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Research Paper / Araştırma Makalesi

Assessment of Health Risks Associated with the Presence of Phthalate Esters in Tea Samples: A Comprehensive Analysis

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ABSTRACT

This study presents novel insights into the occurrence of phthalate esters (PAEs) in tea samples consumed in Turkey. Employing multiple reaction monitoring (MRM) mode with precursor-product ion transitions, quantitative and qualitative analyses of PAEs were conducted using an LC-MS/MS system. A dispersive solid-phase extraction (dSPE) technique was utilized to determine the PAE contents in the tea samples. The limit of detection for all PAEs ranged from 0.350 to 1.882 ng/mL, while the limit of quantification ranged from 1.165 to 6.273 ng/mL. Moreover, strong correlations were observed with R^2 values exceeding 0.996 for all PAEs, indicating robustness. Recovery studies demonstrated satisfactory results, falling within the range of 92.20% to 97.24%, indicating effective retrieval. The relative standard deviation values for the target PAEs ranged from 3.22% to 5.54%. The study findings indicate that PAE levels in the tea samples generally comply with permissible limits set by EU regulations, except for DBP (1807.70±1478.86 ng/mL). Notably, DBP and DINP (941.44±852.80 ng/mL) were identified as the predominant plasticizers in the tea samples. Health risk assessment, conducted through hazard quotient and hazard index calculations across various age groups, consistently yielded values below 1 for DEP, DEHP, DINP, BBP, and DBP, suggesting minimal non-carcinogenic health risks associated with tea consumption across all age groups. Furthermore, the intake of individual phthalate esters, including DEP, DEHP, DINP, BBP, and DBP, remained below acceptable daily limits defined by the EFSA. Although some tea products exhibited slightly elevated PAE levels, overall, the study emphasizes the importance of continuous monitoring and regulations to ensure the safety of packaged tea and mitigate potential long-term exposure risks linked to PAEs.

Keywords: Tea bags, Health risk assessment, Phthalates, LC-MS/MS

Çay Örneklerindeki Fitalat Esterlerinin Varlığına İlişkin Sağlık Risklerinin Değerlendirilmesi: Kapsamlı Bir Analiz

ÖZ

Bu çalışma, Türkiye'de tüketilen çay örneklerinde fitalat esterlerinin (PAE'lerin) varlığına ilişkin yeni perspektifler sunmaktadır. Çoklu reaksiyon izleme (MRM) modu ve öncü-ürün iyon geçişleriyle, LC-MS/MS sistemi kullanılarak PAE'lerin nicel ve nitel analizleri gerçekleştirilmiştir. Çay örneklerindeki PAE içeriklerini belirlemek için dağıtıcı katı faz ekstraksiyonu (dSPE) tekniği kullanılmıştır. Tüm PAE'ler için saptama limiti (LOD) 0.350 ile 1.882 ng/mL arasında değişirken, nicemleme limiti (LOQ) 1.165 ile 6.273 ng/mL arasında değişmektedir. Ayrıca, tüm PAE'ler için 0.996'dan büyük R^2 değerleriyle güçlü korelasyonlar gözlemlenmiş, bu da sağlamlığı işaret etmektedir. Kurtarma çalışmaları, etkili kurtarma gösteren %92.20 ila %97.24 aralığında tatmin edici sonuçlar vermiştir. Hedef PAE'ler için göreceli standart sapma değerleri %3.22 ila %5.54 arasında değişmektedir. Çalışmanın bulguları, çay örneklerindeki PAE seviyelerinin genellikle AB düzenlemeleri tarafından belirlenen izin verilen sınırlarla uyumlu olduğunu göstermektedir, ancak DBP (1807.70±1478.86 ng/mL) için değil. Özellikle, DBP ve DINP (941.44±852.80 ng/mL) çay örneklerinde belirgin plastikleştiriciler olarak belirlenmiştir. Çeşitli yaş grupları arasında yapılan tehlike oranı (HQ) ve tehlike indeksi (HI) hesaplamalarıyla gerçekleştirilen sağlık riski değerlendirmesi, çay tüketiminin tüm yaş gruplarında minimal kanserojen olmayan sağlık riskleri taşıdığını gösteren DEP, DEHP, DINP, BBP ve DBP için 1'den düşük değerler sağlamıştır. Ayrıca, EFSA tarafından belirlenen kabul edilebilir günlük limitler dahilinde DEP, DEHP, DINP, BBP ve DBP gibi bireysel fitalat esterlerinin alımı devam etmektedir. Bazı çay ürünlerinin hafif yüksek PAE seviyeleri göstermesine rağmen, genel olarak, çalışma, paketli çayın güvenliğini sağlamak ve PAE'lerle ilişkilendirilen potansiyel uzun vadeli maruziyet risklerini en aza indirmek için sürekli izleme ve düzenlemelerin önemini vurgulamaktadır.

Anahtar Kelimeler: Çay poşetleri, Sağlık risk değerlendirmesi, Fitalatlar, LC-MS/MS

INTRODUCTION

Tea, originating from the leaves of the plant species *Camellia sinensis* L., is one of the most extensively consumed beverages on a global scale [1]. Despite lacking official endorsement from the US Food and Drug Administration (FDA), the consumption of hot water tea extracts, commonly known as tea infusions, is believed by some to confer beneficial effects on human health [2], while also being rich in proteins, amino acids, vitamins, and minerals [1], and showing potential in reducing the risk of chronic diseases such as heart disease [3], diabetes [4], arthritis [3], and cancer [6]. Due to its perceived therapeutic efficacy and the assumption of minimal or negligible toxic side-effects when consumed in significant quantities compared to synthetic drugs, consumers may regard tea as a safe beverage. However, concerns may arise regarding the presence of poly-fluoroalkyl substances (PFAS) [1], heavy metals [2], phthalate esters (PAEs) [7] in tea and their potential adverse health effects. PAEs, chemical compounds commonly employed as plastic softeners, have been well-established as endocrine disruptors [8]. Exposure to high doses of certain PAEs has been associated with reproductive and developmental toxicities in both males and females [9]. Several human studies have explored potential links between PAEs and alterations in semen quality [10], shortened gestation [11], reduced anogenital distance in newborn boys [12], and premature breast development in girls [13]. Although the inherent PAEs contamination in tea leaves is typically minimal, the tea production process, encompassing various stages such as harvesting, transportation, transformation, and packaging [14], can introduce PAEs into the final tea product [2]. Notably, the principal source of PAEs in commercial tea products appears to be the plastics used for packaging or the plastic lining present in filter paper-based tea bags [15]. In this context, the present study aims to investigate the presence of PAEs in tea products available in the Turkish market. The study aims to assess the potential migration of PAEs from the packaging materials into the tea and its potential implications for consumer health.

MATERIALS and METHODS

Chemicals and Reagents

In this research, we employed high-purity chemicals and standards for the analyses. Formic acid (>98%), acetic acid (100% glacial), and acetonitrile (>99.9%) were sourced from Merck (Darmstadt, Germany), while di-isodecyl phthalate (DIDP), methanol (≥99.9%), and di-isononyl phthalate (DINP) were obtained from Sigma-Aldrich (St. Louis, USA). The standard mixture, PAE 2000 µg/mL, was acquired from Dr. Ehrenstorfer (Augsburg, Bavaria, Germany). To facilitate filtration, a 0.45 µm PTFE filter from ISOLAB (Wertheim, Germany) was utilized. For the extraction process, Q-sep dSPE extraction salt and Q-sep dSPE tubes were provided by RESTEK (Bellefonte, USA).

Sampling Method

In this comprehensive research, our core objective was to investigate the occurrence of PAEs in various tea samples and assess their potential impact on consumer exposure. To accomplish this, we collected a total of 64 tea samples from both local and international markets, encompassing eight distinct types of tea: black, Import black, green, Import green, earl grey, Import earl grey, herbal, and fruit. All teas examined within the scope of the research are composed of teas packaged in tea bags for sale. The samples were analyzed for PAE content, focusing on potential variations among different tea products. The insights gained from this study provide valuable information regarding PAE exposure through tea consumption, aiding in the establishment of consumer safety measures. Understanding the presence and potential health implications of PAEs in tea is crucial for ensuring food safety and promoting public health.

Sample Extraction Procedure

In this study, we employed a dispersive solid-phase extraction (dSPE) technique to determine the PAE contents in the tea samples, following the protocol outlined by Isci et al [16]. The tea samples were first prepared by brewing them in glass beakers, following the instructions provided on the tea packaging. Subsequently, a combination of ultrapure water and acetonitrile, along with dSPE extraction salt, was added to the tea samples. After vigorous vortexing and centrifugation, the supernatant, primarily containing acetonitrile, was separated. Next, the extraction tube underwent a vacuum-assisted drying process, and PSA powder and cleaning salts were added. After another round of vortexing and centrifugation, the resulting supernatant was carefully filtered through a 0.45 µm filter before being injected into the LC-MS/MS system for analysis.

Instrument

The liquid chromatography (LC) system utilized in this study was an advanced triple quadrupole instrument

(Agilent Model no: K6460) manufactured by Agilent Technologies, a prominent company based in Loveland, CO, USA. This system was equipped with cutting-edge MS/MS (Mass Spectrometry) capabilities, ensuring precise and reliable analyses. The setup also incorporated other crucial components, including a highly efficient Vacuum Degasser (Agilent Model No: G1322A 1200 Series), a versatile Quaternary Pump (Agilent Model No: G4204A), a modern Infinity Autosampler 1260 Series (Agilent Model No: G4226A), and a thermostatted column oven 1200 Series (Agilent Model No: G1316A). To achieve optimal separation and detection performance, we carefully selected a Poroshell 120 SB-C18 column (3.0 mm, 100 mm, 2.7 µm) from Agilent Technologies as the chromatographic column.

LC-MS/MS Analysis

In this study, the quantitative and qualitative analysis of PAEs was conducted using the multiple reaction monitoring (MRM) mode with precursor-product ion transitions. The LC-MS/MS system employed in this analysis utilized a mobile phase A, composed of 0.1% formic acid and 5 mM ammonium formate in water, with an injection volume of 5 µL and a flow rate of 0.3 mL/min. Additionally, mobile phase B, containing 0.1% formic acid in methanol, was also used. To establish the calibration curve, a series of known concentrations of PAEs ranging from 1 to 250 ng/mL were prepared and injected twice into the LC-MS/MS system. To ensure the reliability of the method, we evaluated several important parameters, including the relative standard deviation (RSD) to measure precision, the limit of detection (LOD) to establish the lowest detectable concentration, the limit of quantification (LOQ) to determine the lowest accurately quantifiable concentration, the correlation coefficient (R^2) to assess calibration curve linearity, and the recovery (%) to measure the accuracy of the method by determining the amount of analyte successfully recovered from the samples. The results of these evaluations are presented in Table 1, demonstrating the method's robustness and suitability for analyzing PAEs in tea samples.

Study Population

The Turkey Nutrition and Health Survey (TBSA) is a resource prepared with the aim of ensuring that individuals living in Turkey have a healthy and balanced diet. This guide includes information on dietary habits and nutritional status in Turkey. The TBSA was jointly prepared by the Ministry of Health. This guide has been created based on scientific research and the opinions of nutrition experts [17]. According to TBSA, the daily tea consumption for males is as follows: 221.5 ± 261.9 mL day^{-1} for ages 15-18, 570.3 ± 473.2 mL day^{-1} for ages 19-64, and 423.5 ± 308.5 mL day^{-1} for ages >65. For females, the daily tea consumption is 142.6 ± 174.2 mL day^{-1} for ages 15-18, 459.1 ± 401.6 mL day^{-1} for ages 19-64, and 316.52 ± 261.7 mL day^{-1} for ages >65. The exposure calculations in the study were based on these recommended consumption values (Table 1).

Table 1. Parameters used for EDI and HQ calculation in different age groups (mean±standard deviation)

Age (years)	Body weight (kg)	Tea consumption (mL/day)
	Male	
15-18	66.30±14.50	221.50±261.90
19-64	81.20±15.30	570.30±473.20
>65	78.80±13.50	423.50±308.50
	Female	
15-18	58.60±14.30	142.60±174.20
19-64	71.60±15.80	459.10±401.60
>65	73.60±16.00	316.20±261.70

Non-carcinogenic Risk Assessment of PAEs

In order to evaluate the potential health risks associated with PAEs in tea, we focused on estimating the daily intake (EDI) of these compounds based on average concentration levels (C) in ng/mL. To achieve this, we took into consideration various essential factors, including the daily volume of tea consumed (IV) in milliliters (mL), the individual's body weight (Bw) in kilograms (kg), and their respective age groups, as specified in the study population. Utilizing the established Equation (1), we calculated the estimated daily intake (EDI) of PAEs, providing a comprehensive assessment of the exposure levels related to tea consumption and its potential health implications.

$$EDI = (C \times IV)/B_W \quad \text{Eq. (1)}$$

To determine the estimated daily intake (EDI) of PAEs, a comprehensive calculation was conducted, yielding results expressed in µg/kg Bw/day.

Hazard Index (HI) and Hazard Quotient (HQ)

The assessment of potential health risks, including both carcinogenic and non-carcinogenic effects, related to tea consumption involved the computation of the HQ for individual PAEs, as detailed in Equation (2). Additionally, the HI was calculated for multiple PAEs using Equation (3), following the methodology established by the US Environmental Protection Agency [18].

$$HQ = EDI/RfD \quad \text{Eq. (2)}$$

An HQ and HI values below 1 indicates a safe level of health risk, while an HQ and HI values equal to or greater than 1 suggests a potential non-carcinogenic health risk [18].

$HI = \sum HQ$DEP + HQBBP+ $HQDBP$ + $HQDEHP$ +HQDINP Eq. (3)

The RfD (Reference Dose) values in (Eq. 2) are $2x10^{-1}$,810^{-1}, $1x10^{-1}$, $8x10^{-1}$, $2x10^{-2}$ and mg/kg Bw/day for BBP, DINP, DBP, DEP, DEHP, respectively [12].

Carcinogenic Risk Assessment of PAEs

Within the scope of this study, our assessment of cancer risk (CR) was specifically centered on DEHP and BBP. We utilized the corresponding cancer slope factor (CSF) values provided by the USEPA, which were reported as $1.4x10^{-2}$ mg/kg/day for DEHP and $1.9x10^{-3}$ mg/kg/day for BBP. This approach allowed us to delve into the potential cancer risks associated with these specific compounds and their exposure levels [19]. The daily intake (EDI) value, expressed in μg/kg Bw/day, represents the calculated intake of PAEs resulting from tea consumption.

CR = CSF × EDI Eq. (4)

The CR assessment, following US EPA guidelines, evaluates potential oral health risks related to specific substances. CR values are classified into three levels: high cancer risk ($CR>1\times10^{-4}$), indicating significant health risk; acceptable cancer risk (CR: $1x10^{-6}$ to $1x10^{-4}$), within an acceptable range; and negligible cancer risk ($CR<1x10^{-6}$), indicating minimal risk [19].

Statistical Analysis

The analysis of the obtained data involved statistical procedures such as ANOVA, analysis of variance, performed using SPSS version 26.0 (IBM, Chicago, IL, USA). In the case of tea samples, ANOVA was utilized to examine the average PAE values, with a significance level set at $p<0.05$. To ascertain the statistical variances among tea samples, Duncan's multiple tests were applied. Integral percentiles, including the mean, P5, P50 (median), and P95, were pivotal in evaluating the data.

RESULTS and DISCUSSION

Quality Control and Quality Assurance

As shown in Table 2, the LOD for all PAEs ranged from 0.350 to 1.882 ng/mL, while the LOQ ranged from 1.165 to 6.273 ng/mL. Moreover, the R^2 exceeded 0.996 for all PAEs, indicating a strong correlation. Additionally, the recovery studies demonstrated satisfactory results, falling within the range of 92.20% to 97.24%, thus indicating effective recovery. The RSD values for the target PAEs ranged from 3.22% to 5.54%, further affirming the suitability of the extraction method within the tea matrix.

Table 2. Method verification parameters

Analytes	Retention time (min)	Spiking level (ng/mL)	Linearly range		Recovery (%)	RSD (%)	Quantification	
			(ng/mL)	R^2	Tea	Tea	LOD (ng/mL)	LOQ (ng/mL)
DMP	2.62	100	1-100	0.998	94.75	5.27	1.882	6.272
DEP	3.58	100	1-100	0.998	95.44	4.57	0.350	1.165
BBP	5.16	100	1-100	0.998	94.40	3.22	0.517	1.723
DBP	5.25	100	1-100	0.999	92.20	4.78	0.567	1.891
DEHP	7.92	100	1-100	0.999	97.24	5.27	1.176	3.920
DNOP	8.02	100	1-100	0.999	95.42	5.27	1.347	4.490
DINP	8.24	100	1-100	0.996	95.74	5.54	1.064	3.546
DIDP	8.51	100	1-100	0.999	95.42	4.70	0.474	1.581

PAEs Levels in Samples

PAE levels of BBP, DBP, DEHP, DMP, DIDP, DNOP, DEP, and DINP of 64 tea samples with different types which are available in Turkey are shown in Table 3. Among the samples, all PAEs were detected. The mean DEHP levels across the different types were detected at 743.29 (ND-4507.84) ng/mL. Statistical analysis indicated that there were no significant differences in DEHP levels among the various types (P>0.05). The highest average DEHP levels was found in I. Black (1199.62 ng/mL), while the lowest average level was found in herbal (327.35 ng/mL). Under Commission Regulation (EU) No 10/ (2011) [20], maximum allowable specific migration limit (SML) value of 1.5 mg/kg has been set for DEHP. The mean DEHP levels in all samples do not exceed the established SML value. Contrary to this study, the literature lacks research on the migration of PAEs from tea bags. However, there are some studies related to tea and tea cultivation. In comparison, Tang et al. [21] reported lower DEHP levels in Chinese black tea samples, ranging from 11.73 μg kg^{-1} to 28.33 μg/kg. Additionally, Troisi et al.[2] observed a median DEHP concentration of 9.4 (8–10.8) ng/mL in black tea and 8.6 (8.3–10.4) ng/mL in green tea from Southern Italy. Liu et al.[22] reported an average DEHP range of 10.7 μg kg^{-1} to 353.3 μg kg^{-1} in fresh tea leaves sourced from China. Similarly, Li et al. [23] detected mean DEHP concentrations spanning from ND to 9.34 mg/kg in Chinese tea plantation soils. The utilization of nylon or polyethylene terephthalate (PET) tea bags is linked to the migration of micro- and nanoparticles [24]. These PET tea bags may contribute to the migration of PAEs. The analysis revealed that the mean concentrations of DEP across various tea types ranged from 26.52 (ND-143.36) ng/mL. Among the different tea types, herbal tea samples exhibited the highest average DEP levels (38.50 ng/mL), while e. green tea samples showed the lowest average levels (13.15 ng/mL). Despite these variations, statistical analysis indicated no significant difference in DEP levels among the different tea types (P>0.05). Notably, Alnaimat et al. [15] conducted a study in which DEP was not detected in any of the tea bag infusions that were analyzed. In our study, we found that the average concentrations of DBP varied among various types of tea, ranging from 1807.70 (60.98-5905.38) ng/mL. Notably, herbal tea samples displayed the highest average DBP levels (2400.83 ng/mL), whereas I. Earl Grey tea samples exhibited the lowest average levels (1436.37 ng/mL). The DBP was determined as the most abundant plasticizer in tea samples. It is important to highlight that Commission Regulation No 10/(2011) sets the SML for DBP at 0.3 mg/kg. However, upon thorough assessment of the tea samples, it was observed that the average migration of DBP from tea bags significantly exceeded the established SML value. Alnaimat et al. [15] reported significantly lower values compared to the findings of this study, indicating the presence of DBP in all analyzed tea bag infusions, with concentrations ranging from 12.6 to 51.7 ng/mL. The observed disparity between these two studies is believed to originate from the variance in the materials used to produce tea bags. In this study, the mean DINP levels across distinct tea types were determined to be 941.44 (19.11-2682.09) ng/mL. Among the different tea types, fruit tea samples exhibited the highest average DINP levels (1121.93 ng/mL), while e. black samples showed the lowest average levels (788.93 ng/mL). Similarly, the mean DIDP levels across the various tea types were detected at 104.23 (1.23-526.74) ng/mL. Notably, black tea samples displayed the highest average DIDP levels (145.99 ng/mL), while I. black samples exhibited the lowest average levels (79.89 ng/mL). Despite these variations, the statistical analysis indicated no significant difference in DINP and DIDP levels among the different tea types (P>0.05). According to Commission Regulation No 10/ (2011), the SML for both DINP and DIDP is established at 9 mg kg^{-1}. In this study, the levels of DINP and DIDP in the tea samples were found to be below the established limits. The mean DNOP levels among different tea types were measured at 383.43 (ND-2558.91) ng/mL. The statistical analysis demonstrated no significant variations in DNOP levels across the different types (P>0.05). The highest average DNOP concentration was observed in I. black tea samples (638.96 ng/mL), while the lowest average level was identified in herbal tea samples (147.69 ng/mL). The analysis showed no significant differences in DMP and BBP levels among the different types (P>0.05). The mean DMP and BBP concentrations across various tea types were 3.15 (ND-122.26) ng/mL and 2.19 (ND-61.30) ng/mL, respectively. Notably, the levels of BBP in the tea samples remained below the established limit of 30 mg/kg according to Commission Regulation No 10/(2011). The study highlights the significance of considering sample collection methods, analytical techniques, and regional differences when interpreting results. Variations in PAE concentrations among samples may be attributed to factors like production methods, additives, machinery, and packaging materials. Notably, except for DBP, all tested tea samples in this study remained below the SML defined by Commission Regulation (EU) No 10/2011 for PAEs, confirming their safety for consumers. Nonetheless, continuous monitoring of PAE levels is vital, especially for food-contact plastics, to ensure compliance with safety regulations and minimize potential risks associated with PAE exposure.

Table 3. The PAE's level of different tea samples (ng/mL) (mean±standard deviation)

Tea Types	BBP	DMP	DBP	DEHP	DEP	DINP	DIDP	DNOP
Black	1.27±3.35 (ND-9.55)	<LOD	2249.77±2009.14 (372.30-5905.38)	1015.15±1528.42 (ND-4507.84)	20.79±15.57 (ND-45.36)	1067.54±1116.42 (29.24-2682.09)	145.99±184.04 (6.57-526.74)	553.72±872.40 (ND-2558.91)
I. Black	2.56±4.98 (ND-14.41)	17.19±42.69 (ND-122.26)	1499.84±1560.80 (60.98-4493.06)	1199.62±1164.25 (ND-3267.07)	25.15±49.62 (ND-143.36)	788.93±757.61 (19.32-2066.27)	79.89±101.67 (1.65-258.15)	638.96±679.83 (18.19-1846.78)
Green	7.85±21.60 (ND-61.30)	<LOD	1479.98±1013.33 (299.38-2826.26)	834.80±1289.17 (ND-3930.93)	31.28±39.60 (ND-101.44)	842.01±845.49 (25.51-2265.40)	87.33±106.30 (1.23-261.70)	429.06±739.00 (ND-2231.16)
I. Green	2.01±2.85 (ND-6.79)	<LOD	1685.39±1186.79 (175.81-3097.68)	833.48±932.76 (10.64-3001.58)	13.15±20.36 (ND-61.76)	893.78±828.27 (116.09-2080.09)	99.07±110.02 (5.26-265.31)	426.31±539.29 (0.24-1701.87)
Earl Grey	0.92±0.86 (ND-2.11)	<LOD	2055.37±1742.92 (121.78-4117.24)	806.58±1102.86 (8.07-3311.63)	26.23±19.98 (2.39-58.83)	940.32±845.70 (53.15-2332.40)	105.63±112.05 (2.05-303.20)	420.43±635.14 (1.07-1878.42)
I. Earl Grey	1.12±1.78 (ND-5.32)	2.12±4.01 (ND-9.95)	1436.37±1455.50 (113.17-4287.38)	327.91±313.13 (ND-771.97)	38.01±40.90 (ND-106.71)	895.68±881.01 (19.11-2192.18)	92.33±113.29 (6.23-264.51)	166.58±155.89 (ND-431.34)
Herbal	1.18±1.71 (ND-4.42)	2.69±3.41 (ND-8.02)	2400.83±1725.56 (306.76-4370.22)	327.35±377.29 (ND-866.21)	38.50±33.68 (ND-104.64)	981.31±928.59 (89.23-2333.21)	108.40±116.34 (14.67-281.88)	147.69±159.33 (ND-332.11)
Fruit	0.59±0.86 (ND-2.17)	<LOD	1654.06±1230.09 (292.34-3720.87)	601.42±763.06 (34.02-2333.87)	19.05±27.75 (ND-81.63)	1121.93±932.96 (137.14-2362.95)	115.22±119.86 (5.31-286.08)	284.67±429.04 (18.36-1319.69)
Mean± SD (min. - max.)	2.19±7.91 (ND-61.30)	3.15±15.41 (ND-122.26)	1807.70±1478.86 (60.98-5905.38)	743.29±1001.46 (ND-4507.84)	26.52±32.17 (ND-143.36)	941.44±852.80 (19.11-2682.09)	104.23±117.50 (1.23-526.74)	383.43±571.51 (ND-2558.91)

ND, Not Detected; I, Import

Health Risk Assessment

Hazard Quotient (HQ) and Hazard Index (HI)

The analysis presented in Table 4 and Table 5 offers valuable insights into the non-carcinogenic health risks linked to tea consumption across different age groups. Notably, all calculated HQ and HI values for BBP, DEP, DBP, DEHP, and DINP were found to be below 1, indicating that the levels of these substances in tea do not pose significant non-carcinogenic health risks to the population (USEPA, 2019). Population exposure to DEP, DBP, DEHP, and DINP from various tea brands and types in Turkey was assessed using both average and P95 sample amounts, as shown in Table 4. When comparing the results with the TDI values recommended by EFSA [25] 0.5, 0.5, 0.15, 0.05, and 0.01 mg/kg/day for BBP, DEP, DINP, DEHP, and DBP, respectively. it becomes evident that the exposure levels for all age groups remain below the established TDI values. These findings suggest that consuming tea available in the Turkish market does not pose a health risk. While limited researchers have explored tea consumption-related exposure assessments, a study by Li et al.[23] revealed that the mean HQ associated with DEHP, DiBP, DOP, DEP, and DMP were all below 1. Additionally, Alnaimat et al. [15] reported that HQ values were consistently below 1 as well. This comprehensive evaluation contributes to a more accurate understanding of potential health risks related to PAE exposure from various food sources.

Table 4. Consumers exposure to PAEs from different tea consumption (mean±standard deviation)

Analytes	Age (years)	Female				Male			
		Exposure (µg/kg Bw/day)	P95	P50 (Median)	P5	Exposure (µg/kg Bw/day)	P95	P50 (Median)	P5
BBP	15-18	0.01±0.00	0.02	2.96E-03	6.18E-03	0.01±0.00	0.03	4.10E-03	0.00
	19-64	0.01±0.01	0.05	0.01	8.10E-03	0.02±0.01	0.06	0.01	8.94E-03
	>65	0.01±0.01	0.03	0.01	8.58E-03	0.01±0.01	0.04	0.01	6.82E-03
DBP	15-18	4.36±0.85	5.80	4.03	3.47	6.05±1.18	8.04	5.59	4.81
	19-64	11.52±1.24	15.31	10.64	9.16	12.72±2.47	16.89	11.75	10.11
	>65	7.72±1.50	10.25	7.13	6.13	9.70±1.89	12.89	8.96	7.71
DEHP	15-18	1.79±0.48	2.90	1.98	0.79	2.49±0.67	4.02	2.75	1.10
	19-64	4.74±1.28	7.65	5.23	2.09	5.23±1.41	8.44	5.77	2.30
	>65	3.17±0.86	5.12	3.50	1.40	3.99±1.08	6.44	4.40	1.76
DEP	15-18	0.06±0.02	0.09	0.06	0.03	0.09± 0.03	0.13	0.09	0.06
	19-64	0.17±0.05	0.24	0.16	0.08	0.19±0.05	0.27	0.18	0.03
	>65	0.11±0.03	0.16	0.11	0.06	0.14±0.04	0.20	0.14	0.07
DINP	15-18	2.27±0.21	2.71	2.21	1.90	3.15±0.29	3.75	3.07	2.64
	19-64	6.00±0.55	7.15	5.85	5.02	6.62±0.60	7.89	6.46	5.55
	>65	4.02±0.37	4.79	3.92	3.36	5.05±0.46	6.02	4.92	4.23

Table 5. Estimation of HQ, HI and CR values of PAEs exposure due to tea consumption

Analytes	Age groups (years)	Consumers exposure			
		Female		Male	
		HQ	CR	HQ	CR
BBP	15-18	2.64E-05	1.00E-05	3.66E-05	1.39E-05
	19-64	6.97E-05	2.65E-05	7.70E-05	2.92E-05
	>65	4.67E-05	1.77E-05	5.87E-05	2.23E-05
DEHP	15-18	8.97E-02	2.51E-02	0.12	3.48E-02
	19-64	2.37E-01	6.63E-02	0.26	7.32E-02
	>65	1.59E-01	4.44E-02	1.99E-01	5.59E-02
DEP	15-18	7.95E-05	*	1.10E-04	*
	19-64	2.10E-04	*	2.32E-04	*
	>65	1.41E-04	*	1.77E-04	*
DINP	15-18	2.84E-03	*	3.94E-03	*
	19-64	7.50E-03	*	0.01	*
	>65	5.02E-03	*	6.31E-03	*
DBP	15-18	4.36E-02	*	0.06	*
	19-64	1.15E-01	*	0.13	*
	>65	7.72E-02	*	0.10	*
HI	15-18	0.14		0.19	
	19-64	0.36		0.40	
	>65	0.24		0.30	

*Not calculated for CR; HQ, Hazard Quotient

Carcinogenic Risk (CR) Assessment

CR indicates a risk assessment and classification system developed by the US Environmental Protection Agency [19]. In this system, grade A (CR>$1x10^{-4}$) indicates high cancer risk, grade B (CR: $1x10^{-6}$ to $1x10^{-5}$) indicates acceptable cancer risk, and grade C (CR<$1x10^{-6}$) indicates a negligible cancer risk. In this context, carcinogenic risk assessment among age groups were calculated for DEHP and BBP, whose CSF values were given by the authorities. The carcinogenic risk assessment results for tea consumers of different ages are summarized in Table 5. Carcinogenic risk factors for BBP in age and gender groups consuming tea are in the range of $1.00x10^{-5}$- $2.92x10^{-5}$. So, the carcinogenic risk factor for BBP was determined at the acceptable cancer risk level (grade B) for all age and gender groups. Furthermore, the carcinogenic risk assessment for DEHP among different age and gender groups consuming tea revealed a range of $2.51x10^{-2}$ to $7.32x10^{-2}$. As a result, the carcinogenic risk factor for DEHP was classified at a high cancer risk level (Grade A) across all age and gender categories. In the context of the existing literature, only one study was identified concerning the estimated cancer risk factor in tea samples. Li et al. [23] conducted a study where they determined that the average cancer risk (CR) value for DEHP through dietary exposure surpassed the threshold of 10^{-6}, measuring specifically at $7.20x10^{-6}$ for adults and $2.84x10^{-6}$ for children. These findings raise concerns regarding the potential carcinogenic risk associated with DEHP exposure. In contrast, the present study focused on the consumption of steeped tea from tea bags and indicated an acceptable level of cancer risk.

CONCLUSION

This study provides valuable insights into the presence of plasticizers (specifically, phthalate esters or PAEs) and their potential impact on consumers in tea samples within Turkey. The findings indicated detectable levels of PAEs in the tea samples, although tested compounds except for DBP remained below the SML established by the EU Regulation. Among the analyzed PAEs, DINP, and DBP were identified as the most prevalent plasticizers in the tea samples. To assess potential health risks, researchers calculated HQ and HI values based on tea consumption patterns across various age and gender groups. The results revealed that all HQ and HI values for DEHP, DINP, DEP, and DBP were below 1, indicating minimal non-carcinogenic health risks associated with tea consumption across different age groups. Furthermore, the dietary intake levels of each phthalate ester, including BBP, DEHP, DINP, DEP, and DBP, were found to be well below the TDI values established by the EFSA. The study offers reassurance that the detected concentrations of PAEs in tea samples, whether from local or international brands in Turkey, do not pose a health risk to consumers. However, it does raise concerns about the possibility of long-term consumption of packaged tea leading to PAE exposure exceeding TDI values. Notably, packaged tea products generally exhibited slightly elevated PAE levels, resulting in marginally increased exposure for consumers of such products. In conclusion, this research underscores the significance of ongoing monitoring and regulatory measures to ensure the safety of packaged tea and mitigate potential risks associated with long-term PAE exposure.

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