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Published in:
Chemosphere

Link to article, DOI:
[10.1016/j.chemosphere.2012.01.003](https://doi.org/10.1016/j.chemosphere.2012.01.003)

Publication date:
2012

[Link back to DTU Orbit](#)

Citation (APA):

Hansen, K. M. S., Willach, S., Mosbæk, H., & Andersen, H. R. (2012). Particles in swimming pool filters – Does pH determine the DBP formation? *Chemosphere*, 87(3), 241-247. DOI: 10.1016/j.chemosphere.2012.01.003

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Particles in swimming pool filters – Does pH determine the DBP formation?

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Abstract

The formation was investigated for different groups of disinfection byproducts (DBPs) during chlorination of filter particles from swimming pools at different pH values and the toxicity was estimated. Specifically, the formation of the DBP group trihalomethanes (THMs), which is regulated in many countries, and the non-regulated haloacetic acids (HAAs) and haloacetonitriles (HANs) were investigated at $6.0 \leq \text{pH} \leq 8.0$, under controlled chlorination conditions. The investigated particles were collected from a hot tub with a drum micro filter. In two series of experiments with either constant initial active or initial free chlorine concentrations the particles were chlorinated at different pH values in the relevant range for swimming pools. THM and HAA formations were reduced by decreasing pH while HAN formation increased with decreasing pH. Based on the organic content the relative DBP formation from the particles was higher than previously reported for body fluid analogue and filling water. The genotoxicity and cytotoxicity estimated from formation of DBPs from the treated particle suspension increased with decreasing pH. Among the quantified DBP groups the HANs were responsible for the majority of the toxicity from the measured DBPs.

Keywords: Chlorination; swimming pool; pH; particles; DBPs; Genotoxicity

1 Introduction

Swimming pools are used around the world for recreational, rehabilitation and physical activity and therefore it is imperative that the water and air quality are safe for the health of the bathers. Chlorination is by far the most applied method to control pool water quality and to prevent spreading of pathogens from swimmers because of its residual disinfection effect (WHO, 2006). Chlorine exhibits a pH and temperature dependent-equilibrium between the hypochlorous acid (HOCl) and the hypochlorite ion (OCl⁻) ($\text{pK}_{\text{a}, 25^{\circ}\text{C}} = 7.5$), with the sum of the two commonly known as free chlorine (White, 1992). HOCl is the main active species responsible for the disinfection effect of chlorine. Therefore it is crucial to closely monitor and control pool water pH to ensure disinfection effectiveness (White, 1992). Chlorine reacts with the natural organic matter (NOM) found in the source water and the organic material deposited by the swimmers. A part of the organic material is mineralized (Judd and Bullock, 2003) while the rest cause formation of chlorinated organic compounds commonly known as disinfection by-products (DBPs). Currently, more than 600 different DBPs have been detected in chlorinated drinking water (Richardson, 2011) but the identified compounds only comprise approximately 30–50% of the total organic halogen (Krasner et al., 2006; Richardson et al., 2007). In a survey of 50 pools from France approximately 50% of the total organic halogen are covered by the four groups of DBPs: Trihalomethanes (THMs), haloacetic acids (HAAs), haloacetonitriles (HANs), and chloral hydrate (Brunet et al., 2010).

THMs were the first carbon based DBP group to be detected in chlorinated drinking water (Bellar et al., 1974; Rook, 1974) and linked to NOM for their formation. Other DBP groups include HAAs (Heller-Grossman et al., 1993; Cowman and Singer, 1996), HANs (Oliver, 1983), halonitromethanes (Thibaud et al., 1987), and halo ketones (Suffet et al., 1976) have later been

detected in chlorinated drinking water. These organic DBPs have been identified in swimming pool water as well (Richardson et al., 2010), together with inorganic nitrogenous DBPs like trichloramine (NCl_3) (Hery et al., 1995). The major concern regarding DBP formation in swimming pools is their effect to human health because some are carcinogenic (Richardson et al., 2007) while others are suspected to cause asthma (Thickett et al., 2002; Goodman and Hays, 2008), and irritations to the eyes and mucous membrane (Chiswell and Wildsoet, 1989; Erdinger et al., 1998). Furthermore, a large study on bladder cancer has found an increased risk associated with swimming in pools (Villanueva et al., 2007).

Regulations of DBPs in swimming pool water around the world have focused only on THMs and combined chlorine (chloroamines), which are easily analyzed. However, recent research has shown that other chlorinated molecules such as cyanogen halides, HAAs and HANs (Glauner et al., 2005; Zwiener et al., 2007) are much more relevant DBPs in the pool water since they are directly linked to cancer risk and are generally more toxic than the regulated DBPs. The cyto- and genotoxic potency of HANs is higher than HAAs which is higher than THMs (Plewa et al., 2008). In a recent pool conference proceedings paper, two HANs, dibromoacetonitrile and bromochloroacetonitrile were found to be important contributors to the overall toxicity of pool water (Kramer et al., 2009). In a recent study, seven public pools with different disinfection and source water treatment practices had higher genotoxic potency than their supply water because of DBP formation (Liviatic et al., 2010).

One way to limit the formation of some DBPs is to reduce the chlorine concentration as well as the pool water pH to slightly more acidic conditions, so HOCl concentration is maintained thus maintaining the disinfection efficiency since HOCl is a stronger disinfectant than OCl^- .

Inspired by the pH dependency of disinfection efficiency of chlorine (defined by HOCl concentration) and the German standards use of lower chlorine concentrations ($0.3\text{--}0.6 \text{ mg L}^{-1}$) combined with lower pH ($6.5\text{--}7.5$) a Danish full scale study experimentally operated a public indoor swimming pool at pH 6.7 and $0.4 \text{ mg chlorine L}^{-1}$ compared to the traditional pH 7.3 and 1.5 mg L^{-1} of chlorine. The study showed a decrease in THM, absorbable organic halogen (AOX) and combined chlorine while microbiological quality was retained (Kristensen et al., 2007). Based on that study it has been officially suggested in Denmark to change the regulations for swimming pools to promote running of the pools at lower pH, specifically changing the lower limit for the pH from 7.0 to 6.8, while lowering the pH even to 6.0 has been discussed. Though this may be beneficial by decreasing the formation of THM, AOX and combined chlorine, a recent study shows that formation of HAN and NCl_3 increases with decreasing pH (Hansen et al., 2011) when chlorine reacts with an artificial analog of the mixture of soluble compounds that bathers pollute the swimming pool water (known as body fluid analogue, BFA).

Besides the dissolved compounds the swimmers release particles consisting mainly of hair and skin cells. In traditional pool water treatment systems these particles are retained on sand filters, where they are exposed to the chlorinated pool water until they are removed by back flushing the sand filter. Back flushing frequencies depend on the rise in backpressure of the filter and local regulations, but in practice the frequency varies from daily to biweekly. During the exposure to

chlorinated water the particles are hydrolyzed and react with chlorine which results in DBP formation. THM and HAN formation from hair and skin at pH 7 are reported (Kim et al., 2002) but to our knowledge the pH effect on the formation of DBPs has not been investigated.

A few swimming pools in Denmark use drum filters based on a weaved microsieve (cutoff 10 or 20 μm) which removes the particles from the pool water fast by washing the filter as the filter cloth turns out of the water stream (See Fig. SM-1 in Supplementary Materials (SM)). This removes the particles from contact with the pool water much faster than traditional sand filters. Depending on the load and pool type backwashing frequencies and thus maximal water contact time for collected particles can be less than 15 min.

In this study particles were collected from a microsieve filter from a hot tub which was used to investigate the effect of pH on DBP formation from particles which would typically be trapped in the filter. Specifically, the impact of pool water pH on the formation of 4 THMs, 6 HAAs, 4 HANs, NCl_3 , trichloronitromethane, dichloropropanone, and trichloropropanone (see Table 1 for the complete list) was investigated at fixed pH values under controlled chlorination conditions. Further, the measured DBP concentration was used with literature values for toxic potency of each compound to estimate the overall cytotoxicity and genotoxicity of the chlorinated particle suspension at the different pH levels.

2 Material and method

2.1 Reagents

All chemicals and standard solutions were analytical grade purchased from Sigma-Aldrich.

2.2 Analysis of THMs and HANs

Free chlorine in THM and HAN samples was quenched by adding 200 μL of ammonium chloride solution (50 g L^{-1}) to 40 mL borosilicate glass vials before it was filled head-space-free with the sample. The samples were analyzed the same day by Purge and Trap (Velocity XPT Purge and Trap Sample Concentrator, Teledyne Tekmar, with autosampler: AQUATek 70, Teledyne Tekmar) coupled with a GC (HP 6890 Series GC System, Hewlett Packard) with mass spectrometer (5973 Mass selective detector, Hewlett Packard). This method was also used for the detection of trichloronitromethane, dichloropropanone, and trichloropropanone. For more information refer to SM.

2.3 Analysis of Haloacetic Acids

For the analysis of the haloacetic acids a modified version of the EPA 552.2 method was used. Sulfuric acid, sodium sulfate, surrogate standard (2-bromobutanoic acid) and methyl-tert-butyl ether (MtBE) was added to the samples and extracted. The MtBE phase was transferred to a test tube and acidified methanol was added. The samples were placed in an oven at 60 $^{\circ}\text{C}$ for 2 h to methylate the haloacetic acids and subsequently neutralized by adding saturated sodium bicarbonate solution. The MtBE phase was then transferred to a GC vial and analyzed on a GC (7890A GC System, Agilent

Technologies) coupled to a mass spectrometer (5975C, Agilent Technologies). Details on the method can be found in the SM.

2.4 Analysis of Chlorine, Combined Chlorine and Trichloramine

The residual chlorine and the solution pH were measured at the beginning and the end of each experiment. The hypochlorite stock solution (~10% w/w, Sigma-Aldrich) and the free and total chlorine of the samples were measured with a photometer (DR 2800, Hach Lange) using the diethyl-p-phenyldiamine method from a cell test kit (LCK 310, Lange).

The trichloramine was measured by the method described by Lützenkirchen and Breuer (2007) and Hery et al. (1995). Trichloramine was stripped from the water by aerating the sample for 20 min and trapping the released trichloramine on a filter impregnated with arsenite (As_2O_3) which reduced trichloramine to chloride. The filter was subsequently placed in MilliQ water to dissolve the chloride which was measured by ion chromatography (ICS-1500, Dionex). A more detailed description of the method can be found in SM.

2.5 Samples of particles

Particles were collected from a drum micro filter on a hot tub in the indoor public swimming pool of Køge Municipality, Zealand, Denmark. The filter collects particles larger than 10 μm . The hot tub has a hydraulic retention time of 6 min and the filter was flushed every 6 min which results in an average contact time with chlorine for the particles of less than 15 min. The particle suspension from flushing the filter was collected on the 6th of July 2010. Immediately after collection residual chlorine was quenched with sodium sulfite before subsamples were frozen in glass bottles until used for experiments (less than 14 d).

In order to scale the dose of particles used in experiments the hydrolysable carbon from the particle suspension was estimated by treating a subsample of particles at pH 2 (phosphoric acid) for 12 h before the subsample was filtered and DOC was measured with the total organic carbon analyzer (TOC-V WP, Shimadzu) using UV and persulphate for mineralization.

2.6 DBP formation tests

The formation of DBPs from particles from swimming pool was investigated by DBP formation tests. Similar test parameters have been used in other studies to investigate NCl_3 formation (Schmalz et al., 2011) and THM and HAA formation (Kanan and Karanfil, 2011).

Principally, excess chlorine compared to the dose required to theoretically completely oxidize the NOM was added to the water. When experiments were made with constant free chlorine ($\text{HOCl} + \text{OCl}^-$) concentration the initial concentration of Cl_2 was 35 mg L^{-1} . Specifically, at constant free chlorine (35 mg L^{-1}) the HOCl concentration was 34 mg L^{-1} at pH 6 and 9.1 mg L^{-1} as Cl_2 at pH 8.

In experiments where the concentration of active chlorine (HOCl) was constant the chlorine was added at a calculated initial concentration of HOCl of 26.6 mg L^{-1} by adding hypochlorite adjusted according to the speciation at each given pH value. Specifically, at constant active chlorine (26.6 mg L^{-1}) the free chlorine concentration was 27 mg L^{-1} at pH 6 and 102 mg L^{-1} at pH 8.

The ratio between the initial Cl_2 and DOC in this formation test is much higher than the ratios between measured Cl_2 and DOC in swimming pools. This does not mean that the experimental conditions are unrealistic since most of the DOC in pool water has been in the water a long time and reflects the fraction of carbon added over a long time which is not reactive to chlorine.

2.7 Experiments performed

All the chlorination experiments were carried out as batch experiments. The particle suspension was homogenized by shaken it well before taking sample for experiment and was dosed equivalent to $125 \mu\text{g}$ acid dissolved DOC L^{-1} ($10.4 \mu\text{mol L}^{-1}$) in freshly made reverse osmosis water.

For the investigation of formation of THM, HAN and HAA the diluted particle suspension was buffered with phosphate buffer at pH 6.0, 6.5, 7.0, 7.5, and 8.0 and the chlorination were applied either as constant initial concentration of free chlorine ($\text{HOCl} + \text{OCl}^-$) or active chlorine (HOCl) as described in the previous section. The experiments were performed in headspace-free borosilicate glass bottles sealed with caps with a PTFE (polytetrafluoroethylen) seal (SCHOTT DURAN) to avoid loss of the volatile compounds. The bottles were kept at 25°C for 48 h, and then samples were taken for analysis of THM, HAN, HAA and chlorine residues and for measuring the solution pH. Table 1 contains the compounds analyzed and their abbreviation.

The experiment for trichloramine detection was carried out as headspace-free duplicate in 100 mL borosilicate glass bottles at pH 6.0 buffered with phosphate buffer. The diluted particle suspension was added 35 mg L^{-1} free chlorine. To avoid any degradation of NCl_3 due to UV-radiation the bottles were wrapped with aluminum-foil and allowed to react for 24 h at 25°C .

Table 1. List of investigated compounds and chemical structures.

	Compound	Abbreviation	Chemical structure
THMs	Chloroform	TCM	CHCl_3
	Bromodichloromethane	BDCM	CHBrCl_2
	Dibromochloromethane	DBCM	CHBr_2Cl
	Bromoform	TBM	CHBr_3
HAAs	Chloroacetic acid	CAA	CH_2ClCOOH
	Bromoacetic acid	BAA	CH_2BrCOOH
	Dichloroacetic acid	DCAA	CHCl_2COOH
	Bromochloroacetic acid	BCAA	CHBrClCOOH
	Dibromoacetic acid	DBAA	CHBr_2COOH
	Trichloroacetic acid	TCAA	CCl_3COOH
HANs	Dichloroacetonitrile	DCAN	CHCl_2CN
	Bromochloroacetanotile	BCAN	CHBrClCN
	Dibromoacetonitrile	DBAN	CHBr_2CN
	Trichloroacetonitrile	TCAN	CCl_3CN
Others	Trichloronitromethane		CCl_3NO_2
	Dichloropropanone		$\text{CHCl}_2\text{COCH}_3$
	Trichloropropanone		$\text{CCl}_3\text{COCH}_3$
	Trichloramine		NCl_3

3 Calculations

Based on the measured concentration of the different DBPs, the cyto- and genotoxicity was estimated as the sum of the concentration of each compound divided by its EC_{50} (Eq. 1).

$$\sum_1^i \frac{C_i}{EC_{50,i}} \quad (1)$$

All the EC_{50} values were used as reported in the literature (Plewa et al., 2002; Muellner et al., 2007; Plewa et al., 2008). These references were chosen because all the investigated compounds were tested in the same assay, except dichloropropanone and trichloropropanone which were not detected in the experiments of this study. The assay used was an *in vitro* cellular toxicological assays based on Chinese hamster ovary cells and the cytotoxicity was measured as the reduction in cell density while the genotoxicity was measured by single cell gel electrophoresis (Plewa et al., 2002; Muellner et al., 2007; Plewa et al., 2008). The EC_{50} values used for the estimations are given in Table SM-2.

4 Results and Discussion

4.1 Effect of pH on DBP formation

Reaction of chlorine with the collected particles resulted in formation of DBPs. The control experiment with freshly made reverse osmosis water at pH 7 and 35 mg L⁻¹ chlorine resulted in very low concentration of chloroform (0.039 μmol L⁻¹ (5.0 μg L⁻¹)), dichloroacetonitrile (DCAN, 0.0040 μmol L⁻¹ (0.44 μg L⁻¹)) and trichloroacetonitrile (TCAN, < 0.016 μmol L⁻¹ (2.4 μg L⁻¹)). These concentrations were negligible compared to the ones obtained in the experiments with particles. The chlorinated organic DBPs: THMs, HAAs, and HANs were detected (Fig. 1). The chlorination approach (constant free versus constant active chlorine concentration) did not have a significant effect on the formation of THMs, HAAs and HANs, except for TCAN at pH 8. At pH 8 a high concentration of chlorine was needed to have 26.6 mg L⁻¹ of active chlorine and that caused high formation of TCAN.

The effect of pH combined with the effect of the chlorination approaches on the DBP formation differed for the investigated DBP groups. Particularly, the lowest chloroform formation was found at pH 6 and the formation increased with increasing pH-level (Fig. 1a). The same pH dependency was observed during chlorination of BFA, which simulates sweat and urine contamination from swimmers, (Hansen et al., 2011) as well as drinking water (Liang and Singer, 2003; Bougeard et al., 2008). In these studies the precursor material had different characteristics. The drinking water contains NOM while the BFA includes dissolved organic matter from sweat and urine, and the particles consisting of hair and skin cells. The main differences between the DBP-precursors are that the anthropogenic DBP-precursors (BFA and particles) have higher nitrogen to carbon molar ratio compared to NOM. However, despite the difference in the DBP-precursors they exhibit the same pH dependency regarding THM formation.

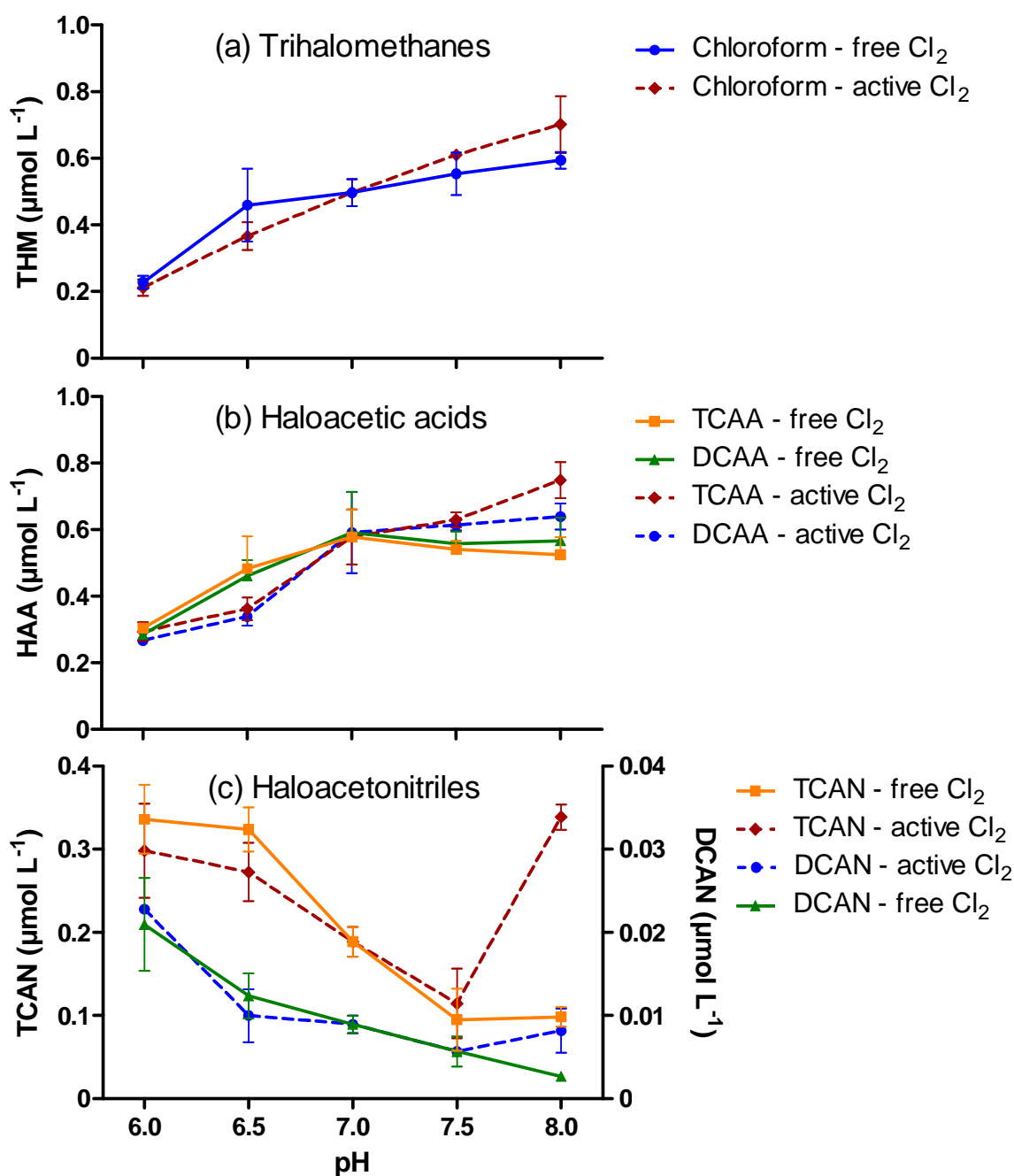


Figure 1. Effect of pH on THM (a), HAA (b) and HAN (c) formation from filter particles with constant free chlorine and active chlorine. Brominated species were not detected. The error bars represent the standard deviation of three replicates. Reaction time = 48 h, temperature = 25 °C and particle suspension = 125 $\mu\text{g DOC L}^{-1}$. 1 μmol correspond to 119 μg chloroform, 163 μg TCAA, 129 μg DCAA, 144 μg TCAN or 110 μg DCAN.

The formation of HAAs was lowest at pH 6 and increased with pH-levels (Fig. 1b). Since there are limited studies on the effect of pH on DBP formation in swimming pool water our findings were

compared with studies on chlorination of drinking water. Some studies reported increasing HAA concentration with decreasing pH (Cowman and Singer, 1996; Liang and Singer, 2003) while others found contradictory pH dependencies for two different types of drinking water (Bougeard et al., 2008). Furthermore, a study on chlorination of BFA observed no pH effect (Hansen et al., 2011). Based on these findings, it can be concluded that the pH effect on the HAA formation strongly depends on the precursor material.

Generally, TCAN formation was favored over DCAN (Fig. 1c) which was generally formed in approximately 20 times higher concentration. The highest formation of HANs was observed at pH 6 and the formation decreased with increasing pH for the constant free chlorine experiments and the experiments with constant active chlorine except for TCAN in the latter experiment which increased again from pH 7.5 to 8. The increase may be due to the high free chlorine concentration needed to obtain constant active chlorine concentration at pH 8.0 (26% HOCl and 74% OCl⁻) and has been observed as well in previous studies (Hansen et al., 2011). HANs are the most toxic DBP group examined in our study (Plewa et al., 2008) and HANs have also been reported to be the DBP group that contributes the most to the toxicity of chlorinated pool water (Kramer et al., 2009). Therefore, in order to limit their formation it is imperative to identify the conditions where HANs are produced. To the best of our knowledge, a recent study was the first to identify the conditions under which they are formed during chlorination of body fluid analogue (Hansen et al., 2011) and the findings were similar to the findings in this study with chlorination of filtered particles.

Previous studies have found that the highest formation of NCl₃ occurs at low pH (Palin, 1950; Schmalz et al., 2011). Therefore NCl₃ from particle suspension was initially tested at pH 6.0 only and the formation was found negligible around the limit of quantification for the method (0.8 μmol L⁻¹ (96 μg L⁻¹)). The experiment with the particles suspension had a total THM level at 0.23 μmol L⁻¹ (27 μg L⁻¹ as chloroform) which was higher than the BFA study (0.077 μmol L⁻¹ (9 μg L⁻¹ as chloroform)) and in the BFA study the NCl₃ formation was 31 μmol L⁻¹ (3700 μg L⁻¹), which is much higher than LOQ = 0.8 μmol L⁻¹ (96 μg L⁻¹) (Hansen et al., 2011). Consequently, NCl₃ formation was not investigated for the entire pH range 6.5-8.0 of this study. Based on our findings it appears that trichloramine is not formed from particles in the traditional sand filter in swimming pool treatment. This fits well with the results in a recent published paper (Schmalz et al., 2011) where urea is found to be the main precursor for NCl₃ during an investigation of different amides, amino acids, and amines. Urea is soluble and will not be caught in the filters. The collected hair and skin cells mainly consist of the three amino acids cysteine (17.5%), serine (11.7%) and glutamic acid (11.1%) (McElwee, 2011). Schmalz et al. (2011) found that by reaction with chlorine 95% of urea was transformed to NCl₃ at pH 5.9 but only 19% of glutamic acid and 15% of serine was found as NCl₃. Cysteine was not investigated, however, like alanine, it is substituent at the alpha-carbon contrary to glycine and cysteine so it is expected to form very little NCl₃ like alanine (5.4% at pH 5.9).

In addition to the abovementioned compounds, trichloronitromethane, dichloropropanone, and trichloropropanone were also monitored in all the experiments. However, their formations were too close to the method detection limit (detection limit of trichloronitromethane $6.1 \cdot 10^{-9}$ M (1.0 μg L⁻¹),

dichloropropanone $4.9 \cdot 10^{-9}$ M ($0.6 \mu\text{g L}^{-1}$), and trichloropropanone $8.3 \cdot 10^{-11}$ M ($0.01 \mu\text{g L}^{-1}$) and therefore, conclusions on the effects of pH and chlorination approaches cannot be made.

4.2 Relative DBP formation

So far, the load of particles per bather measured as TOC has not been determined and thus it is difficult to compare how much DBPs originate from particles compared to the soluble pollution from one person. Therefore each group of DBPs was normalized to the acid dissolved DOC as $\Sigma\text{DBPs}/\text{DOC}$ and a comparison of the normalized formation was made (Table 2). At pH 7.0 the HAAs had the highest formation at $9.6 \mu\text{mol (mg DOC)}^{-1}$, followed by THMs at $4.0 \mu\text{mol (mg DOC)}^{-1}$ with HANs having the lowest formation at $1.6 \mu\text{mol (mg DOC)}^{-1}$. Kim et al. (2002) have tested DBP formation from hair and skin collected from a 25-yr-old man. However, the results are not directly comparable because they performed their formation experiments in water with high TOC levels which in most cases resulted in higher level of DBP in their control than their experiments.

Table 2. Relative DBP formation at pH 7.0 from different studies.

	THM $\mu\text{mol (mg DOC)}^{-1}$	HAA $\mu\text{mol (mg DOC)}^{-1}$	HAN $\mu\text{mol (mg DOC)}^{-1}$	HAA/THM	HAN/THM
Particles	4.0	9.6	1.6	2.41	0.40
BFA ^a	0.077	0.27	0.025	3.48	0.33
BFA ^b	0.25	0.51		2.03	
Filling water ^b	0.62	0.26		0.42	

^aHansen et al., 2011 ^bKanan and Karanfil, 2011

The formation of DBPs from chlorination of the BFA suggested by Judd and Bullock (2003) has previously been investigated. Hansen et al. (2011) reported the formation of DBPs as $0.27 \mu\text{mol HAA (mg DOC)}^{-1}$, $0.077 \mu\text{mol THM (mg DOC)}^{-1}$ and $0.025 \mu\text{mol HAN (mg DOC)}^{-1}$. Kanan and Karanfil (2011) reported with higher chlorine to DOC ratios formation at $0.25 \mu\text{mol THM (mg DOC)}^{-1}$ and $0.51 \mu\text{mol HAA (mg DOC)}^{-1}$ for BFA and for the filling water the formation is reported at $0.62 \mu\text{mol THM (mg DOC)}^{-1}$ and $0.26 \mu\text{mol HAA (mg DOC)}^{-1}$. For the particles and the BFA the formation of HAAs was higher than the formation of THM, while the opposite is the case for the filling water. However, it appears that the particles have a higher potential to form DBPs in swimming pool water relative to the organic carbon content than the BFA and the filling water. This suggests that the organic material in the filtered particles creates more DBPs than other types of organic material tested. However, it is also possible that this is due to the fact that chlorine can react with more of the organic material in the particle suspension than is estimated by determining the DOC after hydrolysis at low pH.

When the DBP formation is normalized to the THM formation for each type of organic carbon discussed above, as shown in Table 2, it can be seen that the HAA/THM ratio was between 2 and 3.5 for material of human origin while for the NOM from the filling water the ratio was 0.4. The HANs were only measured in one study besides this and the HAN/THM ratio from chlorination of

BFA was quite similar at 0.33 compared to 0.40 for particles in this study, which can be explained by the two materials containing similar nitrogen rich molecules mainly based on amino acids.

4.3 Cytotoxicity and Genotoxicity

To evaluate the effect of DBP formation on the bathers' health, the cytotoxicity of the chlorinated particle suspension at the different pH values and chlorination approaches was estimated as described in Sec. 3.

The highest cytotoxicity was found at pH 6.0 and 6.5 in the case with constant free chlorine (Fig. 2a) and at pH 6.0 for constant active chlorine (Fig. 2b). In both cases the estimated cytotoxicity decreased with increasing pH, but for constant active chlorine the toxicity increased again at pH 8.0. The contribution of THMs to the overall solution toxicity was negligible compared to the other groups. However, the absolute values of THM toxicity increased with increasing pH which relates to the increasing THM concentration shown in Fig. 1a. Likewise, the HAA and HAN contribution to toxicity were dependent of the solution pH, as were the overall concentrations (Fig. 1b and c). The toxicity of the HANs comprise 63–92% of the total estimated cytotoxicity, which make the HANs the most toxicity relevant group of DBPs measured in this study followed by the HAAs, and with the THMs contributing the least to the toxicity.

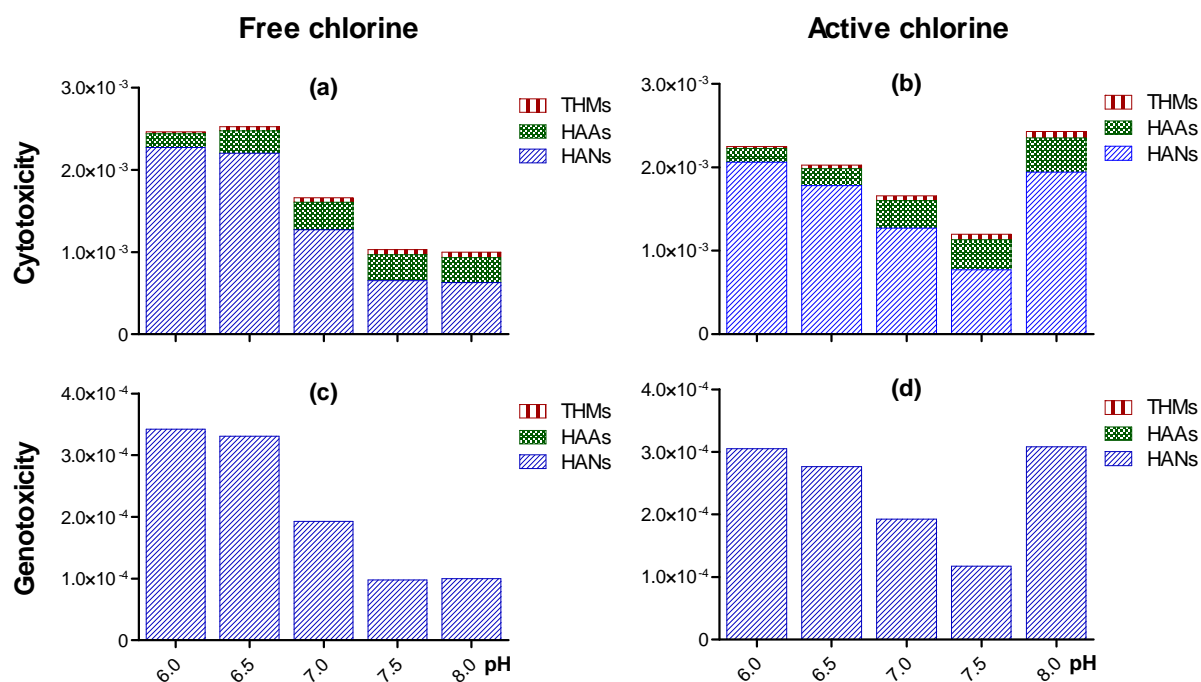


Figure 2. Estimated cytotoxicity (a, b) and genotoxicity (c, d) of chlorinated particle water with constant free and active chlorine, at $6.0 \leq \text{pH} \leq 8.0$.

The genotoxicity of the chlorinated particle suspension in each experiment was estimated in the same way as the cytotoxicity. The genotoxicity follows similar trends as the cytotoxicity. The highest toxicity was found at pH 6.0 with decreasing genotoxicity with increasing pH-levels, except

for pH 8.0 at constant active chlorine (Fig. 2c and 2d). Contradictory to the cytotoxicity, for the DBPs measured in this study, the HANs are the only contributor to the estimated genotoxicity since chloroform, DCAA, TCAA were not genotoxic in the assay used by Plewa et al. (2002) and Plewa et al. (2008).

Based on the above, the HANs contribute most to the toxicity of the DBPs measured and the group is thus predicted to be an important contributor for the cyto- and genotoxicity of the treated pool water. Similar results have been obtained when chlorinating body fluid analog at different pH values (Hansen et al., 2011) and was also reported in a preliminary study of real pool water (Kramer et al., 2009).

5 Conclusions

This study investigated the effect of pH and chlorination approach on the formation of three DBP groups: THMs, HAAs and HANs. The pH affects the formation of each investigated DBP group differently, while there was no real difference between the two chlorination approaches. A decrease in concentration of THMs which is regulated in many countries and the non-regulated HAAs was observed for decreasing pH, while the concentration of the HANs increased. The particles were more reactive to chlorine than body fluid analogue for THMs, HAAs, and HANs and the relative DBP formation was higher.

Furthermore, the cyto- and the genotoxic potency of the chlorinated particle suspension were estimated by calculations. At pH 6.0 the highest cyto- and genotoxicity was found which decreased with increasing solution pH. The HANs are the most significant toxic compounds detected in this study though they are found at low concentration. However, it is not known how much the HANs contribute to the overall mixture toxicity. Based on the above, the pH level of pool water affects the formation of DBPs from particles in swimming pool filters. Therefore caution is warranted if the pH-level in swimming pool waters can be decreased to levels less than 7.0 in order to reduce the concentration of THMs, since the concentration of the more toxic HANs appear to increase.

Acknowledgements

The authors thank DTU Environment for funding and the staff at Køge public swimming pool and Henning Hammerich from Rambøll Consulting Engineers for assistance with particle collection. Sinh Hy Nguyen at DTU Environment is acknowledged for technical assistants. Maria Antoniou at DTU Environment is acknowledged for assistance with preparing this manuscript. The work was supported by the Graduate School Urban Water Technology (www.urbanwatertech.dk).

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Supplementary Materials

Particles in swimming pool filters – Does pH determine the DBP formation?

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1 System description of the hot tub

The volume of the hot tub is 3 m³ with hydraulic flow of 30 m³/h which results in a hydraulic retention time of 6 min. This design follows the regulation in Denmark. The chlorine level is 1.2 mg/L. The drum filter collects particle larger than 10 µm.

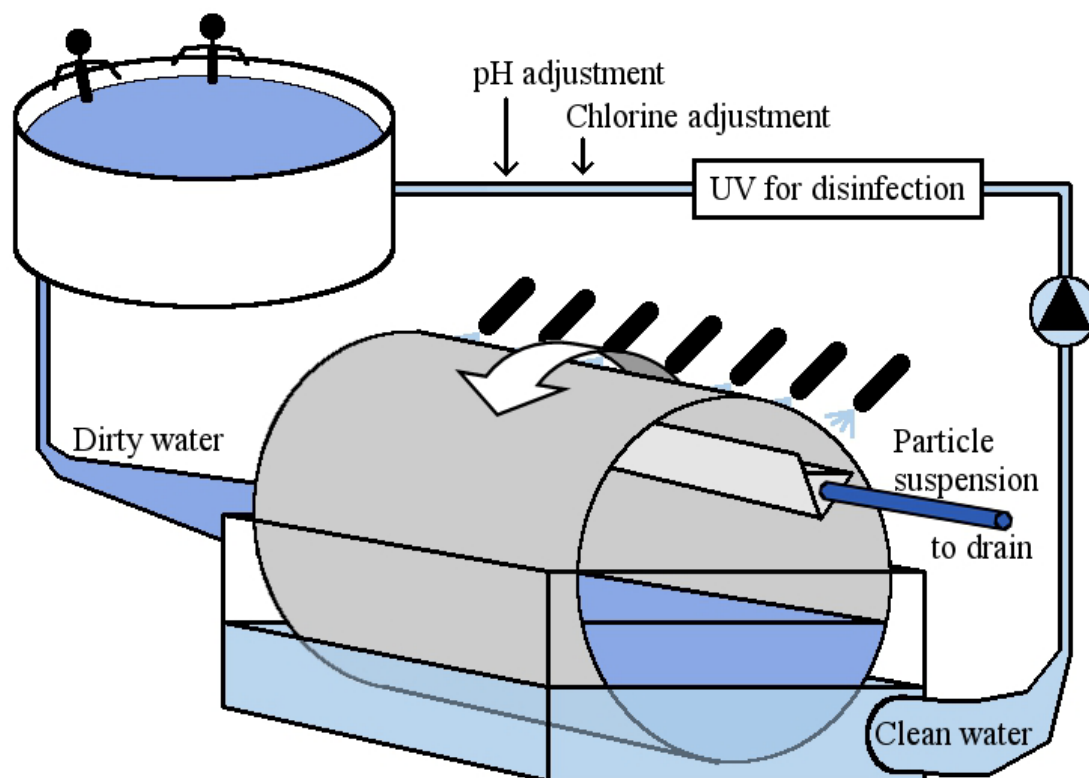


Figure SM-1. Schematic of water treatment of the hot tub.

The drum filter rotates when the resistance to water flow through the filter is so high that the water level inside the filter raises and thus fresh filter cloth is put into the water while dirty filter cloth is back flushed. The flushing occurs 100 – 150 times a day and with 12 h of opening it results in a back flush approximately every 6 min. The particle suspension from the back flush is led to the drain.

2 Detailed experimental section

2.1 Analysis of THMs and HANs

Free chlorine in THM and HAN samples was quenched by adding 200 µL ammonium chloride solution (50 g/L) to 40 mL borosilicate glass vials before it was filled head-space-free with the sample and were analyzed the same day. An autosampler (AQUATEk 70, Teledyne Tekmar) with a 5 mL loop was used to transfer the samples to the purge cell (Velocity XPT Purge and Trap Sample Concentrator, Teledyne Tekmar). The sample was purged for 11 minutes by bubbling nitrogen with a flow rate of 44 mL/min. The compound was adsorbed on a trap, VOCARB 3000, Telmark®. The sample was desorbed for 3 minutes at 250°C from the trap. Simultaneously, with desorption from the trap, the GC (HP 6890 Series GC System, Hewlett Packard) was started with a flow rate of helium at 2.5 mL/min, which was set to 1 mL/min after the 3 minutes. The analysis was performed

in split mode with a ratio of 3:1. To clean the trap and minimize carryover it was baked at 260 °C with a flow rate of 200 mL/min for 5 minutes. The compounds were separated with a fused silica capillary column (30.0 m x 0.25 mm i.D., 1.5 µm film thickness; VOCOL, Supelco). The initial temperature of the oven was 45 °C for the first 3 minutes and then it increased with a rate of by 25 °C/min until it reached 230 °C. The compounds were detected with a mass spectrometer (5973 Mass selective detector, Hewlett Packard) set in SIM mode. The ions monitored during SIM mode is given in Table SM-1.

2.1.1 Preparation of standards

For each run a new calibration curve was prepared, with a range of 1 - 100 µg/L and if found necessary it was extended to 0.1 – 200 µg/L. Standard solutions for HANs were made from the EPA 551B Halogenated Volatiles Mix (2000 µg/mL) standard mixture dissolved in acetone. The THMs standard solution (2000 µg/mL) was prepared by mixing the 4 individual THMs in methanol, based on their density.

Table SM-1. Detection and quantification limit for the trihalomethanes, haloacetonitriles, halopropanones and trichloronitromethane based on their quantifier and qualifier ions.

Compound	Quantifier ion (m/z)	Qualifier ions (m/z)	Detection limit (mol/l)	Quantification limit (mol/l)
Trichloromethan (TCM)	83	85, 47	$4.2 \cdot 10^{-9}$	$1.4 \cdot 10^{-8}$
Bromodichloromethane (BDCM)	129	83, 85	$1.7 \cdot 10^{-9}$	$5.8 \cdot 10^{-9}$
Dibromochloromethane (DBCM)	129	127, 131	$5.0 \cdot 10^{-9}$	$1.7 \cdot 10^{-8}$
Tribromomethane (TBM)	173	171, 175, 252	$5.6 \cdot 10^{-10}$	$7.6 \cdot 10^{-10}$
Dichloroacetonitrile (DCAN)	74	76, 84	$7.7 \cdot 10^{-10}$	$2.6 \cdot 10^{-9}$
Bromochloroacetanotile (BCAN)	76	74, 155	$1.9 \cdot 10^{-8(*)}$	$1.9 \cdot 10^{-8(*)}$
Dibromoacetonitrile (DBAN)	120	118, 199	$1.1 \cdot 10^{-8}$	$1.5 \cdot 10^{-8}$
Trichloroacetonitrile (TCAN)	108	110, 73, 82	$1.6 \cdot 10^{-8}$	$1.6 \cdot 10^{-8}$
Trichloronitromethane (TCnitro)	117	119, 121, 82	$6.1 \cdot 10^{-9(*)}$	$6.1 \cdot 10^{-9(*)}$
Dichloropropanone (DCprop)	63	83	$4.9 \cdot 10^{-9}$	$1.6 \cdot 10^{-8}$
Trichloropropanone (TCprop)	125	127, 97	$8.3 \cdot 10^{-11}$	$8.3 \cdot 10^{-10}$

(*) Detection and quantification limit are given based on the lowest detected standard.

2.2 Analysis of haloacetic acids

For the analysis of the haloacetic acids a modified version of the EPA 552.2 method was used. To quench the residual chlorine of the samples 200 µL of 50 g/mL sodium sulfite were filled into each P&T vial, following by the addition of 30 mL of sample. The analysis was subdivided into four main steps: extraction, methylation (i.e., derivatization), neutralization and finally analysis in the GC-MS.

In order to extract the haloacetic acids, a stepwise acidification of the water sample with 3 mL of concentrated H₂SO₄, was performed in order to avoid sudden changes in the solution temperature. In addition, the samples were cooled-down in an ice bath. 100 µL of the surrogate standard, 2-bromobutanoic acid (0.1 g/L in methanol) and approximately 9.5 g of sodium sulfate were added to

the samples. Following this, the P&T vials were sealed with a teflon-lined screw cap and were hand-shaken in order to make a saturated salt solution. Finally, 2 mL of MtBE were added to the samples. The samples were then sealed with the caps and placed on a mechanical shaking table for another 30 min.

Following that, time was given, so the two solution phases (water vs. MtBE phase) were separated, and the MtBE phase was transferred into a conical tube. The control derivatization standard was prepared by adding 15 μ L of the EPA 552.2 standard mix and 100 μ L of surrogate standard to 1.5 mL of MtBE. 0.5 mL of acidified methanol (10% H₂SO₄) was then added to each conical tube. The tubes were tightly sealed with a Teflon-lined screw cap and placed into the oven at 60 °C for 2 h.

The tubes were left to cool-down before the caps were removed. The samples were neutralized by adding 2 mL of saturated sodium bicarbonate (50 g NaHCO₃ in 400 mL deionized water). After the neutralization the MtBE phase was partly transferred to a GC vial, sealed with a silicone/PTFE screw cap and analyzed at the same day.

The samples were analyzed in a GC-MS (7890 A GC System and 5975 C VLMSD, Agilent Technologies). The compounds were separated in a fused silica capillary column (30 m x 0.25 i.D. 1.5 μ m film thickness; VOCOL, Supelco). The carrier gas was helium with a flow rate of 1.3 mL/min. The starting temperature was 45 °C for 1 min and then it increased with a rate of 20 °C/min until 140 °C were reached. Then the temperature rate was reduced to 10 °C/min until 155 °C. The third ramp was set at 5 °C/min until 170 °C were reached, followed by a rate of 15 °C/min until 190 °C. Finally a rate of 40 °C/min was used to reach 230 °C, which was held for 2 min.

2.2.1 Preparation of standards

For each run a new calibration curve was prepared, with a range of 2 – 100 μ g/L and if found necessary it was extended to 200 μ g/L. Standard solutions were made from EPA 552.2 Halogenated Acetic Acids Mix (2000 μ g/mL each component in methyl tert-butyl ether, Supelco). The standards were treated like the samples i.e. extracted, derivatized, neutralized and analyzed with the GC-MS.

Table SM-2. Detection and quantification limit of haloacetic acids based on the quantifier and qualifier ions.

Compound	Quantifier ion (m/z)	Qualifier ions (m/z)	Detection limit (mol/L)	Quantification limit (mol/L)
Chloroacetic acid (CAA)	108	79, 77	$1.1 \cdot 10^{-7(*)}$	$1.1 \cdot 10^{-7(*)}$
Bromoacetic acid (BAA)	93	121	$3.6 \cdot 10^{-8(*)}$	$3.6 \cdot 10^{-8(*)}$
Dichloroacetic acid (DCAA)	83	85	$3.0 \cdot 10^{-9}$	$9.0 \cdot 10^{-9}$
Bromochloroacetic acid (BCAA)	129	127	$1.6 \cdot 10^{-9}$	$4.8 \cdot 10^{-9}$
Dibromoacetic acid (DBAA)	173	171, 175	$1.1 \cdot 10^{-10}$	$3.2 \cdot 10^{-10}$
Trichloroacetic acid (TCAA)	82	84, 59	$1.5 \cdot 10^{-8}$	$4.5 \cdot 10^{-8}$
Surrogate standard	152	101	-	-

(*) Detection and quantification limit are given based on the lowest detected standard.

2.3 Analysis of trichloramine

The filter for the trichloroamine analysis consisted of two 37-mm quartz fiber filters (with one of them being a back-up filter) impregnated with 500 μ L of a solution of arsenic (III) oxide (8 g/L As_2O_3), sodium carbonate (106 g/L Na_2CO_3) and glycerol (40 g/L $\text{C}_3\text{H}_8\text{O}_3$). The two filters were placed in a sampling cassette separated by polypropylene supporting pad and in front of the filter a tube with impregnated silica gel (1.25g sulfamic acid/50g silica gel) was placed to prevent airborne water droplets of chloride, monochloramine and dichloramine from being included in the sample. After sampling, the impregnated filters were desorbed in 10 mL Milli-Q water, sonicated for 15 min, and left to stand alone for 30 min before filtering them with a syringe filter (0.45 μ m nylon membrane syringe filter, PALL Life Sciences). The chloride concentration was measured by ion chromatography (ICS-1500, Dionex).

2.4 EC_{50} value of cyto- and genotoxicity

The EC_{50} value of cyto- and genotoxicity used for the estimation of sample toxicity are given in Table SM-3.

Table SM-3. The EC_{50} values for cyto- and genotoxicity taken from Plewa et al. (2002), Muellner et al. (2007) and Plewa et al. (2008).

		Cytotoxicity EC_{50} (mol/L)	Genotoxicity EC_{50} (mol/L)
THM	Chloroform (TCM)	$9.1 \cdot 10^{-3}$	*
	Bromodichloromethane (BDCM)	$9.1 \cdot 10^{-3}$	*
	Dibromochloromethane (DBCM)	$5.2 \cdot 10^{-3}$	*
	Bromoform (TBM)	$4.0 \cdot 10^{-3}$	*
HAN	Dichloroacetonitrile (DCAN)	$5.8 \cdot 10^{-5}$	$2.8 \cdot 10^{-3}$
	Trichloroacetonitrile (TCAN)	$1.7 \cdot 10^{-4}$	$1.0 \cdot 10^{-3}$
	Bromochloroacetanotile (BCAN)	$8.4 \cdot 10^{-6}$	$3.2 \cdot 10^{-4}$
	Dibromoacetonitrile (DBAN)	$2.9 \cdot 10^{-6}$	$3.0 \cdot 10^{-5}$
HAA	Chloroacetic acid (CAA)	$9.0 \cdot 10^{-4}$	$4.1 \cdot 10^{-4}$
	Bromoacetic acid (BAA)	$9.8 \cdot 10^{-6}$	$1.6 \cdot 10^{-5}$
	Dichloroacetic acid (DCAA)	$7.2 \cdot 10^{-3}$	*
	Trichloroacetic acid (TCAA)	$2.3 \cdot 10^{-3}$	*
	Bromochloroacetic acid (BCAA)	$8.4 \cdot 10^{-4}$	$3.7 \cdot 10^{-3}$
	Dibromoacetic acid (DBAA)	$5.2 \cdot 10^{-4}$	$1.7 \cdot 10^{-3}$

Trichloronitromethane (TCnitro)	$5.2 \cdot 10^{-4}$	$9.1 \cdot 10^{-5}$
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* The compounds were not found genotoxic in the assay used.

Muellner, M.G., Wagner, E.D., McCalla, K., Richardson, S.D., Woo, Y.T., Plewa, M.J., 2007. Haloacetonitriles vs. regulated haloacetic acids: Are nitrogen-containing DBPs more toxic?. *Environ. Sci. Technol.* 41, pp. 645-651.

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