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Graphic abstract

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Formation of regulated and unregulated disinfection byproducts during chlorination of algal organic matter extracted from freshwater and marine algae

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18   **Abstract**

19   Seasonal algal blooms in freshwater and marine water can increase the input of algal organic

20   matter (AOM) to the pool of dissolved organic matter. The impact of bromide (Br$^-$) and iodide (I$^-$

21   ) on the formation of regulated and unregulated disinfection byproducts (DBPs) were studied

22   from chlorination of AOM solutions extracted from three species of cultured isolates of

23   freshwater and marine algae (*Microcystis aeruginosa* (MA), *Synechococcus* (SYN), and

24   *Alexandrium tamarense* (AT)). Comparable concentrations of DBPs were formed from three

25   types of AOM. In the absence of Br$^-$, trihalomethanes (THMs), haloacetic acids (HAAs), and

26   haloacetaldehydes (HALs) were the main groups of DBP formed, and haloacetonitriles (HANs)

27   were formed at lower concentrations. In contrast, the formation of iodinated THMs was < 8 nM

28   (1.7 µg/L) since most of initial I$^-$ was oxidized to iodate. Increasing initial Br$^-$ concentrations

29   increased the formation of THMs and HANs, while concentrations of total organic halogen and

30   HAA remained stable. On the contrary, total HAL concentrations decreased due to the instability

31   of bromated HALs. Decreasing the specific UV absorbance (SUVA) value of AOM favours

32   bromine substitution since bromine more preferentially reacts with low reactivity organic matter

33   than chlorine. Increasing the pH enhanced the formation THMs but decreased the formation of

34   HANs. Concentrations of HAN and HAL decreased at high pH (e.g., 9.0), high initial chlorine

35   concentration and long reaction time due to the decomposition. Based on the cytotoxicity

36   calculations, unregulated HANs and HALs were the main contributors for the total toxicity of

37   DBP measured, even though based on the weight regulated THM and HAA predominated.

38   *Keywords:* disinfection byproducts, algal organic matter, bromide, iodide, total organic halogen,

39   bromine substitution

1. Introduction

The frequent occurrence of algal blooms in freshwater and marine water worldwide poses a challenge to water supply (Chapra et al. 2017, Paerl and Huisman 2008). In an algal bloom event, the addition of oxidants (e.g., ozone, chlorine, and permanganate) to untreated water prior to coagulation has been shown to increase the removal of algae since these oxidants alter the surface charge of algae thus improving their removal during coagulation (Henderson et al. 2008a, Qi et al. 2016b). However, the oxidants can also damage or lyse the algae cells, resulting in the release of bulk algal organic matter (AOM) (Coral et al. 2013, Daly et al. 2007, Qi et al. 2016a, Xie et al. 2013). AOM is comprised of a wide variation of proteins, carbohydrates, lipids, nucleic acids and other dissolved organic substances (Henderson et al. 2008b, Her et al. 2004). Thus, AOM exhibits a more hydrophilic character and less aromatic carbon content, as evidenced by much lower specific UV absorbance (SUVA) values (<2.0 L/(mg m)) and higher heterogeneity (Fang et al. 2010b, Li et al. 2012, Nguyen et al. 2005), in contrast with terrestrial natural organic matter (NOM) which is derived from lignin and contains a high aromatic content (Leenheer and Croué 2003). The hydrophilic organic carbon fraction is less prone to coagulation and is recalcitrant to conventional treatment process (Lee and Westerhoff 2006, Widrig et al. 1996). Therefore, the fraction of AOM over the bulk dissolved organic matter (DOM) might increase after a conventional treatment train.

Chlorine disinfection after conventional treatment is commonly used to provide hygienically safe drinking water. However, chlorine reacts with DOM to produce toxic halogenated disinfection byproducts (DBPs) (Liu et al. 2017, Richardson and Postigo 2015, Wagner and Plewa 2017). In addition to four trihalomethanes (THM4) and five haloacetic acids (HAA5), which are currently regulated by the United States Environmental Protection Agency (US EPA)

63 (U.S. Environmental Protection Agency 2001), unregulated DBPs including haloacetaldehydes

64 (HALs), haloketones (HKs), haloacetonitriles (HANs), and halonitromethanes (HNMs) were of

65 particular concern due to their much higher toxicity even though their concentrations are much

66 lower than regulated DBPs (Plewa et al. 2017).

67   Bromide (Br⁻) and iodide (I⁻) levels in fresh surface waters which are highly variable range

68 from <10 to >1000 µg/L and 0.5 to 20 µg/L, respectively (Liu et al. 2014, Liu et al. 2012).

69 However, the median concentrations of Br⁻ and total iodine (including I⁻ and iodate) are much

70 higher in sea water (e.g., ca. 66 mg/L and 60 µg/L, respectively) (Heeb et al. 2014, Liu et al.

71 2014). In addition, anthropogenic activities such as hydraulic fracturing, coal-fired power plants,

72 and wastewater effluent discharge may lead to the elevated halide concentrations in the

73 downstream surface waters (Good and VanBriesen 2016, Harkness et al. 2015, Vidic et al.

74 2013). For example, discharge from hydraulic fracturing has high concentrations of Br⁻ (up to 1.9

75 g/L) and I⁻ (up to 54 mg/L) (Harkness et al. 2015), leading to an increase in Br⁻ concentrations in

76 surface waters in Pennsylvania, USA (Vidic et al. 2013). In the algal bloom event and source

77 water impacted by high concentration of Br⁻ and I⁻, the blending/introduction of bromide and

78 iodide-enriched water to algal-impacted surface waters may bring elevated levels of Br⁻ and I⁻

79 and AOM to potable water.

80   During chlorination process, naturally occurring Br⁻ and I⁻ can be oxidized to hypobromous

81 and hypoiodous acids (HOBr and HOI, respectively). They can react with terrestrial NOM to

82 form brominated and iodinated DBPs (Br-DBPs and I-DBPs), respectively, which are more

83 cytotoxic and genotoxic, as a group, than their chlorinated analogues (Liu and Croue 2016,

84 Plewa et al. 2004, Richardson et al. 2008, Wagner and Plewa 2017). Previous studies indicated

85 that AOM could serve as important precursors of DBPs under formation potential conditions

4

86   (Fang et al. 2010a, Fang et al. 2010b, Hoehn et al. 1980, Hong et al. 2008, Huang et al. 2009, Li

87   et al. 2012, Nguyen et al. 2005, Plummer and Edzwald 2001, Tomlinson et al. 2016, Wert and

88   Rosario-Ortiz 2013). Among these, a few have studied the formation and speciation of THM and

89   HAA at single initial Br^- concentration (100 μg/L (Wert and Rosario-Ortiz 2013) and 480 μg/L

90   (Huang et al. 2009)). However, high initial concentrations of chlorine employed in these studies

91   result in a low $[Br^-]/[Cl_2]$ ratio. There is limited information regarding to the formation and

92   speciation of DBPs from AOM under various initial Br^- concentrations, especially corresponding

93   to high $[Br^-]/[Cl_2]$ ratios. Due to the distinct nature of AOM with NOM, it is unclear how Br^- and

94   I^- will affect formation and speciation of regulated and unregulated DBPs from AOM. An

95   understanding of how the halogen competes for the AOM to produce halogenated DBPs still

96   lacks.

97   The objectives of this study were to investigate the effect of Br^- and I^- on the formation and

98   speciation of regulated THMs and HAAs, unregulated iodinated THMs (I-THMs), HALs, HANs,

99   HKs, and HNMs, and total organic halogen (TOX) during chlorination of AOM derived from

100   three freshwater and marine algae. Furthermore, the effect of AOM type, pH, initial chlorine

101   concentration, and reaction time on the formation of DBPs was studied. Based on the

102   information of measured DBP, the theoretical cytotoxicity assessment was performed to evaluate

103   the significance of DBPs with respect to their potential contribution to toxicity.

104

105   **2. Materials and methods**

106   **2.1.   Reagents**

107 All chemical solutions were prepared from reagent grade chemicals or stock solutions using

108 deionized Milli-Q (MQ) water (18.2 MΩ·cm, Millipore). The descriptions of all other standards

109 and reagents used in this study are provided in Text S1 of the supplementary material.

110 **2.2.   Selection and Culturing of Algae and Preparation of AOM Solutions**

111 Three algae species, *Microcystis aeruginosa* (MA, freshwater cyanobacteria), *Synechococcus*

112 (SYN, seawater cyanobacteria) and *Alexandrium tamarense* (AT, seawater dinoflagellate) were

113 selected in this study based on their occurrence, potency to produce algal bloom and

114 environmental importance. MA is one of the most popular and problematic algae species in fresh

115 water (Li et al. 2012). SYN is among the most common type of picoplankton existing in the open

116 sea (Wang et al. 2011), and AT which can cause red tide is one of the most studied marine

117 dinoflagellate groups (John et al. 2014). MA (Strain: LB 2061) and SYN (Strain: LB 2380) were

118 purchased from Culture Collection of Algae at the University of Texas, Austin, TX, USA, while

119 AT (Strain: CCAP 1119/32) was provided by Culture Collection of Algae and Protozoa,

120 Scotland, UK. They were cultured in lab conditions to simulate the algal bloom in freshwater and

121 marine water. Additional information regarding the algae, culturing conditions, and extraction

122 and characteristics of AOM can be found in the supplementary material (Text S2, and Tables S1-

123 2).

124 **2.3.   Analytical methods**

125 The analytical methods of residual oxidants, total organic carbon, total dissolved nitrogen, AOM

126 characterization, concentrations of anions (Cl$^-$, Br$^-$, and I$^-$, etc), TOX (specifically, chlorine,

127 bromine, and iodine, i.e., TOCl, TOBr, and TOI, respectively), and DBPs can be found in detail

128 in Text S3 of the supplementary material. Four THMs (chloroform (TCM),

129 bromodichloromethane (BDCM), dibromochloromethane (DBCM), and bromoform (TBM)),

130 nine HAAs (monochloro-, dichloro-, trichloro-, monobromo-, dibromo-, bromochloro-,

131 bromodichloro-, dibromochloro-, and tribromo-acetic acids (MCAA, DCAA, TCAA, MBAA,

132 DBAA, BCAA, BDCAA, DBCAA, and TBAA, respectively)), six I-THMs

133 (dichloroiodomethane (DCIM), bromochloroiodomethane (BCIM), dibromoiodomethane

134 (DBIM), chlorodiiodomethane (CDIM), bromodiiodomethane (BDIM), and iodoform (TIM)),

135 six HANs (monochloro-, dichloro-, trichloro-, monobromo-, dibromo-, and bromochloro-

136 acetonitrile (CAN, DCAN, TCAN, BAN, DBAN, and BCAN, respectively), four HALs

137 (trichloro-, bromodichloro-, dibromochloro-, and tribromo-acetaldehyde (TCAL, BDCAL,

138 DBCAL, and TBAL, respectively)), two HKs (1,1-dichloro-2-propanone (1,1-DCP), and 1,1,1-

139 trichloroacetone (1,1,1-TCP)), and one HNMs (trichloronitromethane, TCNM) were quantified.

140 **2.4.   Experimental Setup and Procedures**

141 The role of AOM type, initial chlorine and Br^- concentrations, pH and reaction time on the

142 formation of DBPs from various AOM (dissolved organic carbon (DOC) = 2 mg/L) in the

143 presence of 0.4 μM I^- was investigated. Initial concentrations of chlorine and Br^- ranged from 14

144 to 224 μM (i.e., 1.0-16.0 mg/L as Cl_2) and 0 to 10 μM (i.e., 0-800 μg/L), respectively. The pH

145 was adjusted to 6.0, 7.5 and 9.0 with HNO_3 or NaOH solutions in the presence of a 10.0 mM

146 tetraborate or phosphate buffer.

147 All experiments were conducted in the dark in 250 mL of capped amber bottles under

148 headspace-free conditions at room temperature (21±1 °C). Reactions were initiated by the

149 injection of an aliquot of chlorine stock solution, to the buffered solutions containing AOM in

150 the presence or absence of Br^- and I^-. Samples were withdrawn at preselected time points

151 (ranging from 0.5 to 72 h), and then samples were analysed for residual oxidant (i.e., sum of free

7

152   chlorine, bromine or iodine) concentrations. An equimolar ascorbic acid was added immediately

153   to quench the residual for DBP measurements. Each experiment was performed in duplicate.

154   **2.5.   Calculations of bromine substitution factor**

155   Bromine substitution factor (BSF, between 0 and 1) is defined as the ratio of the molar

156   concentration of bromine incorporated into a given class of DBP to the total molar

157   concentrations of chlorine and bromine in that class (Hua et al. 2006), as shown in Equation 1.

$$BSF = \frac{\sum Br_{DBP}}{\sum Br_{DBP} + \sum Cl_{DBP}}$$

158   1

159   When THM was used as an example, molar concentrations of bromine and chlorine could be

160   calculated by Equations 2 and 3, respectively.

$$\sum Br_{THM} = [BDCM] + 2[DBCM] + 3[TBM]$$

161   2

$$\sum Cl_{THM} = 3[TCM] + 2[BDCM] + [DBCM]$$

162   3

163   **2.6.   Calculations of theoretical cytotoxicity**

164   The theoretical cytotoxicity assessment was performed by dividing measured molar

165   concentrations of each group of DBPs by concentrations (i.e., LC 50 value) determined in

166   toxicological assays and assuming that toxicity is additive (Yeatts et al. 2010). The LC 50 value

167   is the concentration of each individual DBP inducing a 50% reduction in the density of Chinese

168   Hamster Ovary cells for 72h (Wagner and Plewa 2017). The LC 50 values of individual DBPs

169   (THMs, HAAs, HANs, HAL, I-THMs, and HNM) were available in the literature (Wagner and

170   Plewa 2017), which are also presented in the Table S3 of supplementary material. This approach

171   has been used to assess toxicity of measured DBPs and evaluate the contribution of individual

8

172 DBPs to the total DBP-associated toxicity (Chuang and Mitch 2017, Krasner et al. 2016b, Smith

173 et al. 2010).

174

175 **3. Results and discussion**

176 **3.1.   Effect of AOM type on DBP formation**

177 *3.1.1. THMs*

178 Figure 1 shows the concentrations of DBP formed from AOM extracted from three types of

179 algae species in the presence of various initial Br⁻concentrations, and Figure S1 of supplementary

180 material presents the residual oxidant after 24 h reaction. Residual oxidant concentrations were

181 in the ranges of 8.0-22.7 μM (i.e., 0.6 – 1.6 mg/L Cl_2), indicating that there is enough oxidant to

182 allow the reaction to produce DBPs. Generally, increasing initial Br⁻ concentration led to an

183 enhanced chlorine demand since bromine reacts much faster than chlorine (Westerhoff et al.

184 2004). The concentrations of THMs in the presence of MA AOM solution, ranging from

185 74.1(±1.1) to 174.8(±6.9) nM (i.e., from 8.8 (±0.2) to 36.3 (±1.4) μg/L), are slightly lower than

186 those produced from two seawater algae, i.e., SYN (113.1(±1.0)-202.0(±40.8) nM) and AT

187 (111.8(±1.8)-192.9(±3.6) nM). This can be ascribed to more THM precursors available in AOM

188 extracted from SYN and AT.

189   Increasing the initial Br⁻ concentration increased the formation of total THMs (Figure 1a),

190 which is similar to previous studies on the THM formation from natural waters (Hu et al. 2010,

191 Hua et al. 2006). For example, increasing the initial Br⁻ concentration from 0 to 10 μM, the

192 formation of total THMs from MA AOM increased from 74.1±1.1 nM (i.e., 8.8 ±0.2 μg/L) to

193 174.8±6.9 nM (i.e., 36.3 ±1.4 μg/L), respectively. In the absence of Br⁻ (only for MA) only

194 TCM was formed from the chlorination of AOM. Increasing initial Br⁻ concentration led to less

9

195 TCM formation but enhanced the formation of brominated THMs. At an initial Br^- concentration

196 of 2.5 µM, chlorine concentration ($[HOCl]_0$= 55 µM) was 20 times higher than the formed

197 bromine. However, the formed BDCM was close to that of DBCM (16.1 nM vs 17.6 nM),

198 indicating that the ability of bromine to produce THMs from AOM is at least 20 times higher

199 than chlorine.

200

201 *3.1.2. HAAs*

202 MA AOM produced higher concentrations of total HAAs than the two seawater algae (Figure

203 1b). For example, total HAA concentrations in the presence of MA AOM ranged from 112.5±3.3

204 nM to 118.6±5.5 nM, corresponding to concentrations ranging from 16.8 ±0.5 to 22.6 ±0.9 µg/L,

205 while those for SYN and AT were 74.3(±7.4) - 87.1(±8.0) and 75.7(±7.4)- 85.4(±5.5) nM,

206 respectively. It was reported that increasing the aromaticity of NOM (i.e., SUVA) enhanced the

207 formation of THMs and HAAs (Liu and Croue 2016). However, the similar trend was not

208 observed for AOM. For example, MA has the highest SUVA among the three AOM solutions,

209 produced higher HAAs, but lower THMs. It was reported that THM and HAA can be formed

210 from different precursors (Hua and Reckhow 2007). Therefore, more HAA precursors in MA

211 AOM led to the higher formation of HAAs. In terms of DOC, NOM comprised of > 50% of

212 humics which contain aromatic/phenolic and carboxyl group contents and are the THM/HAA

213 precursors (Leenheer and Croué 2003, Liu and Croue 2016). However, humic substances only

214 account for 14%-22% of the total DOC in AOM (Figure S2, supplementary material). Therefore,

215 no relationship was observed between SUVA and DBPs produced for AOM. Considering also

216 the narrow SUVA range of AOM, SUVA does not appear to be a good surrogate to characterize

217 the DBP formation.

218   When the initial Br^- concentration increased total HAA concentrations remained stable for

219   any algae species, but the formation of HAAs was shifted from chlorinated species to brominated

220   analogues. All nine HAA species were measured, and their concentrations vary according to the

221   initial Br^- concentration. Nine species of HAAs were grouped as the mono- (MXAA, including

222   MCAA and MBAA), di- (DXAA, including DCAA, DBAA, and BCAA), and tri-halogenated

223   acetic acids (TXAA, including TCAA, BDCAA, DBCAA, and TBAA) since each group of HAA

224   may be formed through similar chemical pathways. Interestingly, the ratios of MXAA, DXAA

225   and TXAA over total HAAs were relatively stable, irrespective of initial Br^- concentrations, as

226   shown in Figure S3 of supplementary material, in agreement with a previous study on HAA

227   formation from NOM (Cowman and Singer 1995). Generally, TXAA accounted for comparable

228   fraction (ranging from 40% to 50%) to total HAA pool with DXAA, while MXAA only

229   accounted for c.a. 20% of total HAAs. This indicates that TXAA and DXAA were the main

230   species, while MXAA is the minor species.

231   *3.1.3. HANs*

232   AOM extracted from three algae produced comparable total HANs (ranging from 25 to 27 nM)

233   at lower initial Br^- concentrations (Figure 1c). When $[Br^-]_0 = 10\ \mu M$, however, concentrations of

234   total HANs from MA (e.g., 42.9±1.7 nM) were higher than SYN (e.g., 36.7±8.7 nM) and AT

235   (e.g., 31.1±2.2 nM), likely due to more precursor for brominated HANs available from MA

236   AOM. Based on a DBP survey in Europe, the weight ratio of DCAN to TCM was ~10% on a

237   median basis (Krasner et al. 2016a). In this study, this ratio ranged from 22%-28%, which could

238   be attributed to the presence of more N-containing organics (e.g., amino acids) in AOM than

239   NOM.

11

240   Increasing the initial Br⁻ concentration increased the formation of total HANs. For example,

241   increasing the initial Br⁻ concentration from 0 to 10 μM, the formation of total HANs from MA

242   AOM increased from 25.7±0.3 nM (i.e., 2.7 ±0.1 μg/L) to 42.9±1.7 nM (i.e., 6.4 ±0.3 μg/L).

243   This indicates that bromine favours the formation of HANs, as compared to chlorine.

244   Dihalogenated acetonitrile (DHAN) was the major species of HANs, while mono-halogenated

245   acetonitrile (MHAN) was the minor species, and tri-halogenated acetonitrile was not even

246   detected.

247   *3.1.4. HALs*

248   HALs were reported to be third largest group of DBPs based on weight, following THMs and

249   HAAs (Krasner et al. 2006). However, AOM produced comparable molar concentrations of

250   HALs with total THMs in this study at lower initial Br⁻ concentrations (Figure 1d). At ambient

251   Br⁻ concentration, SYN produced higher concentrations of HALs (92.7±8.7 nM, i.e., 14.5 ±1.4

252   μg/L) than MA and AT did (74.0±5.7 and 57.4±3.3 nM, respectively), probably because SYN

253   has the highest dissolved organic nitrogen among the three types of AOM (Table S2,

254   supplementary material). It was reported that amino acids can serve as the precursors of TCAL

255   (Trehy et al. 1986). Again, there is no trend between SUVA and HAL formation.

256   Different from other groups of DBPs (THM, HAA, and HAN). Increasing the initial Br⁻

257   concentration decreased the concentration of total HALs, due to the decomposition of

258   brominated HALs. The stability of HALs at neutral pH follows the sequence: TCAL> BDCAL >

259   DBCAL> TBAL (Koudjonou and LeBel 2006, Xie 2016). THM was the corresponding

260   hydrolysis product (Barrott 2004). Due to its instability, concentrations of TBAL were < 3 nM

261   (i.e., 0.8 μg/L) in this study.

262   *3.1.5. I-THMs, HKs, and HNMs*

12

263   It was seen from Figure 1e that concentrations of I-THMs were relatively low (< 8 nM, i.e., 1.7

264   µg/L), and only DCIM and CDIM were measured. Reaction between HOI and DOM to form I-

265   THMs is in competition with the oxidation of HOI by chlorine or bromine (Bichsel and von

266   Gunten 1999, Criquet et al. 2012). However, in chlorination system (in the presence of Br$^-$),

267   the latter pathway is much faster. Based on kinetic model calculations (Liu et al. 2014), the

268   calculated times for the transformation of 90% of initial I$^-$ to IO_3^- are 24, 14, 6, 4, and 2 min for

269   $[Br^-]_0$ = 0, 0.5, 2.5, 5.0, and 10 µM, respectively. This time range was certainly not enough for

270   HOI reactions with AOM to produce a significant amount of I-DBPs, and therefore iodate was

271   the main sink. Iodate is non-toxic, and is therefore the desired sink for iodine in drinking waters

272   (Burgi et al. 2001).

273   Concentrations of HKs (e.g., 1,1,1-TCP and 1,1-DCP) and HNMs (e.g., TCNM) in the

274   presence of any AOM were below 5 nM (data not shown). Therefore, the formation of HKs and

275   HNMs was relatively negligible for the AOM tested in this study, and the brominated analogues

276   were not measured.

277   *3.1.6. TOX*

278   Figure 1f shows the formation of TOX, TOCl and TOBr during chlorination of AOM from three

279   species of algae. MA produced the highest TOX (2.7-2.8 µM) among the three AOM, followed

280   by SYN (2.2-2.7 µM) and AT (2.1-2.4 µM). For any algae species, TOX formation is generally

281   stable irrespective of initial Br$^-$ concentrations due to the constant concentrations of precursors.

282   However, increasing initial Br$^-$ concentrations shifted the formation of TOX from chlorinated

283   species to brominated analogues. It was noted that TOI was below 0.079 µM (i.e., 10 µg/L as I)

284   and iodate was the main sink of total iodine, which agrees with the insignificant formation of I-

285   THMs shown in Figure 1e.

286   Figure S4 of supplementary material shows the fraction of each group of DBP of total TOCl

287   and TOBr. At lower initial Br$^-$ concentration, THM, HAL and HAA are the main groups of

288   DBPs in the total TOCl, while at higher initial Br$^-$ concentrations, THM are the main groups of

289   DBPs. For the TOBr pool, the main group of DBPs formed is THM. Unknown TOCl/TOBr was

290   calculated from the difference between the TOCl/TOBr and the sum of halogen-equivalent

291   concentrations of measured specific DBPs. Unknown TOCl/TOBr accounted for > 60% of total

292   TOCl/TOBr due to the hydrophilic nature of AOM. Generally increasing the initial Br$^-$

293   concentration decreased the percentage of unknown TOBr, indicating that the formation of

294   known brominated DBPs (e.g., THM, HAN, etc) was favoured in bromination process.

295   *3.1.7. BSF*

296   Figure 2 presents the comparison of BSF among THM, DHAN, DHAA, THAA, HAL, and TOX.

297   As expected, increasing the initial Br$^-$ concentrations increased the BSF of any class of DBPs.

298   Also, increasing the SUVA generally decreased the BSF of any class of DBPs. This indicates

299   that bromine preferentially reacts with organic matter moiety with low reactivity, which can be

300   explained by the difference in the reactivity of chlorine and bromine toward AOM. Bromine is a

301   much stronger substuting halogen than chlorine (Westerhoff et al. 2004). AOM with a low

302   reactivity (i.e., SUVA) enriched in electron withdrawing groups, e.g., amino acid, would lead to

303   a higher degree of bromination than chlorination. When the BSF values of THM formed from

304   AOM were compared with those obtained from chlorination of surface water with higher SUVA

305   (from 2.1 to 5.1) at similar conditions (Ersan et al. 2018), AOM exhibits higher BSF (especially

306   when $[Br^-]_0 \leq 5.0$ μM) than NOM does (Figure S5, supplementary material). This further

307   confirms that bromine preferentially reacts with DOM with lower SUVA, leading to a higher

308   BSF.

309   Three trihalogenated DBP classes exhibited distinct BSF patterns with increasing initial Br⁻

310   concentrations. The BSFs of THM was higher than those of THAA and HAL for three types of

311   AOM (Figure S6, supplementary material). It indicates that bromine substitution into THM is

312   more effective than into THAA and HAL. Also, there is a high linearity (R^2=0.976) between

313   BSFs of THM and BSFs of HAL, probably because THM is the decomposition product of HAL

314   (Barrott 2004).

315

316   **3.2.   Effect of pH under various initial bromide concentrations**

317   *3.2.1. THM*

318   Considering that three types of AOM showed comparable DBP formation pattern and MA was

319   one of the most popular and problematic algae species in fresh water, leading to harmful algae

320   bloom in many large lakes worldwide (Paerl and Paul 2012), AOM from MA was selected to

321   investigate the effect of pH (6-9) on the formation and speciation of DBPs (Figure 3). Figure S7

322   of supplementary material presents the concentrations of residual oxidants after 24 h reaction.

323   Residual oxidant concentrations ranged from 9.5 to 22.7 μM, with the highest chlorine demand

324   at pH 9. Increasing the pH increased the total concentrations of THMs at any initial Br⁻

325   concentration. For example, at initial Br⁻ concentration of 10 μM, THM4 concentrations were

326   89.8±1.3, 174.8±6.9, and 288.6±4.1 nM for pH 6.0, 7.5, and 9.0, respectively. The enolization of

327   the carbonyl moiety of AOM or the hydrolysis of intermediate (e.g., trihalocarbonyl) to THM

328   may be catalysed by hydroxide ion (Liu and Croue 2016) These reactions may play more

329   important roles in determining pH effects on THM formation.

330   *3.2.2. HAA*

15

331   Total HAA concentrations increased as the pH increased from 6.0 to 7.5 and then decreased

332   within pH range of 7.5-9.0 (Figure 3b). Among the nine species of HAA, DHAA increased from

333   pH 6.0 to 9.0, while the optimum formation of THAA was at pH 7.5. For example, DCAA

334   formation in the absence of Br^- was 28.6±0.5, 44.0±3.4, and 53.4±0.5 nM, in contrast to the

335   TCAA concentrations of 25.7±1.1, 51.6±2.0, and 17.7±1.8 nM at pH 6.0, 7.5 and 9.0,

336   respectively. This can be ascribed to the fact that DHAA formation was also hydroxide

337   catalysed, even though the catalytic effect was not pronounced as that for THM. However,

338   formation of THAA in the higher pH range (≥9.0) was inhibited, therefore, lower pH will favour

339   the oxidation of THAA precursor (such as $R-CO-CX_3$) to produce THAA if the R group is a

340   readily oxidizable functional group capable of donating an electron pair to the rest of the

341   molecule (Liang and Singer 2003). In addition, THAA may undergo the decomposition to THM

342   at higher pH (Zhang and Minear 2002).

343   *3.2.3. HAN*

344   For HAN, the effect of pH is complex (Figure 3c). At lower initial Br^- concentrations, in which

345   DCAN was the major species of HAN, HAN concentrations significantly decreased with the

346   increasing pH. In contrast, at higher initial Br^- concentrations, HAN concentrations only

347   decreased slightly with the increasing pH (e.g., 47.3±1.1, 42.9±1.7, and 41.8±0.5 nM for pH 6.0,

348   7.5 and 9.0, respectively at $[Br^-]_0 = 10$ μM). It was noted that at $[Br^-]_0 = 10$ μM, DBAN

349   concentrations increased significantly from 11.7±1.4 nM at pH 7.5 to 31.7 nM at pH 9.0, which

350   compensated the loss of total HANs. DCAN and DBAN were formed from the same precursors

351   and reaction pathway. Their distinct concentrations at higher pH can be attributed to the stability

352   in chlorinated solutions. HAN can undergo the reactions with chlorine or hydrolysis to its

353   respective haloacetamide and further to HAA and the rate constants follow the sequence:

354 TCAN> DCAN > BCAN> DBAN> MCAN> MBAN (Yu and Reckhow 2015). The fact that

355 TCAN was not detected in this study is in line with this sequence.

356 *3.2.4. HAL*

357 Figure 3d shows the effect of pH on the formation and speciation of HALs. Increasing the pH

358 from 6.0 to 7.5 increased the formation of HALs, and a further increase in pH from 7.5 to 9.0 led

359 to a decreasing HAL formation. At pH 9.0, brominated HALs were not detected owning to the

360 decomposition of brominated HALs at alkaline conditions (Xie 2016).

361 *3.2.5. TOX*

362 The concentrations of TOCl, TOBr and TOX from pH 6.0 to 9.0 are presented in Figure S8 of

363 supplementary material. The highest TOX formation (3.5-3.9 μM) was observed at pH 6.0, and

364 there was no significant difference between pH 7.5 and 9.0. This may be ascribed to the higher

365 reactivity of halogens in their molecular form. Again, increasing the initial Br^- concentration did

366 not change the total TOX formation, only shifting the formation from TOCl to TOBr. TOI was

367 below 0.079 μM (i.e., 10 μg/L as I) from pH 6.0 to 9.0. UTOX decreased as the increasing pH

368 (Figure S9, supplementary material), due to the enhanced formation of known species, i.e.,

369 THM, which is the major class of DBP identified at higher pH.

370 *3.2.6. BSF*

371 Figure S10 of supplementary material depicts the effect of pH and initial Br^- concentration on

372 chlorination DBP BSFs. For THM and DHAN, no significant difference was observed for the

373 BSFs between pH 6.0 and 7.5. However, a significant increase of BSF from pH 7.5 and 9.0

374 indicated that bromine substitution was favoured at higher pH. In addition, the lesser stability of

375 DCAN than DBAN would contribute to the higher BSF at pH 9.0. Increasing the pH also

376 increased BSFs of DHAA, THAA and HAL. This indicated that bromine outcompetes chlorine

377 at higher pH.

378

379 **3.3.   Effect of initial chlorine concentration**

380 Figure 4 shows concentrations of formed DBPs at various initial chlorine concentrations (14-224

381 μM, i.e., 1.0-16.0 mg Cl_2/L). Residual oxidant after 24 h is presented in Figure S11 of

382 supplementary material. No residual was observed for initial chlorine concentrations of 14 and

383 28 μM, while at higher initial chlorine concentrations, residual oxidant concentrations are > 19.3

384 μM. Increasing the initial chlorine concentrations from 14 to 112 μM increased THM4 from

385 194.9$\pm$6.5 to 379.9$\pm$86.4 nM (i.e., 47.6$\pm$1.6 to 90.9$\pm$20.5 μg/L), but further increasing chlorine

386 concentrations from 112 to 224 μM did not increase THM4 due to the limited precursor

387 available. Increasing the initial chlorine concentrations also increased the formation of

388 chlorinated THMs where TCM and BDCM were the major THM species.

389   Total HAA concentrations increased (5.5$\pm$0.2 to 275.3$\pm$15.9 nM, i.e., 0.6 to 43.6$\pm$3.2 μg/L)

390 with increasing initial chlorine concentrations from 14 to 224 μM (Figure 4b). Again, increasing

391 the initial chlorine concentrations also increased the formation of chlorinated HAAs where

392 MCAA, DCAA and TCAA were the major HAA species because high concentrations of chlorine

393 facilitates the chlorination of AOM moieties/intermediates.

394   Total HANs increased from 0 to 56.0$\pm$1.0 nM (i.e., 6.9$\pm$0.2 μg/L) when initial chlorine

395 concentrations increased from 14 to 112 μM (Figure 4c). However, increasing initial chlorine

396 concentration from 112 to 224 μM decreased the HAN formation to 35.8$\pm$1.1 nM (i.e., 4.3$\pm$0.2

397 μg/L), due to the reaction between HAN and chlorine or the hydrolysis of HAN (Yu and

398 Reckhow 2015). Again, increasing the initial chlorine concentrations also increased the

18

399   formation of chlorinated HANs. Therefore, CAN, DCAN and BCAN were the major HAN

400   species, while BAN and DBAN were the minor species.

401   Increasing initial chlorine concentration from 14 to 56 μM increased the formation of HAL

402   which afterwards remained stable for initial chlorine concentration from 56 to 224 μM, probably

403   due to the limited HAL precursor available (Figure 4c). The formation of chlorination HALs

404   increased when total HAL remained stable due to the increased ratio of $[Cl_2]/[Br^-]$.

405   The concentrations of TOCl, TOBr and TOX are presented in Figure S12 of supplementary

406   material. Increasing the initial chlorine concentrations from 14 to 224 μM (i.e., 1 to 16 mg/L Cl_2)

407   increased the formation of TOCl. In contrast, the formation of TOBr remained relatively stable

408   (ranging from 0.6 to 0.8 μM) owing to the constant initial Br^- concentration. TOI at $[Cl_2]_0 = 14$

409   μM was 0.13 μM (i.e., 16.6 μg/L as I). Increasing initial Cl_2 concentrations from 28 to 224 μM

410   decreased the TOI to < 0.079 μM, since high Cl_2 exposure facilitated the transformation of I^- to

411   IO_3^- (Liu et al. 2014).

412   Figure S13 of supplementary material shows the effect of initial chlorine concentration on

413   the BSFs of chlorination DBPs. Increasing the initial chlorine concentrations from 14 to 28 μM

414   (i.e., 1 to 2 mg/L Cl_2) significantly increased the BSFs of DBPs. For example, BSF of THM

415   increased from 0.23 to 0.57, and BSF of other DBPs increased from 0 to >0.5. When applied

416   chlorine dose was below 28 μM, AOM concentration was in excess of oxidant (no residual after

417   24 h). HOCl or HOBr reacts with DOM via oxidation (releasing Cl^- or Br^-) and halogen

418   substitution (forming DBPs) (Liu and Croue 2016). The recycling of Br^- to HOBr in the presence

419   of chlorine would increase the bromination of AOM (i.e., BSF). However, when oxidant was in

420   excess of organic matter ($[Cl_2]_0 > 56$ μM), further increase in the initial chlorine concentrations

19

421   gradually decreased BSF. This can be related to the increasing ratio of chlorine over formed

422   bromine.

423

424   **3.4.   Effect of reaction time**

425   Figure 5 shows the concentrations of DBPs at different reaction times. Residual oxidants were

426   available from 0.5 to 72 h (residual oxidant at 72 h= 4.2 μM, 0.3 mg Cl$_2$/L) (Figure S14,

427   supplementary material). Increasing the reaction time gradually increased the formation of THM,

428   HAA, and HAN. In contrast, HAL concertation decreased from 24 to 72 h of reaction time. The

429   concentration of DBPs depends on their formation and stability (i.e., decomposition or

430   oxidation). THMs and HAAs which were relatively stable were the final products during

431   chlorination of organic matter (Reckhow et al. 1990). Their concentrations, therefore, gradually

432   increased as a function of reaction time. Even HAN can undergo decomposition or degradation

433   (Yu and Reckhow 2015), concentrations of total HANs increased from 24 to 72 h, primarily

434   owning to the increase in BCAN and DBAN which are stable at neutral pH and low chlorine

435   concentration in a short reaction time. However, concentrations of BDCAL and DBCAL

436   decreased significantly due to the hydrolysis (Xie 2016), leading to a decrease in total HAL

437   concentration from 24 to 72 h (Figure 5d).

438   The concentrations of TOCl, TOBr and TOX are presented in Figure S15 of supplementary

439   material. Increasing the reaction time from 0.5 to 72 h increased the formation of TOCl (1.1 to

440   2.8 μM), TOBr (0.6 to 1.3 μM), and TOX (1.7 to 4.1 μM). TOI was below 0.079 μM (i.e., 10

441   μg/L as I). Figure S16 of supplementary material shows the BSFs of chlorination DBPs under

442   different reaction times. The BSFs of DBPs (except for HALs) slightly increased with reaction

20

443 time. Owning to the decomposition of brominated HALs from 24 to 72 h of reaction, the

444 corresponding BSF decreased significantly.

445

446 **3.5.   Evaluation of theoretical cytotoxicity of measured DBPs**

447 *3.5.1. Effect of initial bromide concentration in the presence of three AOM*

448 The influence of initial Br^- concentrations in the presence of three AOM on theoretical

449 cytotoxicity based on the measured DBPs is shown in Figure 6a. Increasing initial Br^-

450 concentration increased the calculated cytotoxicity of DBP measured. HAN and HAL are the

451 major contributors (>90%) to the cytotoxicity. Increasing the initial Br^- concentration increased

452 the formation of total HANs and led to the formation of brominated species. Therefore, total

453 cytotoxicity increased significantly. Even THM contributes to the similar or even higher fraction

454 of total DBPs formed based on molar concentration, because of its low cytotoxicity index

455 (Wagner and Plewa 2017), there was no significant contribution from THM. HAA is another

456 important class DBP formed based on molar concentration, but HAA only constituted < 10% of

457 cytotoxicity. Among the three types of AOM, MA and SYN present slightly higher toxicity than

458 AT at higher initial Br^- concentration. Since HAN and HAL are the controlling agent for the

459 calculated cytotoxicity, no relationship between toxicity and SUVA was found. AOM produced

460 less THM and HAA than terrestrial NOM due to the hydrophilic nature of AOM. However,

461 AOM may form higher or comparable unregulated DBPs (HAN and HALs) which controls the

462 toxicity of DBPs produced. Currently only THM and HAA are regulated, these findings indicate

463 the necessity to quantify and control the unregulated DBPs, especially in the seasonal algal

464 bloom.

465   The formation of TOI (<10 µg/L) and I-THMs (<1.7 µg/L) was low (Figure 1), and IO_3^-

466   which is non-toxic was expected to be the main sink in this study. Therefore, even iodinated

467   DBPs are more cytotoxic than their chlorinated and brominated analogues (Plewa et al. 2004,

468   Richardson et al. 2008, Wagner and Plewa 2017), I-THMs did not contribute to the total toxicity.

469   When source water is threatened by high concentration of Br^- and I^- during algal bloom events,

470   to mitigate the formation of highly toxic I-DBPs, the chlorine exposure should be optimized to

471   allow the full conversion of I^- to IO_3^- meanwhile minimize the formation of chlorinated and

472   brominated DBPs, thereby reducing the total toxicity of DBP formed.

473   *3.5.2. Effect of pH*

474   Figure 6b presents the influence of pH on total theoretical cytotoxicity from DBPs measured. In

475   the absence of Br^-, chlorinated HAN controls the total toxicity. Increasing the pH decreased the

476   concentrations of HAN due to the decomposition at higher pH, thereby decreasing the calculated

477   toxicity. At lower initial Br^- concentration (0.5 -2.5 µM), the maximum toxicity was at pH 7.5

478   due to the enhanced formation of brominated HALs. However, due to the decomposition of

479   brominated HALs at pH 9.0, the cytotoxicity decreased from pH 7.5 to 9.0. At higher initial Br^-

480   concentrations HAN is the controlling agent for the cytotoxicity. Brominated HAN such as

481   DBAN, which is relatively stable, dominates the toxicity. Increasing formation of DBAN led to

482   the increased calculated cytotoxicity.

483   *3.5.3. Effect of initial chlorine concentration*

484   The influence of initial chlorine concentrations on calculated cytotoxicity of DBP measured is

485   shown in Figure 6c. When applied chlorine dose was ≤ 28 µM (AOM concentration was in

486   excess of oxidant), increasing the initial chlorine concentration significantly increased the

487   calculated toxicity where HAN is the controlling agent. Further increasing the initial chlorine

488   concentration from 56 to 224 µM decreased the calculated toxicity due to the less contribution

489   from HAL. Increasing the initial chlorine concentration shifted the formation of HAL from

490   brominated species to TCAL. The LC 50 of brominated HALs was 2 orders of magnitude lower

491   than TCAL (Table S3, supplementary material). This may indicate that excess amount of

492   chlorine may favour the reduction of toxicity since DBP speciation was shifted from brominated

493   species to chlorinated analogues and toxic HANs and HALs can be degraded by chlorine.

494   *3.5.4. Effect of reaction time*

495   Figure 6d shows that the calculated cytotoxicity of measured DBPs increased as a function of

496   reaction time. Again, HAN is the controlling agent for the calculated toxicity due to its low LC

497   50 (Plewa et al. 2017). The increase in toxicity from HAN led to an increasing toxicity of

498   measured DBPs as a function of reaction time. From 24 to 72 h, even the contribution for the

499   toxicity from HAL decreased owning to the decomposition of HALs, the significant increase

500   from HAN compensates the loss of toxicity from HAL.

501   It should be noted that the theoretical cytotoxicity was calculated based on the measured

502   DBPs, which however only accounts for partial TOX. Therefore, it is of great importance to

503   consider the toxicity of all formed DBPs. Experimental evaluations of cyto-toxicity and geno-

504   toxicity of DBP mixture for selected water samples may be addressed in future studies.

505

4. Conclusions

506

507   Comparable DBP formation was observed from chlorination of AOM extracted from three

508   species of freshwater and marine algae. THMs, HAAs, HALs, and HANs were the main groups

509   of DBPs formed, even though HANs were formed at relatively lower concentrations than the

510   former three groups in the absence of Br⁻. In contrast, the formation of other DBPs (e.g., I-

23

511 THMs, HKs and HNMs) was low (<8 nM). Increasing the initial Br$^-$ concentration decreased the

512 formation of HAL but increased HAN and THM. Unregulated HANs and HALs were relatively

513 less stable than regulated THMs and HAAs. Therefore, their concentrations decreased at higher

514 pH and chlorine residual and longer reaction time. When measured DBPs were weighed against

515 their toxicity index, regulated THMs and HAAs only account for < 10% of total calculated

516 toxicity, and unregulated HANs and HALs were the controlling agents for the calculated

517 toxicity.

518

523 **Supplementary data**

524 Supplementary data related to this article can be found on line.

References

Barrott, L. (2004) Chloral hydrate: Formation and removal by drinking water treatment. Journal of Water Supply: Research and Technology - Aqua 53(6), 381-390.

Bichsel, Y. and von Gunten, U. (1999) Oxidation of iodide and hypoiodous acid in the disinfection of natural waters. Environmental Science & Technology 33(22), 4040-4045.

Burgi, H., Schaffner, T. and Seiler, J.P. (2001) The toxicology of iodate: A review of the literature. Thyroid 11(5), 449-456.

Chapra, S.C., Boehlert, B., Fant, C., Bierman, V.J., Henderson, J., Mills, D., Mas, D.M.L., Rennels, L., Jantarasami, L., Martinich, J., Strzepek, K.M. and Paerl, H.W. (2017) Climate Change Impacts on Harmful Algal Blooms in U.S. Freshwaters: A Screening-Level Assessment. Environmental Science & Technology 51(16), 8933-8943.

Chuang, Y.-H. and Mitch, W.A. (2017) Effect of Ozonation and Biological Activated Carbon Treatment of Wastewater Effluents on Formation of N-nitrosamines and Halogenated Disinfection Byproducts. Environmental Science & Technology 51(4), 2329-2338.

Coral, L.A., Zamyadi, A., Barbeau, B., Bassetti, F.J., Lapolli, F.R. and Prévost, M. (2013) Oxidation of Microcystis aeruginosa and Anabaena flos-aquae by ozone: Impacts on cell integrity and chlorination by-product formation. Water Research 47(9), 2983-2994.

Cowman, G.A. and Singer, P.C. (1995) Effect of Bromide Ion on Haloacetic Acid Speciation Resulting from Chlorination and Chloramination of Aquatic Humic Substances. Environmental Science & Technology 30(1), 16-24.

Criquet, J., Allard, S., Salhi, E., Joll, C.A., Heitz, A. and von Gunten, U. (2012) Iodate and iodo-trihalomethane formation during chlorination of iodide-containing waters: Role of bromide. Environmental Science & Technology 46(13), 7350-7357.

Daly, R.I., Ho, L. and Brookes, J.D. (2007) Effect of Chlorination on Microcystis aeruginosa Cell Integrity and Subsequent Microcystin Release and Degradation. Environmental Science & Technology 41(12), 4447-4453.

Ersan, M., Liu, C., Amy, G. and Karanfil, T. (2018) The interplay between natural organic matter and bromide on bromine substitution. In preparation.

Fang, J., Ma, J., Yang, X. and Shang, C. (2010a) Formation of carbonaceous and nitrogenous disinfection by-products from the chlorination of Microcystis aeruginosa. Water Research 44(6), 1934-1940.

Fang, J., Yang, X., Ma, J., Shang, C. and Zhao, Q. (2010b) Characterization of algal organic matter and formation of DBPs from chlor(am)ination. Water Research 44(20), 5897-5906.

Good, K.D. and VanBriesen, J.M. (2016) Current and Potential Future Bromide Loads from Coal-Fired Power Plants in the Allegheny River Basin and Their Effects on Downstream Concentrations. Environmental Science & Technology 50(17), 9078-9088.

Harkness, J.S., Dwyer, G.S., Warner, N.R., Parker, K.M., Mitch, W.A. and Vengosh, A. (2015) Iodide, Bromide, and Ammonium in Hydraulic Fracturing and Oil and Gas Wastewaters: Environmental Implications. Environmental Science & Technology 49(3), 1955-1963.

Heeb, M.B., Criquet, J., Zimmermann-Steffens, S.G. and von Gunten, U. (2014) Bromine production during oxidative water treatment of bromide-containing waters and its reactions with inorganic and organic compounds: A critical review. Water Research 48(1), 15-42.

Henderson, R., Parsons, S.A. and Jefferson, B. (2008a) The impact of algal properties and pre-oxidation on solid–liquid separation of algae. Water Research 42(8–9), 1827-1845.

Henderson, R.K., Baker, A., Parsons, S.A. and Jefferson, B. (2008b) Characterisation of algogenic organic matter extracted from cyanobacteria, green algae and diatoms. Water Research 42(13), 3435-3445.

Her, N., Amy, G., Park, H.-R. and Song, M. (2004) Characterizing algogenic organic matter (AOM) and evaluating associated NF membrane fouling. Water Research 38(6), 1427-1438.

Hoehn, R.C., Barnes, D.B., Thompson, B.C., Randall, C.W., Grizzard, T.J. and Shaffer, P.T.B. (1980) Algae as Sources of Trihalomethane Precursors. Journal of American Water Works Association 72(6), 344-350.

Hong, H.C., Mazumder, A., Wong, M.H. and Liang, Y. (2008) Yield of trihalomethanes and haloacetic acids upon chlorinating algal cells, and its prediction via algal cellular biochemical composition. Water Research 42(20), 4941-4948.

Hu, J., Song, H. and Karanfil, T. (2010) Comparative Analysis of Halonitromethane and Trihalomethane Formation and Speciation in Drinking Water: The Effects of Disinfectants, pH, Bromide, and Nitrite. Environmental Science & Technology 44(2), 794-799.

Hua, G. and Reckhow, D.A. (2007) Characterization of Disinfection Byproduct Precursors Based on Hydrophobicity and Molecular Size. Environmental Science & Technology 41(9), 3309-3315.

Hua, G., Reckhow, D.A. and Kim, J. (2006) Effect of bromide and iodide ions on the formation and speciation of disinfection by-products during chlorination. Environmental Science & Technology 40, 3050–3056.

Huang, J., Graham, N., Templeton, M.R., Zhang, Y., Collins, C. and Nieuwenhuijsen, M. (2009) A comparison of the role of two blue–green algae in THM and HAA formation. Water Research 43(12), 3009-3018.

John, U., Litaker, R.W., Montresor, M., Murray, S., Brosnahan, M.L. and Anderson, D.M. (2014) Formal revision of the Alexandrium tamarense species complex (Dinophyceae) taxonomy: the introduction of five species with emphasis on molecular-based (rDNA) classification. Protist 165(6), 779-804.

Koudjonou, B.K. and LeBel, G.L. (2006) Halogenated acetaldehydes: Analysis, stability and fate in drinking water. Chemosphere 64(5), 795-802.

Krasner, S.W., Kostopoulou, M., Toledano, M.B., Wright, J., Patelarou, E., Kogevinas, M., Villanueva, C.M., Carrasco-Turigas, G., Santa Marina, L., Fernandez-Somoano, A., Ballester, F., Tardon, A., Grazuleviciene, R., Danieviciute, A., Cordier, S., Costet, N., Righi, E., Aggazzotti, G., Stephanou, E.G., Kargaki, S. and Nieuwenhuijsen, M.J. (2016a) Occurrence of DBPs in Drinking Water of European Regions for Epidemiology Studies. Journal of American Water Works Association 108(10), E501-E512.

Krasner, S.W., Lee, T.C.F., Westerhoff, P., Fischer, N., Hanigan, D., Karanfil, T., Beita-Sandí, W., Taylor-Edmonds, L. and Andrews, R.C. (2016b) Granular Activated Carbon Treatment May Result in Higher Predicted Genotoxicity in the Presence of Bromide. Environmental Science & Technology 50(17), 9583-9591.

Krasner, S.W., Weinberg, H.S., Richardson, S.D., Pastor, S.J., Chinn, R., Sclimenti, M.J., Onstad, G.D. and Thruston, A.D., Jr. (2006) Occurrence of a new generation of disinfection byproducts. Environmental Science & Technology 40(23), 7175-7185.

Lee, W. and Westerhoff, P. (2006) Dissolved organic nitrogen removal during water treatment by aluminum sulfate and cationic polymer coagulation. Water Research 40(20), 3767-3774.

Leenheer, J.A. and Croué, J.-P. (2003) Peer Reviewed: Characterizing Aquatic Dissolved Organic Matter. Environmental Science & Technology 37(1), 18A-26A.

Li, L., Gao, N., Deng, Y., Yao, J. and Zhang, K. (2012) Characterization of intracellular & extracellular algae organic matters (AOM) of Microcystic aeruginosa and formation of AOM-associated disinfection byproducts and odor & taste compounds. Water Research 46(4), 1233-1240.

Liang, L. and Singer, P.C. (2003) Factors Influencing the Formation and Relative Distribution of Haloacetic Acids and Trihalomethanes in Drinking Water. Environmental Science & Technology 37(13), 2920-2928.

Liu, C. and Croue, J.-P. (2016) Formation of bromate and halogenated disinfection byproducts during chlorination of bromide-containing waters in the presence of dissolved organic matter and CuO. Environmental Science & Technology 50(1), 135–144.

Liu, C., Olivares, C.I., Pinto, A.J., Lauderdale, C.V., Brown, J., Selbes, M. and Karanfil, T. (2017) The control of disinfection byproducts and their precursors in biologically active filtration processes. Water Research 124, 630-653.

Liu, C., Salhi, E., Croué, J.-P. and von Gunten, U. (2014) Chlorination of iodide-containing waters in the presence of CuO: Formation of periodate. Environmental Science & Technology 48(22), 13173-13180.

Liu, C., von Gunten, U. and Croué, J.-P. (2012) Enhanced bromate formation during chlorination of bromide-containing waters in the presence of CuO: Catalytic disproportionation of hypobromous acid. Environmental Science & Technology 46(20), 11054-11061.

Nguyen, M.-L., Westerhoff, P., Baker, L., Hu, Q., Esparza-Soto, M. and Sommerfeld, M. (2005) Characteristics and Reactivity of Algae-Produced Dissolved Organic Carbon. Journal Of Environmental Engineering 131(11), 1574-1582.

Paerl, H.W. and Huisman, J. (2008) Blooms like it hot. Science 320(5872), 57-58.

Paerl, H.W. and Paul, V.J. (2012) Climate change: Links to global expansion of harmful cyanobacteria. Water Research 46(5), 1349-1363.

Plewa, M.J., Wagner, E.D. and Richardson, S.D. (2017) TIC-Tox: A preliminary discussion on identifying the forcing agents of DBP-mediated toxicity of disinfected water. Journal of Environmental Sciences.

Plewa, M.J., Wagner, E.D., Richardson, S.D., Thruston, A.D., Woo, Y.T. and McKague, A.B. (2004) Chemical and biological characterization of newly discovered Iodoacid drinking water disinfection byproducts. Environmental Science & Technology 38(18), 4713-4722.

Plummer, J.D. and Edzwald, J.K. (2001) Effect of Ozone on Algae as Precursors for Trihalomethane and Haloacetic Acid Production. Environmental Science & Technology 35(18), 3661-3668.

Qi, J., Lan, H., Liu, R., Miao, S., Liu, H. and Qu, J. (2016a) Prechlorination of algae-laden water: The effects of transportation time on cell integrity, algal organic matter release, and chlorinated disinfection byproduct formation. Water Research 102, 221-228.

Qi, J., Lan, H.C., Miao, S.Y., Xu, Q., Liu, R.P., Liu, H.J. and Qu, J.H. (2016b) KMnO4-Fe(II) pretreatment to enhance Microcystis aeruginosa removal by aluminum coagulation: Does it work after long distance transportation? Water Research 88, 127-134.

Reckhow, D.A., Singer, P.C. and Malcolm, R.L. (1990) Chlorination of humic materials: byproduct formation and chemical interpretations. Environmental Science & Technology 24(11), 1655-1664.

Richardson, S.D., Fasano, F., Ellington, J.J., Crumley, F.G., Buettner, K.M., Evans, J.J., Blount, B.C., Silva, L.K., Waite, T.J., Luther, G.W., McKague, A.B., Miltner, R.J., Wagner, E.D. and Plewa,

M.J. (2008) Occurrence and mammalian cell toxicity of iodinated disinfection byproducts in drinking water. Environmental Science & Technology 42(22), 8330-8338.

Richardson, S.D. and Postigo, C. (2015) Recent Advances in Disinfection By-Products, pp. 189-214, American Chemical Society.

Smith, E.M., Plewa, M.J., Lindell, C.L., Richardson, S.D. and Mitch, W.A. (2010) Comparison of Byproduct Formation in Waters Treated with Chlorine and Iodine: Relevance to Point-of-Use Treatment. Environmental Science & Technology 44(22), 8446-8452.

Tomlinson, A., Drikas, M. and Brookes, J.D. (2016) The role of phytoplankton as pre-cursors for disinfection by-product formation upon chlorination. Water Research 102, 229-240.

Trehy, M.L., Yost, R.A. and Miles, C.J. (1986) Chlorination byproducts of amino acids in natural waters. Environmental Science & Technology 20(11), 1117-1122.

U.S. Environmental Protection Agency (2001) National Primary Drinking Water Regulations., Washington D.C.

Vidic, R.D., Brantley, S.L., Vandenbossche, J.M., Yoxtheimer, D. and Abad, J.D. (2013) Impact of Shale Gas Development on Regional Water Quality. Science 340(6134).

Wagner, E.D. and Plewa, M.J. (2017) CHO cell cytotoxicity and genotoxicity analyses of disinfection by-products: An updated review. Journal of Environmental Sciences 58, 64-76.

Wang, K., Wommack, K.E. and Chen, F. (2011) Abundance and distribution of Synechococcus spp. and cyanophages in the Chesapeake Bay. Applied And Environmental Microbiology 77(21), 7459-7468.

Wert, E.C. and Rosario-Ortiz, F.L. (2013) Intracellular Organic Matter from Cyanobacteria as a Precursor for Carbonaceous and Nitrogenous Disinfection Byproducts. Environmental Science & Technology 47(12), 6332-6340.

Westerhoff, P., Chao, P. and Mash, H. (2004) Reactivity of natural organic matter with aqueous chlorine and bromine. Water Research 38(6), 1502-1513.

Widrig, D.L., Gray, K.A. and McAuliffe, K.S. (1996) Removal of algal-derived organic material by preozonation and coagulation: Monitoring changes in organic quality by pyrolysis-GC-MS. Water Research 30(11), 2621-2632.

Xie, P., Ma, J., Fang, J., Guan, Y., Yue, S., Li, X. and Chen, L. (2013) Comparison of Permanganate Preoxidation and Preozonation on Algae Containing Water: Cell Integrity, Characteristics, and Chlorinated Disinfection Byproduct Formation. Environmental Science & Technology 47(24), 14051-14061.

Xie, Y. (2016) Disinfection byproducts in drinking water: Formation, analysis, and control, CRC press.

Yeatts, S.D., Gennings, C., Wagner, E.D., Simmons, J.E. and Plewa, M.J. (2010) Detecting departure from additivity along a fixed-ratio mixture ray with a piecewise model for dose and interaction thresholds. Journal of agricultural, biological, and environmental statistics 15(4), 510-522.

Yu, Y. and Reckhow, D.A. (2015) Kinetic Analysis of Haloacetonitrile Stability in Drinking Waters. Environmental Science & Technology 49(18), 11028-11036.

Zhang, X. and Minear, R.A. (2002) Decomposition of trihaloacetic acids and formation of the corresponding trihalomethanes in drinking water. Water Research 36(14), 3665-3673.

Figure 1. Formation of (a) THMs, (b) HAAs, (c) HANs, (d) HALs, (e) I-THMs, and (f) TOX during chlorination of AOM derived from three species of algae in the presence of various initial bromide concentrations. Experimental conditions: MA, SYN and AT AOM. [DOC] = 2.0 mg C/L, [HOCl]$_0$ = 56 μM, [Br$^-$]$_0$ = 0-10 μM, [I$^-$]$_0$ = 0.4 μM, pH= 7.5, T =21±1 °C, reaction time = 24 h. TOI was below 0.079 μM (i.e., 10 μg/L as I).
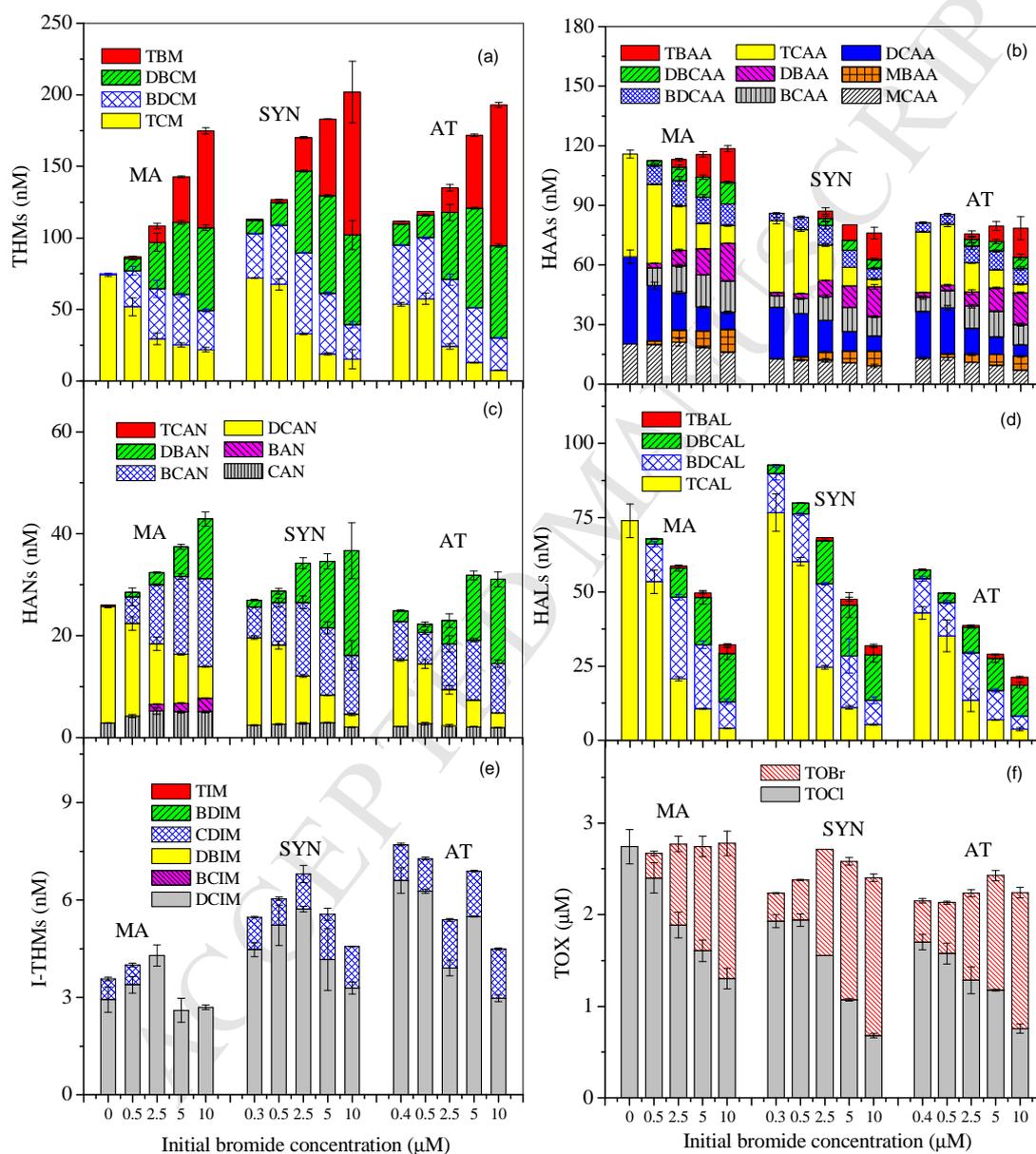
Figure 2. Effect of AOM type on the BSF of (a) THM, (b) DHAN, (c) DHAA, (d) THAA, (e) HAL, and (f) TOX during chlorination. Experimental conditions: MA, SYN and AT AOM. [DOC] = 2.0 mg C/L, $[HOCl]_0 = 56$ μM, $[Br^-]_0 = 0\text{-}10$ μM, $[I^-]_0 = 0.4$ μM, pH= 7.5, T =21±1 °C, reaction time = 24 h. Ambient Br$^-$ concentrations were 0, 0.3, and 0.4 μM for MA, SYN and AT, respectively.
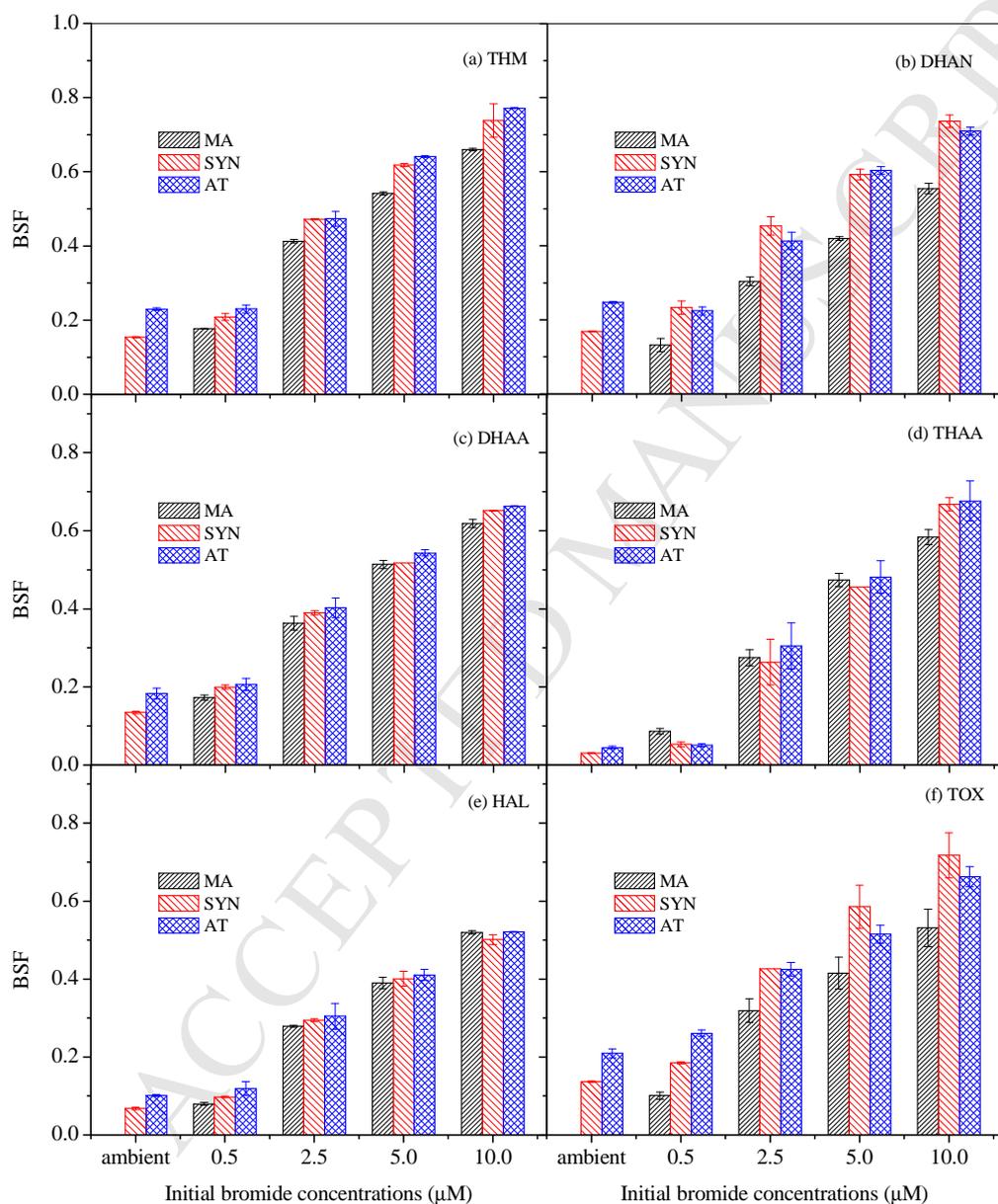
Figure 3. Effect of pH on formation of (a) THMs, (b) HAAs, (c) HANs, and (d) HALs during chlorination of MA AOM. Left, middle, and right column of each initial bromide concentration stands for pH 6.0, 7.5, and 9.0, respectively. Experimental conditions: [DOC] = 2.0 mg C/L, $[HOCl]_0 = 56$ µM, $[Br^-]_0 = 0$-10 µM, $[I^-]_0 = 0.4$ µM, pH= 6-9, T =21±1 °C, reaction time = 24 h.

3
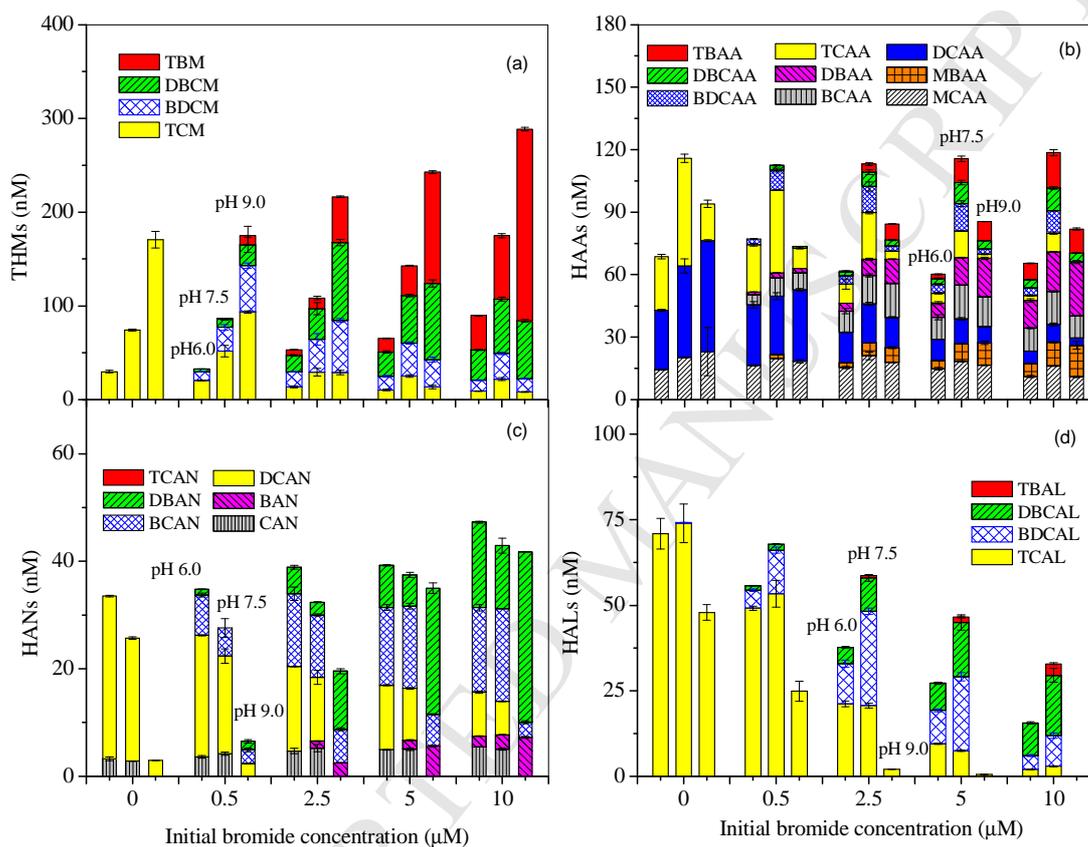
Figure 4. Effect of initial chlorine concentration on the formation and speciation of (a) THMs, (b) HAAs, (c) HANs, and (d) HALs during chlorination of MA AOM. Experimental conditions: [DOC] = 2.0 mg C/L, $[HOCl]_0$ = 14-224 µM (i.e., 1.0-16.0 mg/L), $[Br^-]_0$ = 2.5 µM, $[I^-]_0$ = 0.4 µM, pH= 7.5, T =21±1 °C, reaction time = 24 h.
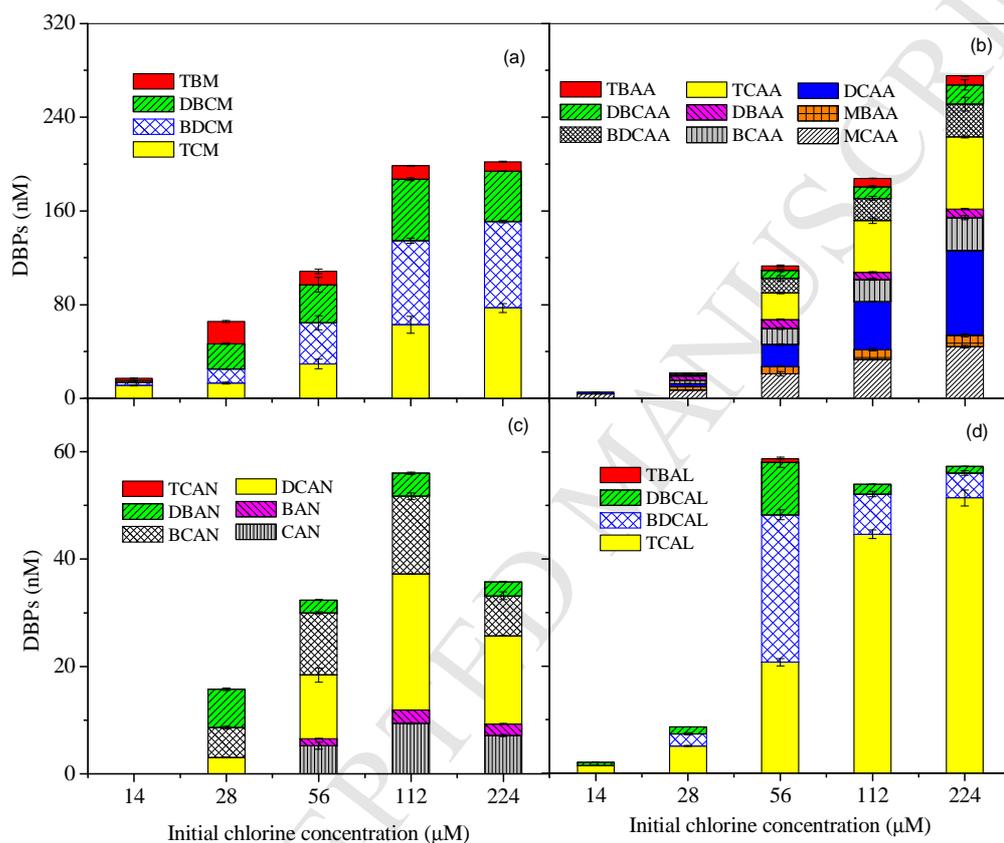
Figure 5. Effect of reaction time on the formation and speciation of (a) THMs, (b) HAAs, (c) HANs, and (d) HALs during chlorination of MA AOM. Experimental conditions: [DOC] = 2.0 mg C/L, [HOCl]$_0$ = 56 μM, [Br$^-$]$_0$ = 2.5 μM, [I$^-$]$_0$ = 0.4 μM, pH= 7.5, T =21±1 °C, reaction time = 0.5-72 h.
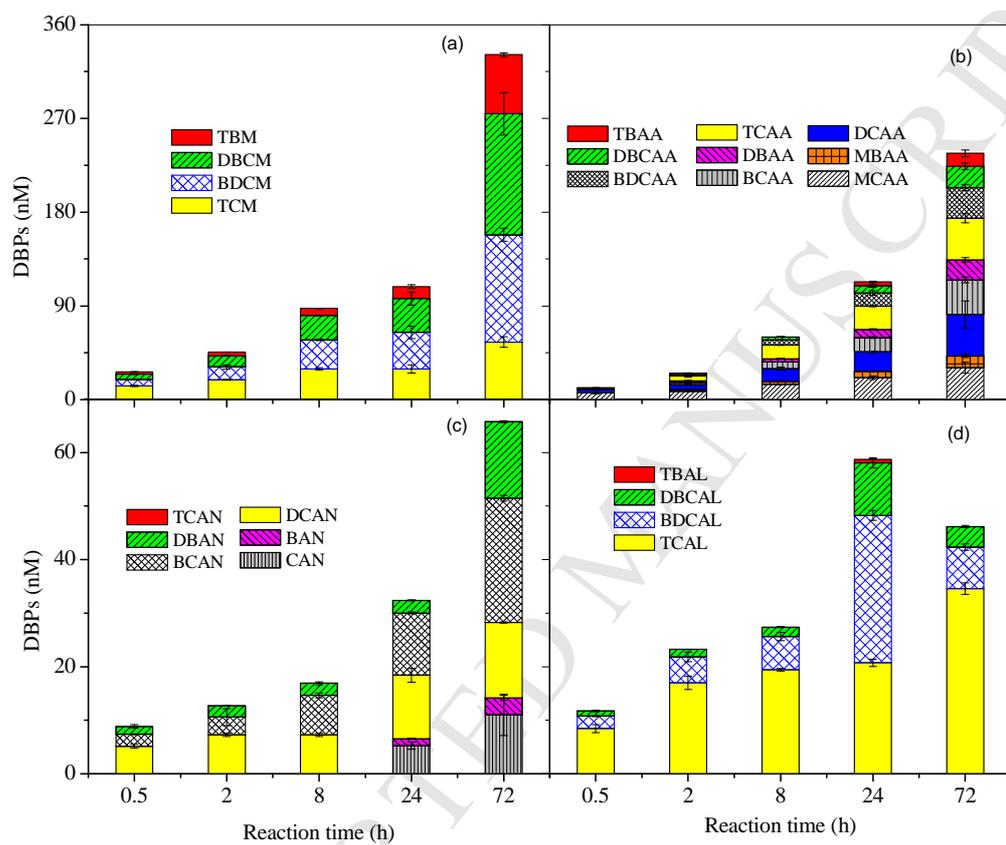
Figure 6. Effect of (a) AOM type under various initial bromide concentrations, (b) pH under various initial bromide concentrations (left, middle, and right column of each initial bromide concentration stands for pH 6.0, 7.5, and 9.0, respectively), (c) initial chlorine concentrations, and (d) reaction time on calculated toxicity of various DBPs measured. Experimental conditions: [DOC] = 2.0 mg C/L, $[I^-]_0 = 0.4$ μM, T $= 21 \pm 1$ °C. (a) MA, SYN and AT AOM, $[HOCl]_0 = 56$ μM, $[Br^-]_0 = 0\text{-}10$ μM, pH= 7.5, reaction time = 24 h; (b) MA AOM, $[HOCl]_0 = 56$ μM, $[Br^-]_0 = 0\text{-}10$ μM, pH= 6-9, reaction time = 24 h; (c) MA AOM, $[HOCl]_0 = 14\text{-}224$ μM (i.e., 1.0-16.0 mg/L), $[Br^-]_0 = 2.5$ μM, pH= 7.5, reaction time = 24 h; and (d) MA AOM, $[HOCl]_0 = 56$ μM, $[Br^-]_0 = 2.5$ μM, pH= 7.5, reaction time = 0.5-72 h.
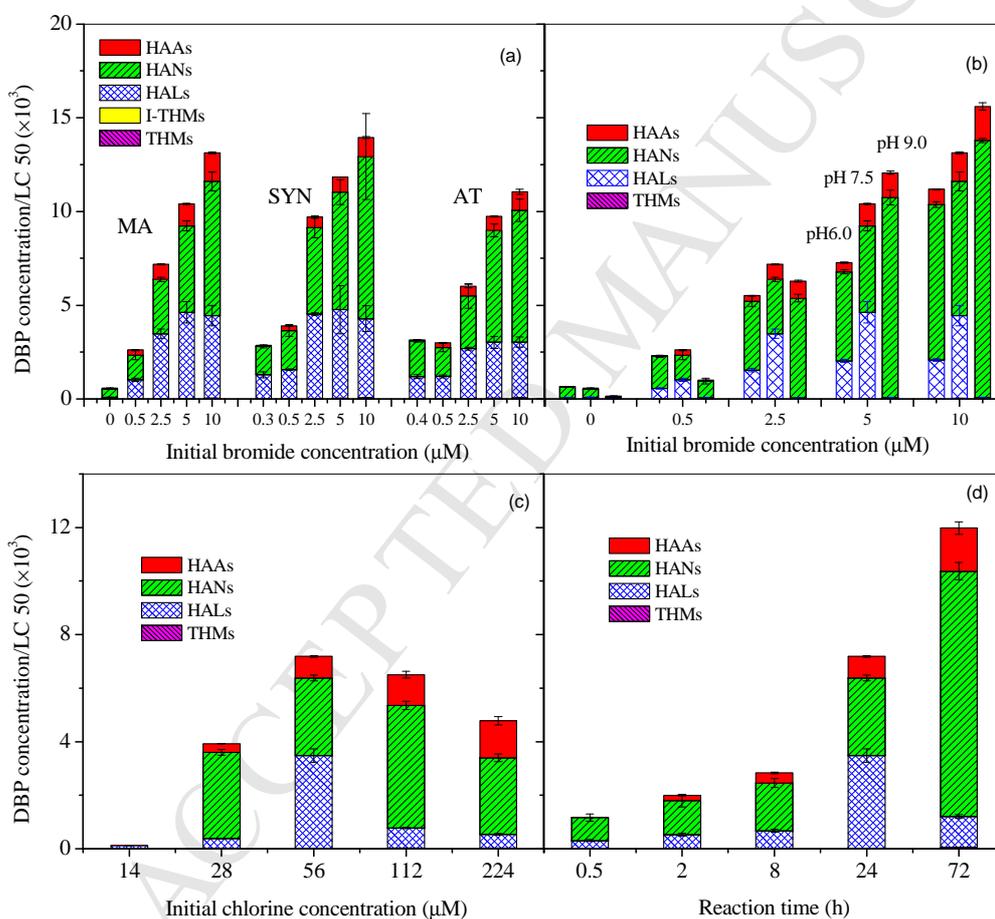
Highlights

- High levels of Br⁻ enhanced formation of THM and HAN, not for HAA, HAL and TOX
- Three types of algal organic matter exhibited comparable DBP formation
- Lower SUVA of algal organic matter showed higher bromine substitution
- Br-HAN is more stable than Cl-HAN, but the opposite is true for HAL
- Unregulated HAN and HAL were primary contributors to calculated cytotoxicity