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Formation and estimated toxicity of trihalomethanes, haloacetonitriles, and haloacetamides from the chlor(am)ination of acetaminophen

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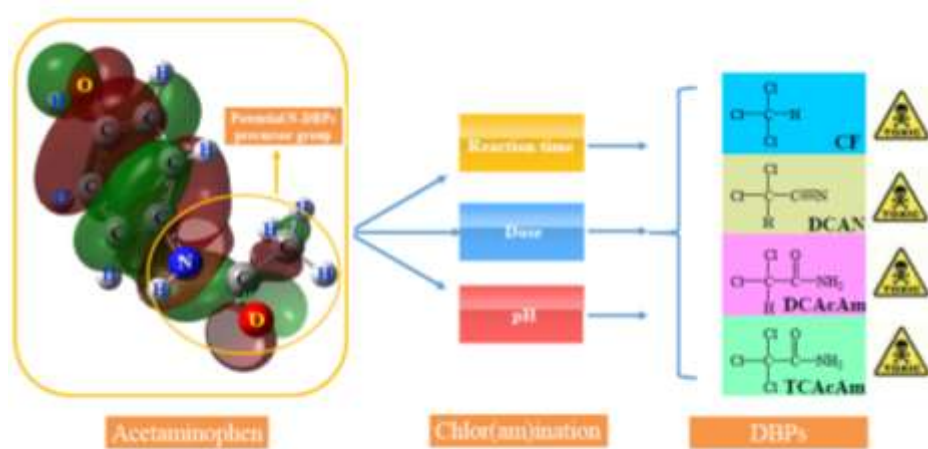
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Graphical abstract



Highlights

- Acetaminophen formed CF, DCAN, DCACAm, and TCACAm during chlor(am)ination.
- Yields of all DBPs were higher during chlorination than that chloramination.
- Lower pH increased N-DBP formation and decreased CF formation.
- Two formation pathways for CF, DCAN, DCACAm, and TCACAm were presented.
- DBP toxicity from chlorination was higher than from chloramination.

Abstract

The occurrence of pharmaceuticals and personal care products (PPCPs) in natural waters, which act as drinking water sources, raises concerns. Moreover, those compounds incompletely removed by treatment have the chance to form toxic disinfection byproducts (DBPs) during subsequent disinfection. In this study, acetaminophen (Apap), commonly used to treat pain and fever, was selected as a model PPCP. The formation of carbonaceous and nitrogenous DBPs, namely trihalomethanes, haloacetonitriles, and haloacetamides, during chlor(am)ination of Apap was investigated. Yields of chloroform (CF), dichloroacetonitrile (DCAN), dichloroacetamide (DCAcAm), and trichloroacetamide (TCAcAm), during chlorination were all higher than from chloramination. The yields of CF continuously increased over 48 h during both chlorination and chloramination. During chlorination, as the chlorine/Apap molar ratios increased from 1 to 20, CF yields increased from $0.33 \pm 0.02\%$ to $2.52 \pm 0.15\%$, while the yields of DCAN, DCAcAm and TCAcAm all increased then decreased. In contrast, during chloramination, increased chloramine doses enhanced the formation of all DBPs. Acidic conditions favored nitrogenous DBP formation, regardless of chlorination or chloramination, whereas alkaline conditions enhanced CF formation. Two proposed formation mechanisms are presented. The analysed DBPs formed during chlorination were 2 orders of magnitude more genotoxic and cytotoxicity than those from chloramination.

Abbreviations:

PPCPs, Pharmaceuticals and personal care products; DBPs, Disinfection byproducts; CF, Chloroform; DCAN, Dichloroacetonitrile; DCAcAm, Dichloroacetamide; TCAcAm, trichloroacetamide; C-DBPs, Carbonaceous disinfection by-products; N-DBPs, Nitrogenous

disinfection by-products; GC/ECD, Gas chromatography/electron capture detection GCMS, Gas chromatograph/mass spectrometry; DWTPs, Drinking water treatment plants; WWTPs, Wastewater treatment plants.

Keywords

Acetaminophen; Disinfection byproducts; Haloacetonitriles; Haloacetamides; Trihalomethanes

1. Introduction

Pharmaceuticals and personal care products (PPCPs) have become important emerging contaminants [1, 2]. They are frequently encountered in both environmental and drinking waters, due to their incomplete removal by various wastewater and drinking water treatment processes, including biological processes, filtration, coagulation, flocculation, and sedimentation [3, 4]. Acetaminophen (Apap) (Table SM1), also known as Paracetamol, is the most commonly used medication to treat pain and fever in the United States, Europe and many other regions [5, 6]. It is also on the WHO Model List of Essential Medicines [7]. Previous studies have showed that the worldwide annual production of Apap is 1.45×10^5 tons, and since Apap is incompletely metabolized in the human body, it therefore enters into wastewater [8, 9]. Moreover, Apap is ineffectively removed by the conventional treatment processes in wastewater treatment plants (WWTPs), and thereby has been detected in WWTP effluents in many regions, including Greece, German, Korea, and China, at concentrations of up to 0.5, 6.0, 6.8, and 5.3 $\mu\text{g/L}$, respectively [10-13]. Therefore, Apap eventually reaches surface or underground waters that may be used as source waters of drinking water treatment plants (DWTPs) [14, 15]. The median concentration, maximum concentration, and frequency of detection of Apap was 0.11 $\mu\text{g/L}$, 10.0 $\mu\text{g/L}$, and 23.8%, respectively, in 139 streams across 30 states in U.S. during 1999 and 2000 [4]. Apap has also been detected in many natural waters all over the world and concentrations have reached up to 69.6 $\mu\text{g/L}$ in the river Tyne in England [9, 16-19].

The frequent detection of Apap in drinking water sources has raised concerns because it has the potential to affect finished drinking water quality with respect to bacterial resistance [20]. As Apap can largely pass through the conventional treatment process in DWTPs, it can react with

disinfectants (typically chlorine or chloramines) to form disinfection byproducts (DBPs) [21]. A previous study found 8 of the 20 pharmaceuticals had nitrosamine DBP molar yields higher than 1% during chloramination [22]. It has also been reported that the antibiotic chloramphenicol and two of its analogues have substantial haloacetamide (HAcAm) yields during chlorination [23]. Since Apap is nitrogenous, it has the potential to form nitrogen-containing DBPs (N-DBPs). The unregulated N-DBPs such as haloacetonitriles (HANs), haloacetamides (HAcAms), and emerging aromatic halogenated N-DBPs have been reported to be much more cytotoxic and genotoxic than the regulated carbon-containing DBPs (C-DBPs) such as trihalomethanes (THMs) and haloacetic acids (HAAs) [24-28]. For example, HAcAms are $142 \times$ more cytotoxic and $12 \times$ more genotoxic than regulated HAAs, based on the median toxicity index value of all of the individual members of a single class of DBPs. And, amino acids were usually selected as the precursors of THMs, HANs and HAcAms in the previous studies [29, 30]. However, little is known about whether Apap can form regulated C-DBPs and unregulated N-DBPs at conditions relevant to drinking water.

Therefore, the objective of this study were to investigate the effects of contact time, chlor(am)ine dose, and solution pH on the formation of regulated C-DBPs (represented by THMs) and unregulated N-DBPs (HANs and HAcAms) during the chlorination and chloramination of Apap. Moreover, the estimated toxicity caused by THMs, HANs and HAcAms was also examined. The formation of THMs, HANs and HAcAms mainly focused on the The results in the study will help to better understand the formation of DBPs from PPCPs, which is conducive to the simultaneous control of Apap, analogues of Apap and DBPs in drinking water.

2. Materials and methods

2.1. Materials

Dichloroacetamide (DCAcAm, 98.5%) and trichloroacetamide (TCAcAm, 99%) were obtained from Alfa Aesar (Karlsruhe, Germany). EPA 551A Halogenated Volatiles Mix, containing chloroform (CF), and 551B Halogenated Volatiles Mix, containing dichloroacetonitrile (DCAN), were purchased from Sigma-Aldrich (St Louis, Missouri, USA). Apap (> 99%) and acetamide (> 99.5%) were purchased from Aladdin Industrial Inc. (Shanghai, China). All other chemical reagents were at least analytical grade and obtained from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China) unless otherwise noted. Stock solutions (1 mM) of Apap were prepared by dissolving 75.6 mg Apap in 500 mL ultrapure water, and were stored at 4 °C. Free chlorine stock solution was prepared from sodium hypochlorite solution (active chlorine > 5%). The preparation of monochloramine (NH₂Cl) solution followed previous studies [31, 32]. All solutions were prepared in ultrapure water produced from Millipore Milli-Q Gradient water purification system (18 MΩ·cm, Billerica, MA, USA) and all tests were undertaken in triplicate. Error bars in all the figures represent the relative standard deviation of the three replicates.

2.2. Experimental procedures

All experiments were performed in 40 mL brown glass volumetric bottles under headspace-free conditions in the dark at 25.0 ± 0.5 °C. Chlor(am)ination experiments were undertaken by applying a specified chlorine or chloramine dose (0.05, 0.15, 0.25, 0.50, or 1.00 mM) to Apap solution (0.05 mM) buffered at pH 5-8 (10 mM phosphate buffer) or pH 9 (10 mM carbonate buffer) for a designated reaction time (1, 6, 12, 24, or 48 h). It should be noted that 24 h was selected as the basis

reaction time in the study, considering the delivery time for water from DWTPs to users is generally not more than 24 h. Periodically, 10 mL of the aqueous solution was withdrawn and immediately extracted without the addition of quenched reagent to analysis HAcAms and THMs. A separate 40 mL aqueous solution was quenched by ascorbic acid at the initial molar concentration of the chlorine or chloramine added and to analyze the formation of HANs.

2.3. Analytical methods

Free chlorine was measured by a portable photometer (Hach Pocket ColorimeterTMII, USA). HAcAms and THMs were extracted at the end of the predetermined contact time by adding 2 g anhydrous sodium sulfate and 2 mL MTBE to 10 mL aqueous sample, which was then shaken using a IKA oscillator (Staufen, German) at 2800 r/min. Extracted samples were analyzed soon after (less than 3 h) by gas chromatography equipped with electron capture detection (GC/ECD, QP2010plus, Shimadzu Corporation, Japan) using a previously detailed analytical method [33, 34]. HANs were analyzed with a purge & trap sample concentrator (eclipse4660, OI, USA) and gas chromatograph/mass spectrometry (GC/MS, QP2010, Shimadzu Corporation, Japan). The detailed information of analyses about HANs is available on previous studies [34, 35]. The detection limits for CF, DCAN, DCAcAm, TCAcAm were 0.07, 0.11, 1.27, and 1.62 µg/L, respectively. Intermediate products were analysed by gas chromatography/mass spectrometry (GC/MS, QP2020, Shimadzu Corporation, Japan), with detailed information is available on supporting data. DBPs yields are defined in % mol/mol as the molar ratio of the produced DBPs to the initial Apap (Eq. (1)).

$$\text{DBPs yields} = \frac{\text{Formed DBPs molar concentration}}{\text{Initial Apap molar concentration}} \times 100\% \quad (1)$$

2.4. Frontier electron density calculation

Electronic structure calculations and geometric optimizations, frequencies, and structural parameters of Apap were carried out by using the Gaussian 09 software package (Gaussian, Inc. USA) and the M062X hybrid functional at 6-311+G(d,p) level, respectively [31]. Based on the wave function, the frontier electron density (FED²) was analyzed by the software MultiWFN 3.37 [36].

2.5. Determinations of predicted toxicity

The measured DBPs concentration were divided by the previous published genotoxicity potencies in the Chinese hamster ovary (CHO) comet assay (4 h exposure), which is the dose required to induces a Tail DNA value and presented in Table S2, to elicit the predicted genotoxicity of the measured DBPs (i.e., THMs, HANs, and HAcAms), which is a unitless value [24, 37, 38].

Similarly, the predicted cytotoxicity was calculated by dividing the measured concentration by the published LC₅₀ values, which is the dose required to induces 50% viability of the cells as compared to the concurrent negative control for CHO cells (72 h exposure) and presented in Table S3 [24, 37, 38].

3. Results and discussion

3.1. Effects of reaction time on the formation of CF, DCAN, and HAcAms

[Figure 1]

Fig. 1a and Fig. 1b present the changes in the yields of CF, DCAN, DCaAm, and TCaAm versus reaction time during the chlor(am)ination of the Apap. CF yield steadily increased from $0.22 \pm 0.02\%$ to $2.25 \pm 0.18\%$ as the reaction time increased from 1 to 48 h during chlorination. Meanwhile, the yield of CF during chloramination was far lower than from chlorination and was undetectable (limit of detection = $0.07 \mu\text{g/L}$) at a contact time of 24 h. DCAN yields increased from $0.02\% \pm 0.001\%$ at 1 h to $0.66 \pm 0.04\%$ at 24 h, then gradually decreased to $0.54 \pm 0.03\%$ at 48 h. DCaAm yields also exhibited the trend of increasing first and then decreasing, with values increasing from $0.06\% \pm 0.003\%$ at 1 h to $0.63 \pm 0.04\%$ at 24 h and then decreasing to $0.51 \pm 0.03\%$ at 48 h, whereas the yields of TCaAm increased gradually to $0.20 \pm 0.01\%$ at 48 h. However, the yields of DCaAm and TCaAm both were increased from 1 h to 48 h during the chloramination of Apap, as shown in Fig. 1b.

The time-dependent trends of these DBPs are dependent on both their formation and stability. CF is relatively stable in the presence of chlorine or chloramines and commonly regarded as the final product during the chlor(am)ination of organic matter, thus yields of CF typically increase with reaction time [39, 40]. Previous studies have also indicated that a shift from chlorination to chloramination resulted in the decrease of the formation of THMs, including CF [41, 42]. DCAN and DCaAm tend to hydrolyze at neutral pH and degrade in the presence of chlorine, resulting in the decrease of their concentrations after a long reaction times [43-45]. In contrast, yields of DCAN and DCaAm during chloramination increased steadily, but dramatically decreased during chlorination, which agrees with a previous study comparing the yields of DCAN and other DBPs

during the chlorination and chloramination of L-aspartic acid [46]. It has also been reported previously that chlorination favored the formation of DCAN over DCACAm and chloramination formed more DCACAm than DCAN [47]. However, chlorination and chloramination both favored the formation of DCACAm over DCAN in this study. Although TCACAm has a faster hydrolysis rate than DCACAm under the same chlorination condition [48], the yield of TCACAm increased continually as the yield of DCACAm decreased with increasing time, implying that TCACAm formation was slower than DCACAm during the chlorination of Apap. As shown in Table SM5, the DBP yields from other typical model compounds were also listed. Although the highest CF yield (2.6%) from Apap was lower than aromatic compound (11.9% from resorcinol), Apap were significant precursor for HACams compared with the listed typical precursor compound [29, 46, 47, 49].

3.2. Effects of chlor(am)ine dose on the formation of CF, DCAN, and HACams

Fig. 1c and Fig. 1d show the variation in DBPs yields at different chlor(am)ine dose after 24 h contact. Yields of CF steadily increased from $0.33 \pm 0.02\%$ to $2.52 \pm 0.15\%$ as Cl_2 dose increased from 0.05 to 1.0 mM after 24 h contact, whereas CF was not detected when the molar ratio of $\text{NH}_2\text{Cl}/\text{Apap}$ was ≤ 10 . As can be seen in Fig. 1c, the yields of DCAN, DCACAm, and TCACAm exhibited a common trend of increasing first and then decreasing with the increase of chlorine dose after 24 h contact. The maximum yields of DCAN, DCACAm, and TCACAm were $0.66 \pm 0.04\%$, $0.81 \pm 0.06\%$, and $0.21 \pm 0.01\%$, respectively. However, yields of DCAN, DCACAm, and TCACAm all present a trend of increasing with the increase of monochloramine dose after 24 h contact and were in all cases lower than in equivalent chlorination experiments.

As CF is stable in the presence of chlorine and it is a terminal chlorination product, its concentrations increased with increasing chlorine dose, which is consistent with previous research [50]. Conversely, concentrations of unstable DBPs, which includes DCAN, DCaAm, and TCaAm, depend on relative contributions of their formation and decomposition rates. Previous studies have indicated that the degradation of DCAN, DCaAm, and TCaAm all increased with chlorine dose [45, 48]. Therefore, DCAN and DCaAm both increased with chlorine dose, before decreasing at the higher doses. The formation of TCaAm followed the same trend as DCAN and DCaAm, while previous research reported that increasing chlorine dose was more favourable for TCaAm formation than that for DCaAm formation [44, 51]. The yields of DCAN, DCaAm, and TCaAm were all gradually increased with increasing chloramine dose, which can be explained by their degradation rates being low in the presence of NH_2Cl .

3.3. Effects of pH on the formation of CF, DCAN, and HAcs

Fig. 1e and Fig. 1f show the effect of pH on the formation of CF, DCAN, DCaAm, and TCaAm during the chlorination and chloramination of Apap. The yield of CF steadily increased from $0.63 \pm 0.04\%$ to $2.58 \pm 0.17\%$ as the pH increased from 5 to 9 during the chlorination of Apap, and was hardly detected during chloramination was hardly detected unless at pH 9. The formation of DCAN during chlorination was highest at pH 6, when $0.80 \pm 0.05\%$ was formed, and decreasing with increasing pH. During chloramination, DCAN steadily decreased with increasing pH until it was below the limit of detection ($\leq 0.11 \mu\text{g/L}$) at pH 8. The formation of DCaAm and TCaAm exhibited a common trend of decreasing with increasing pH from 5 to 9 during both chlorination and chloramination.

The pH-dependent variation of CF in this study was consistent with previous research that has shown CF formation increasing with pH [52, 53]. However, the pH also affects the stability of DCAN, DCaAm, and TCaAm, which undergo base-catalyzed hydrolytic decomposition [45, 48, 54]. In addition, the degradation of DCAN, DCaAm, and TCaAm accelerates with increasing pH [45, 48]. The formation of DCAN at pH 5 being lower than at pH 6 during chlorination was probably ascribed to that the residual hypochlorite as a catalyst accelerating the degradation of DCAN [55, 56]. More DCaAm was formed than DCAN during chlorination, which likely was due to that the degradation of DCAN producing DCaAm [45]. The lower DCaAm and TCaAm yields under alkaline conditions than acidic conditions were probably due to their faster degradation under alkaline conditions than acidic conditions. Trichloroacetonitrile (TCAN) was not detected in this study, implying that TCaAm was barely produced from TCAN degradation.

3.4. Proposed pathway for the formation of CF, DCAN, and HAcAms

[Figure 2]

Fig. 2 showed the results of the FED² of Apap. The frontier orbital theory indicated that electrophilic substitution reaction between reactant and electrophilic reagent (chlor(am)ine) is most likely to occur at the atom with the highest FED² in the HOMO. For Apap, electrophilic substitution reactions are expected to proceed on the phenolic ring in ortho and/or meta position to the phenol functionality [57, 58]. Deborde and von Gunten determined the most probable sites of chlorine attack in the aromatic ring by calculating the sum of Hammett constant values for the substituents present [58]. In this method, benzene was used as a reference compound (i.e., $\sum \sigma_{o,p,m} = 0$) and assumed $\sigma_o \approx \sigma_p$

[59]. The site with the minimum $\sum\sigma_{o,p,m}$ is the most frequently attacked by chlor(am)ine. By comparing the $\sum\sigma_{o,p,m}$ of unsubstituted ortho and para position(s) to the phenolic function (sites 1 and 2) as shown in Fig. SM1, we determined that the most probable site(s) of chlor(am)ine attack were the ortho positions to the phenolic function, which also consistent with the finding that the FED² of C7 (7.39%) and C8 (7.60%) were somewhat higher than the C5 (7.06%) and C6 (5.99%). In addition, evidence from a previous study using mass spectrometry indicated the formation of products chloro-4-acetamidophenol and dichloro-4-acetamidophenol [57]. The amide group in the para position to the phenol moiety, is electron-withdrawing, which makes the hydrogen atoms (H17-19) on the methyl group more acidic, thus increasing chlor(am)ine substitution [58]. It induces the successive replacement of hydrogen by chlor(am)ine and subsequently produces CF via the haloform reaction [58, 60]. Chlorine substitution on the aromatic ring also could lead to CF formation [61]. The mass spectral evidence of the formation of several products with molecular weights over 280 from previous study also supports this proposed pathway [62].

As shown in Fig. 2, carbon atom (C4), which is connected to the acetyl amino group, has a highest FED² of 15.22% in Apap, implying that the C4 is more easily to be substituted by chlor(am)ine. The hydrogen atoms (H17-19) of the methyl group can be substituted by chlorine followed by breaking the C4-N3 bond to form HAcAms. Further, cleavage of the C4-N3 bond to form acetamide may directly precede chlorine substitution reaction on the phenol ring or/and methyl group. There was also mass spectral evidence for the formation of acetamide and 2,4,6-trichlorophenol, as shown in Fig. SM2. A previous study indicated that the chlorination of acetamide can form THMs [63]. We also measured the formation of DCAN, DCAcAm, and TCACAm during the chlorination of acetamide to elucidate this pathway (Condition: reaction time = 24 h; pH = 7;

$\text{Cl}_2/\text{acetamide}$ molar ratio = 3). Respective yields for DCAN, DCACAm and TCACAm were 0.03%, 0.11%, and 0.01%, respectively. Equivalent yields from Apap under the same experimental conditions were 0.03%, 0.82%, and 0.32%, respectively. Therefore, chlorination of acetamide cannot account for N-DBP formation by itself and the former pathway is likely to be the major contributor. Another relevant may be that the phenol OH can interact with the amide nitrogen by resonance, making Apap more reactive (to chlorine substitution) than acetamide. The detailed proposed pathways for the formation of CF, DCAN, and HACams were shown in scheme 1 and 2 in Fig. SM3. The above hypothesis is only directed at chlorination disinfection. As known, for chlorination (Cl_2), only Apap supplied the nitrogen during HAN and HACAm formation. However, for chloramination (NH_2Cl), a portion of N in N-DBPs were also probably supplied by NH_2Cl , as the previous studies have reported that NH_2Cl could supply the nitrogen for the formation of HANs and HACams [49, 64].

3.5. Estimated toxicity of DBPs at different disinfection scenario

[Figure 3]

[Figure 4]

Fig. 3 and Fig. 4 present the estimated genotoxicity and cytotoxicity of the sum of the measured DBPs, namely CF, DCAN, DCACAm, and TCACAm, during the chlor(am)ination of Apap. As shown, the estimated genotoxicity and cytotoxicity during chlorination presented a trend of increasing first and then decreasing with time, while the toxicity constantly increased during

chloramination with reaction time. The measured DBPs formed during chlorination were 2 orders of magnitude more genotoxic and cytotoxic than DBPs formed during chloramination. As the chlorine dose increased, the predicted genotoxicity and cytotoxicity both increased to a maximum (Cl_2/Apap molar ratio = 10), before decreasing at higher chlorine doses. During chloramination, the estimated genotoxicity and cytotoxicity both steadily increased as $\text{NH}_2\text{Cl}/\text{Apap}$ molar ratios increased. Results shown in Section 3.3 indicate that acidic conditions favor the formation of DCAN, DCACAm, and TCACAm, which were 1-2 orders of magnitude more genotoxic and cytotoxic than CF. Therefore, disinfection under acidic conditions result in increased toxicity due to the measured DBPs.

It should be noted that one PPCP cannot represent the whole precursor compound in aqueous environment and the measured DBPs are not the only driver of toxicity. In authentic drinking water additional DBPs will be present and the synergistic mixture effects also need to be considered. Nonetheless, this approach is a useful starting point for evaluating how toxicity is impacted by different disinfection scenarios. Further research also need to investigate the impact of different water matrices on the formation of HANs and HACams.

4. Conclusions

The worldwide detection of PPCPs in natural water and even drinking water raises concerns over the removal of these compounds by conventional drinking water treatment during recent years. The possibility for concurrent formation of CF, DCAN, and HACams during subsequent disinfection has become another significant concern for delivered drinking water quality because of their genotoxicity and cytotoxicity. In this study, we report that Apap, commonly used medication to treat

pain and fever, can form the CF, DCAN, and HAcAms during chlor(am)ination. Yields of all four DBPs during chlorination were higher than from chloramination at the same oxidant doses. Yields of CF and TCACAm increased over 48 h contact during chlorination and chloramination. Conversely, yields of DCAN and DCACAm first increased then decreased during chlorination and no significant concentrations of DCAN and DCACAm were formed from chloramination. During chlorination, as the chlorine/Apap molar ratio increased from 1 to 20, CF yields increased from $0.33 \pm 0.02\%$ to $2.52 \pm 0.15\%$ and the yields of DCAN, DCACAm, and TCACAm all first increased then decreased. In contrast, during chloramination, increased chloramine doses enhanced the formation of all DBPs. Acidic conditions favored DCAN, DCACAm, and TCACAm during both chlorination and chloramination, whereas alkaline conditions enhanced the formation of CF. The measured DBPs formed during chlorination were 2 orders of magnitude more genotoxic and cytotoxicity than during chloramination. As known, there are so many PPCPs in the water, a “Big database” for the formation of DBPs from PPCPs need to be established to help the simultaneous control of multifarious PPCPs and DBPs in drinking water. The present study also will contribute the particular data and information for the “Big database”, whereas more research should be carried out in the DBP formation from more representative PPCPs.

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Figure captions

Fig. 1. Effect of reaction time (Fig. 1a and Fig. 1b), chlor(am)ine dose (Fig. 1c and Fig. 1d), and pH (Fig. 1e and Fig. 1f) on the formation of DBPs during the chlor(am)ination of Apap (Conditions: initial Apap conc. = 0.05 mM; Cl_2 or NH_2Cl dose = 0.5 mM; reaction time = 24 h; and pH = 7.0 ± 0.2 , unless otherwise noted).

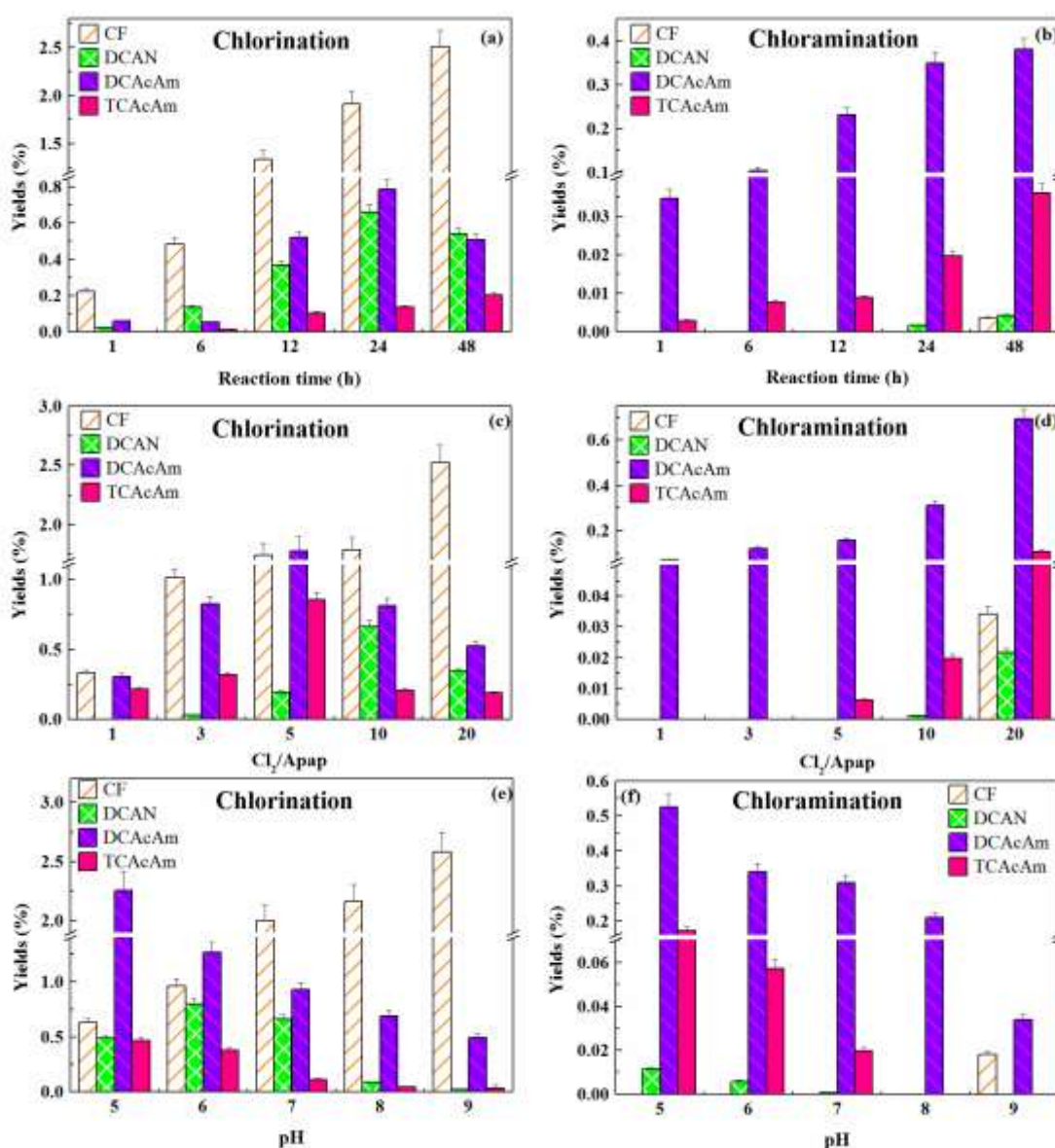


Fig. 2. Structural model and frontier electron densities (FED²) in HOMO on atoms of Apap.

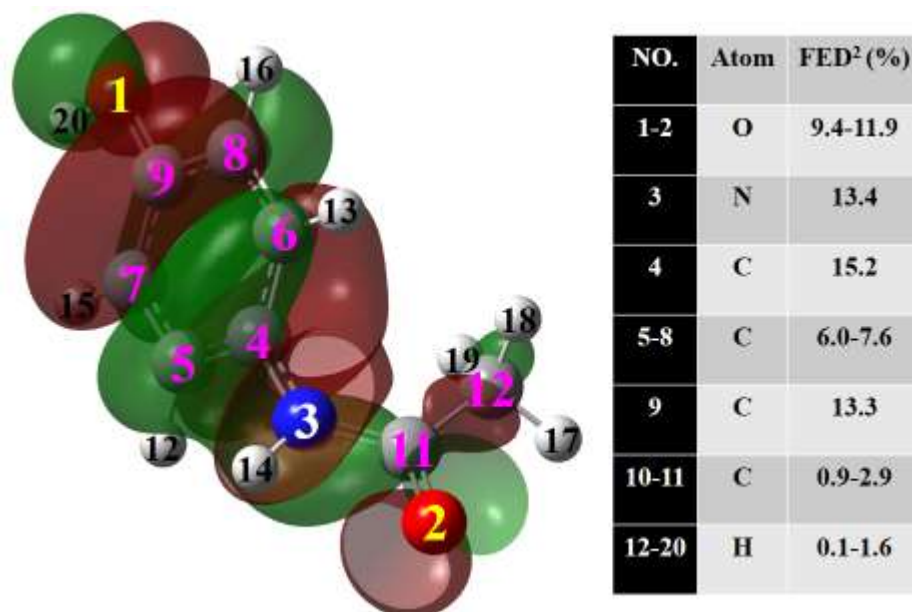


Fig. 3. Estimated genotoxicity of the sum of the measured DBPs during the chlor(am)ination of Apap at different reaction time (a), Cl/Apap molar ratios (b), and pH (c).

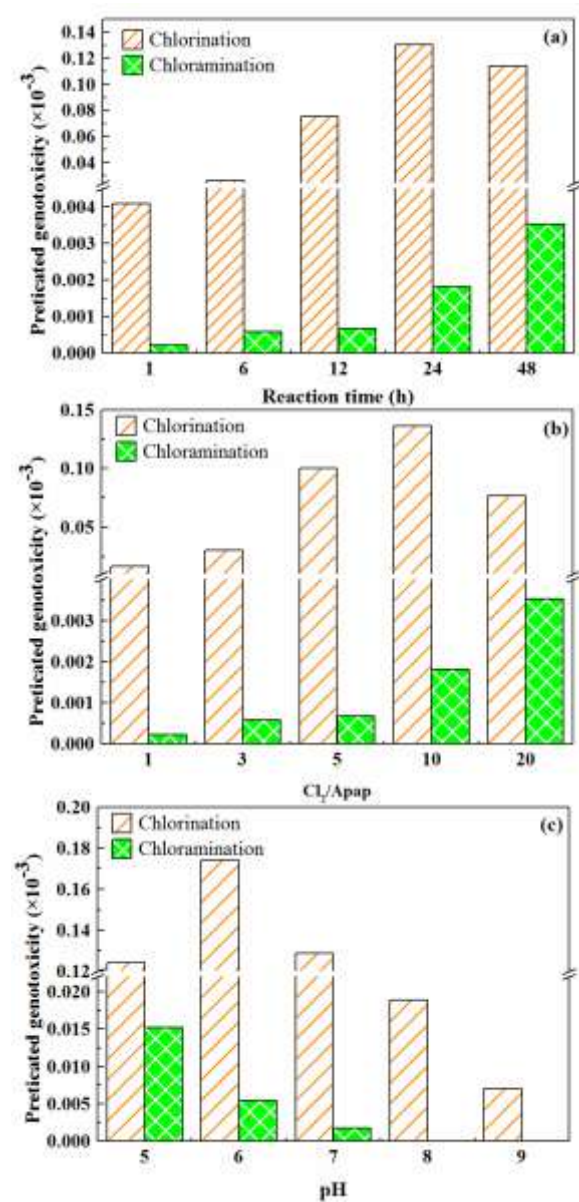


Fig. 4. Estimated cytotoxicity of the sum of the measured DBPs during the chlor(am)ination of Apap

at different reaction time (a), Cl/Apap molar ratios (b), and pH (c).

