



Environmental and personal determinants of the uptake of disinfection by-products during swimming

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ABSTRACT

Background: Trihalomethanes (THMs) in exhaled breath and trichloroacetic acid (TCAA) in urine are internal dose biomarkers of exposure to disinfection by-products (DBPs) in swimming pools.

Objective: We assessed how these biomarkers reflect the levels of a battery of DBPs in pool water and trichloramine in air, and evaluated personal determinants.

Methods: A total of 116 adults swam during 40 min in a chlorinated indoor pool. We measured chloroform, bromodichloromethane, dibromochloromethane and bromoform in exhaled breath and TCAA in urine before and after swimming, trichloramine in air and several DBPs in water. Personal determinants included sex, age, body mass index (BMI), distance swum, energy expenditure, heart rate and 12 polymorphisms in *GSTT1*, *GSTZ1* and *CYP2E1* genes.

Results: Median level of exhaled total THMs and creatinine adjusted urine TCAA increased from 0.5 to 14.4 $\mu\text{g}/\text{m}^3$ and from 2.5 to 5.8 $\mu\text{mol}/\text{mol}$ after swimming, respectively. The increase in exhaled brominated THMs was correlated with brominated THMs, haloacetic acids, haloacetoneitriles, halo ketones, chloramines, total organic carbon and total organic halogen in water and trichloramine in air. Such correlations were not detected for exhaled chloroform, total THMs or urine TCAA. Exhaled THM increased more in men, urine TCAA increased more in women, and both were affected by exercise intensity. Genetic variants were associated with differential increases in exposure biomarkers.

Conclusion: Our findings suggest that, although affected by sex, physical activity and polymorphisms in key metabolizing enzymes, brominated THMs in exhaled breath could be used as a non-invasive DBP exposure biomarker in swimming pools with bromide-containing source waters. This warrants confirmation with new studies.

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Abbreviations: BMI, Body mass index; BrCIAA, Bromochloroacetic acid; BrDCIM, Bromodichloromethane; BrHAAs, Brominated haloacetic acids; BrTHMs, Brominated THMs; CHBr₂CN, Dibromoacetonitrile; CHBr₃, Bromoform; CHCl₃, Chloroform; C₃H₃Cl₃O, 1,1,1-Trichloropropanone; CHCl₂CN, Dichloroacetonitrile; CHBrClCN, Bromochloroacetonitrile; CYP2E1, Cytochrome P450 2E1 gene; DBrAA, Dibromoacetic acid; DBrCIM, Dibromochloromethane; DBP, Disinfection by-product; DCIAA, Dichloroacetic acid; DCIBrAA, Dichlorobromoacetic acid; DMNA, Dimethylnitramine; FCI, Free chlorine; *GSTT1*, Glutathione S-transferase theta 1 gene; *GSTZ1*, Glutathione S-transferase zeta 1 gene; HAA, Haloacetic acid; HAN, Haloacetonitrile; HK, Haloketone; IQR, Interquartile range; METs, Metabolic equivalent tasks; MHR, Maximum heart rate; NCl₃, Trichloramine; NDMA, Nitrosodimethylamine; NH₂Cl, Monochloramine; NHCl₂, Dichloramine; NPOC, Non-purgeable organic carbon; SNP, Single-nucleotide polymorphism; TCAA, Trichloroacetic acid; THAAs, Total haloacetic acids; THM, Trihalomethane; TTHMs, Total trihalomethanes; TOBr, Total organic bromine; TOC, Total organic carbon; TOCl, Total organic chlorine; TOI, Total organic iodine; TOX, Total organic halogen

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1. Introduction

Swimming in pools is a popular and healthy activity that involves high exposure to disinfection by-products (DBPs). Mixtures of chemicals produced during disinfection process with different toxicities are present in treated drinking water. An increased bladder cancer risk has been consistently associated with long-term exposure to residential trihalomethanes (THMs) (Villanueva et al., 2004, 2015) and an association with pool attendance has also been suggested (Villanueva et al., 2007). Polymorphisms in key metabolizing enzymes, including glutathione S-transferase (*GSTT1*, *GSTZ1*) and cytochrome P450 (*CYP2E1*), have been suggested to modify DBP-associated bladder cancer risk (Cantor et al., 2010). Slight increased risks for some reproductive outcomes may also be related to DBP exposure (Villanueva et al., 2015). The exposure to trichloramine, a volatile irritant DBP in indoor pools, has been associated with an increased risk of respiratory effects in highly exposed populations (Villanueva and Font-Ribera, 2012).

Hundreds of DBPs have been identified in swimming pool water (Richardson et al., 2010; Xiao et al., 2012), which has a different composition than tap water. Nitrogenous organic matter from bathers (e.g. sweat, urine) produces nitrogenous DBPs such as chloramines, haloacetonitriles (HANs) or nitrosamines (Chowdhury et al., 2014), which are more cytotoxic and genotoxic than THMs and haloacetic acids (HAAs) (Richardson et al., 2007). The route of exposure depends on the chemical properties of each DBP. The major exposure route for volatile DBPs such as THMs or chloramines is inhalation (Erdinger et al., 2004; Marco et al., 2015), whereas less volatile and skin permeable compounds such as HAAs (Kim and Weisel, 1998) can enter the body through accidental ingestion or inhalation of aerosol (Cardador and Gallego, 2011).

Only THMs and HAAs have been measured in the human body as indicators of internal dose. The majority of studies assessing DBPs exposure in swimmers have measured THMs in exhaled breath (Caro and Gallego, 2008; Aggazzotti et al., 1998; Font-Ribera et al., 2010), blood (Aggazzotti et al., 1998, 1990) or urine (Aprea et al., 2010; Caro and Gallego, 2007, 2008). Only a few studies have measured specific HAAs in urine after swimming (Cardador and Gallego, 2011). The uptake of THMs during swimming has been related to the levels in water (Caro and Gallego, 2007; Font-Ribera et al., 2010; Aggazzotti et al., 1990) and air (Font-Ribera et al., 2010; Caro and Gallego, 2008; Aggazzotti et al., 1990), and the level of trichloroacetic acid (TCAA) in urine was related to the level in water (Cardador and Gallego, 2011). However, the correlations between these exposure biomarkers with other more toxic and rarely measured DBPs in swimming pools are not known. Individual characteristics of swimmers such as age, sex or body mass index (BMI) did not show to affect the uptake of these exposure biomarkers (Font-Ribera et al., 2010; Cardador and Gallego, 2011). However, intensity of physical activity during swimming has been shown to increase the uptake of THMs (Marco et al., 2015).

In this study, we aimed to further characterize exposure to DBPs among swimmers. Specifically, (1) to describe how well two internal dose biomarkers (THMs in exhaled breath and HAAs in urine) reflect the levels of several DBPs in water and trichloramine in air; and (2) to identify the individual characteristics that affect these internal dose biomarkers, including sex, BMI, body surface area, the polymorphisms of specific metabolizing genes and several measures of physical activity. These results will help understanding how exposure to DBPs occurs in swimming pools and will be especially useful to improve methodological aspects in epidemiological studies assessing short-term health effects after swimming in pools.

2. Materials and methods

2.1. Study design

In total, 116 non-smoking non-professional swimmers 18–40 years old swam for 40 min in a single, indoor, 25 m-long chlorinated swimming pool in Barcelona, Spain. Four participants were evaluated individually per day, between 9 am and 2 pm (before lunch) in June and between September and December 2013, with a total of 30 experimental days. Participants were asked to swim during a fixed time (40 min) at a free pace, resting as much as they wanted. Before and after swimming, biological samples and measurements were obtained in a room inside the sports centre separated from the swimming pool area. Participants had not attended swimming pools during the week before, did not take a shower in the morning of the experimental day and DBP-free bottled water was provided during the study period.

2.2. Internal dose biomarkers

Four THMs (chloroform (CHCl_3), bromodichloromethane (BrDCIM), dibromochloromethane (DBrCIM) and bromoform) were measured in exhaled breath before the swimmers entered the swimming pool and right after they left the pool, using the Bio-VOC™ Sampler (Markes International Ltd, UK). After breathing deeply three times, the subjects retained the air for 10 s and then breathed continuously into the disposable cardboard mouthpiece until the end of a quiet breathing to obtain the alveolar air retained by the Bio-VOC™ Sampler. Once 150 mL alveolar air had been collected, a screw-in plunger was used to steadily discharge the sample into a sorbent tube trap. This process was repeated four times resulting in a total exhaled breath volume of 600 mL. After collection, sorbent tubes were capped with brass storage caps fitted with PTFE ferrules and were stored at 4 °C in a solvent-free environment until analyses. THMs were desorbed from sorbent tubes and concentrated in a thermal desorption (TD) unit equipped with a Unity Series 2 Thermal Desorber and an Ultra 50:50 Multi-tube Auto-sampler (Markes International Ltd.). The THMs were transferred to a Gas Chromatograph 7890 (Agilent Technologies) coupled to Mass Spectrometer 5975C Inert XL MSD with a Source in Electronic Impact Mode (Agilent Technologies). Ten mL urine samples were collected before and 30 min after swimming for TCAA and creatinine analyses. Urine samples were stored at –20 °C and shipped on dry ice to the laboratory. TCAA concentrations in the five mL urine samples were measured using solid phase extraction followed by liquid chromatography tandem mass spectrometry (LC-MS-MS). Methods have been described previously (Salas et al., 2014). Creatinine was also measured in order to adjust for dilution, and TCAA concentration was expressed as creatinine adjusted levels ($\mu\text{mol TCAA/mol creatinine}$).

2.3. Environmental measurements

Free chlorine (FCI), three chloramines, pH, four THMs, nine HAAs, four HANs, three halo ketones (HKs), a nitrosamine, a nitramine, total organic carbon (TOC), and total organic halogen (TOX) in water were measured. Water samples were collected at two different locations of the pool while participants were swimming, and the samples were stored at 4 °C until laboratory analyses. FCI, chloramines, THMs, HANs, and HKs were measured for each participant, TOC was measured twice per day, TOX, and HAAs once per day and nitrosamines and nitramines only for selected days. For THM analyses, 3–5 mg $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5 \text{H}_2\text{O}$ and 30–50 mg KHSO_4 were added to a 20 mL air-tight headspace-free glass vial with crimp cap. For HAA, HAN and HK analyses, 10 mg NH_4Cl were added to 100 mL amber glass vials with Teflon faced screw

caps. The analyses of THMs and HANs was conducted by head-space method coupled with gas chromatography/mass spectrometry (HS-GCMS) (GC: Agilent 7890A, MS: Agilent 5975C, column (Restek-VMS): 60 m length, 0.25 mm ID, 1.4 μ m film thickness) (Schmalz et al., 2011). A modified EPA 552.3 method was used for HAA analysis, which was in general separated into three steps: liquid-liquid extraction, sample methylation and analysis. HKs were analyzed directly after the extraction step. HAA and HK were analyzed by Agilent 6890 GC coupled with Agilent 5973 MS using a column: J & W Scientific DB-5MS (5% phenyl-95% dimethyl polysiloxane, 30 m length, 0.25 mm ID, 0.25 μ m film thickness). FCI, monochloramine and dichloramine in water were measured *in situ* by N,N-diethyl-p-phenylenediamine (DPD) procedure (Pallin, 1957; SMWW 4050-Cl G, 2005) with a portable photometer (DINKO Instruments, Inc., Barcelona, Spain). Notably, besides monochloramine and dichloramine, other organic chloramines might also form in chlorinated swimming pool water, and the DPD method cannot differentiate inorganic chloramines from organic chloramines and therefore gives a sum signal for both. Generally the inorganic chloramines are more abundant (Shang and Blatchley III, 1999; Li and Blatchley III, 2007). A membrane introduction mass spectrometer (MIMS) might be used to accurately measure monochloramine and dichloramine in the pool water, but unfortunately this instrument was not available when this study was undertaken. Fifteen mL glass vials previously acidified were used to collect water for TOC analysis. Non-purgeable organic carbon (NPOC) was measured with a TOC analyzer (multi N/C[®] 3100 analyzer, Analytik Jena, Germany). Sodium thiosulfate was added to 100 mL amber glass vials with Teflon faced screw caps for TOX analysis. TOX was differentiated into total organic chlorine (TOCl), total organic bromine (TOBr), and total organic iodine (TOI). It was measured according to Standard Method 5320B (Eaton et al., 2012), except that an off-line ion chromatograph was used as a halide separator and detector for the measurements of TOCl and TOBr (Li et al., 2010, 2011; Liu and Zhang, 2013), and the Waters ultra-performance liquid chromatography/electrospray ionization-triple quadrupole mass spectrometry was used for the measurement of TOI (Pan and Zhang, 2013). Ascorbic acid was added to 2 L plastic fluorine-lined containers for nitrosamine and nitramine analysis by a modified version of EPA Method 521 (Walse and Mitch, 2008). Air samples were collected every day to measure trichloramine. Air was collected with a sampling pump at a constant flow rate of 1.2 L/min for 115 ± 32 min ($n=26$), within 1 m from the pool edge and at a height of 60 cm above the water level. Trichloramine in air was measured following the method described by Hery et al. (1995); further details are available elsewhere (Jacobs et al., 2007). The temperature of water and air and the number of swimmers in the pool were recorded.

2.4. Personal information of participants

Weight and height were measured with standard procedures and BMI and body surface area (Mosteller, 1987) were calculated. Physical activity was assessed with different parameters. A technician sitting in front of the pool counted the number of laps and the time swimming by each participant with a counter and a chronometer. Energy expenditure (in kcal) was estimated using the swimming speed and the weight of the participant, assuming that swimming at 46 m/min equals 8.3 metabolic equivalent tasks (METs; kcal per kg per hour) (Ainsworth et al., 2011). Subjective exertion and shortness of breath were evaluated before and after swimming using the Borg scale (Borg, 1970), and the changes were calculated for each parameter. Heart rate during the 40 min was measured using a heart rate monitor (Polar RCX5, Polar Electro Oy, Kempele, Finland), and the intensity of the physical activity was calculated in relation to the individual theoretical maximum heart

rate (MHR) (Gulati et al., 2010). The heart rate < 50% of MHR, between 50% and 69% of MHR, and > 69% of MHR were considered as low, moderate and high intensities, respectively. Questionnaires were used to collect information on sociodemographic data, frequency of swimming pool attendance and other physical activities. The question "Did you swallow some water during swimming? (I'm sure I didn't; I'm not sure; Yes, some; Yes, quite a lot)" was used to assess accidental water ingestion.

2.5. Gene selection and genotyping

Blood was collected in EDTA tubes and stored at -80°C until DNA extraction. DNA was extracted using QIAamp DNA Blood Mini Kit (QIAGEN) and was measured with NANODROP 8000 (Thermo-Scientific). All samples were diluted to a fixed concentration of 2.5 ng/mL. Germ line variations in candidate genes known to participate in DBP detoxification were examined. Specifically, twelve different polymorphisms were genotyped: three single-nucleotide polymorphisms (SNPs) in the glutathione S-transferase zeta 1 (*GSTZ1*) gene, eight SNPs in the *CYP2E1* gene and a common deletion in the glutathione S-transferase theta 1 (*GSTT1*) gene by Taqman allele discrimination assay (Life Technologies). For quality control purposes, duplicate samples (5% of the total numbers of samples) were repeated for each SNP, and no template controls (NTCs) were included in each plate. All polymorphisms could be successfully analyzed in the 116 subjects (the quality control of genotypes was assured with > 99% of concordance). All polymorphisms were also in Hardy-Weinberg equilibrium and minor allele frequencies were similar to those described in the International HapMap Project for European individuals (International HapMap Consortium, 2003).

2.6. Statistical analyses

The distribution of each compound was evaluated for normality by evaluating skewness and kurtosis. Mean or median values were reported accordingly to describe central tendencies. Among the exhaled breath samples before swimming, 9%, 3%, 19% and 4% were under the detection limits for chloroform (0.108 $\mu\text{g}/\text{m}^3$), BDCIM (0.009 $\mu\text{g}/\text{m}^3$), DBCIM (0.004 $\mu\text{g}/\text{m}^3$) and bromoform (0.007 $\mu\text{g}/\text{m}^3$), respectively; 20% of water samples were under the detection limit for dibromoacetic acid (DBrAA) (1 $\mu\text{g}/\text{L}$). These samples were imputed half the respective detection limit. The compounds with undetectable levels in all the samples (chloroacetic acid, bromoacetic acid, dibromochloroacetic acid, tribromoacetic acid, trichloroacetonitrile, chloropicrin, 1,1-dichloropropanone and TOI) were excluded from the statistical analyses. The concentrations of BDCIM, DBCIM and bromoform were summed up and reported as brominated THMs (BrTHMs). The change in levels of exhaled chloroform, BrTHMs, total THMs (TTHMs), and TCAA in urine after swimming were calculated and used as exposure biomarkers. Since exposure biomarkers and several DBPs in water did not follow a normal distribution, Spearman correlations were used to assess relationships. The p -value threshold for statistical significance was < 0.05. To test associations between exposure biomarkers and personal categorical variables (i.e., sex and accidental water ingestion), the Kolmogorov-Smirnov test and the Kruskal-Wallis test were applied, respectively. The chi-square test (1 degree of freedom), with a type-I error threshold set at $\alpha=0.05$, was used to verify whether the genotypes were in Hardy-Weinberg equilibrium. Linear regression models were fitted to calculate the variance of the increase in the concentration of each exposure biomarker explained by different personal and environmental determinants. All covariates were tested in each model, and only those that were statistically significant were retained in the multivariate models. All the statistical analyses were performed with

the statistical package STATA 12.0 (StataCorp., College Station, TX, USA).

3. Results

The study involved 116 participants (48.3% males), with a median age of 23.9 years (Table 1). Mean swimming distance was 1.1 km (range 0.2–2.1), and 53% of participants swallowed some water during swimming. The mean time between leaving the pool and exhaled breath sampling was 4.3 min (standard deviation (SD)=1.8; range=0–11).

Median TTHMs concentration in exhaled breath increased from 0.5 to 14.4 $\mu\text{g}/\text{m}^3$ (with an interquartile range (IQR) of 10.0–18.4) after swimming (Fig. 1). Median level of TCAA in urine increased from 24.0 to 75.5 nmol/L after swimming, equivalent to 2.5–5.8 $\mu\text{mol}/\text{mol}$ of creatinine adjusted TCAA (Fig. 1).

The concentrations of DBPs in water are shown in Table 2. In summary, median TTHMs was 48.5 $\mu\text{g}/\text{L}$ (IQR=43.6–54.7) and total HAAs (THAAs) was 111.2 $\mu\text{g}/\text{L}$ (IQR=95.5–129.3). CHCl_2CN was the HAN with the highest concentration (7.3 $\mu\text{g}/\text{L}$). Nitrosodimethylamine (NDMA) and dimethylnitramine (DMNA) were detected in the ng/L range, TOCl was found at 0.45 mg/L as Cl and TOBr at 0.060 mg/L as Br. TOI was below the detection limit ($< 4 \mu\text{g}/\text{l}$ as I) in all samples. Trichloramine in air had a median level of 473 $\mu\text{g}/\text{m}^3$ with considerable variability (SD=134 $\mu\text{g}/\text{m}^3$, range=249–858 $\mu\text{g}/\text{m}^3$). No seasonality was observed in the DBP levels along the study period (Supplementary material Fig. S1).

Chloroform and TTHMs in water were poorly correlated to the level of other DBPs, with statistically significant correlations with few compounds (10 and 8 out of 29 respectively, Table S1 in Supplementary material). In contrast, BrDCIM, DBrCIM and BrTHMs were significantly correlated to several other DBPs (21 out of 29 parameters for BrTHMs), positively to brominated DBPs and negatively to chlorinated DBPs. Similarly, TOBr was correlated positively to brominated DBPs (e.g., CHBr_3 , BrTHMs, DBrAA, and

CHBr_2CN) and negatively to chlorinated DBPs (e.g., CHCl_3 , DCIAA, TCIAA, $\text{C}_3\text{H}_3\text{Cl}_3\text{O}$, and CHCl_2CN), while TOCl was correlated negatively to brominated DBPs. Trichloramine in air showed moderately significant correlations with some DBPs and parameters in water, i.e., 0.47 with TTHMs, 0.37 with pH and –0.17 with THAAs.

The increase in BrTHMs in exhaled breath was significantly correlated to TOC (Spearman correlation=–0.22) and several DBPs occurring in pool water such as BrTHMs (0.48), THAAs (–0.34), CHCl_2CN (–0.40), $\text{C}_3\text{H}_3\text{Cl}_3\text{O}$ (–0.42), chloramines, TOCl and TOBr (Table 3). The increase in exhaled chloroform after swimming was only significantly correlated to $\text{C}_3\text{H}_3\text{Cl}_3\text{O}$ in pool water (–0.20) and to the temperatures of air and water. The level of trichloramine in air was correlated to BrTHMs in exhaled breath (Spearman correlation=0.27), but not to exhaled chloroform or TTHMs. The increase in creatinine adjusted TCAA in urine was only correlated to the level of brominated HAAs (BrHAAs), THAAs, and monochloramine in water (–0.19, –0.23 and +0.26, respectively) (Table 3). The patterns for bromodichloromethane, dibromochloromethane and bromoform were similar to that for BrTHMs (not shown in tables).

Males had a significantly higher uptake of THMs during swimming than females as shown by chloroform and TTHMs levels in exhaled breath, while females had a higher uptake of TCAA indicated by urine concentrations (Table 4). Age, BMI, and body surface area did not influence the uptake of the internal dose biomarkers, while exercise did. Swimming distance and energy expenditure were positively related to chloroform and BrTHMs and TTHMs uptakes and negatively to TCAA uptake. The percentage of time at high activity assessed by heart rate, subjective exertion, and shortness of breath were significantly related to THM uptake but not to TCAA in urine. Reported accidental water ingestion was not significantly related to the increase in any internal dose biomarker.

Differences in the increase of the internal dose biomarkers were detected according to specific polymorphisms of the three selected genes (Table 5). Subjects with CT/TT genotypes of

Table 1
Characteristics of the study population (N=116).

		N	%
Subject characteristics			
Sex	Males	56	48.3
	Females	60	51.7
Current pool attendance ^a	Yes	33	28.4
	No	83	71.5
	Median	IQR	
Age, years		23.9	21.3–28.9
Body mass index, kg/m^2		23.0	21.3–25.5
Body surface area, m^2		1.7	1.6–1.9
Exercise performed during the 40 min in the pool			
Distance swum, km		1.1	0.8–1.2
Time swimming, min		35.3	29.9–38.7
Energy expenditure, kcal		204.5	166.9–254.6
High intensity ($> 69\%$ MHR), percentage time		70.6	33.1–88.2
Exertion ^b		3	2–4
Shortness of breath ^b		2	1–3.75
Accidental water ingestion			
		N	%
I'm sure I didn't		23	19.8
I'm not sure		31	26.7
Yes, some		58	50.0
Yes, quite a lot		4	3.4

IQR=Interquartile range. MHR: maximum heart rate.

^a At least once a month.

^b Difference in the Borg scale (0–10 points), measured before and after swimming.

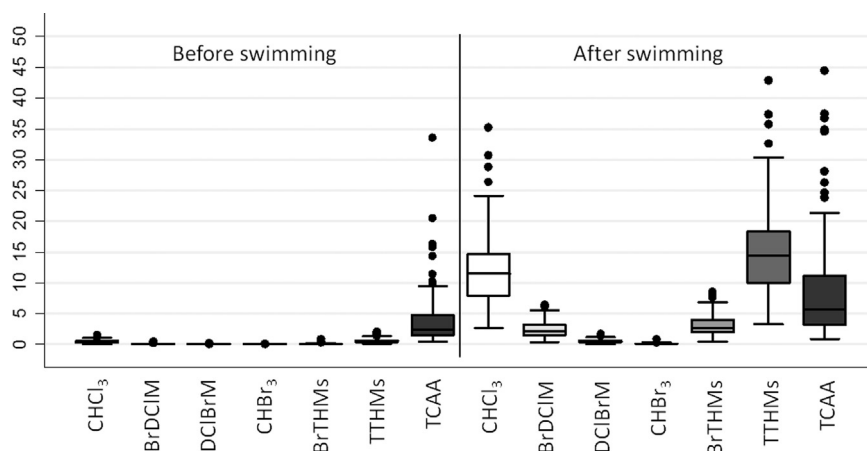


Fig. 1. Levels of trihalomethanes (THMs) in exhaled breath ($\mu\text{g}/\text{m}^3$) and creatinine adjusted trichloroacetic acid (TCAA) in urine ($\mu\text{mol}/\text{mol}$) before and after swimming for 40 min. CHCl_3 : Chloroform; BrDCIM: Bromodichloromethane; DBrCIM: Dibromochloromethane; CHBr_3 : Bromoform; BrTHMs: Brominated trihalomethanes; TTHMs: Total trihalomethanes; TCAA: Trichloroacetic acid. THMs in exhaled breath were measured immediately (4 min) after swimming. Urine was collected 30 min after swimming.

Table 2

Levels of disinfection by-products and related parameters measured in the swimming pool water during the 30 experimental days.

		N	Median	IQR	Min	Max
Trihalomethanes ($\mu\text{g}/\text{L}$)	CHCl_3	108	37.3	32.9–43.8	24	61.6
	BrDCIM	108	7.1	5.4–8.6	3.8	12.9
	DBrCIM	108	2	1.6–3.0	0.9	4.7
	CHBr_3	108	0.7	0.5–1.3	0.2	1.9
	BrTHMs	108	9.5	7.9–12.6	5.7	19.4
	TTHMs	108	48.5	43.6–54.7	30.7	74.7
	TCAA	30	29.4	22.3–37.7	15.4	51.7
Haloacetic acids ($\mu\text{g}/\text{L}$)	DCIAA	30	62.9	57.4–70.2	39.2	83.4
	BrCIAA	30	4.9	4.2–6.2	2.4	8.8
	DBrAA	30	1	1.0–1.8	0.5	3.1
	DCIBrAA	30	11.8	9.4–15.4	4.8	23.4
	THAA	30	111.2	95.5–129.3	73.3	144.3
	CHBr_2CN	27	1.3	1.2–1.4	1.1	3.6
	CHCl_2CN	108	7.3	5.9–8.7	3.8	12.2
Haloacetonitriles ($\mu\text{g}/\text{L}$)	CHBrClCN	103	3	2.7–4.2	1.8	4.7
	$\text{C}_3\text{H}_3\text{Cl}_3\text{O}$	108	2.1	1.7–2.6	1.4	5.8
	NDMA	12	10.6	10.1–11.1	8.0	13.6
Nitrosamines/nitramines (ng/L)	DMNA	10	2.1	1.6–2.4	0.9	3.2
	NPOC	57	2.4	2.2–2.7	1.8	10.1
	TOCl (as Cl)	24	0.45	0.42–0.50	0.39	0.55
Total organic carbon (mg/L)	TOBr (as Br)	24	0.06	0.05–0.07	0.05	0.08
	TOX (as Cl)	24	0.48	0.45–0.52	0.42	0.57
	Free chlorine	114	1.3	1.0–1.6	0.6	2.0
Chlorine (mg/L as Cl_2)	Total chlorine	114	2.2	1.9–2.3	1.6	2.7
	NH_2Cl	114	0.2	0.1–0.4	0.0	0.7
Chloramines (mg/L as Cl_2)	NHCl_2	112	0.3	0.0–0.4	0.0	0.7
	NCl_3	114	0.0	0.0–0.6	0.0	1.6
Other parameters	pH	115	7.40	7.3–7.6	7.1	8.0
	Water temperature ($^{\circ}\text{C}$)	115	28.0	27.8–28.2	27.1	28.6
	Number of swimmers	115	23.0	16.3–34.3	9.0	120.5
	Air temperature ($^{\circ}\text{C}$)	115	28.5	27.8–29	26.5	29.5
	NCl_3 in air ($\mu\text{g}/\text{m}^3$)	26	472.6	381.4–533.5	248.5	858.3

All samples were under the limit of detection for chloroacetic acid ($< 3 \mu\text{g}/\text{L}$), bromoacetic acid ($< 2 \mu\text{g}/\text{L}$), dibromochloroacetic acid ($< 2 \mu\text{g}/\text{L}$), tribromoacetic acid ($< 5 \mu\text{g}/\text{L}$), trichloroacetonitrile ($< 0.5 \mu\text{g}/\text{L}$), chloropicrin ($< 1 \mu\text{g}/\text{L}$), dichloropropanone ($< 2 \mu\text{g}/\text{L}$), and total organic iodine ($< 4 \mu\text{g}/\text{L}$ as I). IQR (Interquartile range, percentile 25–percentile 75); CHCl_3 : Chloroform; BrDCIM: Bromodichloromethane; DBrCIM: Dibromochloromethane; CHBr_3 : Bromoform; BrTHMs: Brominated trihalomethanes; TTHMs: Total trihalomethanes; DCIAA: Dichloroacetic acid; TCAA: Trichloroacetic acid; BrCIAA: Bromochloroacetic acid; DBrAA: Dibromoacetic acid; DCIBrAA: Dichlorobromoacetic acid; THAAs: Total haloacetic acids; CHBr_2CN : Dibromoacetonitrile; CHCl_2CN : Dichloroacetonitrile; CHBrClCN : Bromochloroacetonitrile; $\text{C}_3\text{H}_3\text{Cl}_3\text{O}$: 1,1,1-Trichloropropanone; NDMA: Nitrosodimethylamine; DMNA: Dimethylnitramine; NPOC: Non-purgeable organic carbon; TOCl: Total organic chlorine; TOBr: Total organic bromine; NH_2Cl : Monochloramine; NHCl_2 : Dichloramine; NCl_3 : Trichloramine.

rs1046428 in *GSTZ1* had a 2.5 times higher increase in TCAA in urine than those with CC genotype. Subjects with *GSTT1* null genotype had a higher increase of THMs in exhaled breath and TCAA in urine compared to those with the enzymatic activity. Two SNPs in the *CYP2E1* gene were also associated with different increases in exhaled BrTHMs. Other analyzed SNPs in *GSTZ1* or *CYP2E1* were not associated with the internal dose biomarkers.

In multivariate models, sex, distance swum, and air temperature explained between 12% and 15% of the variance of the exhaled THM increase (Supplementary material Table S2). Adding the variable $\text{C}_3\text{H}_3\text{Cl}_3\text{O}$ (1,1,1-trichloropropanone) level in water increased the R-squared of the chloroform and TTHMs models (to 0.14 and 0.16, respectively), while BrTHMs concentration in water increased the explained variance for BrTHMs uptake model (R-

Table 3

Spearman correlation between disinfection by-products in swimming pool water and the increase in the internal dose biomarkers (levels after swimming^a – levels before swimming).

	CHCl ₃ in exhaled breath	BrTHMs in exhaled breath	TTHMs in exhaled breath	N ^b	Creatinine adjusted TCAA in urine	N ^b
CHCl ₃	0.13	−0.06	0.09	108	−0.14	105
BrTHMs	0.11	0.48**	0.20*	108	−0.05	105
TTHMs	0.16	0.16	0.17	108	−0.12	105
TCAA	−0.16	−0.36**	−0.21*	116	−0.14	113
BrHAAs	0.04	−0.01	0.03	116	−0.19*	113
THAAs	−0.09	−0.34**	−0.14	116	−0.23*	113
CHCl ₂ CN	< −0.01	−0.40**	−0.09	108	0.07	105
C ₃ H ₃ Cl ₃ O	−0.20*	−0.42**	−0.25**	108	−0.04	105
NDMA	−0.03	−0.06	−0.03	47	−0.26	47
DMNA	0.18	−0.27	0.09	39	−0.31	39
FCI	0.01	0.14	0.04	114	0.02	111
NH ₂ Cl	0.16	0.36**	0.09	114	0.26**	111
TOCl	0.06	−0.31**	−0.02	93	0.09	90
TOBr	−0.03	0.38**	0.06	93	0.18	11
TOC	−0.02	−0.22*	−0.06	114	−0.18	112
pH	0.07	0.28**	0.12	115	−0.01	112
Water temperature	−0.20*	−0.13	−0.19*	115	−0.11	112
Number of swimmers	0.16	−0.06	0.11	115	0.00	112
Air temperature	−0.25**	−0.24*	−0.25**	115	−0.08	112
Trichloramine in air	0.15	0.27**	0.18	102	−0.12	100
CHCl ₃ exhaled	–	0.82**	0.99**	116	−0.04	113
BrTHMs exhaled	–	–	0.88**	116	−0.02	113
TTHMs exhaled	–	–	–	116	−0.04	113

CHCl₃: Chloroform; BrTHMs: Brominated trihalomethanes; TTHMs: Total trihalomethanes; DCIAA: Dichloroacetic acid; TCAA: Trichloroacetic acid; THAAs: Total haloacetic acids; CHCl₂CN: Dichloroacetone; C₃H₃Cl₃O: 1,1,1-Trichloropropanone; NDMA: Nitrosodimethylamine; DMNA: Dimethylnitramine; FCI: Free chlorine; NH₂Cl: Dichloramine; TOCl: Total organic chlorine; TOBr: Total organic bromine; TOC: Total organic carbon.

* p-value < 0.05.

** p-value < 0.01.

^a THMs in exhaled breath were measured immediately (4 min) after swimming. Urine was collected 30 min after swimming.

^b Number of observations included in each model.

squared=0.32). Distance swum, monochloramine or TCAA levels in water were the variables that most explained the variance in TCAA uptake (maximum R-squared=0.11).

4. Discussion

The increase in exhaled BrTHMs after swimming was correlated to the concentration of several DBPs in water and to trichloramine in air, the irritant DBP potentially related to respiratory effects in swimmers. Such correlations are not detected for the increase in exhaled chloroform, exhaled TTHMs or urine TCAA. Intensity of physical exercise increased the THM uptake and decreased the urine TCAA level. Men showed a larger increase in exhaled THM levels, while women had a larger increase in creatinine adjusted TCAA in urine. Age, BMI, body surface area or self-reported accidental water ingestion did not influence the uptake of THMs and, unexpectedly of TCAA. Polymorphisms in *GSTT1*, *GSTZ1* and *CYP2E1* were associated with different increases in the internal dose biomarkers.

Significant increases in the DBP internal dose biomarkers were detected after swimming for 40 min in an indoor chlorinated pool. TTHMs in exhaled breath increased by 24 times after swimming (around 13.8 µg/m³) and creatinine adjusted TCAA in urine

increased by 2.3 times. Previous studies detected different increases in exhaled THMs after swimming, from 4.5 to 138 µg/m³ (Aggazzotti et al., 1998; Fantuzzi et al., 2011; Caro and Gallego, 2008; Aprea et al., 2010; Font-Ribera et al., 2010). The study that measured the change in TCAA in urine in 27 swimmers and 25 pool lifeguards, detected an increase after 1 h swimming in an indoor or in an outdoor pool, and after working for 2 h in the indoor pool, but not in the outdoor pool (Cardador and Gallego, 2011).

The concentrations of DBPs in pool water found in the present study were in the range reported previously (Chowdhury et al., 2014). As expected (Villanueva et al., 2012), we detected both positive and negative correlations between different classes of DBPs. The levels of chloroform or TTHMs in water are often used as an overall DBP indicator in epidemiological studies (Villanueva and Font-Ribera, 2012). We have shown that in swimming pools TTHMs concentrations do not necessarily correlate with concentrations of other classes of DBPs. Therefore, controlling the TTHMs levels in swimming pools may not necessarily mean controlling all other classes of DBPs (Zwiener et al., 2007).

We found that DBP levels in water and trichloramine in air were much more correlated to the measured increase in exhaled BrTHMs after swimming, than the measured increase in exhaled chloroform, exhaled TTHMs or TCAA in urine. This higher correlation between environmental and exhaled BrTHMs compared to chloroform is consistent with the results from two studies assessing THM exposure after a shower (Weisel et al., 1999; Silva et al., 2012). Two reasons may explain this difference. On one hand, BrTHMs have a lower elimination rate in blood than chloroform, due to higher lipophilicity (Silva et al., 2012). We measured THMs in exhaled breath as soon as possible after swimming, i.e. around four min after leaving the pool. This time could have been enough for chloroform to be more excreted and less representative of the actual uptake during swimming than BrTHMs. A second reason could be that the relative contribution of dermal absorption compared to inhalation may be higher for BrTHMs than for chloroform due to differences in volatility and skin permeability (Xu et al., 2002). Dermal absorption may be a route leading to less variation in internal exposure than inhalation (Silva et al., 2012), which is strongly affected by external factors such as water temperature, air ventilation and fresh air intake in the pool environment. This higher correlation between environmental DBP levels and exhaled BrTHMs than compared to exhaled chloroform is also in accordance with previous studies detecting short-term changes in respiratory (Font-Ribera et al., 2010) and genotoxic biomarkers (Kogevinas et al., 2010) after swimming related only to levels of exhaled bromoform and not exhaled chloroform.

A low correlation between TCAA uptake and HAAs in water is expected, since accidental ingestion is the main route of exposure (Cardador and Gallego, 2011) and is probably more variable than dermal absorption or inhalation. In the present study, the majority of participants recognized that they swallowed some water during swimming, but self-reported accidental ingestion did not predict TCAA uptake. This is unexpected given that HAAs are not volatile or skin permeable, thus ingestion is supposed to be the major exposure route. Our results may suggest that among typical adult swimmers in indoor swimming pools, inhalation of aerosol could be more relevant than accidental ingestion. This hypothesis would be supported by studies measuring significant HAAs levels in aerosol (Sá et al., 2012). In addition, without having an explanation for this, the increase in urine TCAA was negatively correlated to TCAA and THAAs in water.

Age, body surface area or BMI did not influence the uptake of THMs and HAAs. Males had higher uptake of THMs, also after adjusting for distance swum, maybe due to generally larger volumes of the lungs. Women had a higher increase in TCAA in urine

Table 4
Spearman correlation between individual determinants and the increases in the internal dose biomarkers (levels after swimming^a – levels before swimming). For sex and accidental water ingestion, median values are shown (N=113).

	CHCl ₃ in exhaled breath (μg/m ³)	BrTHMs in exhaled breath (μg/m ³)	TTHMs in exhaled breath (μg/m ³)	Creatinine adjusted TCAA in urine (μmol/mol)
Subject characteristics				
Sex				
Males	11.4	3.0 [*]	15.0	1.8 ^{**}
Females	10.7	2.5 [*]	12.7	4.0 ^{**}
Age	0.02	0.04	–0.05	0.13
Body mass index	0.07	0.07	0.07	–0.05
Body surface area	0.11	0.17	0.13	–0.09
Exercise performed during the 40 min in the pool				
Distance swum	0.26 ^{**}	0.21 [*]	0.25 ^{**}	–0.32 ^{**}
Time swimming	0.05	0.14	0.07	–0.21 [*]
Energy expenditure	0.27 ^{**}	0.24 ^{**}	0.27 ^{**}	–0.33 ^{**}
High intensity (> 69% MHR)	0.16	0.21 [*]	0.19 [*]	–0.14
Exertion ^b , change after swimming	0.22 [*]	0.37 ^{**}	0.26 ^{**}	0.03
Shortness of breath ^b , change after swimming	0.21 [*]	0.34 ^{**}	0.24 ^{**}	0.03
Self-reported accidental water ingestion^c				
I'm sure I didn't	10.1	2.3	12.3	2.3
I'm not sure	9.1	2.7	12.9	3.0
Yes, some	11.4	2.7	14.2	3.2
Yes, quite a lot	13.5	3.3	16.9	1.5

CHCl₃: Chloroform; BrTHMs: Brominated trihalomethanes; TTHMs: Total trihalomethanes; TCAA: Trichloroacetic acid. MHR: Maximum heart rate. P-values from the spearman correlations, except for sex (Kolmogorov-Smirnov test) and accidental water ingestion (Kruskal-Wallis test).

*p-value < 0.05. **p-value < 0.01.

^{*} p-value < 0.05.

^{**} p-value < 0.01.

^a THMs in exhaled breath were measured immediately (4 min) after swimming. Urine was collected 30 min after swimming.

^b Borg scale (0–10).

^c Answer to the question “Did you swallow some water?”

than men.

There is limited evidence in humans about the role of genetic polymorphisms on exposure and associated health effects and we provide hereby one of the first examples showing the influence of genetic variants on DBP exposure. We selected genes known to modulate metabolism of major DBPs in experimental studies and related to different DBP-associated bladder cancer risk (Cantor et al., 2010). These genes have several polymorphisms, often with a functional effect on the metabolism of xenobiotics (Norppa, 2003). Although our analysis is hampered by the small study population and results should be cautiously interpreted, our findings are partly consistent with previous knowledge. *GSTZ1* metabolizes some haloacids and *GSTZ1*-depleted rats were shown to have lower body clearance of dihaloacetic acids (Saghir and Schultz, 2005). We found that subjects with the CT/TT allele in the rs1046428 SNP had a higher increase in TCAA in urine, which is consistent with the findings that subjects with a T allele in this SNP have a lower enzymatic activity (Blackburn et al., 2001) and a higher DBP-associated bladder cancer risk (Cantor et al., 2010). *GSTT1* activates BrTHMs to mutagens and the null form of the allele is associated with lack of enzymatic activity (Cantor et al., 2010). Humans carrying the *GSTT1* null allele have been shown to have higher blood levels of unmetabolized BrDCIM after BrDCIM exposure (Leavens et al., 2007) and to have no DBP-associated bladder cancer risk (Cantor et al., 2010). We found that subjects with the *GSTT1* null allele had a higher increase in THMs and TCAA, although the difference in the BrTHMs increase was small and not statistically significant. *CYP2E1* metabolizes a variety of low molecular weight compounds including chloroform (Guengerich et al., 1991). However, we did not identify effects of *CYP2E1* polymorphisms on exhaled THM levels, except a minor modulation

played by rs2515641 on exhaled BrTHMs. Our results need confirmation on larger samples. The role of genetic variations would contribute to disentangle the molecular mechanisms of DBPs, the biological plausibility of epidemiological associations in previous studies, and the identification of susceptible subgroups.

The degree of physical activity during swimming was related to the increase in both THMs and TCAA. Physical activity increases the uptake of THMs by increasing the inhalation rate, blood pressure and surface capillary perfusion, which was previously seen in 48 subjects (Marco et al., 2015). The negative correlation to TCAA in urine is more difficult to explain, but we hypothesize that it could be related to changes in the proportion of TCAA bounded to plasmatic proteins that affect the TCAA elimination rate in the urine (US EPA, 2011). Different dimensions of physical activity were assessed, including objective and subjective measures. Although all of them were associated with some internal dose biomarkers, energy expenditure seems a good indicator since it was the one with the highest correlations with the exposure biomarkers and it is simple to estimate.

Some methodological issues are worth discussion. This study used a semi-experimental design and was conducted during 30 days from June to December, with four subjects evaluated per day. Although we studied several parameters, we could only explain a low portion of the variances of the increases in the internal dose biomarkers (from 11% in urine TCAA to 32% in exhaled BrTHMs). However, this study reflected a real-life scenario, where the environmental and personal factors evaluated were not controlled by the researchers but only measured as accurately as possible. Some potentially relevant factors affecting THMs uptake, such as the degree of ventilation, could have been missed. This could explain why the correlations between water and exhaled THMs detected

Table 5Increases in the internal dose biomarkers after^a swimming by selected polymorphisms.

		N	CHCl ₃ in exhaled breath (μg/m ³)		BrTHMs in exhaled breath (μg/m ³)		TTHMs in exhaled breath (μg/m ³)		Creatinine adjusted TCAA in urine (μmol/mol)	
			Median	IQR	Median	IQR	Median	IQR	Median	IQR
GSTZ1	rs1046428									
	CC	70	11.4	7.6–16.3	2.7	1.9–4.1	13.9	10.0–19.5	2.1	0.7–4.1
	CT/TT	46	10.6	7.4–13.6	2.6	1.9–3.3	13.0	9.3–17.0	5.3	1.5–7.2
	p-value ^b		0.052		0.175		0.095		0.003	
	rs7972									
	GG	96	10.7	7.6–14.1	2.6	1.9–3.9	13.0	10.0–18.0	3.1	1.1–6.1
	GA/AA	20	11.7	6.4–14.5	2.8	1.8–3.4	15.1	8.4–17.7	3.0	0.9–5.9
	p-value ^b		0.356		0.797		0.250		0.969	
	rs7975									
	GG	48	10.3	7.4–13.3	2.6	1.9–4.0	12.5	9.3–17.2	3.2	1.4–6.6
GA/AA	68	11.4	7.6–15.0	2.7	1.9–3.9	14.4	10.0–18.5	2.5	0.6–5.7	
p-value ^b		0.158		0.877		0.305		0.452		
GSTT1	GSTT1									
	Null	24	13.8	8.9–15.5	3.1	2.4–4.0	16.9	11.8–18.8	5.2	3.5–6.4
	Positive	92	10.7	7.4–13.6	2.6	1.8–3.9	13.0	9.3–17.7	2.2	0.9–5.7
	p-value ^b		0.022		0.200		0.031		0.002	
CYP2E1	rs8192766									
	TT	92	11.1	7.7–14.5	2.7	2.0–3.9	13.7	10.1–18.3	2.6	1.1–6.1
	TG/GG	24	9.2	5.6–13.3	2.4	1.2–3.1	11.3	6.8–16.6	4.1	0.7–6.9
	p-value ^b		0.322		0.096		0.214		0.461	
	rs2070676									
	CC	89	11.1	7.6–14.5	2.7	1.9–3.9	13.9	10.1–18.5	3.0	1.0–5.7
	CG/GG	27	10.3	6.0–13.7	2.5	1.4–2.9	12.6	8.1–16.2	4.4	1.4–9.4
	p-value ^b		0.413		0.041		0.437		0.247	
	rs2031920									
	CC	107	11.0	7.5–14.4	2.6	1.9–3.9	13.5	10.0–17.7	3.1	1.1–6.1
	CT/TT	9	9.6	7.6–14.1	2.3	1.7–3.4	11.9	9.3–17.7	1.1	–0.2–5.7
	p-value ^b		0.957		0.714		0.932		0.170	
	rs2249695									
	CC	80	11.1	7.5–14.5	2.8	1.9–4.0	13.7	10.0–18.3	3.1	1.0–6.1
	CT/TT	36	10.8	7.6–14.0	2.4	1.7–3.1	13.1	9.0–17.4	2.9	1.3–6.9
	p-value ^b		0.962		0.115		0.891		0.842	
	rs915906									
	TT	88	11.1	7.7–14.5	2.9	1.9–3.9	13.7	10.1–18.0	3.2	1.0–6.1
	TC/CC	28	10.8	5.9–14.0	2.3	1.4–2.9	13.1	7.8–17.4	2.2	1.4–5.1
	p-value ^b		0.553		0.072		0.540		0.642	
rs915907										
CC	89	10.7	7.5–13.8	2.6	1.9–3.9	13.0	9.4–17.6	3.0	1.1–6.2	
CA/AA	27	12.0	8.7–15.5	2.7	1.8–3.9	16.0	10.5–18.7	3.2	0.4–5.7	
p-value ^b		0.261		0.985		0.324		0.830		
rs2070673										
TT	79	11.2	7.8–14.9	2.9	2.0–4.1	14.1	10.1–18.6	3.0	1.0–5.9	
TA/AA	37	10.1	6.0–13.7	2.3	1.3–3.0	12.4	7.4–17.1	3.2	1.4–7.1	
p-value ^b		0.288		0.079		0.278		0.717		
rs2515641										
CC	90	11.1	7.6–14.5	2.8	1.9–4.1	14.0	10.0–18.5	3.1	1.0–5.7	
CT/TT	26	10.2	7.5–13.0	2.5	1.7–2.8	12.5	8.8–16.0	3.4	1.4–9.4	
p-value ^b		0.484		0.016		0.217		0.404		

CHCl₃: Chloroform; BrTHMs: Brominated trihalomethanes; TTHMs: Total trihalomethanes; TCAA: Trichloroacetic acid. MHR: Maximum heart rate.^a THMs in exhaled breath were measured immediately (4 min) after swimming. Urine was collected 30 min after swimming. The major versus the two minor allele frequencies are compared. P-values for Kolmogorov-Smirnov test.^b P-value from the Kolmogorov-Smirnov equality-of-distributions test.

in this study are lower than those detected in blood after a 10 min shower with controlled water and air temperature (Silva et al., 2012). Similarly, our results cannot be extrapolated to outdoor swimming pools. Although the lack of measurement of THMs in air may be a limitation, the added value of those measurements would be small given the correlation between water and air levels in indoor swimming pools (Lourencetti et al., 2012). The number of samples analyzed for each compound varied between the DBP classes (from around 110 for THMs and HANs to 10 for DMNA), which may have affected the power to detect significant

correlations. The strengths of the study that represent an improvement from previous literature are the large sample size (116 subjects) and a more accurate exposure measurement, including several classes of DBPs and different parameters of physical activity. We evaluated for the first time how polymorphisms of specific metabolizing genes affect the DBP internal dose biomarkers after swimming.

5. Conclusions

In summary, we have shown for the first time that the increase

in exhaled brominated trihalomethanes after swimming is correlated to the levels of several DBPs in swimming pool water and to trichloramine in air. Such correlations are not detected for exhaled chloroform, exhaled TTHMs or urine TCAA. Despite occurring at low levels, these findings suggest that brominated trihalomethanes in exhaled breath could be a non-invasive biomarker of recent DBP exposure in swimming pools with bromide-containing source water, although affected by sex, the degree of physical activity and some metabolizing genes. These results require replication in other settings for confirmation.

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The study was approved by the ethics committee of the research centre following the international regulations, and all volunteers signed an informed consent before participation.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at <http://dx.doi.org/10.1016/j.envres.2016.05.013>.

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