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Detection of Haloacetic Acid in Swimming Pool in Haikou and Its Influencing Factors

Huang Xiao-Juan

International School of Public Health and One Health, Hainan Medical University, Haikou, 571199, China

Guan Qing (Corresponding Author)

Haikou Center For Disease Control and Prevention, Haikou, 571199, China Email: 774589136@qq.com

Yu Chun-Wei

School of Tropical Medicine, Hainan Medical University, Haikou, 571199, China Key Laboratory of Tropical Translational Medicine of Ministry of Education, NHC Key Laboratory of Control of Tropical Diseases, School of Tropical Medicine, Hainan Medical University, Haikou, Hainan, 571199, China



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Abstract

Chlorine disinfection is a commonly applied disinfection products due to its effectiveness, strong disinfection ability and low cost. The objective of this study is to investigate the occurrence of haloacetic acids (HAAs), a group of disinfection byproducts, in swimming pool and spa water, and analyze its influencing factors, so as to provide reference to understand the pollution status of haloacetic acid (HAAs) disinfection byproducts in swimming pool water in Haikou, for the formulation of hygienic standards and sanitation management of HAAs in swimming pool water. The samples were collected from18 swimming places with sanitary licenses in Haikou. For a period from April to July in 2022. High performance liquid chromatography -tandem mass spectrometry (HPLC-MS) was used to detect the concentration of HAAs in swimming pool water, and its influencing factors were also analyzed. Results indicated that the levels of average concentration of HAAs in indoor and outdoor swimming pools were 176.20 μ g·L⁻¹ and 241.53 μ g·L⁻¹, respectively. There were differences in the levels of HAAs in indoor and outdoor swimming places (p<0.05). The Pearson correlation analysis showed that the concentration of HAAs was positively correlated with free chlorine and urea (p<0.05). The water quality and sanitation of the swimming pools in this survey were somewhat poor. It is necessary to formulate the regulation of HAAs in the swimming pool sanitation standards, and strengthen the water quality sanitation management, and take further effective measures to reduce the concentration of HAAs to protect swimmers. **Keywords:** Disinfection by-products (DBPs); Swimming pool; Haloacetic acid (HAAs).

1. Introduction

Swimming is a popular fitness sport, and its water quality and safety has received more and more attention. In order to effectively disinfect and killing pathogenic microorganisms in swimming pool water and to prevent the spread of water-borne infectious diseases, common disinfection methods for swimming pools were adopted including chlorine, ozone, and ultraviolet rays [1]. Chlorine disinfection is a commonly applied disinfection products due to its effectiveness, strong disinfection ability and low cost. However, during the disinfection process, chlorinecontaining disinfectants can react with various organic precursors in water to form a series of disinfection byproducts (DBPs) that are potentially harmful to human [2]. The amount and type of DBPs generated in swimming pool water are related to many factors, including not only natural organic matter in tap water, but also additional bodily fluids (such as urine, sweat, saliva) carried by swimmers [3, 4], and personal care products released from human skin or urine [5]. By far, more than 100 DBPs have been detected in swimming pools [6]. Among them, haloacetic acid (HAAs) is one of the most abundant chlorinated disinfection by-products [7], mainly including monochloroacetic acid (MCAA), dichloroacetic acid (DCAA), trichloroacetic acid (TCAA), monobromoacetic acid (MBAA), dibromoacetic acid (DBAA), tribromoacetic acid (TBAA), bromochloroacetic acid (BCAA), bromodichloroacetic acid (BDCAA), and chlorodibromoacetic acid (CDBAA). It has been attracted extensive attention because of its abundant content in water and its potential carcinogenicity, mutagenicity and teratogenicity [8-10].

In recent years, researchers have investigated the concentration level of HAAs in swimming pool water, and studied the influencing factors and related health risks leading to the formation of HAAs. For example, Wang et al examined six indoor pools and nine outdoor pools in the United States and reported the HAA5 concentration were 1440 μ g·L⁻¹ and 1610 μ g·L⁻¹ [11]. Dehghani et al detected HAA5 in 15 indoor swimming pools in Iran ranging from 148 to 3488 μ g·L⁻¹, with an average concentration of 1045.26 μ g·L⁻¹ [12]. In a study of 54 swimming pools, average concentration of HAA9 was reported at 412.9 μ g·L⁻¹, and there were significant differences in the concentration of HAA9 in swimming pools in the same urban water supply network in Canada [13]. Zhang et al investigated that the average concentration of HAA9 was 109.1 μ g·L⁻¹ in 14 public pools in Beijing [14]. Zhao et al reported that the

average concentration HAA5 was 241 μ g·L⁻¹ in Shanghai, where DCAA and TCAA were the main ones [15]. Studies have shown that the concentration of HAAs in swimming pool water are affected by various factors, including the amount of chlorine, natural and man-made organic precursors, pH, water temperature, urea, free chlorine and other factors [16, 17]. By comparing the reported results of HAAs, it was found that the overall concentration of HAAs varied greatly in different countries and regions, which may be due to the different health management standards for swimming pools, such as the free chlorine is stipulated 1-5 mg·L⁻¹ in the range by the the United States Environmental Protection Agency [18]. In China, swimming pool water quality standard requires that the range of free chlorine is 0.3-1 mg·L⁻¹ [19]. The exposure routes of haloacetic acid include gastrointestinal ingestion and skin absorption during swimming, which may cause related health risks [20]. Fantuzzi et al proved that the prevalence of eye, skin and gastrointestinal tract in swimming staff was higher than that in other non-swimming staff [21]. With the increasing popularity of swimming, traditional hygiene indicators for swimming pool water quality are difficult to fully reflect the pollution factors. It should pay attention to DBPs in swimming pool water method was set for HAAs in swimming pool water. Most researchs have focused on HAAs in drinking water and sewage. There are few studies on the relevant exposure levels of HAAs in the swimming environment.

Based on the above considerations, this study developed a simple and sensitive high-performance liquid chromatography-mass spectrometry (HPLC-MS/MS) for simulataneous the detection of HAAs. The purpose of this study was to understand the pollution status of HAAs in swimming pool water, and to explore the influencing factors between water quality parameters and HAAs. The results will provide reference for the hygiene management and standard formulation of swimming places.

2. Materials and Methods

2.1. Instruments and Reagents

AB SCIEX liquid chromatography-mass spectrometer Q trap 5500 was purchased from AB SCIEX, USA; Water UPLC HSS T3 ($3.0 \times 100 \text{ mm} \times 1.8 \mu\text{m}$) chromatographic column was purchased from Waters Corporation; Millipore-Q ultrapure water machine was purchased from Merck, USA; HAA9 mixed standard solution (each concentration was 1000 µg/mL) purchased from Beijing Tanmo Quality Inspection Technology Co., Ltd.; Acetic acid and methanol used in the experiment were HPLC grade purchased from Shanghai Aladdin Company; Needle filter: polyvinylidene fluoride (PVDF) (pore size 0.25 µm).

2.2. Analytical Methods

2.2.1. Standard Preparation

The mixed standard stock solution of haloacetic acid (1000 μ g/mL) was prepared with methanol: pure water (V/V=1:1) as a solvent to prepare an intermediate standard working solution of 100 μ g/mL, and further diluted to prepare 250.0, 125.0, 100.0, Standard solutions of 50.0, 25.0, 5.0, 1.0 μ g/L. The above solutions were stored at 4 °C in the dark.

2.2.2. Liquid Chromatography and Mass Spectrometry Conditions

Water UPLC HSS T3 (3.0 mm \times 100 mm \times 1.8 µm) was used the column, mobile phase A was ultra-high purity water containing 0.05% acetic acid, mobile phase B was methanol, gradient elution in ultra-high purity water, elution flow rate was 300 µL/min, the column temperature was 40°C, and the injection volume was 10 µL. The standard sample was directly injected after filtering the membrane.

HPLC-AB SCIEX Q trap 5500 was scanned by negative ion mode, which operated in electrospray ionization (ESI) and multi-reaction monitoring (MRM) detection. Flow injection analysis (FIA) was used to optimize ion source conditions for 9 mixtures, as follows: Curtain gas (CUR): 30 psi; Ionspray voltage (IS): -4500 V; Ion source temperature (TEM): 350 °C; Atomized gas (GSI): 40 psi; Auxiliary heating gas (GS2): 40 psi. The mass spectrometry parameters of HAA9 in MRM mode were shown in Table 1.

2.3. Sample Collection and Detection

From April to July 2022, a total of 18 normally open public swimming pools were randomly inspected in Haikou, including 11 outdoor swimming pools and 7 indoor swimming pools

All samples were collected according to recommended standards by China [22]. 2 sampling sites were set for swimming pool area $\leq 1000 \text{ m}^2$, and 3 sampling sites were set for swimming pool area $> 1000 \text{ m}^2$. Sample position was set 1 m from the swimming pool wall and 30 cm below the water surface. 5L of water sample were collected from each sampling site. In addition, 0.2 g of sodium ascorbate was added to all samples to remove residual chlorine. The samples were then transported to the laboratory within 30 min and stored at 4°C in the dark, and measured within 24 h. After the sample was filtered through a 0.25 μ m hydrophilic PVDF membrane, about 1 mL of the sample was directly determined.

Haloacetic acid was detected by HPLC-MS method established above. Swimming pool water quality parameters were analyzed according to the Public Place Hygiene Test Standard Methods [23]. Free chlorine was determined by the o-tolidine method (HANNA, HI96734, Italy). Turbidity was detected by a turbidity meter (HACH TU5200, USA). pH was measured by a portable pH meter (S400-uMix-Kit, Mettler Toledo). Urea was determined by diacetylmonoxime-antipyrine spectrophotometry method (DGB-423, China).

2.4. Statistical Analysis

In this study, statistical analysis was processed by SPSS 20.0 software, and descriptive analysis was used for the test results. Differences were evaluated by non-parametric Mann-Whitney test for indoor and outdoor pools with the significance level p < 0.05. Relationship between HAAs and water quality parameters was explored by Pearson correlation analysis with the significance level p < 0.05, p < 0.05 indicated significant correlation.

| Tuble-1. While for reaction monitoring mass spectrometry parameters of 9 haloaceter actus (n=5) | | | | | | | |
|--|-------------------------------|-----------------|------------------------------------|-----------------|--|--|--|
| Compounds | Precurso | r ion | Fragment ion | | | | |
| | Molecular formula | Theoretical m/z | Molecular formula | Theoretical m/z | | | |
| MCAA | $C_2H_2ClO_2^-$ | 93.0 | Cl | 35.0 | | | |
| DCAA | $C_2HCl_2O_2^-$ | 126.9 | CCl ₂ H ⁻ | 82.9 | | | |
| TCAA | $C_2Cl_3O_2^-$ | 160.8 | CCl ₃ ⁻ | 116.8 | | | |
| MBAA | $C_2H_2^{79}BrO_2^{-1}$ | 136.9 | $^{79}Br^{-}$ | 78.9 | | | |
| DBAA | $C_2H^{81}Br_2O_2^-$ | 218.8 | $^{81}Br^{-}$ | 80.9 | | | |
| TBAA | $C^{79}Br_2^{81}Br_2$ | 250.8 | ⁷⁹ Br | 78.9 | | | |
| BCAA | $C_2H^{81}BrClO_2^{-1}$ | 172.9 | CH ⁸¹ BrCl ⁻ | 128.9 | | | |
| BDCAA | $C^{81}BrCl_2^{-}$ | 162.8 | $^{81}Br^{-}$ | 78.9 | | | |
| CDBAA | $C_2^{79}Br^{81}BrClO_2^{-1}$ | 250.8 | ⁷⁹ Br ⁻ | 80.9 | | | |

Table-1. Multi-ion reaction monitoring mass spectrometry parameters of 9 haloacetic acids (n=3)

3. Results and Discussion

3.1. Water Quality Parameters of the Swimming Pools

Swimming pool water quality parameter results were shown in Table 2. Referring to the Public Place Hygiene Index and Limit Requirements standard in China, the pH qualified rate of the outdoor swimming pool was the lowest, only 56.25%, and the qualified rate of turbidity was 90.62%. The water quality of indoor swimming pools had different degrees of unqualified conditions, among which the qualified rate of pH and turbidity was 85%, and the qualified rate of free chlorine was 90%. Meanwhile, the differential analysis of the qualified rate of water quality parameters for outdoor and indoor swimming pools was studied, which was concluded that there was no statistical difference in the qualified rate of pH between outdoor swimming pools and indoor swimming pools (χ^2 =4.501, p > 0.05). High or low pH will reduce the effective activity of disinfectants in swimming pool water, and will also cause swimmers to develop some symptoms such as irritation of the skin and discomfort of eyes. The reasons may be related to the different doses of disinfectant containing-chlorine in some swimming places and the failure to effectively install the pH adjustment device for pool water [24]. The unqualified turbidity may be due to the poor filtering effect of the swimming pool or the large number of swimmers. As one of the pollution indicators, urea in swimming pools mainly came from the excrement and secretions of swimmers, especially sweat and urine with the highest content of urea. The qualified rate of urea in outdoor and indoor swimming pools was measured 100% and 90%, respectively. To avoid urea failure, swimming places should be replenished daily with appropriate new water, strictly control the flow of swimmers, and increase the use of mandatory showers.

| Parameters | Guideline | Outdoor pool | | | Indoor pool | | |
|---------------|-----------|--------------|-----------|----------------|-------------|-----------|----------------|
| | | Mean | Range | Qualified rate | Mean | Range | Qualified rate |
| | | | | | | | Tate |
| pН | 7.0 ~ 7.8 | 7.84 | 7.00 | 56.25% | 7.88 | 6.81~9.74 | 85.00% |
| Free chlorine | 0.3~1 | 0.83 | 0.5~2.9 | 100% | 0.56 | 0.21~2.44 | 90.00% |
| (mg/L) | | | | | | | |
| Turbidity | ≤1 | 0.62 | 0.25~2.97 | 90.62% | 0.925 | 0.5~1.0 | 85.00% |
| (NTU) | | | | | | | |
| Urea (mg/L) | ≤3.5 | 1.14 | 0.1~3.98 | 100% | 0.51 | 0.1~2.24 | 90.00% |

Table-2. Water quality parameters of the swimming pools (n=3)

3.2. Comparison of HAAs Level between Indoor and Outdoor Pool

The concentration of HAAs in swimming pool water was detected, and five HAAs were mainly identified as DCAA, TCAA, BCAA, and DBCAA. The results were shown in Table 3. The average concentration of HAAs in indoor pool and outdoor pool was 176.20 μ g·L⁻¹ and 241.53 μ g·L⁻¹, respectively. The DCAA and TCAA in the indoor swimming pool were 54.89 μ g·L⁻¹ and 103.18 μ g·L⁻¹, respectively, and both of them in the outdoor swimming pool were 75.74 μ g·L⁻¹ and 141.60 μ g·L⁻¹, respectively. Studies reported that DCAA and TCAA were the most important components of HAAs, and these two compounds occupied more than 93% of HAAs [25]. Difference analysis of HAAs concentration levels in indoor and outdoor swimming pool, the disinfection process, the management and operation mode, and the hygiene status of the users are the main reasons for the different distribution of HAAs in different swimming environments [26, 27].

3.3. Comparison HAAs Concentration Levels in Pool Water and Drinking Water

Meanwhile, the concentration of HAAs in drinking water was also measured. The average concentration of HAAs, DCAA and TCAA in drinking water was 18.91 μ g·L⁻¹, 6.97 μ g·L⁻¹ and 8.62 μ g·L⁻¹, respectively, which did not exceed the limit specified in the Sanitation Standard for drinking water [22]. The concentration of HAAs in swimming pool water was much higher than that in drinking water. It may be related to the continuous addition of chlorine disinfectants and the increase in the content of organic load substances carried by the large number of swimmers.

| Table-5. Weak concentrations of 11 h & 5 in Swithining poor and drinking water | | | | | | | |
|--|--------------------|--------|--------|-------|-------|-------|--------|
| Category | Statistical | DCAA | TCAA | BCAA | BDCAA | CDBAA | HAA9 |
| indoor | n | 11 | 11 | 11 | 11 | 11 | 11 |
| | Mean | 54.89 | 103.18 | 7.86 | 3.91 | 6.36 | 176.20 |
| | Minimum | 19.91 | 26.15 | 3.15 | 1.06 | 2.49 | 78.74 |
| | Maximum | 95.84 | 329.40 | 15.17 | 6.83 | 14.93 | 391.70 |
| | standard deviation | 83.16 | 92.80 | 9.63 | 4.54 | 9.72 | 133.69 |
| outdoor | n | 7 | 7 | 7 | 7 | 7 | 7 |
| | Mean | 75.74 | 141.60 | 10.85 | 4.98 | 8.36 | 241.53 |
| | Minimum | 25.69 | 23.67 | 5.13 | 2.77 | 4.26 | 80.74 |
| | Maximum | 113.80 | 369.80 | 19.34 | 8.96 | 16.42 | 348.63 |
| | standard deviation | 63.71 | 162.80 | 9.63 | 5.24 | 12.72 | 191.47 |
| Drinking | Ν | 15 | 15 | 15 | 15 | 15 | 15 |
| | Mean | 6.97 | 8.62 | 1.72 | 0.54 | 1.06 | 18.91 |
| | Minimum | 2.76 | 2.41 | 0.00 | 0.00 | 0.00 | 6.14 |
| | Maximum | 9.02 | 12.15 | 3.05 | 2.98 | 3.28 | 27.74 |
| | standard deviation | 6.05 | 6.36 | 1.45 | 0.39 | 0.97 | 15.21 |

Table-3. Mean concentrations of HAAs in swimming pool and drinking water

n is the number of swimming pools and drink water.

3.4. Associations between HAAs and Pool Water Quality

The Pearson test was used to analyze the correlation between HAAs and water quality parameters of swimming pool. The results were shown in Table 4. According to the results, HAAs in swimming pools were positively correlated with urea and free residual chlorine (p<0.05). With the increase of urea (r=0.353, p<0.05) and free residual chlorine (r=0.249, P<0.05), the concentration of HAAs also increased. It was also consistent with some studies. It showed that with the increase of chlorine dosage, sufficient organic matter in the swimming pool can continuously react with chlorine agent to generate DBPs, among which the growth rate of DCAA concentration was the most obvious [28]. Zhao et al found a significant correlation between HAA9 concentration and free residual chlorine, suggesting that residual chlorine may be a key factor in the formation of DBPs in swimming pools [15]. Some research proved that urea, citric acid, creatinine and other substances were the main precursors for the formation of HAAs [29], especially DCAA and TCAA. Urine and sweat released by swimmers during activity can interact with chlorine disinfectants treated in pool water, resulting in increased levels of HAAs in pool water. Research also proved that 63% of the pollutants in swimming pools came from unsanitary behavior during swimming, of which 31% was caused by entering the pool without a shower, and 32 % was caused by urination. Therefore, swimming places should set showering to reduce the metabolites caused by the human body in swimming pools.

| Table-4. Correlation between swimming pool water quality parameters and HAAs (n=3) | | | | | | |
|---|-------|-----------|--------|---------------|--------|--|
| Parameters | pН | Turbidity | Urea | Free chlorine | HAAs | |
| pН | 1.000 | 0.052 | 0.203 | -0.180 | -0.078 | |
| Turbidity | | 1.000 | -0.219 | 0.289* | 0.279 | |
| Urea | | | 1.000 | 0.121 | 0.353* | |
| Free chlorine | | | | 1.000 | 0.249* | |
| HAAs | | | | | 1 000 | |

Notes: *: P<0.05.

4. Conclusions

This study is related to investigation HAAs concentrations in 18 public swimming pools and to explored the correlation between HAAs and water quality parameters. The results showed that, in indoor and outdoor swimming pools, the sanitary parameters of swimming pools were unqualified to different degrees, and the main unqualified indicators were pH and turbidity. Meanwhile, the concentration of HAAs in swimming pool water was much higher than that in drinking water, which may be related to the chlorination of organic precursors in swimming pool water. Different types of swimming pools may have an impact on the concentrations level of HAAs. The concentration of HAAs in outdoor swimming pools was higher than that in indoor swimming pools, mainly DCAA and TCAA. The results also showed that free chlorine and urea were positively correlated with HAAs. It represented that free chlorine and urea displayed a great influence on the formation of HAAs. As non-volatile substances, HAAs are easy to accumulate in water and can enter the body through ingestion in the digestive tract and skin absorption, and there

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are certain health risks. Therefore, it is recommended that relevant departments should strive to formulate relevant HAAs regulations, strengthen the supervision and management of swimming pool hygiene, and take corresponding measures, such as a shower before swimming, the publicity of hygiene knowledge for swimmers, and the process of removing organic matter, et. al. It also need to reduce the content of disinfection by-products in swimming pool water. Ultimately, the goal of reducing health risks is achieved.

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