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New Halogenated Disinfection Byproducts in Swimming Pool Water and Their Permeability across Skin

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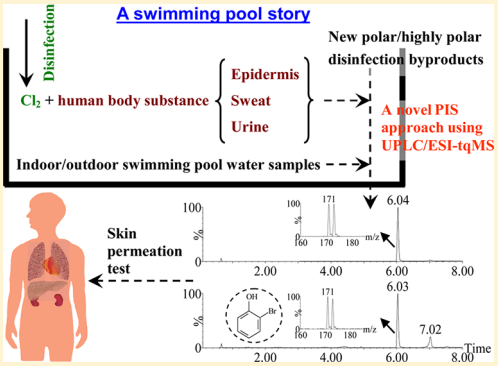
Supporting Information

ABSTRACT: Chlorine is widely used for disinfecting public swimming pool water. The disinfectant chlorine, protecting swimmers from pathogenic infection in swimming, may be responsible for some adverse effects on swimmers' skin and health. In this study, numerous new halogenated disinfection byproducts (DBPs) in chlorinated pool water were detected with a powerful precursor ion scan method using electrospray ionization triple quadrupole mass spectrometry, with or without preseparation with ultra performance liquid chromatography. These new pool DBPs were demonstrated to be mainly halo(nitro)phenols, resulting from chlorination of human body substances (such as urine) in the presence of bromide. Among these new DBPs, 2,4-dibromophenol, 2,4-dichlorophenol, 2-bromophenol, 2,6-dibromo-4-nitrophenol, 2-bromo-6-chloro-4-nitrophenol, and 2,6-dichloro-4-nitrophenol were fully identified or confirmed. For 2,4-dibromophenol, 2,4-dichlorophenol and 2-bromophenol with pure standard compounds available, their permeability values across human skin were measured to be 0.031, 0.021, and 0.023 cm/h, respectively. The effects of chlorine on human skin were also investigated. The interaction of chlorine with epidermis was found to generate many new halogenated DBPs as well as common DBPs; the corneous layer was observed to become rough and even form larger pores after chlorine interaction. It is recommended that swimmers should avoid urinating in pools, and avoid prolonged swimming to reduce chlorine contact and prevent accelerated permeation of DBPs across skin.



INTRODUCTION

Swimming pools are usually disinfected with chlorine to inactivate pathogens. The remaining free chlorine in pools is suggested to range from 1 to 3 mg/L.[1] The health benefits of swimming are numerous. However, the disinfectant chlorine, protecting us from infection, may be responsible for some adverse effects on swimmers' skin and health. Pools have been found to contain halogenated disinfection byproducts (DBPs) that could be carcinogenic, mutagenic, and/or teratogenic.[1−5] Recent studies show that exposure of halogenated DBPs during swimming can cause asthma and bladder cancer and damage genomic DNA.[6−8] Therefore, it is of importance to give a whole picture about pool halogenated DBPs for establishing an updated pool sanitation rule.

Gas chromatography coupled with electron capture detection (GC-ECD) or with mass spectrometry (GC-MS) has been used in detection or identification of halogenated DBPs in pools.[1−3,9] Some volatile or semivolatile DBPs, like trihalomethanes, dichloroacetonitrile, and dichloromethylamine have been found in pool water samples.[1−3,9] However, GC-MS is not suitable for detecting compounds that are polar, nonvolatile, and thermally labile. Liquid direct infusion-MS such as electrospray ionization (ESI)-MS is a logical choice for detecting polar/highly polar halogenated DBPs in pools. A challenging issue involved in using ESI-MS is the differentiation of halogen-containing DBPs from tens of thousands of halogen-free species in pools. Recently, intriguing methods for fast selective detection of polar halogenated DBPs in drinking water have been developed using an ESI-triple quadrupole mass spectrometer (ESI-tqMS).[10−13] The mass spectrometer permits the use of precursor ion scan (PIS) mode, which can detect the entire precursor ions that generate a specific fragment ion, for example, chlorine-containing and bromine-containing DBPs can generate Cl^- and Br^- respectively in the collision induced dissociation chamber of the mass spectrometer, and thus can be selectively detected by performing PISs of m/z 35 and m/z 79 or 81, respectively. In addition, the development of ultra performance liquid chromatography (UPLC) allows achieving higher chromatographic resolution and sensitivity using sub-2 μm particles under a higher pressure (than high performance liquid chromatography). One primary objective of this study

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was to selectively detect polar halogenated DBPs in pools and chlorinated human body substances using (UPLC/)ESI-tqMS PISs.

In fulfilling the aforementioned primary objective of this study, numerous new halogenated DBPs were detected in pools, and several of them were fully identified (as shown later in Results and Discussion) and their purified standard compounds could be acquired, including 2,4-dichlorophenol, 2,4-dibromophenol, and 2-bromophenol. These new halogenated DBPs, especially halophenols, may be toxic. Skin exposure to relatively small amounts of molten 2,4-dichlorophenol (as little as 1% of body surface) may lead to death; eye exposure to 2,4-dichlorophenol can cause severe irritation and corneal injury which can result in permanent impairment of vision or blindness.[14] In addition, 2,4-dichlorophenol has been observed to have endocrine disrupting effects and cause harmful effects in female sexual organs,[15] and have some reproductive toxicity on Wistar-Hannover rats.[16] There are limited data available on the toxicity of mono- and dibromophenols, but brominated DBPs generally are significantly more toxic than their chlorinated analogues.[4] Permeation of DBPs through skin is an important exposure route for swimmers contacting DBPs during swimming.[17−19] The dermal route of exposure is estimated to account for 80% of the chloroform concentration in blood during swimming.[9] However, previous permeability studies are mainly focused on commonly known DBPs, including chloroform, haloacetic acids, and haloketones.[17−19] Accordingly, the other primary objective of this study was to test the permeability of the newly identified DBPs across skin.

Furthermore, as a strong oxidant, chlorine not only inactivates microorganisms but also may damage any living tissues with which it comes in contact. Skin can be viewed as the largest organ in the body, and the reaction between skin and chlorine in pools may be inevitable. Exposure to chlorine in public pools can lead to dry skin or exacerbated cutaneous inflammations for people with atopic dermatitis.[20] Therefore, the reaction between chlorine and fresh human skin was also studied in terms of halogenated DBP formation and skin morphology.

EXPERIMENTAL METHODS

Figure S1 in the Supporting Information (SI) shows the experimental schematic for preparing various samples and characterizing DBPs in them.

Materials. Six halogenated standard compounds were purchased to confirm the identification of newly detected halogenated DBPs, including 2-bromophenol and 4-bromophenol (Sigma-Aldrich), 2,4-dibromophenol, 2,6-dibromophenol, 2,4-dichlorophenol and 2,6-dichlorophenol (Acros). A standard compound of 4-nitrophenol (Acros) was used to synthesize 2,6-dibromo-4-nitrophenol, 2,6-dichloro-4-nitrophenol, and 2-bromo-6-chloro-4-nitrophenol (as described in the SI). Stock solutions containing 2000 mg/L each of nine haloacetic acids or four trihalomethanes were purchased from Supelco. HPLC-grade methanol, acetonitrile, and methyl tert-butyl ether (MtBE) were purchased from Aldrich. A stock solution of hypochlorite was prepared by absorption of ultrahigh-purity chlorine gas with a sodium hydroxide solution and standardized by the DPD ferrous titrimetric method.[21] Ultrapure water (18.2 MΩ/cm) was provided by a NANOpure system (Barnstead).

Original Pool Samples. Original pool samples were collected from an outdoor pool in September 2008 (water temperature 25−29 °C) and an indoor pool in November 2008 (water temperature 23−26 °C). The pool water came from local tap water and was further treated with a liquid bleach containing 12.5% (by weight) NaOCl. The swimmers using the two pools included faculty, staff, and students of an institution. Some chemical characteristics of the pool samples were measured, including pH (∼7.3), total organic carbon (TOC, 2.8 and 3.2 mg/L as C in the outdoor and indoor pools, respectively), total organic chlorine (TOCl, 213 and 246 μg/L as Cl in the outdoor and indoor pools, respectively), total organic bromine (TOBr, ∼4 μg/L as Br), bromide (∼2 μg/L), and chlorine residual (∼1 mg/L as Cl_2). TOCl and TOBr were measured with an automatic quick furnace (AQF-100, Mitsubishi) coupled with ion chromatography (ICS-90, Dionex).[22,23]

Br^--Spiked Pool Samples and Concentrated Tap Water Sample. The bromide concentration in the pool source water was ∼6 μg/L (which was too low to be representative). The Br^- level in raw water averages ∼100 μg/L in the U.S., but it can reach 2000 μg/L in some regions of the world.[24] In this study, NaBr was spiked into the pool samples to a level of 400 μg/L as Br^-. This relatively high bromide level was used to amplify and observe the possible polar brominated DBPs. The Br^--spiked pool samples were disinfected for 24 h with NaOCl at a total dose of 5 mg/L as Cl_2. This higher Cl_2 dose was applied to amplify the pool DBPs for better detection/identification.

To differentiate DBPs whose precursors originated from swimmers, a control test was performed. The tap water, from which the pool water came, was collected at the point of the tap water entering the pool. The tap water sample was then concentrated by a rotary evaporator to an extent that its final TOC level was rather close to those of the pool samples. NaBr was spiked into the concentrated tap water sample to a level of 400 μg/L as Br^-. Then, the sample was disinfected with 5 mg/L NaOCl as Cl_2 for 24 h. The main difference between the pool samples and the concentrated tap water sample was that the organic matter in the former primarily came from swimmers.

Chlorination of Sweat, Urine, Saliva, or Hair. Water containing certain human body substance such as sweat, urine, saliva, or hair was allowed to react with chlorine so that the precursors of DBPs found in the pool samples could be traced. A 28-yr-old man and a 22-yr-old woman were selected as volunteers to donate the human body substances. The volunteers did not take any medicines during the experiment period. To collect sufficient sweat, the volunteers climbed from the bottom to the top of a hill, and their faces were rinsed by ultrapure water. (Before climbing the hill, their faces had been thoroughly cleaned.) The rinsed sweat-containing solution was spiked with 400 μg/L NaBr as Br^-, 90 mg/L alkalinity as $CaCO_3$, and 58.5 mg/L NaCl (ion strength), and was then chlorinated with 5 mg/L NaOCl as Cl_2 for 24 h. Saliva, urine, or hair was added directly to a solution containing 400 μg/L NaBr as Br^-, 90 mg/L alkalinity as $CaCO_3$, and 58.5 mg/L NaCl, and the solution was then chlorinated with 5 mg/L NaOCl as Cl_2 for 24 h. Saliva or urine was added at such an amount that the TOC of the solution was measured to be ∼3 mg/L as C. Hair was added at around 30 threads, taking account of a daily hair loss of 100−140 threads per person.[25]

Chlorination of Epidermis. Fresh human skin specimens were provided by Department of Anatomy, the University of Hong Kong. The 3 mm thick skin layer included epidermis, dermis, and subcutaneous layer. A 10 × 10 cm^2 piece of

epidermis was cut from a skin specimen and then allowed to react with NaOCl (by dosing 2.0 or 4.0 mg/L as Cl_2) for 24 h in a 200 mL solution. The solution also contained 90 mg/L alkalinity as $CaCO_3$, 58.5 mg/L NaCl, and 400 or 1000 μg/L NaBr as Br^-. After 24 h reaction, residual chlorine was quenched by $NaAsO_2$. The haloacetic acids and trihalomethanes generated were analyzed by GC-ECD[26] (Trace GC, Finnigan), as shown in the SI. The epidermis before and after chlorination was scanned by a scanning electron microscope (SEM, JSM 6300, JEOL) to inspect the effects of chlorine on the skin morphology.

Sample Pretreatment and MS Analysis. To determine whether there were any impurities in the reagents or any artifacts in the chlorination and subsequent pretreatment, control samples were generated by repeating the same procedure with aforementioned chlorinated samples without chlorination. All the chlorinated and control samples were quenched immediately with 120% of the requisite stoichiometric amount of $NaAsO_2$, and then pretreated by following the procedure in a previous study.[11] Briefly, a 1 L water sample was adjusted to pH 0.5 with 7:3 (v/v) concentrated sulfuric acid/water and was added with 100 g of Na_2SO_4. Then, the sample was extracted with 100 mL of MtBE. The MtBE layer was rotoevaporated to 0.5 mL. The 0.5 mL solution in MtBE was mixed with 20 mL of acetonitrile, and the mixture was rotoevaporated back to 0.5 mL. The 0.5 mL solution in acetonitrile was stored at 4 °C. It was diluted with ultrapure water to 1 mL prior to (UPLC/)ESI-tqMS analyses.

The pretreated samples were analyzed with a Waters Acquity triple quadrupole mass spectrometer (ESI-tqMS, Waters). The working parameters for the direct infusion ESI-tqMS system can be found previously.[11] Briefly, direct infusion flow rate 10 μL/min, ESI negative mode, capillary voltage 2.9 kV, cone voltage 15 V, source temperature 110 °C, desolvation temperature 300 °C, desolvation gas 650 L/h, cone gas 50 L/h, mass resolution 15 (1-unit resolution), collision energy 15 eV for PISs of m/z 79 and 81, and collision gas (argon) 0.25 mL/min. For all the PISs, the data collection mode was multichannel analysis, which can greatly enhance precursor ion intensities by accumulating multiple scans and eliminate possible ion intensity fluctuation in a single run. To avoid possible overlapping of homologous precursor ions, a Waters UPLC system was coupled to the ESI-tqMS (UPLC/ESI-tqMS, Waters) for preseparation. For the UPLC/ESI-tqMS analysis, 5 μL of a pretreated sample was injected in the UPLC. The chromatographic separation was achieved by an HSS T3 column (2.1 × 50 mm, 1.8 μm particle size, Waters). A gradient eluent of methanol/water was applied at a flow rate of 0.40 mL/min. The composition of methanol/water (v/v) changed linearly from 5/95 to 90/10 in the first 8 min, and then returned in 0.1 min to 5/95, which was held for 3 min. The ESI-tqMS PISs of m/z 79 and 81 were conducted to detect bromine-containing compounds. The parameters for the UPLC PIS were set the same as those for the direct infusion PIS except that higher desolvation temperature (400 °C) and desolvation gas flow (800 L/h) were used. For a brominated molecular ion detected by the PIS, the UPLC/ESI-tqMS selected ion recording (SIR) or multiple reaction monitoring (MRM) mode was applied to confirm the retention time (RT) of the molecular ion; product ion scans were conducted at the specific RT to gain fragment information of the molecular ion for proposing a structure; then, the corresponding standard compound was used to confirm or identify the proposed structure. Notably, in comparison with the UPLC preseparation, direction infusion to the ESI-tqMS system produces intensive PIS spectra, with which it is convenient to find changes in sample compositions.

Skin Permeation Test In Vitro. Fresh human skin was used in this test instead of fixed human skin (soaked in formalin) or animal skin because formalin may interfere with the test and animal skin cannot fully simulate the complex structure like stratum corneum of human skin. A fresh human skin specimen was cut into 12-cm-diameter sections. The sections were then wrapped in aluminum foil and stored at −20 °C. Just before use, the sections were thawed in a phosphate-buffered saline solution to an experimental temperature of 37 °C.[19]

Permeation tests of DBPs across the skin sections were performed with a Hanson Research Dissolution and Transdermal Test System (Hanson Research). Three newly identified DBPs (2,4-dichlorophenol, 2,4-dibromophenol and 2-bromophenol) and a commonly known DBP (chloroform) were chosen for the permeation tests, which were conducted in the absence of chlorine because chlorine may complicate the tests by reacting with the skin to form halogenated DBPs. The donor chamber of the system was filled with the aqueous solution of 50 mg/L each of the halophenols or chloroform. The receiving chamber of the system was filled with the phosphate-buffered saline solution to simulate human blood.[19] The cross-sectional area of skin through which permeation occurred was 20.2 cm^2. The temperature of the receiving chamber was kept at 37 °C to simulate that of a human body. During the test, a small amount of the solution in the receiving chamber was collected periodically to quantify the halophenols and chloroform by the UPLC/ESI-tqMS SIR mode and the GC-ECD, respectively. The donor solution was replaced periodically to maintain roughly constant DBP concentrations.[19] After the test, the skin section was washed thoroughly to remove superficially adsorbed DBPs, and was then placed in a fresh phosphate-buffered saline solution for 24 h under vigorous mixing. This was to quantify the DBPs absorbed within the skin layers. The whole permeation and desorption tests were repeated twice.

RESULTS AND DISCUSSION

Newly Detected/Identified Pool DBPs. Figure 1 shows the chromatograms of the UPLC/ESI-tqMS full scan and PISs of the chlorinated Br^--spiked indoor pool sample. The chromatogram of the UPLC/ESI-tqMS PIS m/z 79 shows the peaks corresponding to the compounds with the bromine isotope ^{79}Br. If these peaks can find their counterparts in the chromatogram of the UPLC/ESI-tqMS PIS m/z 81, which shows the peaks corresponding to the compounds with the bromine isotope ^{81}Br, then they should represent bromine-containing compounds. As shown in Figure 1, there were many pair peaks in the chromatograms of the UPLC/ESI-tqMS PISs m/z 79 and 81. These pair peaks had nearly the same areas at the same RTs (e.g., 0.68, 0.96, 1.07, 1.17, 1.97, 2.43, 3.28, 4.04, 4.51, 5.78, 5.84, 6.05, 7.23, and 8.58 min), so they should correspond to bromine-containing compounds. Then product ion scans of the molecular ions at a specific RT were conducted. By combining all the spectra at the specific RT together, the structure of the bromine-containing compound could be proposed. For instance, there was a pair of strong peaks at RT 6.05 min in the chromatograms of UPLC/ESI-tqMS PIS m/z 79 and 81 (Figure 1); the mass spectra of the peaks

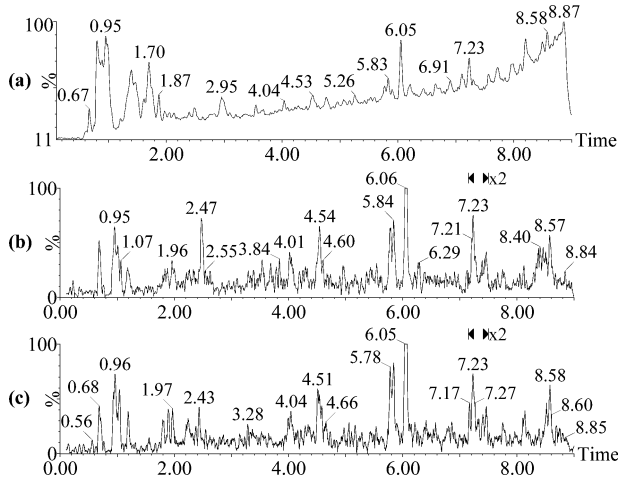

Figure 1. UPLC chromatograms of the chlorinated Br^--spiked indoor pool sample. (a) UPLC/ESI-tqMS full scan, (b) UPLC/ESI-tqMS PIS m/z 79, and (c) UPLC/ESI-tqMS PIS m/z 81. The y-axes are on the same scale.

corresponded to molecular ions m/z 171 and 173, respectively (SI Figure S2), indicating that this compound contains 1Br. SI Figure S2 also shows the UPLC/ESI-MS full scan spectrum (RT 6.05 min) of the chlorinated Br^--spiked indoor pool sample; a clear peak cluster m/z 171/173 with an isotopic abundance ratio of 1:1 matches the theoretical ratio of a compound containing 1Br. Zhai and Zhang[27] have recently reported that the whole RT range of the UPLC was roughly divided into three domains: 0−2.5 min for bromine-containing aliphatic acids, 2.5−6.0 min for bromine-containing benzoic acids, and 6.0−8.0 min for bromine-containing phenols; a bromine-containing compound with more than one functional group could have a shorter RT and move to an adjacent domain. Under the same UPLC setting, this compound had an RT of 6.05 min, so it was tentatively proposed as bromophenol. Then the standard compounds of 2-bromophenol and 4-bromophenol were purchased. Under the UPLC/ESI-MS full scan mode, the chlorinated Br^--spiked indoor pool sample displayed an evident peak in the UPLC chromatogram with the same RT as the standard compound of 2-bromophenol, and the MS full scan spectra at RT 6.04 min were also identical (SI Figure S2). Therefore, this compound with m/z 171/173 in the chlorinated Br^--spiked pool samples was 2-bromophenol. By spiking different levels of the standard compound (SI Figure S3), the concentrations of 2-bromophenol in the chlorinated Br^--spiked indoor and outdoor pool samples were found to be 9.7 and 8.5 μg/L, respectively. With the similar method, 2,4-dichlorophenol and 2,4-dibromophenol were confirmed to exist in the chlorinated Br^--spiked pool samples (SI Figures S4 and S5), and 2,4-dichlorophenol was also confirmed in the original indoor pool sample (6.5 μg/L) and outdoor pool sample (5.3 μg/L). Notably, 2-chlorophenol and 2,4-dichlorophenol have been reported in chlorinated drinking waters at tens of ng/L levels.[28]

More importantly, a few nitrogenous halogenated DBPs were found in the chlorinated pool samples. The characteristic of a nitrogen-containing compound is its even-numbered m/z value in a negative ESI mass spectrum. The compound with m/z 294/296/298 is exemplified here. This compound should contain 2Br because two ion clusters with m/z 294/296 and 296/298 were found in the PIS spectra of m/z 79 and 81, respectively (SI Figure S6), and the isotopic abundance ratios in both precursor and product ion scans followed the theoretical prediction of a compound containing 2Br. The relatively long RT (5.48 min) indicated that this compound might be bromine-containing benzoic acid or phenol,[27] and the product ion scan spectra (no $-CO_2$ loss) precluded the possibility of being benzoic acid. After subtraction of two bromine atoms and one phenol from m/z 294/296/298, the remaining part is 46, for which a reasonable combination should be NO_2 or NH_2OCH_2. Accordingly, this compound should be dibromo-nitrophenol or amino-dibromo-methoxyphenol. To confirm the structure, 2,6-dibromo-4-nitrophenol was synthesized by reacting 4-nitrophenol, Br^- and chlorine (as shown in the SI). SI Figure S7 shows the UPLC/ESI-tqMS MRM chromatograms and spectra of 2,6-dibromo-4-nitrophenol and the chlorinated Br^--spiked indoor pool sample. The same RTs and identical MRM spectra confirmed that ion cluster m/z 294/296/298 at RT 5.48 min was 2,6-dibromo-4-

Table 1. Important Peak Clusters

m/z	formula or structure	RT (min)	sample
161/163/165	2,4-dichlorophenol (confirmed)	6.78	[a] (6.5 μg/L),[b] (5.3 μg/L),[c] (5.7 μg/L),[d] (6.2 μg/L),[e]
171/173	2-bromophenol (confirmed)	6.04	[c] (9.7 μg/L),[d] (8.5 μg/L),[e]
186/188	amino-bromo-phenol (proposed)	4.03	[d]
191/193/195/197	containing 3Cl	1.51	[a,c,d]
206/208/210	2,6-dichloro-4-nitrophenol (confirmed)	5.20	[a,b,c,d,e]
235/237/239	containing 1Br+1Cl	2.35	[c]
249/251/253	2,4-dibromophenol (confirmed)	7.19	[c] (1.6 μg/L),[d] (1.2 μg/L),[e]
250/252/254	2-bromo-6-chloro-4-nitrophenol (confirmed)	5.46	[c,d,e]
255/257/259/261/263	adduct [$Cl_2CHCOOH+Cl_2CHCOO^-$]	1.29	[b]
257/259/261	containing 1Br+1Cl	2.71	[c]
259/261	containing 1Br	2.31	[d]
263/265/267	dibromo-methyl-phenol (proposed)	7.19	[c]
264/266/268	amino-dibromo-phenol (proposed)	5.40	[c]
274/276	containing 1N+1Br+1phenol	6.21	[c]
294/296/298	2,6-dibromo-4-nitrophenol (confirmed)	5.48	[c,d,e]
323/325/327/329/331/333/335	adduct [$Cl_3CCOOH+Cl_3CCOO^-$]	1.32	[a]

[a]Original indoor pool sample. [b]Original outdoor pool sample. [c]Chlorinated Br^--spiked indoor pool sample; [d]Chlorinated Br^--spiked outdoor pool sample; [e]Chlorinated Br^--spiked urine sample.

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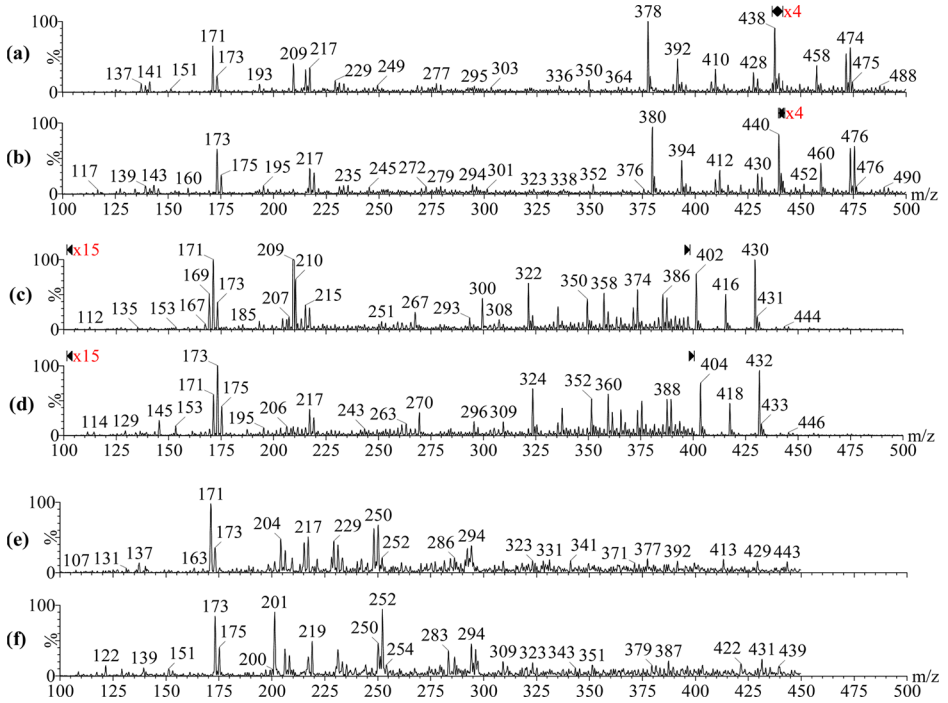

Figure 2. Direct infusion ESI-tqMS PIS spectra of different chlorinated human body substances with the presence of bromide: (a) and (b) are PIS m/z 79 and 81, respectively, of the chlorinated epidermis sample; (c) and (d) are PIS m/z 79 and 81, respectively, of the chlorinated sweat sample; (e) and (f) are PIS m/z 79 and 81, respectively, of the chlorinated urine sample.

nitrophenol, a DBP that has been most recently reported in pool water.[29] Similarly, 2,6-dichloro-4-nitrophenol and 2-bromo-6-chloro-4-nitrophenol were identified in the chlorinated Br^--spiked pool samples (SI Figures S8 and S9), and 2,6-dichloro-4-nitrophenol was also detected in the original pool samples. Nitrogen-containing DBPs have been reported to be generally more toxic than their carbon-based DBP analogues.[4,30]

These identified halo(nitro)phenols, however, were not significantly detected in the chlorinated Br^--spiked concentrated tap water sample with the TOC level similar to the pool samples (e.g., SI Figure S2c). Since the pool water came from the tap water, such a difference implies that precursors of these halo(nitro)phenols should mainly come from swimmers. To verify this, several types of human body substances including urine, hair, saliva, and epidermis were chlorinated to trace their precursors. It turned out that these halo(nitro)phenols were all found in the chlorinated urine sample. The urine release into a pool is estimated to average 27.5 mL and be as high as 77.5 mL per swimmer.[31] Phenol is reported to be 292 mg/L in typical human urine.[32] Interestingly, 4-nitrophenol was found for the first time to exist in urine at 13 μg/L (SI Figure S10). Natural organic matter in surface water contains $\leq$5% of nitrogen,[33] while the organic matter in urine contains ~17% of nitrogen.[34] During chlorination, chlorine or bromine (from the reaction of chlorine with Br^-) can readily substitute onto (nitro)phenol to form halo(nitro)phenols.

Apart from the six identified/confirmed halo(nitro)phenols, a number of other halogenated phenol derivatives in the chlorinated Br^--spiked pool samples were detected by the UPLC/ESI-tqMS. Their UPLC/ESI-tqMS spectra can be found in SI Figures S11–S21. The structures of compounds corresponding to some peaks were proposed in accordance with the isotopic abundance ratios in the full, precursor and product ion scans (as shown in the SI). Table 1 lists important peaks that were detected in chlorinated pool water or human body substance samples. The chromatograms of the UPLC/ESI-tqMS full scan and PISs of the chlorinated concentrated tap water sample are illustrated in SI Figure S22, which basically did not contain any of those important peaks in the chromatograms in Figure 1. Many peak clusters in Table 1 could correspond to halogenated phenol derivatives that have not been previously reported as DBPs in pool water. Besides nitrophenol, other phenol derivatives also exist in human urine and sweat, such as 2-methoxyphenol, 4-methoxyphenol, and 2-methoxy-4-methylphenol.[35,36] These phenol derivatives can readily react with chlorine or bromine to generate halogenated phenol derivatives. Therefore, the proposed halogenated phenol derivatives in Table 1 should be the chlorination products of human body substances (like urine and sweat) in the presence of Br^-. Notably, trihalogenated phenols were barely observed mainly because of the stepwise introduction of halogens through electrophilic aromatic substitution.[37] Ge et al.[38] have reported that mono- and dichlorophenols were the dominant products during chlorination of phenol.

Since the standard compounds for some of the proposed compounds in Table 1 are not available, it is difficult to confirm them at the current stage. On the other hand, five commonly known DBPs including chloroform, bromodichloromethane, dichloroacetic acid, bromochloroacetic acid, and dibromoacetic acid were found in almost all the samples (SI) and are not listed in Table 1.

Chlorination of Human Body Substances. Swimming allows residual chlorine in water to react with skin. Because chlorine is a strong oxidant, the reaction with chlorine may accelerate the skin aging process. SI Figure S23 shows the direct infusion ESI-tqMS spectra of the reaction products of epidermis and chlorine. An increase in chlorine dosage enhanced peak intensities in the ESI-tqMS PIS m/z 79, indicating that the concentrations of brominated DBPs

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increased. Many of these DBPs had high molecular weights (m/z > 300). Haloacetic acids and trihalomethanes generated from chlorination of epidermis (10 × 10 cm^2) were also quantified as shown in the SI (Figure S24). For a man with an estimated skin surface area of 18,900 cm^2 (180 cm in height and 80 kg in weight), this man is estimated to contribute 17 μg chloroform and several μg haloacetic acids to a pool after 2 h swimming (for 400 μg/L Br^- and 2 mg/L Cl_2 dosage).

Chlorine can leave skin feeling dry and itchy, as reported from volunteers. The SEM images of epidermis before and after chlorine interaction are shown in SI Figures S25–S27. Both naked eyes and SEM found that the corneous layer became rough and unhealthily shiny after chlorine interaction. The SEM image also shows that the pores on the skin turned larger after contact with the chlorine-containing solution. Large pores can cause one's complexion to appear dull and uneven.

Figure 2 shows the direct infusion ESI-tqMS PISs of m/z 79 and 81 of the reaction products of chlorine and several human body substances. Many brominated DBPs were generated after chlorination of human body substances, and different types of human body substances formed different compositions and distributions of brominated DBPs. For example, chlorination of urine produced brominated products with m/z values mainly in the range of 100–300 while chlorination of epidermis produced many brominated products with m/z larger than 370. More importantly, many nitrogenous brominated DBPs were generated during chlorination of human body substances in the presence of Br^- (Figure 2): for example, m/z 350/352, 378/380, 392/394, 410/412, 428/430/432, 438/440, 458/460, and 472/474/476 in the chlorinated epidermis sample; m/z 206/208/210, 322/324, 336/338, 350/352, 358/360, 360/362, 374/376, 386/388/390, 402/404, 416/418, and 430/432 in the chlorinated sweat sample; and m/z 206/208/210, 250/252/254, and 294/296/298 in the chlorinated urine sample. It can be inferred that human body substances in pools are important sources of nitrogenous DBPs.

Permeability of DBPs Across Human Skin. Dermal exposure is an important route of exposure for DBPs entering a swimmer's body. A 1 h swimming is postulated to result in a chloroform dose of 65 μg/kg/day.[18] The permeability (P) of the newly identified pool DBPs with corresponding standard compounds available was studied. Figure 3a shows the permeated DBPs across skin with time. P can be evaluated by[39]

$$P = \frac{V_d V_r}{(V_d + V_r)At} \ln\left(1 - \frac{C_r}{C_d}\right)$$

where V_d and V_r are the volumes of the donor and receiving chambers, respectively; A is the cross-sectional area of skin through which permeation occurs; t is the exposure time; C_r and C_d are the DBP concentrations in the receiving and donor chambers, respectively.

For the exposure time of 64 h, the P values (mean ± standard error of the mean) of 2,4-dibromophenol, 2,4-dichlorophenol, and 2-bromophenol were 0.031 ± 0.004, 0.021 ± 0.003, and 0.023 ± 0.003 cm/h, respectively. 2,4-Dibromophenol had a P value of ~1.5 times higher than its chlorinated analogue, and the decrease in the number of bromine atoms on the benzene ring seemed to decrease the P value. As a comparison, chloroform presented a higher permeability (0.077 ± 0.004 cm/h), which was 2.5 times larger than 2,4-dibromophenol. Xu et al.[19] also reported high P values for trihalomethanes, yet the P value for chloroform found in this study is approximately half of the reported value. Compared to halophenols, the higher permeability of chloroform might be ascribed to its smaller molecular size and thus higher diffusion coefficient in skin.

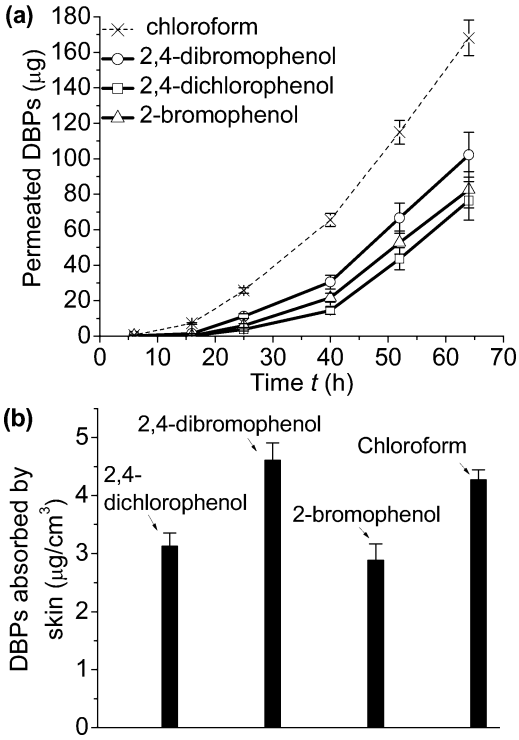

Figure 3. (a) Amount of permeated DBPs across skin as a function of exposure time; (b) Amount of DBPs absorbed by skin after the 64 h permeation test. In both charts, the data are presented as mean ± standard error of the mean.

It needs pointing out that the above P values were the average ones for the whole exposure time of 64 h. As shown in Figure 3a, the permeated DBP amounts across skin generally increased in an accelerated way with exposure time, which means that the permeability of the DBPs was increasing with exposure time. The slow permeation at the beginning might be attributed to the association of the DBPs with the lipid-rich intercellular matrix in the stratum corneum that has a strong affinity with the DBPs and thus become the rate-limiting diffusional barrier. When the association gradually reached equilibrium (i.e., saturation of the DBPs in the stratum corneum), a much higher permeation rate was observed, which should be driven by the concentration difference between the donor chamber and the receiving chamber. During the initial few hours (1–4 h), the permeated DBP amounts could not be quantified because the DBP concentrations in the receiving chamber were below the detection limits. By aggressively assuming that the permeated DBP amounts increased linearly within the initial few hours, the permeated DBP amounts after a 2 h swimming were estimated to be 74, 45, 62, and 247 ng (per 20.2 cm^2 skin) for 2,4-dibromophenol, 2,4-dichlorophenol, 2-bromophenol, and chloroform, respectively. The results suggest that swimmers should avoid prolonged swimming to prevent the accelerated permeation of DBPs across the skin barrier.

Halophenols and chloroform that were absorbed within the skin after the 64-h permeation tests were also quantified. Figure 3b shows the desorption test results. Since the skin specimens

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had been washed carefully to remove superficially adsorbed DBPs before the desorption test, the results imply that the studied halophenols and chloroform stayed within the skin layer (~3 mm). Some halophenols, including 2,4-dichlorophenol, have been found to be tumor promoters in skin.[40] Therefore, swimmers are suggested to take showers frequently to remove DBPs superficially adsorbed on skin, thus preventing them from deeper penetration.

■ ASSOCIATED CONTENT

Supporting Information

Additional details and Figures S1–S27. 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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