase in per capita consumption of bottled water in India is attributed to the rise in health awareness and fear of diseases as an aftermath of drinking contaminated tap water, increase in tourism, ease of availability of bottled water and easy portability and convenience provided by bottled water ***[3].*** India packaged bottled water industry is mainly dominated by the players like, Parle Bisleri, Parle Agro, PepsiCo, the Coca Cola Company, Indian Railways, Tata Global, etcetera.

Plastic bottles, manufactured using polyethylene Terephthalate (PET), produced from ethylene glycol and either terephthalic acid or dimethyl terephthalate, is the most used packaging material for drinking water. The strength and permeability properties of PET, its resistance to chemicals and its high degree of transparency are the main factors that make it more applicable ***[4].***

To enhance the quality and properties of plastic materials various chemical agents, such as plasticizers, stabilizers, pigments, catalyst, and softeners are added in plastic processing. Phthalates and phthalate esters are a large group of such compounds used as liquid plasticizers to increase the plastic flexibility ***[5].***

Phthalates are, however, considered as non-intentionally added substances (NIAS) in plastic bottles, that could originate from source water, production process, raw material of packaging, and even background contamination in the laboratory ***[6].***

Packaging, nevertheless, may represent a source of contamination owing to the potential leaching of substances, such as residues from the polymerization process, degradation products, and plastic additives, into food and drinks. Among the contaminants are the phthalic acid esters, also called phthalate esters or phthalates, a group of compounds of similar chemical structure with lipophilic properties ***[7]***.

According to a research, it was found that the daily phthalates intakes estimated from urinary metabolites to be higher in children than their parents ***[8]***. In children incidence such as Childhood insulin resistance ***[9,10]*** and obesity ***[11,12,13]*** has been found due to exposure of phthalate. In adult both men and women presences of phthalates has been detected. Presence of phthalates was associated with low gestational age of foetus, delayed appearance of pubarche in girls ***[14]***, endometriosis ***[15]***. The "testicular dysgenesis syndrome" is one of the major negative impacts of phthalate toxicity in men's characterized by cryptorchidism, hypospadias, undescended testes, reduced anogenital distance, decrease in sperm count or its quality, increase in sterility and testicular cancer ***[9]***.

2. OBJECTIVE OF STUDY

Exposure to phthalates may cause adverse health effects in humans and wildlife. Studies have been carried out by researchers in different countries to investigate phthalates in PET and its possible adverse impact on human health.

This preliminary investigation has been performed to identify and estimate the content of various phthalates in bottled drinking water, manufactured and marketed in India, when stored at different temperature conditions for different periods with object to ascertain the possible phthalates exposure through bottled water among the Indian population.

The target phthalate esters considered for this study were Di-methyl phthalate (DMP), Di-ethyl phthalate (DEP), Di-n-propyl phthalate (DPrP), Di-butyl Phthalate (DBP), Di-isobutyl phthalate (DiBP), Di-pentyl phthalate (DPP), Bis(2-ethylhexyl) phthalate (DEHP) and Di-octyl phthalate (DOP).

3. LITERATURE SURVEY

Migration of phthalates from plastic containers and contamination of bottled drinking water and contamination in surface and ground water as well as aquatic organic contamination has been a matter of concern all over the world for its possible toxic bearings on human health. Researchers and scientist world over are doing research and studies to assess the quantity of ingress of phthalate esters into the drinking water and beverages and its possible health hazard.

Jasna Bošnir, et al. of Croatia ***[4]*** assessed level of phthalate migration from plastic (PET) containers to soft drinks and mineral water and observed that the rate of phthalate migration appears to be influenced also by the drink pH, i.e., the lower the pH value, the greater the phthalate migration. Dimethyl phthalate showed highest migration to preserved drinks as an acidic medium, which might stimulate modification in the composition of plastic containers according to the type and composition of the product. They observed that the mean pool phthalate level and mean pH value in mineral water without preservatives were 20.22 mu g/L and 5.82 +/- 1.26 and noticed that Dibutyl phthalate (DBP) and di-ethyl-hexyl phthalate (DEHP) showed highest rate of migration to the mineral water (56.04 and 43.42 %, respectively). Samples of PET containers were stored at room temperature (22° C) for 30 days.

In a preliminary case study on phthalates exposure and risk, limited in the Indian context in Tamil Nadu, India, Krishna Kumar Selvaraj et al. ***[5]*** observed a substantial increase in phthalates concentration in bottled water with increased shelf life and concluded that total mean phthalates in packed milk (245 ng/g) and raw milk (134 ng/g) showed potential enrichment during "farm to table" process. The study also showcased that among phthalates, the lowest risk was expected from diethyl phthalate, whereas the highest risk was observed for DEHP with cumulative dietary exposure of 0.23 µg/kg bw/day (median).

The migration of di-(2-ethylhexyl) adipate (DEHA), di-(2-ethylhexyl) phthalate (DEHP) and ethylene glycol from PET bottles into the Iranian yogurt drink was studied by Mehdi Farhoodi et al. ***[6]***. It was concluded that the storage temperature had a great effect on the migration of DEHP, DEHA and ethylene glycol. Also increasing storage time resulted in higher concentrations of migrating substances.

Researchers in South China University of Technology, Guangzhou, China ***[7]*** after analyzing literature on five representative phthalates in bottled water across 21 countries performed a potential risk assessment based on human daily intake and estrogenic effect and concluded that phthalates in bottled waters studied would not pose a serious concern to public health. However, the adverse estrogenic effects of phthalates in bottled water from some countries appeared to be significant and therefore, more efforts should be needed to systematically examine the phthalates-related safety of bottled water**.**

José Santana et al ***[8]*** made toxicological assessment of phthalate esters from drinking water in six PET and one glass (the most consumed) commercial brands of bottled water in Portugal and three phthalates, i.e., di-butyl phthalate (DnBP), di-isobutyl phthalate (DiBP), and di (ethyl-hexyl) phthalate (DEHP) was detected therein. The concentration of DEHP was found to be up to five times higher in PET than in glass bottled water though these concentrations did not represent direct risk to human health.

Albert Guart et al. ***[16]*** carried out a comprehensive assessment of the quality of the Spanish bottled water market in terms of migration of plastic components or additives during bottling and during storage and evaluation of the effect of the packaging material and bottle format on the migration potential and observed that target compounds, i.e., 5 phthalates, di-ethyl hexyl adipate (DEHA), alkylphenols and bisphenol-A (BPA) were detected in 5.6% of the data values, with diethyl-hexyl phthalate (DEHP) and bisphenol-A (BPA) being the most ubiquitous compounds detected.

In another study by Paolo Montuori et al. [17], phthalic acid (PhA), bis(2-ethylhexyl) phthalate (DEHP), dimethyl phthalate (DMP), diethyl phthalate (DEP), di-isobutyl phthalate (DiBP) and dibutyl phthalate (DBP) were analyzed in a large number of Italian bottled water samples for assessing human exposure to phthalic acid and phthalate esters from mineral water stored in polyethylene terephthalate and glass bottles and observed that different concentrations of phthalates were nearly twenty times higher in samples bottled in PET than those from glass bottles with total levels of phthalates of 3.52 µg/l and 0.19 µg/l respectively. In addition, no significant correlation was found between the phthalate concentrations and the physicochemical properties of the different water samples, apart from the still/sparkling water parameter for the PET samples, nevertheless slightly higher concentrations were observed for the PET bottled still water samples than for the sparkling water samples, although no explanation has been found yet.

Szilvia Keresztes et al. ***[18]*** studied the leaching of phthalates into carbonated and non-carbonated mineral water samples bottled in 0.5-L, 1.5-L and 2.0-L polyethylene terephthalate (PET) containers belonging to three different water brands commercialized in Hungary and concluded that any of investigated six phthalate esters, namely, di-isobutyl phthalate, di-n-butyl-phthalate, benzyl-butyl phthalate and di(2-ethyl-hexyl) phthalate (DEHP) could be detected in carbonated mineral water samples and DEHP was the most abundant phthalate in the investigated samples. They also concluded that increase in storage period and higher surface/volume ratio boosted phthalate concentration in the samples. Nevertheless, they observed that any clear trend could not establish for phthalate leaching when water samples were kept at higher temperatures (max. 60 °C) because of improper storage condition but established the importance of the quality of PET raw material used to produce the pre-form (virgin vs. polymer containing recycled PET) from the comparatives of phthalate determination measured in the plastic material as well as in the aqueous phase. The researchers also concluded that bottles made with 20-30% w/w recycled PET contained higher DEHP levels than containers made of virgin PET.

E.O. Dada et al. ***[19]*** reviewed Concentrations of Phthalates and Metals in Commercially Packaged Sachet and Plastic Bottled Water Sold in Lagos, Nigeria and concluded that the concentrations of phthalates in sachet water was higher relative to bottled water, which indicated that drinking sachet water may pose higher risk of phthalates exposure.

Maryam Zare Jeddi et al. ***[20]*** analyzed health risk to children in Iran due to concentrations of Phthalates in Bottled Water and concluded that increase in the temperature and the duration of storage affect phthalate migration, but the level of DEHP in bottled water was always very low and not exceed than 26.83% of the maximum concentration limit (MCL) derived by USEPA though DEHP was the most abundant phthalate. They further noted that phthalate migration was not substantial at low temperatures (<25 ºC) and freezing conditions.

The occurrence and concentrations of six common phthalates were investigated in bottled water produced locally in the Egyptian market by Ghada Zaki, Tamer Shoeib. ***[21]***. Significant positive correlation was obtained between the storage time, temperature and the concentration of phthalate compounds detected in the bottled water, indicating possible migration from the PET plastic material as the source. However, the estimated contribution of bottled water consumption to the tolerable daily intake (TDI) levels of the two most abundant phthalates observed for adults and toddlers were far below their respective TDI values and did not exceed 0.16 and 0.72% for DBP and 0.04 and 0.16% for DEHP respectively.

Ihsan Ustun et al. ***[22]*** determined phthalates in beverages (soda, lemonade, cola, mineral water) sold in Turkish markets. The mean phthalate concentrations were determined to be 0.085 and 0.312 mg/L in mineral water. Bis (2-Ethylhexyl) phthalate (DEHP) showed the highest level of migration into beverages.

Iman Al-Saleh et al. ***[23]*** studied the presence of Dimethyl phthalate (DMP), Diethylphthalate (DEP), Di-n-butyl phthalate (DBP), Benzyl butyl phthalate (BBP) and Di-ethyl hexyl phthalate (DEHP) in 10 different brands of bottled water available in Saudi markets and stored under different conditions. Bottled waters stored at 4^0 C contained higher levels of DMP, DEP, BBP and DEHP than those stored at room temperature and outdoors. On the other hand, the levels of DMP, DEP and BBP were significantly lower in bottled waters stored at room temperature than those outdoor. The Researchers noted that temperature and sunlight play a role in the degradation of phthalates within time. However, regardless of storage conditions, all samples did not exceed the maximum established limit of DEHP.

4. APPROACH

Seventy-five numbers of sealed bottled drinking water samples, packaged in One-Litre transparent polyethylene terephthalate (PET) plastic bottles, all having same batch numbers and dates of manufacturing, of each of five different manufacturers, totaling 375 sample bottles, were procured from the local market in city of Hyderabad in India.

All 375 numbers of sample bottles were handed over in one lot to the Analytical Department of the Centre of Mass Spectrometry, CSIR -

Indian Institute of Chemical Technology (CSIR-IICT), Hyderabad who were entrusted with conducting analyses of these bottled drinking water samples at their laboratory using LC-MS/MS and quantifying the investigated analytes after incubating and storing them up to their projected self-life of 180 days at normal room temperature (27°C) and two extreme temperatures of 4°C (refrigeration temperature) and 45°C (outdoor temperature in summer). Incubation and storage of samples, development of methods for analysis and their validation, and providing validated analysis report, etcetera, were within the scope of the CSIR-IICT.

A set of five sample bottles from each manufacturer were analyzed every time for each temperature and storage conditions. The sample water bottles were discarded after taking out requisite amount of water samples for analysis. As such, for every analysis, new sets of five sealed bottles were taken, and no bottle were repeated.

5. MATERIALS AND METHODS

a. Sample collection and sample storage: One-Litre packaged drinking water of same batch and date of manufacturing from 5 different manufactures were procured from market and handed over to CSIR-IICT. Samples bottles were incubated and stored for 180 days at three different temperature conditions, that are, 4°C, 27°C and 45°C. The presence of phthalate esters was estimated on the 0 day at the three temperature conditions and thereafter after incubation and storage for 30 days, 60 days, 120 days, and 180 days at each of three temperature conditions. In totality, 375 numbers of bottled water samples were used for analysis.

b. Instrumental analysis: The LC-MS/MS analysis was carried out on Agilent 6420 QQQ MS/MS system coupled to Agilent 1290 UPLC pump; and Thermo TSQ Altis coupled to Thermo RSLC 3000 system. The method was validated on both the instruments before using them for analysis. The systems were used based on their availability. The separation of the analytes was carried out on Hypersil Gold C18 column of length 100, 2.1 mm internal diameter, and 1.9 µm particle size. Acetonitrile 70% containing 0.1% formic acid was used as mobile phase in isocratic mode. The samples were ionized in electrospray ionization mode. The capillary voltages were optimized to obtain the maximum sensitivity before injection of the samples. The flow rate of mobile phase was maintained at 600µL/min and the analytes were detected in multiple reaction monitoring mode.

c. Chemicals and reagents: The standard chemicals di-methyl phthalate (DMP), di-ethyl phthalate (DEP), Di-propyl phthalate (DPP), Di-n-proyl phthalate (DPrP), Di-isopropyl phthalate (DIPP), Di-butyl phthalate (DBP), Bis(2-ethylhexyl) phthalate (DEHP) and Di-octyl phthalate (DOP) were purchased from Sigma Aldrich (Bengaluru, Karnataka, India). The UPLC grade solvents Acetonitrile and Methanol were purchased from Biolab (Italy). The LC-MS grade water was obtained from the Direct-Q water purification system (Merck-Millipore, Mumbai, India).

d. Method of extraction: As the analysis is intended for trace level quantification of phthalates, the use of plasticware in the entire work was minimized to the maximum possible extent. 20mL of water sample was transferred in to 50mL round bottom flask and was evaporated to dryness by rotary evaporation at 40°C. The residue was reconstituted in 400µL acetonitrile. From this 200µL was taken LC-MS/MS analysis.

e. Method Validation: The method was developed and validated with ultrapure water obtained from the Millipore water purification system. The stock solutions of standard phthalates were prepared by dissolving 10 mg of each in 10 mL of methanol. The subsequent dilutions of the solutions for required concentrations were prepared by serially diluting the standard solution in water as per the required volume. Triethyl phosphate was used as internal standard. The internal standard solution was also prepared in a similar way as that of the standard chemicals. A constant concentration of 100 ng/L was maintained in the calibration solutions. The water samples were fortified with standard solutions to the concentrations 1, 2, 5, 10, 20, 50, 100, 200, 500, 1000, 2000 ng/L in 20 mL with the internal standard at a concentration of 100 ng/L. Six replicates of samples were prepared at 1, 5, 50 and 500 ng/L concentrations for validation of the method. All the samples were evaporated to dryness using rotary evaporation system and the residue was reconstituted in 400µL acetonitrile. 200µL was used for LC-MS/MS analysis.

Inter-day and Intraday method validation were carried out at four different concentrations 1, 5, 50 and 500 ng/l levels by analyzing six replicates of samples prepared separately. The relative standard deviations (percentage of variance) were calculated to the accuracy value obtained from the back calculated values of concentration using the linear calibration curves obtained from the analysis of standard solutions.

f. Sample preparations for analysis of incubated water samples: As the analysis was intend for trace level quantification of phthalate easters the use of plasticware in the entire work was minimized to the maximum possible extent. 20 ml of water sample was transferred in to 50 ml round water flask and was evaporated to dryness by rotary evaporation at 40^{0} C. The residue was reconstituted in 400 µl acetonitrile and one part was used for LC-MS/MS analysis.

6. FINDINGS

This India specific study was conducted to investigate the migration of phthalates in the five commonly marketed 1-Litre bottles of packaged drinking water in India. Of eight investigated phthalate esters, one or other phthalate easters have been detected at various temperature-storage conditions.

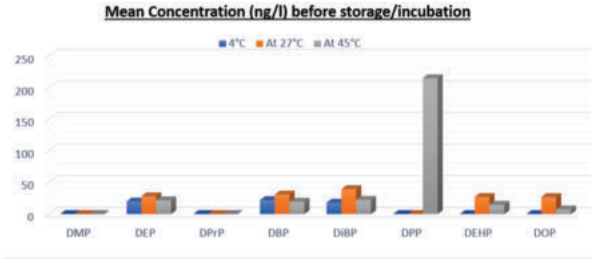
The mean concentration of the eight phthalate esters, in all 75 sets of sampled bottled water collectively for the five manufactures, at the three temperature conditions and five different storage periods (standard deviations in brackets) is compiled in the table-1 below and deliberated separately for each of eight easters hereinafter.

Table-1: Mean Concentration of Phthalate Esters in bottled drinking water samples at various storage (±SD %)

Storage/ Analyte	DAY–0 (ng/l)			DAY–30 (ng/l)			DAY–60 (ng/l)			DAY–120 (ng/l)			DAY–180 (ng/l)		
Temp	**4°C**	**27°C**	**45°C**	**4°C**	**27°C**	**45°C**	**4°C**	**27°C**	**45°C**	**4°C**	**27°C**	**45°C**	**4°C**	**27°C**	**45°C**
DMP	x	x	x	x	x	x	x	347.1 (±67.7)	x	45.4 (±16.4)	84.0 (±116.4)	68.1 (±54.2)	96.4 (±138.7)	158.1 (±360.9)	61.7 (±74.0)
DEP	19.9 (±19.6)	28.3 (±19.1)	21.9 (±21.1)	x	x	x	109.5 (±0.0)	x	166.8 (±35.6)	175.7 (±14.1)	262.3 (±235.8)	318.1 (±250.3)	672.0 (±1173.7)	720.8 (±1144.3)	440.6 (±883.1)
DPrP	x	x	x	x	x	x	x	159.4 (±34.9)	x	x	x	x	131.2 (±224.4)	465.1 (±1198.4)	58.6 (±115.2)
DBP	22.2 (±18.2)	31.0 (±36.0)	19.5 (±14.9)	328.4 (±221.4)	401.0 (±626.6)	145.5 (±66.3)	396.3 (±0.0)	374.7 (±71.6)	225.2 (±105.4)	159.5 (±124.3)	98.7 (±47.8)	129.7 (±73.2)	713.3 (±1808.5)	1209.7 (±2127.8)	394.8 (±681.1)
DiBP	18.2 (±19.3)	39.6 (±40.9)	22.6 (±15.9)	650.3 (±442.7)	524.3 (±840.7)	359.8 (±208.9)	x	x	x	160.2 (±136.1)	99.9 (±50.9)	170.3 (±189.1)	639.4 (±1696.7)	777.8 (±1018.0)	377.0 (±634.2)
DPP	x	x	214.9 (±172.2)	x	x	x	x	x	x	x	x	x	17.6 (±12.4)	151.4 (±311.2)	46.2 (±70.1)
DEHP	x	27.1 (±37.8)	14.7 (±0.0)	177.8 (±0.0)	120.8 (±36.9)	145.3 (±104.0)	x	205.0 (±0.0)	514.7 (±206.0)	x	x	x	13.8 (±12.2)	60.8 (±124.7)	14.9 (±14.3)
DOP	x	27.0 (±40.2)	7.5 (±5.7)	21.4 (±0.0)	115.7 (±16.6)	187.4 (±163.1)	x	171.9 (±49.4)	504.0 (±219.3)	x	x	x	10.8 (±9.5)	58.8 (±119.8)	13.5 (±14.8)

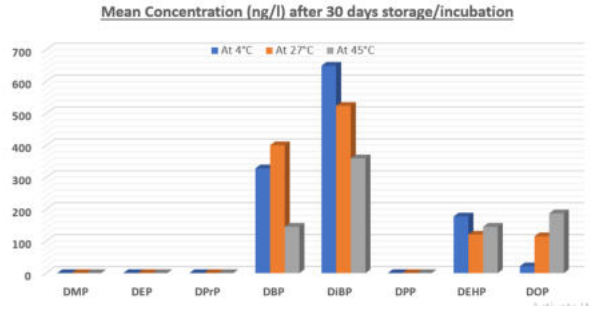
DMP – Di-methyl Phthalate (DMP) was detected in 37.3% of sample sets. Concentration was below quantifiable level in all 15 samples sets (consisting of 5 samples in each set) analyzed on 0 day, and after incubation and storage at 4° C and 45° C for 30-day and 60 days. DMP was detected in 3 of 5 sets (60%) of samples incubated at room temperature (27° C) for 60 days. However, DMP concentration were detected in 10 samples sets (80% of sample sets), incubated at 45° C for 120 days and in all 15 samples sets of samples, incubated at all three temperatures for 180 days.

Figure-1: Mean Concentration of Phthalates Esters in bottled drinking water samples (ng/l) on Zero day



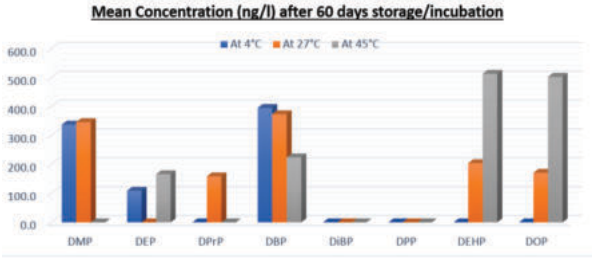
DEP - Di-ethyl Phthalates (DEP) was detected in over half (53.3%) of 75 sample sets analyzed. Presence of DEP was noticed in majority of samples sets (11 of 15 samples sets) even in 0-day analysis at all three temperature conditions. There was no detectable concentration on 30-day incubation and only in 3 samples sets (1 at 4°C and 2 at 45°C) on 60 days, DEP was found in most of sample sets (11 of 15 sets) on 120 days and in all 15 sets of samples on 180 days incubation. The quantity of DEP in samples increased successively with increase in incubation temperature and storage time and was highest after 180 days storage.

Figure-2: Mean Concentration of Phthalates Esters in bottled drinking water samples (ng/l) after 30 days incubation



DPrP - Di-n-propyl Phthalate (DPrP) was the least detected easters amongst the eight phthalate esters investigated in 75 sets of packaged drinking water samples. Still, it was detected in 25.3% of sample sets in totality and was quantified in all 15 sample sets at all three temperature after 180 days incubation. It could be quantified in only 3 sets of samples at room temperature (27°C) after 60 days storage and only in 1 set of samples at 45°C incubation after 120 days storage.

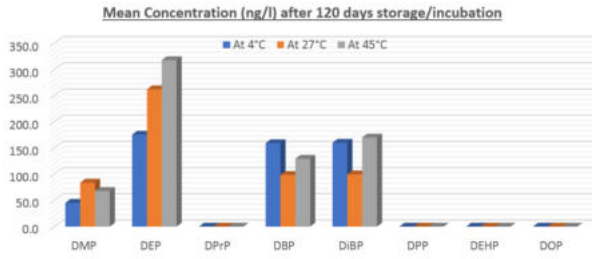
Figure-3: Mean Concentration of Phthalate Esters in bottled drinking water samples (ng/l) after 60 days incubation



DBP - Di-butyl Phthalate (DBP) was found to be most ubiquitous of eight variants of phthalates, which presence in the samples were studied. It was detected in 94.7% samples of 75 samples sets analyzed and was traced at all three temperatures conditions of 4°C, 27°C and 45° C and all five storage durations of 0, 30,60, 120 and 180 days. Though quantities detected were diverse, no linearity in concentrations could be established, nevertheless it was relatively highest on 180 days storage in all three temperatures and the lowest on the 0 day.

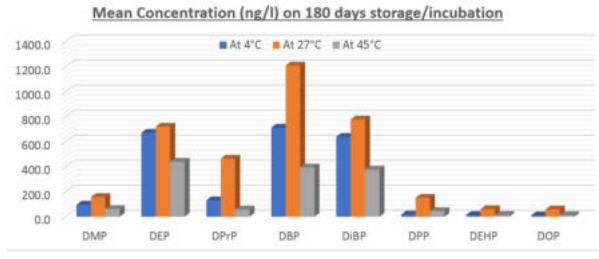
DiBP - Di-isobutyl Phthalate (DiBP), detected in 80% of sample sets, was most profusely found phthalate ester, after DBP in the sample sets analyzed. It was detected in almost all sample sets at all three incubation temperatures and storage periods, other than those analyzed after 60 days storage. Quantities of the analyte was higher at all three temperature conditions incubated for 30 days and 180 days as compared to those incubated for 0 day and 120 days.

Figure-4: Mean Concentration of Phthalate Esters in bottled drinking water samples (ng/l) after 120 days incubation



DPP - Di-propyl Phthalate (DPP) is another variant of Phthalates, which detection was in least number of sample sets. DPP was detected only in 21.3% of sample sets. It was detected in all 15 sample sets incubated for 180 days at all the three temperatures of 4° C, 27° C and 45° C. Presence of DPP concentration was observed on 0 day at 45° C in two of 15 sets of samples. DPP was detected after 180 days storage in 14 of 15 incubated sample sets (93.3%) at three temperature conditions. However, DPP was not quantifiable in any of other sample sets at other storage periods.

Figure-5: Mean Concentration of Phthalate esters in bottled drinking water samples (ng/l) after 180 days incubation



DEHP – Presence of Bis (2-ethylhexyl) Phthalate (DEHP) was detected on 0 day at room temperature (27° C) in 3 of 5 sample sets and in 1 of 5 sample sets at 45° C. Though its concentration was below quantifiable level in samples incubated at all three temperature conditions for 120 days, its presence was noticed in samples incubated at all the three temperature after 180 days storage. Presence of DEHP was detected in 8 of 15 sample sets (53.3%) after 30 days storage, that are, 1 of 5 samples sets incubated at 4° C ,3 of 5 sample sets incubated at 27° C and 4 of 5 sample sets incubated at 45° C. In samples sets incubated for 60 days, presence of phthalates were observed in only 3 of 15 sample sets (20%); that were 1 of 5 sample sets at 27° C and 2 of 5 sample sets at 45° C. The overall percentage of samples sets with presence of DEHP was 40% of 75 sets of samples analyzed.

DOP – Di-octyl Phthalate (DOP) was detected in 38.7% of total 75 sample sets, analyzed. DOP was below quantifiable level in samples sets incubated for 120 days at all three temperature conditions and those sample sets incubated at 4° C on 0 day and after 60 days storage. The presence of DOP was observed in all 15 sample sets incubated at the 3 temperature conditions after 180 days. The quantified concentration in sample sets incubated at 27° C and 45° C on 0 day and after 60 days and at all three temperature conditions in 30 days and 180 days were in varied quantities.

7. DISCUSSION:

Migration of all eight investigated phthalate esters were noticed at all three temperatures conditions on 180 days incubation. One or other phthalate esters were observed in 62% of analysed samples sets at all other temperature and storage combinations, whereas DEP, DBP, DiBP were observed even on the 0-day analysis at all three temperatures.

The presence of all eight phthalate easters measured in the each of five sets of five bottled drinking water samples after incubation at three different temperature conditions on 0 days, 30 days, 60 days, 120 days, and 180 days, were found to be varying in wide range from below detection level to 2727.4 nanogram per litre (ng/l) with standard deviation of ±3605.8%.

The percentage (frequency) of sample bottled water with detectable phthalates esters concentration varied from 21.3% for DPP to 94.7% for DBP. The quantity of detectable concentration varied from minimum concentration of 4.3 ng/L (SD ± 2.4%) for DPrP to maximum concentration of 2727.4 ng/L (SD ± 3605.8%) for DBP. The frequency of detection and the minimum and maximum concentration detected for each of 75 sets of 5 sample bottled drinking water is summarized in the table-2 below :

Table-2 : Frequency and minimum and maximum concentration of Phthalate Esters in the bottled drinking water samples

Serial Number	Phthalate Esters	Number of Sample (each set of five bottled water)	Frequency (Percent) of Samples with detection	Concentration Detected (± SD)	
				Minimum	Maximum
1.	Di-methyl Phthalate (DMP)	75	37.3%	8.8 ng/L (± 0.0%)	384.1 ng/L (± 0.0%)
2.	Di-ethyl Phthalate (DEP)	75	53.3%	9.0 ng/L (± 2.3%)	1508.3 ng/L (±1955.9%)
3.	Di-n-propyl Phthalate (DPrP)	75	25.3%	4.3 ng/L (± 2.4%)	1342.6 ng/L (±2114.1%)
4.	Di-butyl Phthalate (DBP)	75	94.7%	6.1 ng/L (± 5.3%)	2727.4 ng/L (±3605.8%)
5.	Di-isobutyl Phthalate(DiBP)	75	80.0%	5.7 ng/L (± 0.0%)	2440.9 ng/L (±1543.1%)
6.	Di-pentyl phthalate (DPP)	75	21.3%	8.2 ng/L (± 0.0%)	409.3 ng/L (±572.2%)
7.	Bis (2-ethylhexyl) Phthalate(DEHP)	75	40.0%	6.9 ng/L (± 2.9%)	629.9 ng/L (±114.8%)
8.	Di-octyl Phthalate (DOP)	75	38.7%	5.3 ng/L (± 3.1%)	561.7 ng/L (±166.0%)

Based on the above frequency and maximum quantity of each phthalate esters detected, the cumulative ingestion of phthalate esters through bottled drinking water may be 0.213 µg/l. considering daily water intake of 2 litres and body weight of 60 Kg,

8. HEALTH RISK STANDARDS

The World Health Organization (WHO), after due health risk assessment, has established guideline values of 8 µg/l for Di-2-ethylhexyl phthalate (DEHP) for drinking water quality. The Joint Expert Committee on Food Additives (JECFA) of the WHO has recommended that human exposure to this compound in food be reduced to the lowest level attainable and that might be achieved by using alternative plasticizers or alternatives to plastic material containing DEHP ***[24]***.

The US Food and Drug Administration (USFDA) has fixed allowable level of contaminant for DEHP in bottled water as 0.006 mg/l ***[25].***

United States Environmental Protection Agency (USEPA) in 1980 determined the ambient water criterion for the protection of human health from the toxic properties of Phthalate Esters ingested through water and/or contaminated aquatic organisms, as hereunder ***[26]***.

Table-3: Ambient Water Criterion for Phthalate Esters determined by USEPA in 1980 for human health *[26]*

Sl. No.	Phthalate Ester	Ingestion through Water and contaminated aquatic organisms	Ingestion through aquatic organism contamination
1	Di-methyl Phthalate (DMP)	313 mg/l	2.9 g/l
2	Diethyl Phthalate (DEP)	350 mg/l	1.8 g/l
3	Di-butyl Phthalate (DBP)	34 mg/l	154 mg/l
4	Di-2-ethylhexyl phthalate (DEHP)	15 mg/l	50 g/l

United States Environment Protection Agency (USEPA), in 1995, has nevertheless, set maximum contamination level goal (MCLG) in drinking water for phthalate (DEHP) as zero, and set MCL for the same as 6 ppb, believing that as the lowest level to which the water system can reasonably be required to remove contamination from drinking water with existing technology and resources. ***[27].***

The USEPA has further envisioned that short term phthalate exposure beyond this MCL (6 ppb) may potentially cause mild gastrointestinal disturbances, nausea, and vertigo and in long term lifetime exposure of phthalate at levels beyond the MCL (6 ppb) has potential to cause damage to liver and testes, reproductive effects, and cancer ***[27].***

In the European Union, binding food regulatory principles have been established to ensure the safety and quality of the packed food. The Commission Regulation (EC) No 10/2011 ***[28]*** is most comprehensive regulation applicable to food contact materials and articles made of plastics (e.g., bottles made of polyethylene terephthalate, PET or closures made of high-density polyethylene, HDPE). The EU Plastics Regulation prescribes specific migration limit of 7.5 mg/kg and 5 mg/kg food for terephthalic acid monomer and isophthalic acid monomer in the manufacture of PET bottles ***[28].***

In India, the Food Safety and Standards Authority of India (FSSAI) has notified specific migration limits of 1.5 mg/kg for Phthalic Acid (Ph A) and Bis-2-ethylhexyl phthalate (DEHP) from plastic materials intended to be in contact with food products ***[29]***. This implies that prescribed migration limit by FSSAI for Ph A and DEHP into drinking water packaged in one-litre PET bottle of average weight of 25 gms may be 0.038 mg/l.

9. CONCLUSION

This study provides firsthand information on the contamination levels of eight phthalate esters in the representative commercially packaged drinking water in India**.**

The most profusely detected phthalate ester in the analyzed sample of bottled water was Dibutyl phthalate (DBP), out of 75 set of samples, 71 samples set had detectable DBP concentration, followed by Di-isobutyl phthalate (DiBP), which as detectable in 60 of 75 sample bottled water sets. Di-pentyl phthalate (DPP) was least detected phthalate ester with detectable concentration in only 16 of 75 bottled water sample sets and was preceded by Di-n-propyl Phthalate (DPrP) with detectable concentration in 19 of 75 bottled water samples. The order of most abundant esters found was DBP > DiBP > DEP > DEHP > DOP > DMP > DPrP > DPP.

Majority of phthalate esters namely DEP, DPrP, DBP, DiBP and DPP showed increase in leaching with increase of storage period to 180 days irrespective of temperature. The highest concentration range was of Dibutyl Phthalate (DBP) and the lowest was of Dimethyl phthalate (DMP) ester in all the analyzed bottled water samples. The order of the prominence (highest to lowest concentration) of phthalate esters detected was DBP > DiBP > DEP > DPrP > DEHP > DOP > DPP > DMP.

Effect of temperature on leachates, however, could not be established with this study as different bottles were incubated at different temperature and storage period. Nevertheless, on maximum days storage showed more leaching though increase in leaching concentration was not linear. Surprising higher level of concentration of phthalate esters has been observed at some lower temperature. This may be because of phthalates are subjected to more rapid degradation processes at increased temperatures.

A substantial increase in phthalates concentration in bottled water was observed with increased shelf life. The concentration of phthalates obtained in this study for incubation up to 45 ^{0}C and storage up to 180 days are well below the tolerable daily intake (TDI) or maximum contaminant level (MCL) fixed by various authorities for ingestion of phthalates contamination in drinking water. A person drinking water at or below these levels, whether briefly, occasionally, or daily for a lifetime, may have little or no risk of health effect**.** As such, the human health risk may be safe with the observed level of concentration of phthalates in the subject study. However, the accumulation of small individual amounts taken with time may increase the lifelong phthalate exposure and eventually may threaten the exposed person's life.

This study did not consider the source of raw water, process adopted for manufacturing and manufacturing cycle, quality, and type of plastic - virgin or recycled - of PET used for bottling by the manufacturers. These factors may have influence on the detection of phthalate ester, which are probably reflected in detection of some phthalate esters even before incubation/storage of samples (that is, on 0 day) as well as in high standard deviations in some of the result averages.

This being a study with limited parameters in consideration, reports phthalate migration in bottled drinking water in India, warrants further extensive phthalates exposure assessment to understand the effect on public health. It is therefore prudent to have a detailed study with large number of samples establishing relationship with quality of plastic and parameters used for manufacturing of bottled drinking water. With this high consumption of packaged drinking water in India, it is prudent a strong quality assurance norm for phthalate contamination in drinking water is made.

ACKNOWLEDGEMENT

The author acknowledges with thanks the services the CSIR-Indian Institute of Chemical Technology, Hyderabad who kindly agreed to develop methods and conduct necessary analyses as per the protocol on sample drinking water bottles, procured and made available to them from the local market. The author is especially thankful to the team of Centre of Mass Spectrometry of Analytics Department, who conducted the analyses. The author is also thankful to his team at BPA Consulting, both present and past, who have actively participated during investigation and preparation of this study.

FUNDING

This study has not been sponsored by any person, body or association and received no external funding. Use of these data except for scientific reference is prohibited.

CONFLICTS OF INTEREST

This study was undertaken purely with scientific interest and does not intend to malign or promote any or other brand of packaged drinking water, manufactured and marketed in India or anywhere. The author declares no conflicts of interest regarding the publication of this paper

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