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Association between Water Consumption from Polycarbonate Containers and Bisphenol A Intake during Harsh Environmental Conditions in Summer

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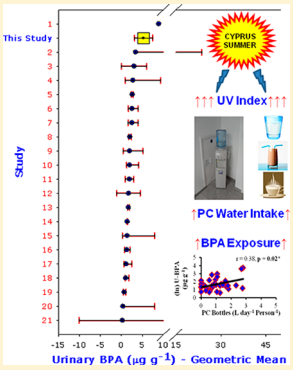
Supporting Information

ABSTRACT: With the exception of polycarbonate (PC) baby bottles, little attention has been paid to bisphenol A (BPA) intake from packaged water consumption (PC water dispensers), especially during summer weather conditions. We determined the magnitude and variability of urinary BPA concentrations during summer in 35 healthy individuals largely relying upon PC packaged water to satisfy their potable needs. We used liquid chromatography−tandem mass spectrometry to measure urinary BPA concentrations. A questionnaire was administered in July/August and a spot urine sample was collected on the same day and 7 days after the completion of the interview (without intervention). Linear regression was performed to assess the association of variables, such as water consumption from different sources, on urinary BPA levels for the average of the two urine samples. A significant positive association (p = 0.017) was observed between PC water consumption and urinary BPA levels in females, even after adjusting for covariates in a multivariate regression model. The geometric mean of daily BPA intake back-calculated from urinary BPA data was 118 ng·(kg bw)$^{-1}$·day^{-1}, nearly double the average intake levels observed in biomonitoring studies worldwide. High urinary BPA levels were partially ascribed to summer's high PC water consumption and weather characteristics (high temperatures, >40 °C; very high UV index values, >8), which could be causing BPA leaching from PC. It is suggested that PC-based water consumption could serve as a proxy for urinary BPA, although the magnitude of its relative contribution to overall daily intake requires further investigation.



1. INTRODUCTION

Bisphenol A (BPA) is one of the most studied high production volume chemicals. The predominant applications of BPA (by volume) are (i) polycarbonate (PC) bottles used for potable purposes, such as large water containers,[1] baby bottles,[2] and water cookers[3] (i.e., plastic electric cooker/kettle for water heating with polycarbonate-based inner lining); (ii) constituent in epoxy resins applied to inner walls of canned food and beverages;[4,5] (iii) in epoxy resin-based relining of urban drinking water pipe network;[6] and (iv) in numerous personal care products (PCP), such as sunscreen, nail polish, body wash/lotion, bar soap, shampoo, conditioner, shaving cream, and face lotion/cleanser.[7] Human exposures to BPA from various consumer products has lately gained intense attention due to its endocrine-disrupting properties and its association with several endocrine-related end points, such as reproductive function studied in the setting of in vitro fertilization;[8,9] thyroid dysfunction via fluctuations in up- or downregulation of thyroid hormones;[10] metabolic syndrome, obesity, and type 2 diabetes mellitus;[11,12] hypertension[13] and cardiovascular conditions.[14]

BPA's widespread use in consumer products makes it vulnerable to seasonal manifestation of extreme weather environmental conditions, such as temperature, UV exposure duration, and frequency of water-contact materials (WCM) reuse,[15] promoting leaching of BPA and other packaging-related constituents into water/foodstuff ready for human consumption. Polycarbonate water dispensers (19 L) may be subject to reuse as much as 50 times after bottle washing with sodium hydroxide and hydrogen peroxide at 75 °C upon each emptying of contents (personal communication with a bottled water company in Limassol, Cyprus). In India, such bottles were used at least 100 times, while cleaning between uses entailed wash cycles with mild soap and sodium hypochlorite followed by hot air drying (personal communication with Ram Das at Sri Krishna Bottling Company in Delhi, India). Under various environmental and cleaning conditions, WCM have

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been found to leach well-known endocrine-disrupting chemicals (EDC) into packaged water, such as BPA, phthalates, perfluorinated compounds, alkylphenols like 4-nonylphenol, adipates, etc.,[2,16,17] and perhaps brominated flame retardants.[1]

During summer, the prolonged occurrence and combined effects of high temperatures, extended UV exposure at high UV index values, and reuse of WCM on promoting BPA leaching from PC-based WCM remain elusive and difficult to study. The situation may be exacerbated in countries facing intense meteorological events, such as prolonged water shortage and very high air temperatures in the summer, including countries surrounding the Mediterranean Sea. Cyprus is a Mediterranean country that belongs to the European Union that can afford the luxury of choosing from more than one water source to satisfy potable needs (tap water, bottled water, etc.). Prolonged water scarcity and misconceptions on tap water quality are primarily responsible for the increasing bottled water consumption pattern in Cyprus, ranking it in the top 20 countries with the highest bottled water consumption worldwide.[18] It was anticipated that summer peaks of water consumption and meteorological conditions in Cyprus, where temperatures >40 °C[19] and very high UV index values (>8)[20] often prevail, could be held responsible for elevated BPA leaching from WCM, reflected in elevated urinary BPA biomarkers. Indeed, the use of polycarbonate-based (BPA-containing) WCM to satisfy potable needs is very common in countries of the Mediterranean region.

Limited information is currently available on the relationship between urinary BPA measurements and daily water consumption from various sources, such as PC and/or polyethylene terephthalate (PET) bottled waters, and different potable water uses, such as plain water and water used to prepare cold/hot beverages (coffee, tea, and juices). Our goal was to conduct a study of this relationship in Cyprus during July and August, the hottest months of the year in Cyprus, often reaching temperatures >40 °C. Our earlier work suggested that increased temperature, UV exposure duration, and frequency of reuse could be held responsible for the leaching of other WCM constituents (antimony) into packaged water.[15] Exposure of WCM to the aforementioned environmental conditions in summer, during WCM production and cleaning, as well as transport and storage before consumption is commonly practiced, because of the lack of harmonized regulatory guidelines to minimize WCM exposure to harsh environmental conditions.

We hypothesized that summer's high temperatures [>40 °C],[19] very high UV exposure index values of above 8 on a generic 10-point scale,[20] and WCM reuse (particularly for the 19 L large water dispensers), that largely prevail in Cyprus in July and August, could result in elevated BPA intake rates as reflected by higher urinary BPA levels. In addition, the degree of association between urinary BPA and other established and potential sources of BPA exposure, such as cosmetics, PCP, vinyl material, and canned food, was also assessed. The main objectives of this study were (i) to investigate the magnitude and variability of human BPA exposures to PC-based WCM but also to other important BPA exposure sources, such as PCP, cosmetics, and canned food; and (ii) to evaluate the degree of association between demographic characteristics, daily water source and water usage habits, PCP, cosmetics, and canned food use and individual urinary BPA measurements.

2. MATERIALS AND METHODS

2.1. Pilot Study Design, Survey, and Sample Collection. Details about the study have been previously presented.[21] In brief, a pilot study was initiated in Cyprus with a young adult group of 35 volunteers (university graduate students and staff) that at least partially depended on PC-based WCM to satisfy their potable needs while at the university or at home. Signed informed consents were individually obtained from all participants adhering to institutional review requirements. A detailed questionnaire was administered as modified from an earlier EDC-based human exposure study at Harvard School of Public Health, based on current understanding of possible BPA exposure sources. The questionnaire took the form of an interview with our trained personnel, after which a spot urine sample was collected. All interviews and subsequent urine sample collection were performed toward the end of July and early August 2010, that is, the warmest months of the year in Cyprus.[19] A second spot urine sample was collected from the same individuals, about 7 days after the collection of the first urine spot sample, without any BPA-related intervention. Collecting repeated sampling was deemed necessary for small molecules with short biological half-lives, like BPA (half-life <6 h),[22] because for molecules with half-lives between 5 and 50 h, sampling frequency should be performed at least once a week in order for biological indicator data to be considered uncorrelated. This is the reason why we chose to have a week between the two urine spot samples in our study, minimizing autocorrelation issues.[23]

Survey questions dealt with demographics (gender, age, weight, residence location and duration, smoking history), water consumption patterns (water sources, plastic bottle types, daily volume of water consumed, weekly frequency of water consumption per water source, water use habits for preparing cold and hot beverages), usage frequency of cosmetics, PCP, and canned food. Specific details were recorded about the weekly use of each of the four major water sources in Cyprus, tap water, bottled water (both PET and PC bottles), groundwater, and mobile water stations (stalls that sell water from mountainous areas); the number of consumed glasses per day from each source (1 glass equals ∼250 mL water); and specific water use characteristics (plain drinking, preparing hot coffee or tea beverages, and preparing cold beverages, such as frappe, green tea, and juice). The per capita daily water consumption rate (liters per day per person) for each water source was calculated from the following formula:

$$\text{daily water consumption rate} = \sum [X_{\text{plain water}} + X_{\text{cold beverages}} + X_{\text{hot beverages}}] \quad (1)$$

where $X = [(\text{glasses}\cdot\text{day}^{-1})(0.25\ \text{L}\cdot\text{glass}^{-1})(\text{days}\cdot\text{week}^{-1})]/(7\ \text{days}\cdot\text{week}^{-1})$. Reported water consumption survey approaches based on NHANES relied on either daily water consumption information or an average of two consecutive days for evaluating associations with urinary BPA.[24,25] The validity of per capita water consumption estimates based on survey questions such as number of glasses of water consumed per day, frequency of consumption in a week in a specific usage form, etc., were qualitatively and quantitatively assessed by both graphic depictions and demonstration kits by a trained interviewer. Similarly, information was collected about the number and usage frequency of cosmetics (foundation, lipstick, eye shadow, eye liner, blush, mascara), PCP (shampoo,

Table 1. Univariate Analyses between Creatinine-Adjusted and Unadjusted Urinary BPA Concentrations with Individual Characteristics of the Study's Participants

characteristic		N	creatinine-unadjusted urinary BPA median (Q1, Q3), ng·mL^{-1}	p^a	creatinine-adjusted urinary BPA median (Q1, Q3), μg·g^{-1}	p^a
overall						
	urine-BPA	35	3.91 (2.99, 7.26)		4.55 (3.37, 8.28)	
	(ln) urine-BPA	35	1.36 (1.10, 1.98)		1.51 (1.21, 2.11)	
gender				0.006**		0.37
	male	13	5.28 (3.92, 8.45)		4.52 (2.99, 5.47)	
	female	22	3.39 (2.64, 5.40)		4.76 (3.54, 9.50)	
age				0.71		0.35
	21–25 years	9	3.91 (2.99, 5.22)		4.96 (4.18, 8.29)	
	26–30 years	14	5.92 (3.19, 8.45)		4.77 (3.61, 8.28)	
	31–35 years	6	3.75 (2.65, 3.92)		3.07 (2.02, 8.74)	
	36–55 years	6	3.94 (3.55, 5.28)		4.18 (3.23, 4.55)	
BMI				0.74		0.24
	<25 kg·m^{-2}	26	3.83 (2.99, 7.26)		4.76 (3.61, 8.29)	
	25–30 kg·m^{-2}	5	5.28 (3.19, 8.45)		4.04 (3.37, 4.58)	
	≥30 kg·m^{-2}	3	4.13 (2.00, 5.22)		3.23 (2.24, 4.55)	
education				0.17		0.93
	college or lower	10	3.13 (2.64, 5.40)		4.54 (3.61, 4.96)	
	postgraduate	25	3.92 (3.41, 7.48)		4.56 (3.37, 8.28)	
smoking status, current				0.26		0.74
	yes	6	6.10 (3.55, 8.60)		5.02 (4.54, 5.92)	
	no	29	3.91 (2.75, 5.97)		4.52 (3.37, 8.29)	
			Exposure Route via Water Sources			
PC bottles				0.032*		0.62
	yes	30	3.75 (2.75, 5.40)		4.55 (3.37, 8.28)	
	no	5	7.26 (6.44, 10.5)		4.04 (3.54, 5.47)	
PET bottles				0.48		0.53
	yes	29	3.92 (2.99, 7.48)		4.56 (3.54, 8.28)	
	no	6	3.83 (3.55, 4.11)		4.36 (2.49, 5.92)	
water board (tap water)				0.96		0.93
	yes	24	3.83 (3.09, 6.21)		4.53 (3.49, 7.10)	
	no	11	4.13 (2.75, 7.48)		4.56 (3.23, 8.28)	
well water				0.17		0.64
	yes	2	7.28 (5.97, 8.60)		4.25 (3.96, 4.54)	
	no	33	3.75 (2.99, 6.44)		4.56 (3.37, 8.28)	
mobile on road water stations				0.42		0.38
	yes	7	3.75 (2.60, 5.22)		4.40 (2.24, 4.96)	
	no	28	3.92 (3.28, 7.37)		4.55 (3.57, 8.28)	
			Other Exposure Routes			
canned food				0.26		0.45
	yes	31	3.91 (3.19, 7.48)		4.56 (3.54, 8.28)	
	no	4	3.29 (2.32, 4.60)		3.52 (2.29, 6.64)	
cosmetics				0.005**		0.22
	yes	21	3.37 (2.64, 5.40)		4.96 (3.61, 9.50)	
	no	14	5.25 (3.92, 8.45)		4.35 (2.99, 5.47)	
personal care products						
	yes	35	3.91 (2.99, 7.26)		4.55 (3.37, 8.28)	
	no	0				
vinyl products				0.95		0.42
	yes	17	3.75 (2.99, 7.48)		4.96 (3.37, 9.50)	
	no	18	3.92 (3.41, 5.97)		4.46 (3.61, 5.92)	
			Exposure Route via Packaged Food			
canned meat				0.17		0.11
	yes	4	2.62 (2.38, 6.57)		5.22 (4.97, 21.4)	
	no	31	3.92 (3.37, 7.26)		4.52 (3.23, 8.28)	
canned seafood				0.17		0.95
	yes	26	4.12 (3.37, 7.48)		4.53 (3.54, 8.28)	
	no	9	3.55 (2.65, 3.92)		4.56 (3.37, 5.92)	

Table 1. continued

characteristic		N	creatinine-unadjusted urinary BPA median (Q1, Q3), ng·mL^{-1}	p^a	creatinine-adjusted urinary BPA median (Q1, Q3), μg·g^{-1}	p^a
Exposure Route via Packaged Food						
canned fruit				0.64		0.98
	yes	7	3.91 (3.51, 5.97)		4.40 (3.61, 8.29)	
	no	28	3.83 (2.70, 7.37)		4.57 (3.11, 7.10)	
canned vegetables				0.31		0.27
	yes	14	4.56 (3.51, 7.48)		4.77 (3.61, 8.29)	
	no	21	3.75 (2.65, 5.97)		4.40 (3.23, 5.47)	
frozen meal				0.84		0.42
	yes	6	5.50 (2.60, 8.31)		4.76 (4.52, 13.6)	
	no	29	3.91 (3.19, 5.97)		4.54 (3.37, 5.92)	

[a] p-value from Wilcoxon or Kruskal–Wallis nonparametric tests as appropriate.

conditioner, body lotion, sunscreen, liquid shower soap, nail polish, perfume, cologne, aftershave), polyvinyl products (vinyl windows, vinyl flooring, vinyl shower curtain), and canned food (meat, seafood, fruits, vegetables). Classification of consumer products as cosmetics or PCP was similar to previous reports.[7,26,27]

Following the completion of the interview-based questionnaire, each participant provided a urine sample in a BPA-free polypropylene cup, while a yes/no answer was recorded for the question whether water consumption from PC-based container occurred during the last 24 h prior to urine sampling. Spot urine collection at different time points within a day showed significant differences in urinary BPA levels.[28] Hence we cautiously attempted to match urine collection time within the day for both sampling points in time. Urine collection and storage was performed following prescribed procedures such as freezing urine samples immediately upon collection, expedited shipping on dry ice (similar to the protocol of Cantonwine et al.[29] for international shipping of urine samples), having minimal standby times during transit and handling, and further storage at −80 °C until analysis in Dr. Snyder's laboratory at the University of Arizona, Tucson, AZ.

2.2. Urinary Bisphenol A Analysis. Creatinine measurements were performed following a colorimetric (picric acid-based) UV/Vis test at 520 nm.[30] Total BPA (sum of free and conjugated forms) in the urine samples were determined after β-glucuronidase/sulfatase hydrolysis.[30] Derivatization agents were used according to a published method.[31] In brief, after 12 h of enzymatic hydrolysis, 50 μL urine samples were transferred to 2 mL amber vials, and then 10 μL of the surrogate $^{13}C_{12}$–BPA (1 mg·L^{-1}) was added. Derivatization was performed by adding into the 2 mL vials 200 μL of aqueous sodium bicarbonate (100 mmol·L^{-1}, pH 10.5) and 200 μL of dansyl chloride (1 mg·mL^{-1} in acetone). The samples were vortex-mixed for 1 min and incubated at 60 °C for 5 min. Then 40 μL of methanol was added, and the samples were analyzed for total BPA by liquid chromatography–tandem mass spectrometry (LC-MS/MS). Analyses were carried out on an Agilent 1290 Infinity UHPLC system (Agilent Technologies, Santa Clara, CA). Separation was achieved with an Agilent Zorbax Eclipse Plus C18 column (1.8 μm; 2.1 mm × 50 mm), and the injection volume was 100 μL. Ultrapure water containing 0.1% formic acid (v/v) (A) and acetonitrile with 0.1% formic acid (v/v) (B) were used as mobile phases, while blanks were carefully prepared to avoid background BPA contamination. In terms of the gradient, the initial 60% B was increased linearly to 100% in 4 min and held for 1 min. Finally the gradient was returned to the initial conditions of 60% B in 0.5 min and held for 2 min to allow for equilibration. The flow rate was 0.4 mL·min^{-1} and the column was maintained at 40 °C.

Mass spectrometry was performed on an Agilent 6460 triple-quadrupole (MS/MS) detector equipped with an Agilent Jet Stream source (Agilent Technologies, Santa Clara, CA). The mass analyzer was operated in positive ionization mode; mass transitions for BPA were 695.2 → 171.1, 156.0 [fragmentor 180 V, collision energy 60 V, 80 V] and for internal standard ($^{13}C_{12}$–BPA) were 707.2 → 171.1, 156.0 [fragmentor 100 V, collision energy 60 V, 80 V]. The optimized detector parameters were as follows: gas temperature, 300 °C; sheath gas heater, 250 °C; capillary voltage, 3500 V; gas flow, 10 L·min^{-1}; sheath gas flow, 11 L·min^{-1}; and nebulilizer, 40 psi. The instrument detection limit was 0.29 ng of BPA·L^{-1} and the method detection limit was 0.03 ng of BPA·L^{-1}. Seven-point analytical calibration curves were spread evenly across 3 orders of magnitude and always resulted in a linear response with r^2 at least 0.99. Method precision for inter- and intraday variability was measured as extent of relative standard deviation (RSD), and the accuracy as degree of closeness was measured by standard deviation. Defined acceptable limit for precision and accuracy was <15% RSD and was verified by analysis of one standard from the standard curve after every five sample injections. Matrix effect was checked based on standard-addition protocol. Recovery from urine samples was 109% ± 11% at 30 μg·L^{-1} BPA spike level and 95% ± 3% at 400 μg·L^{-1} BPA spike level. Quality control of each urine sample batch was conducted with a matrix spike (10 μg·L^{-1}), a procedural blank (deionized water), and an instrument blank.

2.3. Statistical Analysis. Urinary BPA as creatinine-adjusted and unadjusted concentrations were presented as median (interquartile range). The cutoff value of 5 L·day^{-1} represents a rather unrealistic potable water consumption estimate. Only one study participant exceeded this threshold value with a reported tap water consumption of 5.86 L·day^{-1} and this observation was excluded from further statistical analysis. Nonparametric tests such as the Wilcoxon and the Kruskal–Wallis tests were used as appropriate to assess whether there were differences in the urinary BPA levels under the different levels of various variables. Correlations between different variables and urinary BPA were calculated by use of Spearman's correlation coefficients. Urinary BPA levels were first logarithmically (ln) transformed, as they were not normally distributed, and then used in linear regression models in order to assess the effect of several variables such as water

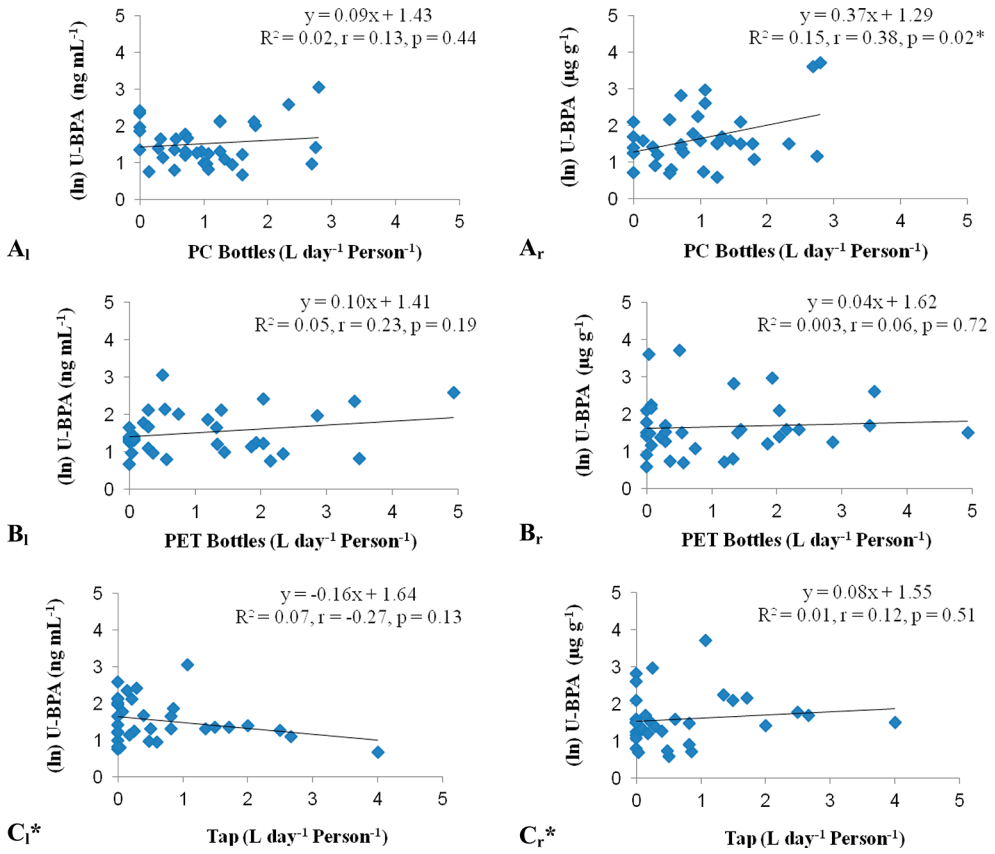

Figure 1. Creatinine-unadjusted [left column (l)] and -adjusted [right column (r)] urinary BPA levels for all study participants (n = 35) in relation to the reported water consumption fractions from (A) polycarbonate (PC) bottles, (B) polyethylene terephthalate (PET) bottles, and (C) tap, including all water uses (plain water, cold and hot beverages). Abbreviation (ln) U-BPA denotes logarithmically transformed urinary BPA values. (*) A female study participant reporting tap water consumption >5 L·day^{-1} was considered as an outlier, and the corresponding urinary BPA data point was excluded from statistical analysis.

consumption from various sources, age, gender, etc., on urinary BPA levels and identify significant predictors of urinary BPA for both unadjusted values of urinary BPA as well as creatinine-adjusted values, after adjusting for covariates. Statistical analyses were performed with SAS 9.2 (SAS Inc., Cary, NC) and all tests were two-sided with $p < 0.05$ considered statistically significant.

3. RESULTS

3.1. Demographics and Urinary Bisphenol A Levels. Characteristics of the participants in this study were previously described in detail.[21] Mean (±SD) age and body mass index (BMI) of the study participants was 30.5 ± 7.5 years and 23.6 ± 4.3 kg·m^{-2}, respectively. In brief, most subjects were females (63%), in the age group 26−35 years (57%), with a normal BMI of <25 kg m^{-2} (74%). Bisphenol A was detected in 100% of the urine samples in the Cypriot pool of subjects for both sampling points in time (taken 1 week apart). Creatinine-adjusted urinary BPA levels at the first and second spot were weakly correlated (r = 0.37, p = 0.03), while no significant correlation was observed for the creatinine-unadjusted urinary BPA values (r = 0.21, p = 0.22). Hence, the urinary BPA average of the two spot urine time points was used in all further analyses for both creatinine-adjusted and unadjusted values. The median urinary BPA (creatinine-adjusted) in the study participants was 4.55 μg·g^{-1} with an interquartile range of 3.37−8.28 μg·g^{-1}, while the median for unadjusted urinary BPA was 3.91 ng·mL^{-1} with an interquartile range of 2.99−7.26 ng·mL^{-1} (Table 1). No significant correlations were observed between age and BMI with either creatinine-adjusted or unadjusted urinary BPA (data not shown). We observed no statistically significant differences in creatinine-adjusted urinary median BPA levels and levels of gender, age, BMI, marital status, education, smoking status, or subject duration in current location (Table 1). Significantly ($p < 0.05$) higher urinary BPA concentrations were observed in males compared to females when unadjusted for creatinine, while a significant ($p < 0.05$) association was also noted for other factors, such as consumption of PC bottled water and cosmetics use (Table 1). However, when the creatinine-adjusted levels of urinary BPA were used, the aforementioned significant differences did not remain ($p > 0.05$), supporting the validity of creatinine normalization to account for potential gender differences with respect to muscle mass, extent of nutrition, hydration, time since last void, total spot urine volume sampled, etc. Urinary BPA levels were logarithmically transformed before used in linear regression models, because of the skewed shape of the distribution. In the overall model of multivariate linear regression analysis, gender, age, PC bottle and tap water consumption, and canned food were not predictive of creatinine-adjusted urinary BPA concentrations, whereas gender was marginally significant (p = 0.011) in the creatinine-unadjusted urinary BPA multivariate regression model (Table SI-1, Supporting Information). Further assessment was made for interaction effects of gender with either PC or tap water consumption on urinary BPA levels.

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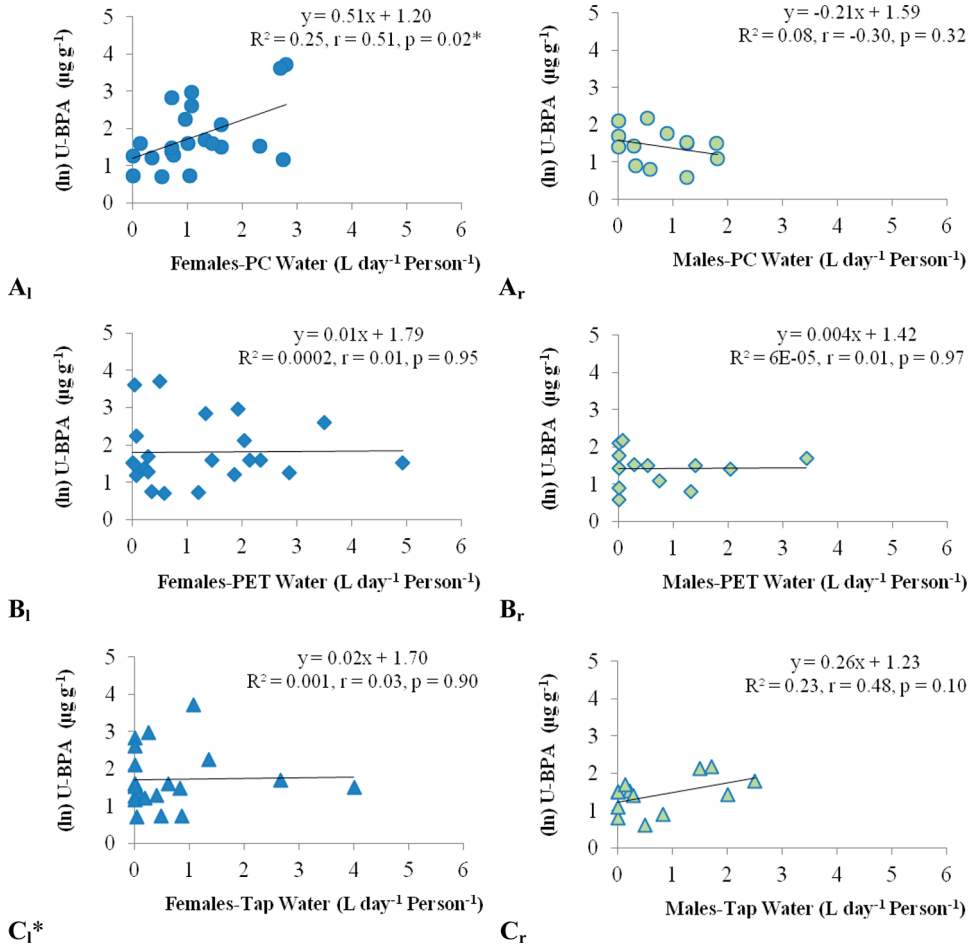

Figure 2. Creatinine-adjusted urinary BPA levels for females [left panel (l)] and males [right panel (r)] in relation to the reported water consumption fractions from (A) polycarbonate (PC) bottles, (B) polyethylene terephthalate (PET) bottles, and (C) tap, including all water uses (plain water, cold and hot beverages). Abbreviation (ln) U-BPA denotes logarithmically transformed urinary BPA values. (*) A female study participant reporting tap water consumption >5 L·day^{-1} was considered as an outlier, and the corresponding urinary BPA data point was excluded from statistical analysis.

3.2. Water Consumption Patterns and Urinary Bisphenol A Levels. Thirty-three out of 35 participants (94%) reported some bottled water consumption (either PET or PC or both), whereas 69% reported tap water consumption, these being the two major sources of potable water in our group of participants (data not shown). Around 85% of the participants used water from WCM in a form other than plain water, such as cold and/or hot beverages (coffee, tea, juices/lemonade), increasing the magnitude of water consumption from 2.20 L·day^{-1}·person^{-1} for plain water use to 3.44 L·day^{-1}·person^{-1} (95% confidence interval 2.75−4.14) when both cold and hot beverage uses were included.[21] Among all study participants, PET and PC bottled water consumption had equal preference (31% and 30%, respectively) followed by tap water (24%), water from a mobile station (14%), and groundwater (2%).[21]

Wilcoxon or Kruskal−Wallis nonparametric tests yielded nonsignificant associations between subjects who did or did not consume water from PC, PET bottles, tap, groundwater, or mobile station (based on a yes or no response) and their individual urinary BPA values (Table 1). Presumably due to the higher water consumption rate, PC water used for plain drinking purposes, but not PC water used for either cold or hot beverages, showed a significant linear association (p = 0.01) with creatinine-adjusted urinary BPA (data not shown). PC water consumption, when plotted as a continuous variable, yielded a significant (p = 0.02) association with creatinine-adjusted urinary BPA in a univariate regression model (Figure 1A$_r$). But, as expected, no significant association was observed for PET bottles (Figure 1B) and tap water consumption (Figure 1C) with urinary BPA, since PET polymeric formulation does not contain BPA and tap water has minimal levels, if any, and observed rarely.[32]

We also observed gender differences in water source and usage habits (Figure 2A−C). A significant interaction effect (p < 0.05) was observed between gender and PC bottle water consumption. This prompted us to perform a gender-stratified linear multivariate regression analysis on urinary BPA levels, following an adjustment for covariables mentioned earlier (Table SI-1, Supporting Information). Interestingly, females exhibited a significant (p = 0.017) linear relationship between PC water consumption and creatinine-adjusted (ln) urinary BPA even after adjusting for age, tap water consumption, and canned food, while men did not (p = 0.726) (Table SI-1, Supporting Information). When no creatinine normalization was used, neither females (p = 0.098) nor males (p = 0.391) showed a significant association between PC bottled water consumption and urinary BPA (Table SI-1, Supporting Information). Contrasting reports are available on gender

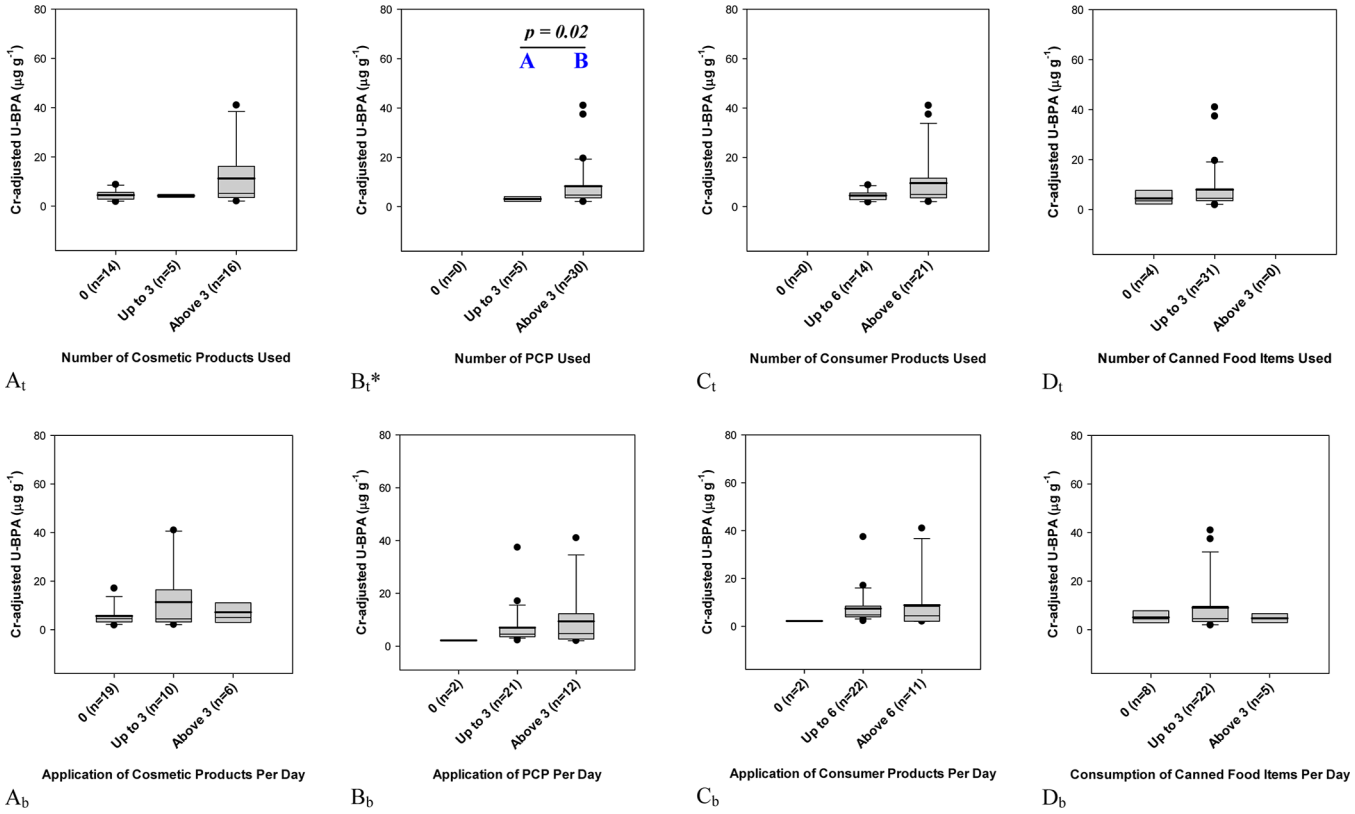

Figure 3. Trends and differences in adjusted urinary BPA levels in all subjects classified on the basis of their usage of (A) cosmetics, (B) personal care products, (C) consumer products, and (D) canned food [number of items [top panel (t)] and frequency of use [bottom panel (b)]]. Differences between groups were tested for significance by the Kruskal–Wallis nonparametric test. (*) The Wilcoxon nonparametric test was used in the case of comparing differences between two groups. Significance in comparisons of treatment medians were represented by different letters. Abbreviation U-BPA denotes urinary BPA levels. The sum of the number of cosmetics and PCP used was represented by the number of consumer products.

effects on urinary BPA levels: (i) no significant difference between men and women,[33] (ii) nonsignificantly higher urinary BPA levels in men,[28] and (iii) significantly higher urinary BPA levels in women (this study). Neither female nor male creatinine-adjusted urinary BPA showed significant association with either PET bottle or tap water consumption (Figure 2B,C). Interestingly, the addition of all water consumption fractions for the three major water sources (PET, PC, and tap), the so-called total water consumption, did not show a significant linear relationship with creatinine-adjusted urinary BPA (Figure SI-1, Supporting Information). This may stimulate discussions on the usefulness of total water consumption estimates in ascertaining associations with BPA daily intake estimates. It seems that only upon inclusion of detailed information on specific water sources and uses could well-informed BPA daily intake estimates with reduced uncertainty be derived.

3.3. Use of Cosmetics, Personal Care Products, Canned Food, and Urinary Bisphenol A Levels. With the exception of one female, all women in this pilot study used cosmetics (such as foundation, lipstick, eye shadow, eye liner, blush, mascara) while none of the male subjects used any. The median number of cosmetics products used by females was 5 with an interquartile range of 3–6. Neither number of cosmetics products used (Figure $3A_t$), nor cosmetics use frequency (number of times applied per day) (Figure $3A_b$) showed a significant association with urinary BPA in a nonparametric Kruskal–Wallis test.

All subjects used at least one of the PCPs included in the questionnaire (shampoo, conditioner, body lotion, sunscreen, liquid shower soap, nail polish, perfume, cologne, aftershave). The median (Q1, Q3) for number of products and use frequency per day of PCP was 5.0 (4.0, 6.0) and 3.0 (2.6, 3.7), respectively. Subjects using more than three personal care products showed significantly higher creatinine-adjusted urinary BPA compared to those using up to three PCP (Figure $3B_t$), which was based on a nonparametric Wilcoxon test for the reason of having only two groups. Neither the number nor the usage rate of consumer products, calculated as the sum of cosmetics and PCP, was significantly associated with creatinine-adjusted urinary BPA (Figure $3C_t$ and $3C_b$). About 52% of the subjects did not use vinyl material, such as vinyl windows, vinyl flooring, or vinyl shower curtain, at the time of this survey; and for those who used vinyl material, it was in the form of only one product (vinyl shower curtain). No significant difference was observed in urinary BPA levels between users and nonusers of vinyl products in this study (Table 1).

Furthermore, the majority of volunteers (89%) consumed canned food (such as meat, seafood, fruits, and vegetables). The median (Q1, Q3) for the number of canned food types and cans consumed per week was 2.0 (1.0, 2.0) and 1 (0.5, 2.5), respectively. The number of canned products used and cans consumed per week did not show any significant relationship with creatinine-adjusted urinary BPA when plotted either as groups ($3D_t$ and $3D_b$) or as continuous variables (data not shown).

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4. DISCUSSION

This study's high daily BPA intake rates were partially ascribed to adverse summer weather conditions where extremely high temperatures were observed [>40 °C],[19] (Figure SI-2, Supporting Information) coupled with prolonged frequency of very high UV index values [>8].[20] This could have resulted in BPA leaching from PC-based WCM that traditionally enjoy big sales on the island during summer months. Indeed, July and August have been traditionally the warmest months in Cyprus, exhibiting average air temperatures of 38−40 °C, while maximum air temperatures on the order of ∼45 °C are frequently observed (Figure SI-2, Supporting Information). Similarly, very high UV index values of >8 are systematically observed in Cyprus during July and August.[20] Everyday water usage for Cyprus and other Mediterranean countries include the use of bottled water (PC, PET) not only for drinking water but also for preparing beverages, like hot and cold coffees/teas and juices. An excess of 1.24 $L{\cdot}day^{-1}{\cdot}person^{-1}$ water consumption, in comparison with the plain water average consumption rate of 2.20 $L{\cdot}day^{-1}{\cdot}person^{-1}$, was attributed to water used for preparing cold and/or hot beverages (coffee, tea, juices). The sum of these values (3.44 $L{\cdot}day^{-1}{\cdot}person^{-1}$) is indeed higher than the average estimate of total water consumption (2.0 $L{\cdot}day^{-1}{\cdot}person^{-1}$) widely used in derivations of pollutant daily intake or individual risk, and it could be partially attributed to high temperatures and increased sales[18] for packaged water during summer.

An interesting finding was that water consumption from PC bottles was significantly associated with females even after adjusting for covariates, but this was not the case with males (Figure 2 and Table SI-1, Supporting Information). These findings were corroborated by the higher mean water consumption from PC bottles by females (1.16 $L{\cdot}day^{-1}{\cdot}person^{-1}$) when compared with that of males (0.78 $L{\cdot}day^{-1}{\cdot}person^{-1}$). Similarly, mean water consumption from three major water sources (bottled, tap, stations) was higher in females (3.28 $L{\cdot}day^{-1}{\cdot}person^{-1}$) compared to males (2.27 $L{\cdot}day^{-1}{\cdot}person^{-1}$). A higher percentage of plain water consumption from tap and bottle water for females in the age group >20 years was reported in the 2005−2008 NHANES survey, although this was not significant when water consumption was reported as cups per day.[34] It was speculated that higher water consumption rates by females could partially explain their higher urinary BPA concentrations and thereby the gender-specific association between PC water consumption and urinary BPA levels. Gender differences were illustrated in biomonitoring-based BPA conjugate complexes but not when total urinary BPA concentrations were used; specifically, males were found with higher urinary BPA glucuronide, while females showed higher urinary BPA sulfate than males.[33] Suggested gender differences in urinary BPA levels may be presumably ascribed to sexually differentiating creatinine metabolism and excretion patterns. We, thus, leaned toward explaining the higher creatinine-adjusted urinary BPA levels in women due to lower muscle mass and differences in hydration, nutrition status, urine volume, and time since last void in comparison with men. Urinary BPA measurements not normalized to creatinine levels revealed higher urinary BPA levels in males, but this trend could reverse upon creatinine adjustment, showing higher urinary BPA in women, as this study and others did.[30,35] The widely used speculation of androgen-related metabolism on BPA needs to be carefully weighed against available data. The pioneering work by Takeuchi and Tsutsumi[36] proposing androgen-related effects on BPA metabolism was based upon serum BPA measurements, when the majority of human biomonitoring BPA studies are based upon urinary biomarkers.

In addition to water consumption from PC-based WCM, other exposure sources unique for females could explain the gender-based BPA differences; for example, increasing number of PCPs used by females was significantly ($p < 0.05$) associated with urinary BPA concentrations (Figure $3B_t$). Numerous personal care products were recently reported to contain BPA.[7] Furthermore, it was shown that canned food was not associated with urinary BPA levels in our study. A comprehensive probabilistic exposure assessment suggested that canned food was the most probable exposure source for BPA in adults; however, PC water consumption from 19 L water dispensers was not included in the risk assessment model other than PC baby bottles and PC water cookers.[3]

The small number of participants ($n = 35$) limited our ability to draw inferences on the findings and their generalization. No data exist on external exposure measurements for BPA in PC bottled water, canned food, and cosmetics marketed in the island, although we currently try to match our internal BPA exposure data (equivalent to urinary BPA levels and back-calculated for BPA intake estimates) with external measurements of BPA in PC-based WCM and tap water. The majority of our study participants (77%) reported water consumption from PC-based containers in the last 24 h prior to urine sample collection, but in univariate analyses performed this was linked to neither (ln) creatinine-adjusted ($p = 0.53$) nor (ln) unadjusted ($p = 0.08$) urinary BPA levels. These results hinted toward coexposures of PC bottled water and other dietary (canned food) or nondietary (PCP, cosmetics) sources. The last 24 h PC-based water consumption question served as an indicator whether to expect some BPA in urine or not, given the observations that (i) exposure to BPA via the oral route yields conjugated metabolites by the liver that are cleared from the body through urine, while nonoral exposures that yield unconjugated BPA may not be excreted; and (ii) almost all of the oral route exposed BPA is excreted through urine within 24 h.[22]

A single spot urine sample may be a good reflection of BPA exposures occurring over a period of 24 h or less,[37] while it may not be a true representation of long-term exposures that (i) vary with time,[38] (ii) occur from nondietary sources,[39] and (iii) result in possible release and excretion of residual BPA stored in lipid tissues.[40] Detailed monitoring of variability in urinary BPA levels (such as between-person, within-person, between-day, and within-day) with sampling approach (such as spot, first morning void, or 24 h collection) showed that within-person and between-day variance was minimal from spot urine collection.[41] The same authors concluded that spot urine may "adequately reflect" average BPA exposures of a general population even when urine samples were randomly collected with respect to dietary exposure times and bladder emptying times.[41]

Daily BPA intake was estimated on the basis of reported body weight, measured creatinine mass, urinary BPA concentrations, and a mean urine excretion volume of 1.5 $L{\cdot}adult^{-1}$ (Table SI-2, Supporting Information). Geometric mean value of internal BPA exposure (estimated from back-calculations with urinary BPA data) in our study participants aged 21−55 years was 104 $ng{\cdot}(kg\ bw)^{-1}{\cdot}day^{-1}$ (urine volume-

based calculation) and 118 ng·(kg bw)$^{-1}$·day^{-1} (creatinine mass-based calculation) (Table SI-2, Supporting Information), which was nearly double the reported average of other biomonitoring studies focused on adult age groups (20+ years); biomonitoring-based daily BPA intake values (nanograms per kilogram body weight per day) were 14,[42] 22,[43] 30,[37] 33,[44] 47,[24] and 60.[45]

Daily BPA intake was higher in our study's males compared to females (133 vs 110 ng·(kg bw)$^{-1}$·day^{-1}), partially explained because of males' greater creatinine mass and body weight. The observed trend was supported by similar data from NHANES 2003−2004 [53.8 ng·(kg bw)$^{-1}$·day^{-1} (males) > 41.0 ng·(kg bw)$^{-1}$·day^{-1} (females)] and 2005−2006 [39.6 ng·(kg bw)$^{-1}$·day^{-1} (males) > 31.2 ng·(kg bw)$^{-1}$·day^{-1} (females)].[24,25] A WHO report on biomonitoring-based median BPA intake values showed a range of 10−50 ng·(kg bw)$^{-1}$·day^{-1} for adults and 20−120 ng·(kg bw)$^{-1}$·day^{-1} for children.[46] Indeed, out of all biomonitoring studies reporting geometric mean (GM) values of measured urinary BPA in various populations around the globe, only one stood higher [GM 8.91 μg of BPA·g^{-1}][47] than the GM reported in this study, 5.27 μg of BPA·g^{-1} (geometric standard deviation 2.13) (Figure SI-3, Supporting Information). The range of reported GM of creatinine-adjusted urinary BPA levels was between 0.24 μg·g^{-1} [48] and 8.91 μg·g^{-1} (Figure SI-3, Supporting Information).[47] This observation further calls for a closer investigation delineating the magnitude and uncertainty of urinary BPA levels in this exposed population. Given the reliance of several Mediterranean countries on packaged water, and the fact that six of them are listed in the global top 20 countries with the highest bottled water consumption,[18] it is warranted that higher than usual contributions of PC bottled water to daily BPA intake estimates are to be anticipated. This may be especially true during elevated temperatures and high UV index values experienced in the region during the summer months.

5. IMPLICATIONS

Results presented highlight the association between PC water consumption by females and urinary BPA levels, even after adjusting for covariates, such as canned food. Females' susceptibility to environmental health risks, especially during pregnancy, coupled with the likelihood of fetal programming and transgenerational health effects,[49−51] could be implicated from the elevated BPA intake values in this study. PC water consumption may significantly contribute to the overall BPA daily intake estimates under certain environmental conditions and practices, but it is not the sole source of exposure. The fact that our study was conducted during the hottest months of the year was reflected in the elevated urinary BPA levels. Handling and storage practices of minimally reused PC containers in ambient temperatures and negligible exposure to sun/UV do not promote leaching of plastic constituents into packaged water. However, this scenario may not hold true for the southern European Union, southwestern United States, and those countries located in tropical or subtropical regions. Sixty percent of the global top 20 countries list for the highest per capita bottled water consumption rates are located either in tropics or in the Mediterranean region.[18] Prolonged manifestation of adverse weather conditions, such as high frequency of days with elevated air temperatures and/or very high UV index values, in conjunction with high frequency of plastic container reuse could accelerate BPA leaching processes into packaged water, elevating consumer exposures to BPA. In the presence of stoichiometric residual chlorine levels, tap water is void of BPA because of rapid BPA degradation to chlorinated BPA congeners of substantial binding affinity to estrogen receptors.[52] However, chlorination of packaged water is not practiced unless a previously chlorinated potable water source is used for packaging. Depending on the magnitude of residual chlorine levels and pH of packaged water, in conjunction with the aforementioned environmental variables, leached BPA could be subject, or not, to degradation reactions, complicating the relationship between leaching processes and the magnitude of human exposures to BPA.

It is warranted that sustainable intervention measures are designed by local bottling/delivery companies with respect to proper use and handling of PC containers. Otherwise, a gradually increasing role of seasonal (summer vs winter) variability in dictating the overall magnitude and variability of daily BPA intake estimates will soon become obvious at a much larger scale. Adverse meteorological conditions in summer often act as a confounder to air pollution effects on human mortality, and similar weather effects are anticipated with respect to plasticizer exposures from water-contact materials. Our findings may have significant implications for other plastics additives such as phthalates, antimony, and nonintentionally added substances that are commonly found in plastic PC and PET containers. It is speculated that the leaching characteristics of these chemicals are similarly impacted by the aforementioned weather conditions and handling practices, thus elevating the magnitude of cumulative exposures to a mixture of plastics additives with variable endocrine-disrupting activities. The seasonal variability of plasticizers has not been yet adequately explored in epidemiological studies, while the increased prevalence of endocrine-related morbidity in the European Union and the United States calls for improved exposure assessment protocols that better capture everyday environmental/lifestyle activities of individuals.

■ ASSOCIATED CONTENT

Supporting Information

Reference for artwork and Figure SI-3 presented; two tables listing urinary BPA multivariate linear regression model estimates and BPA internal exposure estimates [ng of BPA·(kg of body wt)$^{-1}$·day^{-1}]; and three figures showing gender differences in total water consumption and associated urinary bisphenol A levels, meteorological data on air temperatures and UV indices during the study period, and human biomonitoring studies around the globe reporting creatinine-adjusted BPA geometric mean levels. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

BPA bisphenol A
BMI body mass index
PC polycarbonate
PET polyethylene terephthalate
WCM water contact materials

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