

Influence of physical activity in the intake of trihalomethanes in indoor swimming pools

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ABSTRACT

This study describes the relationship between physical activity and intake of trihalomethanes (THMs), namely chloroform (CHCl_3), bromodichloromethane (CHCl_2Br), dibromochloromethane (CHClBr_2) and bromoform (CHBr_3), in individuals exposed in two indoor swimming pools which used different disinfection agents, chlorine (Cl-SP) and bromine (Br-SP). CHCl_3 and CHBr_3 were the dominant compounds in air and water of the Cl-SP and Br-SP, respectively. Physical exercise was assessed from distance swum and energy expenditure. The changes in exhaled breath concentrations of these compounds were measured from the differences after and before physical activity.

A clear dependence between distance swum or energy expenditure and exhaled breath THM concentrations was observed. The statistically significant relationships involved higher THM concentrations at higher distances swum. However, air concentration was the major factor determining the CHCl_3 and CHCl_2Br intake in swimmers whereas distance swum was the main factor for CHBr_3 intake. These two causes of THM incorporation into swimmers concurrently intensify the concentrations of these compounds into exhaled breath and pointed to inhalation as primary mechanism for THM uptake. Furthermore, the rates of THM incorporation were proportionally higher as higher was the degree of bromination of the THM species. This trend suggested that air-water partition mechanisms in the pulmonary system determined higher retention of the THM compounds with lower Henry's Law volatility constants than those of higher constant values. Inhalation is therefore the primary mechanisms for THM exposure of swimmers in indoor buildings.

Key words: Water disinfection, swimming pool, exhaled breath, trihalomethanes, physical exercise, distance swum, bromoform, dichlorobromomethane, dibromochloromethane, chloroform.

1. Introduction

Swimmers, staff personnel and visitors are exposed to trihalomethanes (THMs), e.g. chloroform (CHCl_3), bromodichloromethane (CHCl_2Br), dibromochloromethane (CHClBr_2) and bromoform (CHBr_3), in indoor swimming pools (Aggazzotti et al., 1990; 1993; 1995; 1998; Erdinger et al., 2004; Font-Ribera et al., 2010; Kozłowska et al., 2006; Levesque et al., 1994; 2000; Lindstrom et al., 1977; Lourencetti et al., 2012). They are formed by water chlorination/bromination and reaction with organic matter (Rook, 1974). In addition to these compounds other disinfection by-products have been identified in indoor swimming pool waters (Richardson et al., 2010) but THMs are the most studied. Long term exposure to these compounds is associated with bladder cancer risk increase (Hamidin et al., 2008; Villanueva et al., 2007; 2015).

In a previous study we showed that there are important differences in THM uptake between people swimming, simply bathing (no physical activity) or standing (outside the water) in indoor swimming pools (Lourencetti et al., 2012). In all cases, THM intake was measured from the exhaled breath concentrations. The end-exhaled (alveolar) air gives representative estimates of the concentration of the organic constituents in blood due to the gas exchange in the blood/breath interface of the lungs (Pleil and Lindstrom, 1997). The differences observed between these three groups of swimming pool users were consistent with previous studies showing that besides ingestion, inhalation and dermal absorption may also be significant THM uptake pathways (Backer et al., 2000; Xu, Weisel, 2005; Gordon et al., 2006).

In the present study, we investigate whether the physical activity developed in the pools can be related to THM intake. This is an important aspect for improving the understanding of the processes of THM incorporation in swimmers and for assessment of the possible deleterious effects of THM exposure in these sport practitioners.

CHCl₃ is usually the dominant compound in swimming pools using chlorination. Studies involving THM analysis of exhaled breath-alveolar air, blood or urine in these pools often observe the concentrations of CHCl₂Br, CHClBr₂ or CHBr₃ below limit of quantification (Aggazzotti et al., 1998; Fantuzzi et al., 2001; Caro and Gallego, 2008; Cammann and Hubner, 1995). However, the more brominated THMs are important since they are those showing higher cytotoxic and mutagenic potential (Plewa et al., 2002; Kogevinas et al., 2010) or those involving higher deleterious effects in the respiratory system upon swimming (Font-Ribera et al., 2010).

In the present study, we are using a previously developed method for THM analysis in exhaled breath-alveolar air (Lourencetti et al., 2010) that provides low detection limits. Furthermore, two swimming pools using different systems, chlorination and bromination (Gordon et al., 1997) have been chosen for study. With this approach, the exposure of swimmers to all THM species has been assessed and the relationship between intake of these compounds and extent of physical exercise has been investigated.

2. Materials and Methods

2.1. Study Design

The two indoor swimming pools selected for study were located in Barcelona, in the same area of the city and received tap water from the same supply. Different disinfections agents, chlorine (chlorinated swimming pool, Cl-SP) and bromine (brominated swimming pool, Br-SP) were used. The bromination process uses 1-bromo-3-chloro-5,5-dimethylhydantoin (BCDMH), that is available under the commercial

names DiHalo®, Halobrome®, Aquabrome® and others. In aqueous solution this compound generates HOBr and HOCl, and the latter rapidly combines with NaBr (one end product of BCDMH disinfection) to produce more HOBr (Judd and Jeffrey, 1995). The sizes of the Cl-SP and Br-SP were 33 x 25 x 2 m and 20.9 x 13.5 x 1.3 m, respectively. The floor ceiling heights in the two buildings were 10 m and 5 m, respectively.

All participants (n = 47) were not professional swimmers, non-smokers and within an age range of 23–51, average 31 years. The characteristics of the population including these participants have been described elsewhere (Kogevinas et al., 2010). The influence of physical activity was investigated comparing the THM concentrations in exhaled breath of all subjects. Thirty-nine of them swam during 40 min at their capacity (32 in the Cl-SP and 7 in the Br-SP). In the Cl-SP, 8 subjects were asked to bathe during 40 min without physical activity. Distance of swimming, number of laps, were self-reported for each individual in both pools. The exposure time, 40 min, was selected based on an estimative of the usual swimming time of non-competitive swimmers.

THM intake was assessed from the difference between exhaled breath concentrations after and before swimming or bathing. A portable system collecting the end-exhaled breath (Lourencetti et al., 2010) was employed for this purpose. THM concentrations in swimming pool water and indoor air were monitored during all exposure experiments.

2.2. Sampling

Water, indoor air and exhaled breath samples were collected following the protocol described elsewhere (Lourencetti et al., 2010). Briefly, composite water samples (250

mL) were collected at the four swimming pool corners, and combined in 1 L samples. At least 3 composite samples were collected during each swimming day. After gently mixing, water was transferred to headspace-free 40 mL glass vials with Teflon-faced rubber septa and open-top screw plugs, avoiding bubble formation. The vials contained 3 mg of sodium thiosulfate for quenching residual chlorine and bromine. All samples were stored at 4°C until analysis which was performed no later than 14 days after sampling as recommended by EPA Method 524.2 (US EPA, 1986).

Indoor air samples were obtained by pulling air through 0.5 cm diameter and 9 cm long stainless steel tubes containing 0.18 g of Tenax[®]. After packing, the tubes were conditioned by helium purging and four heating cycles from 60°C to 325°C holding this temperature for 30 min. This packing was activated for 10 min at 325°C before use. The tubes were connected to a constant flow sampling pump (Universal Pump Model 224-PCXR8; 5–5000 mL min⁻¹, SKC Inc., Eighty Four, PA, USA; Woolfenden et al., 1997). An adjustable low flow tube holder dual set was used to collect indoor air samples during 20 min at an average flow rate of 7 mL·min⁻¹. Samples were collected every 20 min during the whole day of human exposure testing. The tubes were situated at distances of 0.60 m from the ground and 1.5 m from the swimming pool edge. The sampling pump was calibrated *in situ* with a Dry-Cal DC-Lite (BIOS, Butler, NJ, USA) prior to sampling and at the end of the sampling day.

THMs in exhaled breath were concentrated in the same tubes described for air sampling using the portable system described in Lourencetti et al. (2010). Participants were requested to provide two exhaled breath samples (1 L each sample), one just before swimming or bathing, and another within 5 min after these activities.

2.3. Chemicals

THMs standards and internal standards for water analysis, 4-bromofluorobenzene and fluorobenzene and Tenax[®] (60/80 mesh) were purchased from Supelco, Inc. (Bellefonte, PA, USA). Sodium thiosulfate (analysis grade) was from Panreac (Barcelona, Catalonia, Spain), while deionized water was obtained from Merck (KGaA, Darmstadt, Germany).

2.4. THM analysis

THMs in indoor air and exhaled air samples were determined by an Automatic Thermal Desorption System (ATD400, Perkin Elmer, Waltham, MA, USA) coupled to an Autosystem gas chromatograph with electron capture detection (GC-ECD; Perkin Elmer). The sampling tubes were thermally desorbed at 300°C for 5 min with a flow rate of 50 mL·min⁻¹ of ultra-pure helium and the target compounds were swept from the tube to a preconcentration cold trap (– 25 °C) made of quartz (16 cm length, 0.4 cm i.d. tube and packed with 0.04 g of Tenax[®] TA between two layers of silanized wool). The cold trap was rapidly heated to 300°C and kept at this temperature for 10 min to transfer the target compounds to the GC-ECD system through a transfer line heated to 225°C. Flow desorption and the inlet and outlet split flows were 50, 210 and 8 mL·min⁻¹, respectively. In these conditions about 10% of the sample was transferred to the GC column and detector. Chromatographic separation was performed on a DB-624 capillary column (0.53 mm i.d., 75 m long, 3 µm film thickness; J&W Scientific, Folsom, CA, USA). The initial GC oven temperature was set to 40°C for 5 min, then ramped at 5°C·min⁻¹ to 160°C, held at this temperature for 1 min, and ramped again to the final temperature of 210°C at 10°C·min⁻¹, where it was held for 5 min. Detector temperature was 290°C. Helium (8 mL·min⁻¹) and nitrogen (34 mL·min⁻¹) were used as carrier and make up gases, respectively. Good correlation coefficients ($r > 0.997$) were obtained

employing calibration curves with external standards ($0.01\text{--}1\ \mu\text{g mL}^{-1}$) for all compounds.

Water samples were analyzed using a SOLATek 72 Multi-Matrix Vial Autosampler coupled to a Purge-and-Trap Concentrator Tekmar 3100 (Tekmar-Dohrmann, Mason, OH, USA) which automatically dispensed aliquots of water samples or standard solution and $5\ \mu\text{L}$ of the internal standard into a 25 mL purging device. These compounds were purged from water samples for 11 min by a stream of helium at $36.5\ \text{mL}\cdot\text{min}^{-1}$ and adsorbed onto a Tenax[®] silica gel-charcoal trap (Supelco) at room temperature. After desorption at 225°C for 4 min, the target compounds were transferred directly to a Trace GC coupled to a Voyager MS (ThermoQuest Finnigan, Waltham, MA, USA) equipped with the same capillary column as described above. The column was held at 35°C during 4 min, ramped to 150°C at $4^\circ\text{C}\cdot\text{min}^{-1}$ and then to 210°C at $11^\circ\text{C}\cdot\text{min}^{-1}$, with a final holding time of 4 min. The injection was operated in splitless mode for 2 min and helium was used as carrier gas, at $5\ \text{mL}\cdot\text{min}^{-1}$ during the first minute and decreasing to $3.5\ \text{mL}\cdot\text{min}^{-1}$ in 45 s. The mass spectrometer was operated in EI mode at 70 eV. The source temperature and GC interface temperature were kept at 200°C and 270°C , respectively. The emission current was $150\ \mu\text{A}$ and the detector voltage was set at 400 V. Calibration, standards and samples were injected following the time scheduled selected ion monitoring (SIM) mode reported in Lourencetti et al. (2010). Quantification was performed with the internal standard method (approximately $0.01\text{--}10\ \text{ng mL}^{-1}$), using fluorobenzene and 4-bromofluorobenzene as internal standards. Good correlation coefficients ($r > 0.999$) were obtained for all THMs.

Limits of detection (LOD) and quantification (LOQ) were calculated from all the laboratory and field blank concentrations using the average values plus three or ten times their standard deviation, respectively. LOD were 0.015, 0.004, 0.005 and 0.011

$\mu\text{g}\cdot\text{L}^{-1}$ for CHCl_3 , CHCl_2Br , CHClBr_2 , CHBr_3 , respectively, in water samples, and 1.7, 0.28, 0.076 and $0.076\text{ ng}\cdot\text{m}^{-3}$, respectively, in indoor air samples. For exhaled breath, LOD values were determined from exhaled breath sampler blanks obtained at the beginning and at the end of each sampling day in both chlorinated and brominated swimming pools. LOD were 0.71 , 0.35 , 0.22 and $0.17\text{ }\mu\text{g}\cdot\text{m}^{-3}$ for CHCl_3 , CHCl_2Br , CHClBr_2 and CHBr_3 , respectively, in the Cl-SP and 0.094 , 0.003 , 0.022 and $0.35\text{ }\mu\text{g}\cdot\text{m}^{-3}$, respectively, in the Br-SP.

2.5. Statistical Analysis

Statistical analyses were performed using a SPSS (Statistical Package for the Social Sciences) version 14.0 (SPSS PC, 2005) and R (R Core Team, 2014). The Shapiro-Wilk Test was used to verify if THM concentrations in indoor air and water samples followed normal distributions. Simple linear regression analysis was used to assess the associations between physical activity and THM intake. Multivariate regression models were used to assess the relationships between THM intake and several covariates, including physical activity (both distance swum or kcal associated to the exercise, depending on the model), THM levels in air and THM levels in water. All variables were standardized (centred at zero and scaled to two standard deviations) to allow direct comparison of the β -coefficients within and between the models and compounds.

3. Results and Discussion

3.1. Main differences between the two pools

The THM concentrations in air and water of these two swimming pools have been described elsewhere (Lourencetti et al., 2012). CHCl_3 and CHBr_3 were the dominant

compounds in the air and water of the Cl-SP and Br-SP, respectively (Table 1). In both cases, a concentration gradient between species of higher or lower degree of chlorination/bromination was observed consistently with the disinfection method used in each swimming pool (Table 1). These gradients were observed when comparing the concentrations in either weight or molar units. Higher total THM (TTHM) aquatic levels were found in the Br-SP when considering weight units, median values 44 and 55 $\mu\text{g L}^{-1}$, respectively, (Table 1) but not molar units, median values 0.28 $\mu\text{mol L}^{-1}$ and 0.22 $\mu\text{mol L}^{-1}$, respectively.

Small amounts of THMs in exhaled breath before exposure in the indoor swimming pools have been observed. Levels ranged between <LOQ–1.4, <LOQ–0.66, <LOQ–0.39 and <LOQ–0.29 $\mu\text{g}\cdot\text{m}^{-3}$ for CHCl_3 , CHCl_2Br , CHClBr_2 and CHBr_3 , respectively, in the Cl-SP and between <LOQ–0.21, <LOD–0.02, <LOQ–0.03 and <LOQ–0.41 $\mu\text{g}\cdot\text{m}^{-3}$ in the Br-SP, respectively. The THM concentrations in the exhaled breath of the volunteers measured before the exposure experiments were similar to those of indoor air, which is consistent with previous reports (Aggazzotti et al., 1998, Caro and Gallego, 2007; Lourencetti et al., 2010).

Comparison of the THM concentrations in exhaled breath collected before and after swimming/bathing showed statistically significant differences for all compounds in both indoor swimming pools (Lourencetti et al., 2012). In the Cl-SP, the exhaled breath of swimmers showed higher proportion of the more chlorinated species and in the Br-SP it was the opposite case, with higher proportion of the more brominated species (Table 1).

There was no significant difference between THM mean values in exhaled breath samples collected during morning (n = 25) and afternoon (n = 7) ($p < 0.05$). No significant difference was also observed for THM concentrations in exhaled breath of

subjects considering sex, age or body mass index (results not shown) which is also in agreement with the studies of Fantuzzi et al. (2001) and Caro and Gallego (2007) who did not find difference by sex, age, weight and height of THMs intake in subjects exposed to THMs in indoor swimming pools.

Total THM concentrations were lower in exhaled breath from subjects swimming in the Br-SP when compared with those swimming in the Cl-SP (average values $3.1 \mu\text{g m}^{-3}$ ($0.013 \mu\text{mol m}^{-3}$) and $6.8 \mu\text{g m}^{-3}$ ($0.047 \mu\text{mol m}^{-3}$) and median values $2.9 \mu\text{g m}^{-3}$ ($0.012 \mu\text{mol m}^{-3}$) and $6.7 \mu\text{g m}^{-3}$ ($0.047 \mu\text{mol m}^{-3}$), respectively; Table 1). In contrast with this difference, subjects in the Br-SP had higher exhaled breath concentrations of CHBr_3 than in the Cl-SP, $2.3 \mu\text{g m}^{-3}$ ($0.0092 \mu\text{mol m}^{-3}$) and $0.33 \mu\text{g m}^{-3}$ ($0.0013 \mu\text{mol m}^{-3}$) for the median concentrations in both pools, respectively. This compound is generally more cytotoxic and mutagenic than the higher chlorine-containing THMs (Plewa et al., 2002).

3.2. Changes of THMs in exhaled breath and physical activity

The THM concentrations in the exhaled breath of the swimming participants were significantly correlated with the THM concentrations in ambient air and water, except for CHBr_3 in water (results described in Lourencetti et al., 2012).

3.2.1. Distance swum

Representation of the exhaled breath concentration differences (after-before exposure) in the swimming pools vs distance swum shows increases of all compounds in both pools, Cl-SP and Br-SP (Figs. 1 and 2). These regression coefficients are significant for all THM species in the Cl-SP (p between <0.014 and <0.0001) and only for CHBr_2Cl and CHBr_3 in the Br-SP ($p <0.01$ and <0.05 , respectively) (Tables 2 and 3). To the best

of our knowledge this is the first time that a relationship between distance swum and increase in exhaled breath THM concentrations is established. Previous studies have reported high concentrations of CHCl_3 intake in competitive swimmers (Aggazzotti et al., 1990; 1993) but the distance swum or the degree of physical effort were not assessed.

The significance values in the Cl-SP are higher than in the Br-SP. This is as a consequence of the larger number of volunteers in the former ($n = 40$ vs $n = 7$, respectively) and of the larger interval of swum distances again in the former (0-1750 m and 211-713 m in the Cl-SP and Br-SP, respectively).

The slopes of the linear regressions between increases in exhaled breath concentrations and distance swum were highest for CHCl_3 in the Cl-SP and CHBr_3 in the Br-SP (Tables 2 and 3; non-standardized models). The highest exhaled breath concentration increases were also observed for these two compounds in their respective swimming pools. These differences are consistent with the disinfection methods in each pool.

Since the exhaled breath concentrations of the volunteers correlated with the THM air concentrations in the swimming pool buildings (Lourencetti et al., 2012), normalization of the observed breath increases to this variable allows to estimating the specific intake of each THM species independently of air concentrations. The results of this normalization study are also shown in Figs. 1-2 and Tables 2 and 3. Again, all THM species exhibit significant trends with distance swum (p-value between <0.01 and <0.0001) in the Cl-SP (Table 2) and all compounds except CHCl_3 (p-value between <0.03 and <0.014) in the Br-SP (Table 3). Now, the slope values of the associations are rather similar and no defined trend between higher degree of chlorination/bromination

of the THM compounds and slope values is observed. All THM species show similar dependence of swimmer assimilation by distance swum.

The lack of significant association for CHCl_3 in the Br-SP is likely due to the low concentrations of this compound in exhaled breath of the swimmers of this pool which, in the context of the number of volunteers and distance swum, does not provide values free of influence from background concentrations.

Further understanding into the associations between distance swum and THM uptake may be obtained from standardization of all variables. As shown in Tables 2 and 3, the significance of the observed associations between exhaled breath THM concentration differences and distance swum do not change after variable standardization. However, there are important relative changes in the slope values. In the standardized models CHBr_3 shows the highest slopes in the Cl-SP both in the case of direct used of exhaled breath concentrations or normalization of these values to air concentration (Table 2). In these models, the results after normalization to air concentrations also show increasing slopes from less to more brominated compounds. The model without air normalization shows the same trend but the slope of the CHCl_3 , 0.43, is a bit higher than that of CHCl_2Br , 0.41. In the Br-SP, the highest slopes are observed for CHCl_2Br but CHBr_3 shows similar values, e.g. 0.88 and 0.77 in the model without air normalization, respectively, and 0.87 and 0.86 in the model with air normalization, respectively.

3.2.2. Energy spent

Energy expenditure during swimming can be calculated assuming that swimming at 46 m min^{-1} equals 11 $\text{kcal kg}^{-1} \text{hr}^{-1}$ (Ainsworth et al., 2000) which in the context of the present study can be expressed by the following equation:

$$ES = WT \times DS \times 11 \text{ kcal kg}^{-1} \text{ hr}^{-1} \times 1 \text{ hr} \times (60 \text{ min} \times 46 \text{ m min}^{-1})^{-1} \quad (\text{eq. 1})$$

where ES is energy spent (kcal), WT is weight of each swimmer (kg) and DS is distance swum (m)

Use of this equation and calculation of the associations with exhaled breath provides similar results like with distance swum (Tables 2 and 3). In the Cl-SP the exhaled breath difference of all THMs is significantly associated with the energy spent during swimming considering both the crude values and the values normalized to air concentrations (Table 2). Nevertheless, in the regressions of crude breath concentration differences with energy calculations, the significance p-values are higher than in the previous distance calculations (less significance) whereas in the regressions of concentration differences normalized to air concentrations both calculations with distance and energy spent have the same p-values (Table 2). Similar results are obtained with the standardized models both in terms of significance and slope differences but with higher p-values when using energy spent.

In the Br-SP, the associations between exhaled breath differences and either energy spent or distance swum are equivalent in terms of significance (Table 3). In these regressions vs energy, the relative differences between slopes of the different THM species remain the same as with distance. Use of the standardized variables provides similar results either using distances swum or energy spent. The good agreement in the results obtained with these two methods of estimation of physical activity provides a strong ground to the observed associations between increase of exhaled THM concentrations and physical activity.

3.3. Multivariate model

As mentioned above, the exhaled breath THM concentrations of swimmers were observed to correlate with the air concentrations of these compounds. In addition, air and water THM concentrations were also correlated (Lourencetti et al., 2012). Accordingly, multivariate models considering distance swum, air THM concentrations in the pool building and pool THM water concentrations have been calculated for assessment of the influence of these variables in the exhaled breath (Table 4). All variables were standardized for these calculations. These multivariate models were only significant in the Cl-SP, the low number of swimmers in the Br-SP did not allow to obtaining statistically significant models with these three variables.

The multivariate models of the Cl-SP show that both distance swum and air THM concentrations were significantly associated with the exhaled breath concentrations of the swimmers in the Cl-SP (Table 4). In contrast, water THM concentrations were not significant.

The model calculations with energy spent instead of distance swum show the same trends. Again, physical exercise (calculated as energy expenditure) and air concentrations are the two significant factors of exhaled breath variation and water concentrations are not significant. However, the coefficients for energy expenditure are lower than those of distance swum. This small difference suggests that distance swum may be a better estimator of physical activity at least in the context of these swimming experiments.

The physical activity coefficients are higher at higher degree of bromination of the THM species (Table 4) which is consistent with the higher slopes at higher degree of THM bromination observed in several of the univariate models (Table 2). These results indicate a higher relative THM uptake at higher degree of bromination. In fact, the

multivariate models show that whereas for the more chlorinated THM, CHCl_3 and CHCl_2Br , the highest coefficients are those of air concentrations, for the more brominated species, CHClBr_2 and CHBr_3 , the highest coefficients are those of distance swum. Calculation of physical exercise from energy spent confirms this trend but only CHBr_3 shows that the highest coefficient is for energy spent and not air concentration. Both series of results consistently indicate a higher relative uptake of the more brominated species vs the more chlorinated THMs.

In principle, THM intake in pools can occur via pulmonary inhalation, percutaneous and oral routes (Aggazzotti et al., 1998; Levesque et al., 1994, Lindstrom et al., 1997; Erdinger et al., 2004). Oral exposure is generally a minor route because THMs are usually inactivated and eliminated by first-pass metabolism in the liver before they can reach the blood circulation. In contrast, dermal exposure or inhalation gives rise to THMs entering into the blood stream directly (bypassing the liver), being distributed throughout the body (Ross, 2004).

Erdinger et al (2004) found higher CHCl_3 intake in swimmers than in swimmers breathing compressed air or subjects walking around the pool with no contact with the water. These authors found similar results for the last two groups and concluded that inhalation is the most important CHCl_3 intake pathway.

The results of both the univariate and multivariate models also suggest that inhalation is the main route of incorporation of THM in swimmers. The well-defined differences between THM uptake according to bromination degree are consistent with the Henry's Law volatility constants of the compounds that decrease with increasing proportion of Br atoms, e.g. $K_H/RT = 0.12, 0.065, 0.035$ and 0.017 for the adimensional constants of $\text{CHCl}_3, \text{CHCl}_2\text{Br}, \text{CHClBr}_2$ and CHBr_3 , respectively ($R = 0.082 \text{ atm L mol}^{-1} \text{ K}^{-1}$, $T = 300^\circ\text{K}$ and K_H values at 20°C ; Batterman et al., 2000). Thus, the less volatile

THMs are incorporated the most. This trend may be related with the retention of these compounds in the pulmonary alveolar exchange between air and blood. Those with lower Henry volatility constants may enter more readily into the blood system (Miles et al., 2002). Alternatively, it could be considered whether dermal incorporation can also be relevant for the observed results.

Physical stress increases intake of THMs and other organic compounds by increasing the pulmonary ventilation, blood pressure and surface capillary perfusion, which decrease the transdermal path length for diffusion of organic compounds and increase the blood volume flow just under the skin (Lefebvre et al., 1990; Lindstrom et al., 1997). Additionally, the rate of dermal absorption is known to increase when the skin is fully hydrated, when the temperature of the skin and solute are elevated, and when 100 % of the body surface is immersed (Brown et al., 1984). However, the lack of significance of the THM water concentrations in the multivariate models suggests that in the context of the present swimming experiments this pathway is not significant.

In any case, the results of the present models and experimental set up document a clear relationship between physical activity measured as distance swum or energy expenditure and THM intake.

4. Conclusions

A clear dependence between physical activity measured as distance swum or energy expenditure and exhaled breath THM concentration increases of swimmers in indoor building pools has been observed. This statistically significant relationship involves higher THM intake at higher intensity of physical exercise. However, air concentrations in the buildings appear to be the major factor determining the intake of CHCl_3 and

CHCl₂Br in swimmers whereas distance swum is the main factor of CHClBr₂ and CHBr₃ intake. These two causes of THM incorporation into swimmers concurrently intensify the concentrations of these compounds into exhaled breath and point to inhalation as primary mechanism for THM uptake. Furthermore, the rates of THM incorporation are proportionally higher as higher is the degree of bromination of the THM species. This trend is again consistent with inhalation as the main pathway of THM uptake in swimmers and suggests that air-water partition mechanisms in the pulmonary system determine higher retention of the THM compounds with lower Henry's Law volatility constants than those of higher constant values.

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Table 1. THM distribution in water, air, and exhaled breath in chlorinated and brominated swimming pool exposure study.

Activity	CHCl ₃			CHCl ₂ Br			CHClBr ₂			CHBr ₃			TTHM			
	EB	Air	Water	EB	Air	Water	EB	Air	Water	EB	Air	Water	EB	Air	Water	
	($\mu\text{g m}^{-3}$)	($\mu\text{g m}^{-3}$)	($\mu\text{g L}^{-3}$)	($\mu\text{g m}^{-3}$)	($\mu\text{g m}^{-3}$)	($\mu\text{g L}^{-3}$)	($\mu\text{g m}^{-3}$)	($\mu\text{g m}^{-3}$)	($\mu\text{g L}^{-3}$)	($\mu\text{g m}^{-3}$)	($\mu\text{g m}^{-3}$)	($\mu\text{g L}^{-3}$)	($\mu\text{g m}^{-3}$)	($\mu\text{g m}^{-3}$)	($\mu\text{g L}^{-3}$)	
Cl - SP Swimming (N = 32)	Mean	3.6	31	15	1.55	14.3	12.4	1.2	13	11	0.34	9.4	6.5	6.8	68	45
	(SD)	(1.7)	(7)	(3.4))	(3.9)	(2.9))	(3.77)	(3.6)	(0.19)	(3.7)	(2.8)	(2.8)	(14.5)	(8.6)
	Median	3.8	31.2	16	1.4	13	12	1.2	12.6	11	0.33	8.1	6.1	6.7	69	44
											<LO					
	Min	0.03	19.5	8.5	0.51	9.5	9.3	0.24	10.2	6.5	Q	4.4	3.0	1.0	47	35
	Max	7.3	48	19	2.8	23	23	2.5	26	23	0.9	22	16	12	104	75
					0.84			0.34						2.3		
	Mean	1.1	27	14	(0.19)	16	19	(0.14)	16	19	0.06	12	11	(0.57)	70	63.5
	(SD)	(0.35)	(8.4)	(3.0))	(2.5)	(3.45))	(2.9)	(3.5)	(0.12)	(2.6)	(3.3))	(10)	(10)
	Median	1.1	30	15.5	0.81	15	20	0.31	15	21	Q	11	11	2.2	71	66.5
										<LO						
	Min	0.52	12	8.4	0.59	14	14	0.15	14	14	Q	8.3	6.85	1.6	52	47
	Max	1.8	36	16	1.2	22	23	0.64	23	23	0.31	16	16	3.1	87	75
Br - SP Swimming (N = 7)	Mean	0.14	3.0	0.16	0.14	2.3	0.30	0.27	6.5	2.3	2.5	64	55	3.1	76	58
	(SD)	(0.11)	(1.5)	(0.10))	(0.4)	(0.09))	(0.37)	(0.20)	(0.9)	(12)	(4.5)	(1.2)	(14)	(4.8)
	Median	0.13	2.0	0.08	0.13	2.2	0.23	0.27	6.6	2.2	2.34	55	52	2.9	66	55
	Min	0.02	1.7	0.08	0.07	1.7	0.22	0.15	6.05	2.1	1.5	53	52	1.8	63	54
	Max	0.36	4.8	0.30	0.25	2.8	0.43	0.43	7.7	2.5	4.5	77	64	5.5	92	67

EB: Difference in the exhaled breath concentration after and before exposure.

Table 2. Summary of the associations between THM concentrations in exhaled breath, distance swum and energy spent in the pool using chlorination (n = 40).

	Increase of THM in exhaled breath												
	range $\mu\text{g}\cdot\text{m}^{-3}$	Distance swum (m)						Energy spent (kcal)					
		Non-standardized model			Standardized model			Non-standardized model			Standardized model		
		slope $\mu\text{g}\cdot\text{m}^{-4}$	R^2	p-value	slope	R^2	p-value	Slope $\mu\text{g}\cdot\text{m}^{-3}\text{kcal}^{-1}$	R^2	p-value	slope	R^2	p-value
CHCl_3	0.034-7.3	0.0016	0.19	<0.01	0.43	0.19	<0.01	0.0049	0.17	<0.05	0.41	0.17	<0.05
CHCl_2Br	0.51-2.8	0.0005	0.17	<0.01	0.41	0.16	<0.01	0.0015	0.15	<0.05	0.38	0.15	<0.05
CHClBr_2	0.15-2.5	0.0007	0.39	<0.0001	0.62	0.39	<0.0001	0.0021	0.32	<0.001	0.57	0.32	<0.001
CHBr_3	0-0.90	0.00029	0.49	<0.0001	0.70	0.49	<0.0001	0.00089	0.41	<0.0001	0.64	0.42	<0.0001

	Increase of THM in exhaled breath/Indoor air concentration												
	range	Distance swum (m)						Energy spent (kcal)					
		Non-standardized model			Standardized model			Non-standardized model			Standardized model		
		slope m^{-1}	R^2	p-value	slope	R^2	p-value	Slope kcal^{-1}	R^2	p-value	slope	R^2	p-value
CHCl_3	0.002-0.24	0.048	0.20	<0.01	0.45	0.18	0.0034	0.00014	0.16	<0.05	0.40	0.16	<0.05
CHCl_2Br	0.039-0.15	0.037	0.30	<0.001	0.55	0.28	<0.001	0.00011	0.26	<0.001	0.51	0.26	<0.001
CHClBr_2	0.011-0.14	0.051	0.41	<0.0001	0.64	0.39	<0.0001	0.00015	0.33	<0.0001	0.58	0.33	<0.0001
CHBr_3	0-0.12	0.034	0.47	<0.0001	0.69	0.46	<0.0001	0.00011	0.46	<0.0001	0.68	0.46	<0.0001

Table 3. Summary of the associations between THM concentrations in exhaled breath, distance swum and energy spent in the pool using bromination (n = 7).

	Increase of THM in exhaled breath												
	range $\mu\text{g}\cdot\text{m}^{-3}$	Distance swum (m)						Energy spent (kcal)					
		Non-standardized model			Standardized model			Non-standardized model			Standardized model		
	slope $\mu\text{g}\cdot\text{m}^{-4}$	R^2	p-value	slope	R^2	p-value	slope $\mu\text{g m}^{-3}\text{kcal}^{-1}$	R^2	p-value	slope	R^2	p-value	
CHCl_3	0.02-0.36	0.00021	0.17	0.37	0.41	0.17	0.36	0.0011	0.20	0.31	0.45	0.20	0.31
CHCl_2Br	0.07-2.5	0.00019	0.52	<0.1	0.72	0.52	<0.1	0.00089	0.55	<0.1	0.74	0.55	<0.1
CHClBr_2	0.15-0.43	0.00041	0.77	<0.01	0.88	0.77	<0.01	0.0019	0.77	<0.01	0.88	0.77	<0.01
CHBr_3	1.5-4.5	0.0037	0.60	<0.05	0.77	0.60	<0.05	0.017	0.62	<0.05	0.79	0.62	<0.05

	Increase of THM in exhaled breath/Indoor air concentration												
	range	Distance swum (m)						Energy spent (kcal)					
		Non-standardized model			Standardized model			Non-standardized model			Standardized model		
	slope m^{-1}	R^2	p-value	slope	R^2	p-value	slope kcal^{-1}	R^2	p	slope	R^2	p-value	
CHCl_3	0.002-0.24	0.076	0.39	0.13	0.63	0.27	0.13	0.0004	0.42	0.12	0.65	0.42	0.12
CHCl_2Br	0.039-0.15	0.070	0.67	<0.05	0.82	0.60	<0.05	0.0003	0.711	<0.05	0.84	0.71	<0.05
CHClBr_2	0.011-0.14	0.056	0.76	<0.05	0.87	0.70	<0.05	0.0003	0.76	<0.05	0.87	0.76	<0.05
CHBr_3	0-0.125	0.054	0.74	<0.05	0.86	0.68	<0.05	0.0002	0.75	<0.05	0.86	0.75	<0.05

Table 4. Multivariate model considering the influence of distance swum/energy spent and THM air and water concentrations in the exhaled breath concentrations of THMs in swimmers of the chlorinated pool.

	Distance swum (m)				Energy spent (kcal)			
	Distance	Air	Water	R ²	Kcal	Air	Water	R ²
CHCl ₃	0.40 **	0.41 *	0.22	0.49	0.36 **	0.42 *	0.20	0.46
CHCl ₂ Br	0.38 **	0.69 ***	-0.17	0.54	0.33 *	0.70 ***	-0.22	0.52
CHClBr ₂	0.54 ***	0.46 **	-0.28	0.49	0.45 **	0.47 **	-0.33	0.42
CHBr ₃	0.70 ***	0.44 **	-0.21	0.56	0.65 ***	0.48 **	-0.25	0.49

*p < 0.05; **p < 0.01; ***p < 0.001

FIGURE CAPTIONS

Figure 1. Representation of the exhaled breath THM concentration differences in volunteers vs distance swum in a pool using chlorination for water disinfection (top). Representation of the same exhaled breath THM concentration differences divided by the average THM air concentrations in the pool building (bottom). The curve fitting parameters of the adjusted equations are discussed in Table 2.

Figure 2. Representation of the exhaled breath THM concentration differences in volunteers vs distance swum in a pool using bromination for water disinfection (top). Representation of the same exhaled breath THM concentration differences divided by the average THM air concentrations in the pool building (bottom). The curve fitting parameters of the adjusted equations are discussed in Table 2.