Mesopotamia Journal of Agriculture

https://magrj.uomosul.edu.iq

DETERMINATION OF PHTHALATES COMPOUNDS MIGRATING FROM PLASTIC CONTAINERS TO AQUATIC AND FOOD PRODUCTS BY USING HPLC AND ESTIMATION OF THEIR DAILY INTAKE

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Article information
Article history:
Received: 12 /3 /2025
Accepted: 1 /9 /2025
Available: 30/9/2025

Keywords:
DEP (diethyl phthalate), DBP (dibutyl phthalate), TB2-AC (tributyl 2-acetyl citrate), DEHP (diethyl hexyl phthalate), DiNP (diisononyl phthalate).

DOI:
https://doi.org/10.33899/mja.2025.158178.1557

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ABSTRACT

The objectives of this study were the determination of the phthalates Diethyl phthalate (DEP), Dibutyl phthalate (DBP), Tributyl 2-acetyl citrate (TB2-AC), Diethyl hexyl phthalate (DEHP), and Diisononyl phthalate (DiNP) in food products packed in plastic containers and to estimate the daily intake and the daily intake per kg body weight for a group of fifty volunteer. Food samples were obtained from markets in the city of Mosul, Iraq, and their chemical compositions, which may affect the migration of phthalates to the foods, were determined. Results showed that all phthalates were found in almost all food products, with the phthalate TB2-AC the most abundant. Water, pickled mango, and sesame tahenia samples contained the highest quantities of phthalates at 3.994, 3.229, and 3.058 ng/g(ml). Sesame tahenia contained the highest amounts of DEP (0.900 ng/g). In contrast, water contained the highest amount of DBP (1.430 ng/ml), pickled mango was highest in TB2-AC (3.015 ng/g), ketchup was highest in DEHP (0.438 ng/g), and lemon juice concentrate was highest in DiNP (0.084 ng/ml). Results also revealed that the highest intake of phthalates was TB2-AC, DBP, and DEP, with 2304.873 ng/day, and the intake per kg of body weight was 433.16 ng. For the daily intake of total phthalates, water participated in the highest intake with 5043.597 ng/day, with a daily intake per kg body weight of 72.570 ng, milk and sesame tahenia resulted in daily intakes of 240.643 and 88.658 ng and a daily intake per kg of body weight of 3.462 and 1.277 ng, respectively.

INTRODUCTION

Phthalates are dialkyl or alkyl aryl esters of phthalic acid within a group of organic chemicals that have applications in many industrial areas and have been used for over 50 years in our daily lives (ZareJeddi *et al.*, 2015; Huang *et al.*, 2021). Phthalate esters (PAEs) have been widely used in many industrial applications as plasticizers in plastic manufacturing since the 1930s. The PAEs are usually added to plastics like chloride polyphenol (PVC), polyethylene terephthalate (PET), polyvinyl acetate (PVA), and polyethylene (PE) in ratios ranging from 10- 60% of the PAEs weight to improve elasticity and polymer formation ability (Giuliani *et al.*, 2020; Fadhil, 2023). One of the phthalates' most important properties is their low-melting and relatively high-boiling points, making them very useful as plasticizers and heat-transferring liquids in the polymers industry. Linear and branched esters are used in plastics manufacturing, where linear esters provide high elasticity at low temperatures

(Haji Harunarashid *et al*., 2017). Phthalate esters PAEs has an economical and commercial importance due to their various applications in plastic-based products, such as construction materials (floors, walls papers, and electric cables), children's toys, clothes, printing inks, packing and covering materials, pesticides, body care and cosmetics products, medicines, medical equipment, food packing materials, and blood bags (Haned *et al*., 2018; Giuliani *et al*., 2020). And it is also used in the manufacture of silicon (Ismaiel and Ibrahim, 2023; Lafta, 2024). In China, a field survey was conducted about the types of phthalates in ready food packages. The phthalate concentration ranged from 0.12-86.1 microgram/gm of food, and the percentage of Diisobutyl phthalate (DiBP), di-n-butyl phthalate (DnBP), and di(2-ethylhexyl) phthalate (DEHP) was 38.7, 37.9, and 15.9% from the total concentration, respectively. The phthalates may be mainly from their addition during package manufacturing or in raw materials. Results show that 7.33, 20.5, and 51.2 % of DEHP, DnBO, and DiBP can be released, respectively (Han *et al*., 2021). Different phthalate compounds can easily transfer to foods and drinks when they are in contact with them during manufacturing, storing, transporting, and preparing. As a result, in the last few years, foods and drinks contaminated with PAEs have been considered the main source of human exposure to phthalates (Fang *et al*., 2017; Abdulsalaam and Akdo, 2023).

Regulations controlling the plastics when in contact with food differ from country to country. In the United Kingdom, the acceptable concentrations for Di(2-ethylhexyl) phthalate DEHP, BBP, DBP, and DEP are 0.05, 0.1, 0.05, and 0.2 mg/kg body weight/day. In the Turkish food institution, the acceptable limits for phthalates DBP, BBP, DEHP, and DiNP not to exceed 0.3, 30, 1.5, and 9 mg/kg of food, respectively (Ustun *et al*., 2015; Mustafa et al., 2023) The European Union 10/2011 regulates the use of plastics in contact with foods and allows the use of phthalates with concentration not exceeding 0.1% in the final product, five types of phthalate esters were allowed in this classification, DEHP, DEP, BBP, DIDP, and DINP. Also, the permissible limits of phthalate esters were discussed and updated in the EFSA panel on food contact materials, enzymes, and processing media (EFSA Panel on Food Contact Materials, 2019).

The phthalate compounds can be released from the containers and ready food wraps to the food and then consumed by the consumer (Xu et al., 2020; Mustafa and Hamza, 2023), where these phthalates have bad effects on the consumers when entering the body in certain concentrations. They could negatively affect the Endocrine gland, growth, and human immune system (Radke *et al*., 2019; Dhaef and Abd Alsalam, 2024). Human exposure to phthalates can increase the risk of diabetes, obesity, and breast cancer (Svensson *et al*., 2011); also, exposure before birth can cause negative effects after birth (Hauser et al., 2016; Han *et al*., 2021). DEHP transfer to food products has been documented with levels ranging from 2.19 to 3.57 mg/kg in samples containing 10% fat and from 4.96 to 6.48 mg/kg in samples containing 50% fat (Jarošováand Bogdanovičová, 2016). In 2012, nine phthalates were discovered in 78 food samples collected from local markets in China (grains, drinks, spices, milk and dairy products, oil, seafood, meat, and meat products). DEP and DIBP were found in more than 94% of the analyzed samples with concentrations ranging from 0.011 ng/gm in bottled water to 572 ng/gm in biscuit. DEHP and DEP were found in 82 and 81% of the samples, respectively. In cake and biscuit, DEP, DIBP, DBP, and DEHP were found in ranges of 0.35-1.97, 24.5-142, 13.7-572, and 45.7-750 ng/gm, respectively (Guo *et al*., 2012). Wrona *et al* (2024) found the plasticizer ATBC in many food products like drinks, fats and oils, dairy products, ice cream, fast food, and baby food (Abd El-Moniem et al., 2025). Ge *et al* (2012) discovered the presence of phthalates in sheep milk used to produce dairy products for infants, and the results showed that they are the main source of infants' exposure to phthalates.

The purpose of the present study was to investigate the presence of five phthalate compounds in some commonly consumed foods and drinks, including water, and also to estimate their daily intake and daily intake per kg body weight for a population of volunteered people.

MATERIALS AND METHODS

Food products

Food products were obtained from the local markets in Mosul city, Iraq, and were packed in plastic bottles and containers at least 3-4 months after the production date. They involved with, sesame tahenia, olive oil, mustard sauce, tomato ketchup (dark container), tomato ketchup (transparent container), mayonnaise (dark container), sauce (dark container), pickled mango, lemon concentrate, full fat milk, white vinegar, lemon vinegar, date molasses, pomegranate molasses, artificial orange beverage, artificial condensed pomegranate beverage, Coca cola (fizzy drink), 7up (fizzy drink), Miranda (fizzy drink), and water.

Determination of phthalate compounds

Phthalate compounds were extracted according to the procedure by Cao *et al* (2015) and Cao *et al* (2021) with some modifications. One gram of each sample was weighed in a 10 ml glass centrifuge tube, then 0.5 gm of Sodium Chloride was added, followed by 5 ml of acetonitrile and then 0.8 $MgSO_4$. The tube was capped with a glass cap, shaken well for 10 min. then centrifuged at 5000 rpm using Triup International Corp (China) for 2 minutes. The clear portion was filtered through a 16x160 mm open-ended tube containing anhydrous Na_2SO_4. Then, 1 ml of acetonitrile was added to ensure all compounds descended from the open-ended tube to the beaker. The filtrate was concentrated to 1 ml using a Barloworld Scientific evaporator (UK) at 45-50°C in a Nitrogen current with a condenser cooled with cold water. The phthalate compounds were estimated using high-pressure liquid chromatography HPLC following the procedure of Shah et al. (2024) and Jandal (2024), with some modifications. After fixing the optimal conditions for separation, a standard curve was made for the pure phthalate compounds (Sigma company, China) as a mixture of phthalate compounds was prepared by mixing 3 µl of each compound in 1 ml acetonitrile in a 2 ml HPLC sample tube. A 5 µl aliquot of phthalate compounds extracted from foods and that of the pure compound's mixture was separated using an HPLC (AL-Behadili, 2024) device from Shimadzu company (Japan) with a UV/Visible spectrometer detector. The compounds were separated using a 4.6x250 mm column containing 5 µm size of Octadecylsilane C_{18} (ODS) and a mobile phase of a methanol-acetonitrile mixture (10:90) with a flow rate of 1 ml/min and temperature of 35°C. The absorbance was measured at a wavelength of 254 nm.

The separated phthalates were identified and quantified by comparing the retention times and the peak areas with the standard compounds.

Proximate analyses

Total crude protein was estimated using a Microkjeldahl device according to the method described in the AOAC, as mentioned by the researcher (Rizvi *et al.*, 2022; Qasim *et al.*, 2024), which stipulated the estimation of crude protein through three stages. First, the digestion stage, in which (10 ml) of concentrated sulfuric acid is added to approximately (0.5 g) of the sample in a glass beaker (Conical flask) with a capacity of (50 ml). The sample is left for three days, then the glass beaker is heated using a glass heater. After boiling for (5-10) minutes, (1-2 ml) of perchloric acid is slowly added to the contents, while heating continues until the color of the sample turns clear. Second, the distillation stage, where after the sample is left to cool, the beaker volume is completed to (50 ml) with distilled water, and (5 ml) of the liquid is taken and transferred to the distillation unit, where (10 ml) of sodium hydroxide solution with a concentration of (10 N) is added to it. The ammonia released from the reaction is received in a receiving medium consisting of (10 ml) of boric acid to which the indicator is added, prepared by adding (10 ml) of the indicator formed by dissolving (1 g) of methyl blue dye with (1.235 g) of methyl red dye in (100 ml) of ethanol, to a liter of boric acid until the color of the indicator changes, and this indicates the formation of ammonium borate. Third, the calibration stage: The receiving medium was calibrated with a standard sulfuric acid (0.014 molar) to determine the volume of acid needed to neutralize the ammonia in the ammonium borate after the original color of the indicator used returned. Then, the percentage of crude protein was calculated by multiplying the percentage of nitrogen by (6.25).

Fat content was estimated using the Soxhlet apparatus according to the method mentioned in the AOAC as mentioned by the researcher (Shin *et al.*, 2013; Watkins, 2023), The dry sample was placed in the cellulose extraction thimble and the petroleum ether solvent was used for (5) hours with heating. After that, the solvent was evaporated and the sample was weighed. The fat percentage was calculated through the weight difference of the sample before and after the extraction process **,** and carbohydrates was measured by difference.

The pH was measured using a pH meter, and the food materials' total acidity was estimated following the method adapted in the AOAC, as explained by the researcher (Tomovska *et al.*, 2016).

The survey: A survey was conducted on 50 peoples (20 males and 30 females), with ages ranging from 19-65 years old and weights ranging from 41-107 kg, to estimate the amounts of the foods consumed per day for nine of the most consumed foods packed in plastic containers. The foods were sesame tahenia , date molasses, full-fat milk, artificial orange juice, artificial pomegranate juice, Coca-Cola, 7up, Miranda (fizzy drink), and water.

Statistical analysis: Statistical analysis was conducted according to Genstat software (18th ed.), 18.1.0.17005, 2015, UK to test the significant differences between means using the Complete Randomized Design (CRD). The Duncan multiple range test was used at $P<0.01$.

RESULTS AND DISCUSSION

Chemical composition of the materials used in the study

Table 1 shows the chemical composition of the food product samples used in the study. It shows that the foods used contain a high percentage of moisture, especially fizzy drinks and types of vinegar and lemon concentrate that had 89-97% moisture, while the drinks had about 87% moisture content. Mayonnaise, ketchup, mustard, and pickled mango had 43.35-81.79% moisture, and the sesame tahenia had 3.71% moisture content. The pH ranged from 1.8-4.5 for vinegar, lemon concentrate, juices, fizzy drinks, pickled mango, mustard, mayonnaise, and date molasses, while it ranged from 5.8-7.3 for sesame tahenia, full-fat milk, olive oil, and water. Acidity values for food samples ranged between 0.08-0.51% for date molasses, fizzy drinks, artificial orange juice, mayonnaise, and full-fat milk, compared to a higher acidity range of 0.9-2.88% in mustard, tomato ketchup, sauce, and artificial pomegranate molasses. Foods containing the highest acidity were pickled mango, vinegar, and artificial pomegranate juice concentrate, with a range of 3.19-8.1%. Results also show moisture, fat, protein, and carbohydrate percentages. The highest fat% was in mayonnaise, sesame tahenia, and olive oil, and had 48.95, 60, and 99.9%, respectively, while mustard, sauce, pickled mango, and full-fat milk had a fat% range of 1.25-3.4%, compared to other foods that didn't have fat in their constituents. Sesame tahenia had the highest fat, followed by mayonnaise, which had 60 and 48.95% fat, respectively, while the fat% in other foods was very low. The protein concentration in sesame tahenia was the highest (20%), while it was 8.37% in mustard and 1.0-7.62% in other food products. The date and pomegranate molasses contained 70.6 and 60.21% for carbohydrates, respectively. Ketchup, sesame tahenia, and pickled mango contained 24.1, 13.9, and 17.8% of carbohydrate, respectively, while Juice and fizzy drinks contained 10.2 to 17.13%.

The variation in the food chemical composition can clearly affect the phthalate transfer from plastic containers to the food. Although phthalate compounds may not be soluble in water, the high moisture percentage in the foods may help transferring the phthalates from the containers to the packed food as they may work as an emulsifier with other food components such as fat, protein, and carbohydrate. Also, the high percentages of fat may largely contribute to transferring phthalates as their compounds are partially soluble in fat, causing them to transfer.

Phthalate compounds in foods

Table (2) shows the amounts of phthalates in food products packed in plastic containers. Five main phthalate compounds have been detected in foods, including DEP, DPB, TB2-AC, DEHP, and DiNP. Results show that the sum of these compounds ranged from 0.05-3.994 ng/gm or ml, with the highest value (3.994 ng/ml) found in water, while sesame tahenia , a highly consumed food product, contained the third largest amount at 3.058 ng/gm. Although pickled mango contained the second largest amount, but it is less important as its consumption is less compared to the other food products. Phthalates are not covalently bound to other plastic compounds, making them easily released to the packed food. Although water is more polar than sesame tahenia, the sterilization processes like UV and Ozone may help transfer these compounds from the plastic containers to the water at a high rate. In addition, sunlight, storage period, shaking during transportation, and other factors may help the immigration of phthalate compounds from the plastic containers to the water.

Table (1): Chemical composition of various used food products

No.	Food product samples	pH	Acidity (%)	Fat	Protein	Carbohydrate	Moisture
				(g/100 g)			
1	Sesame tahenia	5.6		60	20	13.90	3.71
2	Mustard	3.1	0.90	1.25	8.37	5.33	8.79
3	Tomato Ketchup- dark container	3.5	1.60	1.40	3.81	24.10	69.50
4	Tomato Ketchup- transparent container	3.1	1.76	1.40	3.81	24.10	69.50
5	Mayonnaise- dark container	3.2	0.51	48.95	3	3.06	43.35
6	Mayonnaise- transparent container	3.4	0.45	48.95	3	3.06	43.35
7	Sauce	2.9	1.50	1.64	7.62	35.42	53.98
8	Pickled Mango	2.3	3.19	3.48	10.62	17.80	63.45
9	Lemon concentrate	2.3	5.12	0.00	0.00	0.01	92
10	Full fat milk	6.2	0.45	3	3.50	5.50	87.40
11	White vinegar	2.4	4.95	0.00	0.00	0.01	97
12	Lemon vinegar	2.6	8.10	0.00	0.00	0.01	97
13	Dates molasses	4.5	0.34	0.10	5.94	70.60	21.20
14	Pomegranate molasses	1.8	2.88	1.60	7.62	60.21	30
15	Olive oil	5.8	0.00	99.90	0.00	0.00	0.00
16	Artificial orange juice	2.8	0.48	0.00	0.60	12	87
17	Artificial pomegranate juice	1.9	4.32	0.00	0.15	17.13	80
18	Coca Cola	2.3	0.08	0.00	0.00	10.60	89
19	Miranda	2.4	0.32	0.00	0.00	10.60	89
20	7up	3.2	0.16	0.00	0.00	10.20	89.40
21	Water	7.3	0.00	0.00	0.00	0.00	99.99

Sesame tahenia contains a high percentage of fat that helps dissolve these compounds, causing it to be contaminated more than other foods. In addition to the storage period, the surrounding environment, such as temperature and moisture, allows the dissolution and transfer of the phthalate compounds. Ketchup samples in dark and transparent containers included high phthalate compounds, 2.559 and 2.277 ng/gm, respectively. Lemon concentrate contained 2.223 ng/ml of phthalates, not significantly different from ketchup, which may be due to the low pH that can help release phthalates into the acidic medium. Next come date molasses, full-fat milk, and lemon vinegar, containing 1.899, 1.819, and 1.703 ng/mg(ml), respectively. Second in the amounts of phthalates comes mayonnaise in a transparent container, and pomegranate molasses from white vinegar, mustard, sauce, and sauce. Olive oil had 1.155 ng/gm of phthalates- this small amount compared to other high-fat materials could be due to the recently manufacturing date in addition to the presence of antioxidants like phenol that can help to stabilize and prevent the chemical reaction with other materials, including plastics, and as a result, reducing the chance of phthalate compounds migrating to it. Fizzy drinks contained low amounts of phthalates that reached 0.991, 0.473, and 0.051 ng/ml for Miranda, cola, and 7Up, respectively. Although fizzy drinks have low pH, the low amounts of phthalate compounds could be due to the short period between production and consumption, which will not allow the phthalates to migrate from plastic containers to the drinks. Also, fizzy drinks contain compressed CO_2 that may limit the ability of the phthalates to migrate. In addition, the pressure may limit the reaction of the different materials, thus contributing to the low migration of the compounds. The results also show that TB2-AC is the most abundant compound in the food materials, where its sum was 29.832 ng/gm in all food materials, which was statistically different ($P \geq 0.01$) from other phthalate compounds. Pickled mango contained the highest amount of 3.015 ng/gm, where sesame tahenia, ketchup, lemon concentrate, milk, date molasses, and artificial pomegranate juice came second. Sesame tahenia and full-fat milk, the most consumed materials, contained 2.104 and 1.730 ng/gm. Garcia et. al. (2018) mentioned that TB2-AC has been widely used in plastic container manufacturing to replace many phthalates.

DEP comes in second place, with 2.753 ng/gm. Sesame tahenia contained the highest amounts of 0.900 ng/gm, while the water contained the least amount of 0.745 ng/gm. Other materials, like ketchup in a dark container, mayonnaise (two types), sauce, pickled mango, milk, and olive oil, didn't have DEP. In contrast, other materials contained a very small amount of this compound. The existence/non-existence of these compounds in the food may not be only due to the food's chemical composition and the surrounding environment (temperature, sunlight, etc.), but also that the plastic container may not contain a compound or two of these compounds, or that the amount of these compounds is very small in the first place. Sakhi et. al. (2014) showed that the highest migration of DEP was in the product with fat and less in drinks, while it was obvious in full-fat milk. DEP came third in its existence in food materials used in the study, with a total sum of 2.14 ng/gm. Water contained the highest amount of 1.430 ng/ml, while pickled mango came second with a total sum of 0.209 ng/gm, confirming the findings of Van Holderbeke et. al. (2014), that DEP migrates noticeably to many foods. On the other hand, DEHP comes fourth in the food materials used in this research, where its total amount was 1.604 ng/gm. The ketchup in the dark container and the water had the highest amount of DEHP, with 0.438 and 0.410 ng/gm (ml), respectively. These amounts were statistically significant.

Table (2): Concentration values of phthalates in food samples packed in plastic containers (ng/g)

Phthalates / Products	DEP	DBP	TB2-AC	DEHP	DiNP	Total
Sesame tahenia	0.900^{a} ± 0.0320	0.023^{fghi} ± 0.0094	2.104 ^{bc} ± 0.5960	0.026^{ef} ± 0.0006	0.005^{fgh} ± 0.0009	3.058^{b} ±0.6389
Mustard	0.017^{g} ± 0.0041	0.022^{fghi} ± 0.0030	1.064^{ghi} ± 0.0933	0.061^{d} ± 0.0009	0.068^{b} ± 0.0068	1.232^{gh} ±0.1081
Ketchup- dark container	0.270^{c} ± 0.0168	0.039^{cde} ± 0.0074	2.237^{b} ± 0.2463	0.013 ^{ef} ± 0.0019	0.000 ± 0.0000	2.559^{c} ±0.2724
Ketchup- transparent container	0.000^{g} ± 0.0000	0.025 ^{fgh} ± 0.0044	1.807 ^{bcd} ± 0.0076	0.438^{a} ± 0.0325	0.007 ^{fgh} ± 0.0019	2.277^{d} ±0.0464
Mayonnaise- dark container	0.000^{g} ± 0.0000	0.013^{hij} ± 0.0020	1.628 ^{cdef} ± 0.0577	0.007 ^{ef} ± 0.0013	0.010^{ef} ± 0.0004	1.658^{f} ±0.0614
Mayonnaise- transparent container	0.000^{g} ± 0.0000	0.028^{defg} ± 0.0014	1.084 ^{fghi} ± 0.1817	0.031 ^{e} ± 0.0001	0.026 ^{d} ± 0.0037	1.169^{h} ±0.1869
Sause	0.000^{g} ± 0.0000	0.011^{ij} ± 0.0005	1.222^{efgh} ± 0.0467	0.029 ^{ef} ± 0.0001	0.007 ^{fgh} ± 0.0007	1.269^{gh} ±0.048
Pickled mango	0.000^{g} ± 0.0000	0.209 ^{b} ± 0.0084	3.015 ^{a} ± 0.1600	0.001^{f} ± 0.0001	0.004 ^{fgh} ± 0.0014	3.229^{b} ±0.1699
Lemon concentrate	0.104^{d} ± 0.0064	0.054 ^{bc} ± 0.0045	1.955 ^{bcd} ± 0.0093	0.026^{ef} 0.0033±	0.084 ^{a} ± 0.0039	2.223^{d} ±0.0274
Full fat milk	0.000^{g} ± 0.0000	0.013^{hij} ± 0.0021	1.730^{bcde} ± 0.1279	0.016^{ef} ± 0.0030	0.060^{c} ± 0.0008	1.819^{e} ±0.1338
White vinegar	0.067 ^{ef} ± 0.0061	0.028 ^{defg} ± 0.0043	0.866 ^{hij} ± 0.0722	0.390^{b} ± 0.0199	0.016^{e} ± 0.0018	1.367^{g} ±0.1043
Lemon vinegar	0.122 ^{d} ± 0.0217	0.042^{bc} ± 0.0001	1.515 ^{defg} ± 0.1499	0.013 ^{ef} ± 0.0024	0.010 ^{ef} ± 0.0005	1.702^{ef} ±0.1746
Dates molasses	0.002^{g} ± 0.0001	0.021^{ghi} ± 0.0040	1.809 ^{bcd} ± 0.1412	0.005^{ef} ± 0.0020	0.062 ^{bc} ± 0.0040	1.899^{e} ±0.1513
Pomegranate molasses	0.006 ^{g} ± 0.0009	0.020 ^{ghi} ± 0.0016	1.501 ^{defg} ± 0.0934	0.087^{c} ± 0.0013	0.004^{fgh} ± 0.0011	1.618^{f} ±0.0983
Olive oil	0.000^{g} ± 0.0000	0.035^{cdef} ± 0.0030	1.095 ^{fghi} ± 0.0074	0.018 ^{ef} ± 0.0006	0.006 ^{fgh} ± 0.0004	1.154^{h} ±0.0114
Artificial orange juice	0.024^{g} ± 0.0017	0.041^{cd} ± 0.0001	0.986^{ghi} ± 0.0268	0.018^{ef} ± 0.0007	0.010 ^{ef} ± 0.0002	1.079^{i} ±0.0295
Artificial pomegranate juice	0.034 ^{fg} ± 0.0014	0.043 ^{bc} ± 0.0004	1.849^{bcd} ± 0.0542	0.007^{ef} ± 0.0019	0.009^{efg} ± 0.0008	1.942^{e} ±0.0587
Coca cola	0.087 ^{de} ± 0.0123	0.010 ^{ij} ± 0.0004	0.367^{jk} ± 0.0001	0.006^{ef} ± 0.0004	0.003 ^{fgh} ± 0.0004	0.473^{j} ±0.0136
Miranda	0.367^{c} ± 0.0250	0.027^{efg} ± 0.0014	0.595^{ij} ± 0.0135	0.002^{f} ± 0.0003	0.000^{h} ± 0.0000	0.991^{i} ±0.0402
7 up	0.008^{g} ± 0.0017	0.006 ^{j} ± 0.0012	0.035 ^{k} ± 0.0016	0.000^{f} ± 0.0000	0.002^{gh} ± 0.0002	0.051^{k} ±0.0047
Water	0.745^{b} ± 0.0246	1.430^{a} ± 0.0999	1.368^{efgh} ± 0.0380	0.410^{a} 0.0195±	0.041^{c} ± 0.0106	3.994^{a} ±0.1926
Total	2.753^{b} ±0.1548	2.14^{c} 0.1595±	29.832^{a} ±2.1248	1.604^{d} 0.0928±	0.434^{e} ±0.0405	

- Number of replicates 2
- Similar letters in one column are not statistically significant at the 0.0 1significance level.

The third material to have the DEHP was white vinegar, which contained 0.390 ng/ml, while the other materials contained low and variable amounts of this compound, and 7up didn't contain any DEHP. While the DEHP migration results differed from other researchers, Luis et. al. (2021), there is still an agreement that DEHP migrates to food and drinks noticeably with different storage times. DiNP is considered the lesser of the other phthalates in food in this study. The total amount of DiNP was 0.434 ng/gm(ml). Lemon concentrate had the highest amount of 0.084 ng/ml, while mustard had the second highest amount of 0.068 ng/gm, while the amount in other materials was very low. The results of DiNP migration amounts were different from (Sakhi et al., 2014; Al-Fayadh et al., 2024), where they stated that the migrated amounts of DiNP were higher compared to the other compounds in different foods due to the type of plastic containers used in addition to the different phthalate compounds used in manufacturing the plastic containers.

Daily intake and daily intake/kg body weight of phthalate compounds

Table (3) shows the daily intake (ng) and the daily intake per kg body weight (ng/kg body wt/day) from phthalates by the 50 participants (20 male and 30 females with an average weight of 69.5 kg) through consuming of foods. The table shows that the highest intake of these compounds was TB2-AC with 2304.873 ng/day, and the intake per kg of body weight was 433.16 ng. Water participated in 1727.780 ng/day, and the intake per kg body weight was 24.860 ng from consuming 1263 ml/day of water. Full-fat milk resulted in the second-highest amount consumed in one day, with 229.032 ng/day, and the amount consumed per kg/day/kg body weight was 3.295 ng from a consumption of 132.40 ml/day of milk. The consumption of 112.6 and 42.8 ml of artificial orange and pomegranate juice resulted in 111.018 and 79.129 ng of TB2-AC, with a daily consumption of 1.598 and 1.139 ng/day/kg of body weight, respectively. Consuming 29.0 gm of sesame tahenia resulted in an intake of 61.016 ng of the compound daily, while the daily consumption per kg of body weight was 0.878 ng. The other types of food participated in a daily intake of TB2-AC with 1.837-39.248 ng with a daily intake per kg of body weight of 0.026-0.565 ng. DBP was the second most intake compound with a total daily amount, from all foods, of 1817.816 ng with a daily intake per kg of body weight of 26.157 ng. Water participated in 1805.9 ng/day and the daily intake per kg body weight was 25.984 ng and came from a consumption of 1263 ml/day of water. Other types of food were associated with a low daily intake of DBP. DEP was the third highest consumed compound with a total daily intake of 1002.006 ng. Water participated in 941.503 ng/day, and the intake per kg body weight was 13.547 ng. Consuming 29.0 gm of sesame tahenia resulted in an intake of 26.1 ng of the compound per day, while the daily intake per kg of body weight was 0.376 ng.

The 56.0 and 107.0 ml of Miranda and Coca-Cola consumption resulted in 20.53 and 9.33 ng of DEP with a daily consumption of 0.295 and 0.134 ng /kg body weight, respectively. DEHP came forth in total daily intake with 523.079 ng. Water participated in 517.199 ng/day and consumed per day/kg of body weight was 7.442 ng from a consumption of 1263 ml/day of water. Consumption of 132.4 ml full-fat milk and 112.60 artificial orange juice resulted in 2.065 and 2.004 ng/day of DEHP, while the daily consumption per kg of body weight was 0.03 and 0.029 ng, respectively. DiNP compound was the least consumed phthalate in this study, where its total daily intake was 61.929 ng and the daily intake per kg body weight was 0.890 ng. Water participated in 51.215 ng/day, and the intake per kg body weight was 0.737

ng. Date molasses and sesame tahenia participated in daily intake of 0.733 and 0.131 ng, while the daily intake per kg body weight was 0.011 and 0.002 ng, respectively.

For the daily intake of total phthalate compounds, water contributed the highest amount with 5043.597 ng/day, with a daily intake per kg body weight of 72.570 ng, and came from consuming 1263 ml of water daily. Consumption of the amounts of 132.4 ml of full fat milk and 29.0 ml of sesame tahenia per day resulted in daily intake of 240.643 and 88.658 ng and a daily intake per kg of body weight of 3.462 and 1.277 ng, respectively, while the daily consumption of 112.6 ml of artificial orange juice and 42.8 ml of artificial pomegranate juice resulted in daily intake of 121.411 and 83.133 ng which resulted in an intake per kg body weight of 1.747 and 1.196 ng, respectively. Also, a consumed amount of 56.0 ml of Miranda and 107.0 ml of Coca Cola resulted in a total daily intake of 55.427 and 50.612 ng and a daily intake per kg body weight of 0.797 and 0.729 ng, respectively.

Table (3): Daily intake (ng/day) and daily intake/kg body weight (ng/kg body weight/ day) of phthalate compounds

Food products	Daily consumption (gm)	Unit	DEP	DBP	TB2-AC	DEHP	DiNP	Total
Sesame tahenia	29.00	ng/day	26.100	0.660	61.016	0.751	0.131	88.658
	3.6932±	ng/kg Bwt/day	0.376	0.010	0.878	0.011	0.002	1.277
Full fat milk	132.40	ng/day	0	1.668	229.032	2.065	7.878	240.643
	17.2454±	ng/kg Bwt/day	0	0.024	3.295	0.030	0.113	3.462
Dates molasses	12.44	ng/day	0.022	0.259	22.507	0.059	0.773	23.620
	1.8832±	ng/kg Bwt/day	0.000	0.004	0.324	0.001	0.011	0.340
Artificial orange juice	112.60	ng/day	2.652	4.605	111.018	2.004	1.132	121.411
	13.9250±	ng/kg Bwt/day	0.038	0.066	1.598	0.029	0.016	1.747
Artificial pomegranate juice	42.80	ng/day	1.475	1.851	79.129	0.306	0.372	83.133
	9.5081±	ng/kg Bwt/day	0.021	0.027	1.139	0.004	0.005	1.196
Coca cola	107.00	ng/day	9.330	1.081	39.248	0.605	0.348	50.612
	17.4866±	ng/kg Bwt/day	0.134	0.016	0.565	0.009	0.005	0.729
7 up	51.80	ng/day	0.394	0.291	1.837	0	0.080	2.602
	±10.3293	ng/kg Bwt/day	0.006	0.004	0.026	0	0.001	0.037
Miranda	56.00	ng/day	20.530	1.501	33.306	0.090	0	55.427
	±10.4843	ng/kg Bwt/day	0.295	0.022	0.479	0.001	0	0.797
Water	1263	ng/day	941.503	1805.900	1727.78	517.199	51.215	5043.597
	±99.8280	ng/kg Bwt/day	13.547	25.984	24.860	7.442	0.737	72.570
Total		ng/day	1002.006	61817.81	2304.873	9523.07	61.929	
		ng/kg Bwt/day	14.417	26.157	433.16	7.527	0.890	

The results of the daily intake of phthalates compounds per kg of body weight in this study were different from the those found by Chang et. al. (2017), as they found that the daily intake for DEP, DBP, DEHP, and DiNP ranged from 0.067-23.9, 0.003-3.31, 0.262-81.5, and 0.062-3.88 μg/kg body weight, respectively, which are very higher compared to the current study. In general, the results of this study of the daily intake of phthalate compounds of the five studied compounds were less than the allowable limits, except those the daily intake of the compounds that came via water, and this is because of the large amounts of water consumed daily. Bradley et. al. (2013) showed that, based on WHO (2023) that the allowable limits of DEP, DB, BBP, DEHP, DiNP, and DiDP are 0.05, 0.01, 0.5, 0.05, 0.15, and 0.15 mg/kg of body weight/ day, respectively.

CONCLUSIONS

In conclusion, this comprehensive analysis revealed the presence of phthalates, including DEP, DBP, TB2-AC, DEHP, and DiNP, in plastic bottled water and most food products packaged in plastic containers. Variations in the concentrations of these compounds were observed between different samples, with TB2-AC being the most abundant and widespread. Water and sesame tahini samples recorded the highest levels of phthalates compared to the other products examined. When evaluating the average daily intake of these substances and the average daily intake per kilogram of body weight, TB2-AC, DEP, and DBP recorded the highest values, indicating that consumers are exposed to these compounds at levels that warrant attention. Water consumption significantly contributed to the total daily phthalate intake, followed by whole milk and sesame tahini consumption. These results clearly indicate that plastic containers used for packaging and storing water and food represent a source of these chemical contaminants being transferred to consumed products. Based on these findings, it is imperative to emphasize the importance of reducing reliance on plastic containers for storing water and food products as much as possible, especially for long periods, and replacing them with safer packaging alternatives such as glass or other materials. This can reduce consumer exposure to these chemicals, which may have undesirable long-term health effects.

ACKNOWLEDGMENT

The researcher extends thanks and appreciation to the Department of Food Sciences / College of Agriculture and Forestry / University of Mosul for the opportunity to complete the research and assistance in providing materials and laboratories.

CONFLICT OF INTEREST

The authors stated that there are no conflicts of interest with the publication of this work.

تحديد مركبات الفثالات المنتقلة من العبوات البلاستيكية إلى الماء والمنتجات الغذائية باستخدام تقنية HPLC وتقدير المتناول اليومي لها

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الخلاصة

أجريت هذه الدراسة لتحديد كميات ثنائي إيثيل الفثالات (DEP) وثنائي بوتيل الفثالات (DBP) وتريبوتيل 2-أسيتيل سيترات (TB2-AC) وثنائي إيثيل هكسيل الفثالات (DEHP) وثنائي إيزونونيل الفثالات (DiNP) في المنتجات الغذائية معبأة في عبوات بلاستيكية. وكذلك لتقدير المتناول اليومي والمتناول اليومي لكل كغم وزن جسم لمجموعة من خمسين متطوعًا (20 ذكرًا و30 أنثى). تم الحصول على المواد الغذائية من السوق المحلية لمدينة الموصل/ العراق وتم تقدير تركيبها الكيميائي والتي قد تؤثر على انتقال الفثالات إلى الغذاء وتم تقدير الاستهلاك اليومي لتسعة من هذه المنتجات الغذائية. أظهرت النتائج أن جميع مركبات الفثالات وجدت تقريبًا في جميع عينات الأغذية بكميات مختلفة وكان الفثالات TB2-AC هو المركب الأكثر وفرة. احتوت عينات الماء والمانجو المخلل وطحينة السمسم على أعلى كميات من مركبات الفثالات بواقع 3.994 و3.229 و3.058 نانوغرام/غم (مل)، على التوالي. احتوت طحينة السمسم على أعلى كميات منDEP (0.9000 نانوغرام/غم)، وكما احتوى الماء على أعلى كمية من مركب DEP (1.430 نانوغرام/مل)، والمانجو المخلل الأعلى في مركب TB2-AC (3.015 نانوغرام/غم)، وكان الكاتشب الأعلى لمركب DEHP (0.438 نانوغرام/غم) واحتوى عصير الليمون المركز على أعلى كمية من DiNP (0.084 نانوغرام/مل). وكشفت النتائج أيضًا أن أعلى تناول مركبات الفثالات كان في TB2-AC وDBP وDEP بواقع 2304.873 نانوغرام/يوم وكان المتناول لكل كغم وزن الجسم 433.16 نانوغرام. بالنسبة للاستهلاك اليومي لمركبات الفثالات الكلية، فقد أسهم الماء في أعلى كمية بمقدار 5043.597 نانوغرام/يوم مع تناول يومي لكل كغم وزن جسم 72.570 نانوغرام. استهلاك الحليب كامل الدسم وطحينة السمسم أسفر عن تناول يومي بلغ 240.643 و88.658 نانوغرام وتناول يومي لكل كغم وزن جسم بلغ 3.462 و1.277 نانوغرام، وعلى التوالي.

الكلمات المفتاحية: TB2-AC(Tributyl ,DBP (Dibutyl phthalate) ,DEP(Diethyl phthalate) ,2-acetyl citrate) ,DEHP (Diethyl hexyl phthalate) ,DiNP(Diisononyl phthalate).

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