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Letter

# Chlorotyrosines and Oleic Acid Chlorohydrins as Byproducts in Disinfected Conventional Drinking Waters and Potable Reuse Waters

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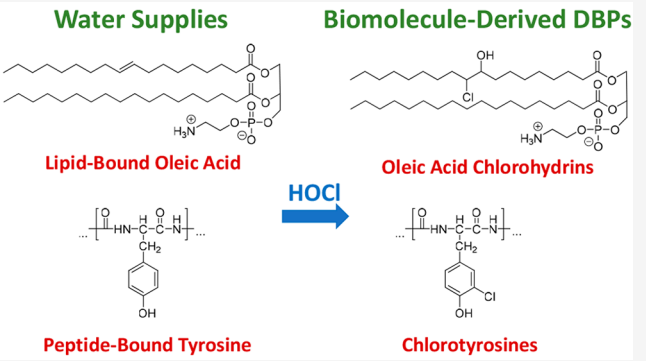
**ABSTRACT:** Recent research indicates that the poorly characterized high-molecular weight disinfection byproduct (DBP) fraction (more than two carbons) contributes more to cytotoxicity than the one- to two-carbon DBPs of current interest. Peptides and lipids contribute to DBP precursors in water supplies. Although partially degraded, a portion of their monomers retain their structures. Using tyrosine and oleic acid as exemplars, this study illustrates the targeted analysis of their chlorinated byproducts as an approach to characterizing high-molecular weight DBPs. After biopolymers had been digested to liberate monomers, oleic acid was detected in four of six secondary effluents from potable reuse facilities at concentrations of up to 91 nM (47 μg/L), while its chlorohydrins were detected in two effluents at concentrations of up to 1.3 nM (0.43 μg/L). Tyrosine was detected in all six effluent samples at concentrations of up to 42 nM (7.6 μg/L). 3-Chlorotyrosine was detected in four samples at concentrations of up to 3.3 nM (0.71 μg/L), and 3,5-dichlorotyrosine was detected in three samples at concentrations of up to 2.1 nM (0.53 μg/L). These DBPs were detected in conventional drinking waters, but at lower frequencies and concentrations. When detected, the contribution of chlorohydrins to cytotoxicity was comparable to some, but not all, of the one- to two-carbon DBP classes.




**KEYWORDS:** *disinfection byproducts, amino acids, fatty acids, proteins, lipids*



## INTRODUCTION

Epidemiological studies have linked chlorine-disinfected drinking water consumption with an increased bladder cancer risk,[1] attributable to byproducts (DBPs) formed from disinfectant reactions with dissolved organic matter (DOM). Although >700 DBPs have been identified,[2] research has focused on one- to two-carbon DBPs.[3] Recent interest has shifted from regulated trihalomethanes (THMs) and haloacetic acids (HAAs) to unregulated one- to two-carbon DBPs (e.g., haloacetonitriles) that are significantly more cytotoxic and genotoxic in Chinese hamster ovary (CHO) cell assays.[4] The calculated additive toxicity (CAT) values obtained by weighting DBP concentrations by concentrations associated with a 50% reduction in CHO cell growth [i.e., lethal concentration or $LC_{50}$ values (eq 1)] indicated that unregulated one- to two-carbon DBPs contribute more to cytotoxicity than regulated DBPs.[5−10] However, a study comparing the one- to two-carbon DBP CAT values to the total cytotoxicity measured by the CHO assay in disinfected conventional drinking waters and potable reuse waters indicated that the one- to two-carbon DBPs accounted for only 16% of cytotoxicity,[11] highlighting the need to identify the poorly characterized pool of higher-molecular weight DBPs (more than two carbons).

$$\mathrm{CAT} = \sum_{i=1}^{n} \left( \frac{[\mathrm{DBP}]_i}{\mathrm{LC}_{50_i}} \right) \quad (1)$$

Key challenges for characterizing DBPs with more than two carbons are that DOM precursors are poorly defined and the number of possible byproducts increases with carbon number. One approach applies nontargeted high-resolution mass spectrometry, which avoids prior assumptions about these structures. Such studies have identified hundreds of halogenated elemental formulae,[12−18] although defining specific structures is laborious. Recognizing that DOM precursors feature aromatic structures, other researchers have

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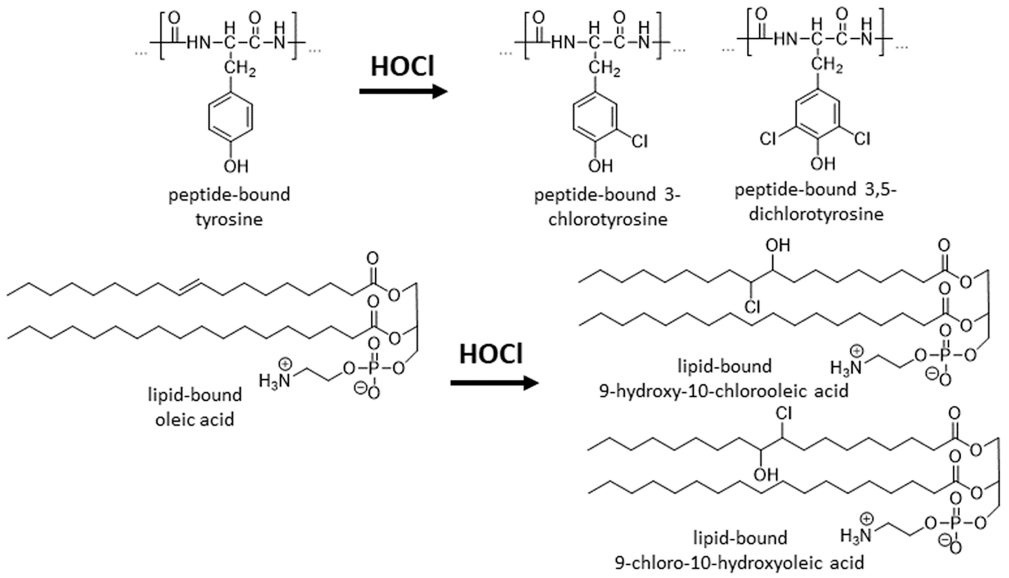


**Figure 1.** Chlorination of peptide-bound tyrosine and lipid-bound oleic acid. Chlorination of peptide-bound tyrosine forms 3-chlorotyrosine and 3,5-dichlorotyrosine. Chlorination of oleic acid within a phospholipid forms two monochlorohydrin products (i.e., 9-hydroxy-10-chlorooleic and 9-chloro-10-hydroxyoleic moieties).

developed novel mass spectrometry techniques to demonstrate the occurrence of halogenated aromatic byproducts (e.g., halobenzaldehydes).[19−24]

A different approach recognizes that biopolymers (proteins, lipids, carbohydrates, and nucleotides) shed by plants, algae, bacteria, and humans contribute precursors to water supplies and wastewaters. Although they are partially degraded by natural watershed or wastewater treatment processes, this approach hypothesizes that a portion of the monomers retain their structures within biopolymers. Research demonstrated that levels of peptide-bound amino acids are higher in wastewaters than in pristine waters. The concentrations of total amino acids were ∼0.25 μM in the pristine MacKenzie River,[25] ∼3 μM in algal- or wastewater-impacted waters,[26] and ∼15−20 μM in wastewaters.[27] The monomers constituting biopolymers are few (e.g., 20 amino acids within proteins), and their well-characterized structures enable the prediction of major chlorination products.

Analyzing byproducts of biopolymer-bound monomers is challenging. The levels of peptide-bound amino acids exceeded those of free amino acids by 10-fold in pristine waters,[25] algal- and wastewater-impacted waters,[26] and wastewaters,[27] suggesting that biopolymer-bound DBPs are important. Research with peptides demonstrated that chlorination forms peptide-bound 3-chlorotyrosine and 3,5-dichlorotyrosine (Figure 1).[28−33] Previous research detected free chlorotyrosines in sewage sludge and anaerobic digestor supernatant exposed to ∼2 g/L chlorine.[34] While halotyrosine-containing dipeptides were detected in disinfected waters,[30−32] the number of possible dipeptides renders quantification difficult. With 20 amino acids, there are ∼40 possible tyrosine-containing dipeptides depending on whether tyrosine is in the N-terminal or C-terminal position. Research with model unsaturated fatty acids demonstrated that chlorine adds across the alkenes to form two chlorohydrin isomers (Figure 1).[35,36] While nontargeted mass spectrometry identified chlorohydrins when a cyanobacterial culture was chlorinated,[37] the number of possible oleic acid-containing lipids inhibits quantification. However, strong acid digestion to liberate peptide-bound chlorotyrosines and lipid-bound oleic acid chlorohydrins enabled their quantification within biopolymers of vegetables chlorinated under post-harvest washing conditions.[38,39]

The objective of this study was to liberate peptide-bound chlorotyrosines and lipid-bound oleic acid chlorohydrins and quantify total chlorotyrosines and oleic acid chlorohydrins in disinfected conventional drinking waters and potable reuse waters. Although total byproduct concentrations include biopolymer-bound byproducts, we expected biopolymer-bound byproducts to dominate based upon the 10-fold higher concentrations of peptide-bound amino acids than free amino acids in wastewaters and drinking waters.[25−27] This study employed whole water extracts generated during a previous study that quantified one- to two-carbon DBPs[11] to compare their contributions to cytotoxicity. Chlorotyrosines and oleic acid chlorohydrins were used as exemplars of peptide-bound and lipid-bound byproducts to illustrate the broader approach of targeted identification of biopolymer-derived DBPs. These DBPs were selected because their standards and CHO cell cytotoxicity $LC_{50}$ values were available.[38,39]

## MATERIALS AND METHODS

Supporting Information Text S1 provides material sources.

**Sample Collection and Extraction.** We evaluated water extracts collected during a previous study that measured one- to two-carbon DBPs and cytotoxicity[11] for which there was sufficient remaining volume. Sample collection and extraction are summarized in Text S1.[11] Briefly, samples (10 L) were collected from five potable reuse facilities and conventional drinking waters supplying the same catchments. Potable reuse facilities A and B (facility letters are those used previously[11]) employed microfiltration (MF) and reverse osmosis (RO). Facilities C−E featured RO-free potable reuse trains using processes such as ozone ($O_3$), biologically active carbon (BAC), granular activated carbon (GAC), and advanced oxidation (AOP). Samples were treated in the laboratory with preformed monochloramine or chlorine followed by chloramines (Text S1). Conventional drinking waters were tap waters derived from groundwater or surface water (facilities


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Table 1. Summary of the Concentrations (nanomolar) of Total Oleic Acid and Tyrosine Precursors and Chlorinated DBPs[a]

| facility | sample | oleic acid | oleic acid 9,10-chlorohydrins | tyrosine | 3-chlorotyrosine | 3,5-dichlorotyrosine |
|---|---|---|---|---|---|---|
| | | | Conventional Drinking Waters | | | |
| A | 1 | <3.7 | <0.13 | <1.4 | <0.37 | <0.64 |
| | 2 | <3.7 | <0.13 | <1.4 | 0.37 | <0.64 |
| B | 1 | <3.7 | <0.13 | <1.4 | <0.37 | <0.64 |
| C | 1 | <3.7 | <0.13 | 2.0 | <0.37 | 1.0 |
| | 2 | <3.7 | <0.13 | <1.4 | <0.37 | <0.64 |
| D | 1 | <3.7 | 0.5 | <1.4 | <0.37 | <0.64 |
| E | 1 | 13 | <0.13 | <1.4 | <0.37 | <0.64 |
| | | | Reuse Waters | | | |
| A | sec. eff. dis. | 53 | <0.13 | 19 | 2.1 | 1.8 |
| | RO eff. | NM | NM | 2 | <0.37 | 1.3 |
| B | sec. eff. | 91 | <0.13 | 44 | 0.73 | 0.81 |
| | sec. eff. dis. | 58 | 0.6 | 47 | 3.3 | 2.1 |
| | RO eff. | <3.7 | <0.13 | <1.4 | <0.37 | <0.64 |
| C1 | sec. eff. | <3.7 | <0.13 | 8.6 | <0.37 | <0.64 |
| | sec. eff. dis. | <3.7 | <0.13 | 3.1 | 0.39 | <0.64 |
| | $O_3$/BAC eff. | <3.7 | <0.13 | <1.4 | <0.37 | <0.64 |
| C2 | sec. eff. | <3.7 | <0.13 | 12 | <0.37 | 0.69 |
| | sec. eff. dis. | <3.7 | <0.13 | 3 | 0.46 | <0.64 |
| | $O_3$/BAC eff. | <3.7 | <0.13 | NM | NM | NM |
| D | sec. eff. | 64 | <0.13 | 9.0 | <0.37 | <0.64 |
| | sec. eff. dis. | <3.7 | <0.13 | <1.4 | <0.37 | <0.64 |
| | AOP eff. | 11 | <0.13 | <1.4 | <0.37 | <0.64 |
| E | sec. eff. | 17 | 1.3 | 1.7 | <0.37 | <0.64 |
| | sec. eff. dis. | NM | NM | 1.4 | <0.37 | <0.64 |
| | GAC Eeff. | 56 | <0.13 | <1.4 | <0.37 | <0.64 |

[a]sec. eff. = secondary effluent prior to disinfectant addition. All other samples were exposed to disinfectant. NM = not measured due to insufficient sample volume.

A−C) or surface water-derived samples collected at treatment plants just prior to disinfection that were treated with chlorine followed by chloramines in the laboratory (facilities D and E). Aliquots had previously been analyzed[11] for one- to two-carbon DBPs using EPA Methods 551.1 and 552.3. The 10 L samples were adjusted to pH 3.7 and extracted by solid-phase extraction (2.5 g of Phenomenex Sepra ZTL), eluted with methanol, concentrated, and exchanged into 200 $\mu$L of DMSO (Text S1).

**Tyrosine and Oleic Acid Analyses.** With one exception (discussed below), chlorotyrosines and oleic acid chlorohydrins were measured only after acid digestion to liberate them from peptides and lipids, thereby measuring total chlorotyrosine and oleic acid chlorohydrin concentrations, without distinguishing free and biopolymer-bound compounds. Komaki et al.[38] and Text S1 provide detailed procedures for acid digestion and total tyrosine, 3-chlorotyrosine, and 3,5-dichlorotyrosine analysis. Briefly, ∼50 $\mu$L of DMSO extracts was treated with methanesulfonic acid at 110 °C to liberate tyrosines from peptides. After neutralization and methanol addition, lipids were extracted into chloroform. The aqueous/methanol phase was evaporated, reconstituted at pH 5, passed over C18 solid-phase extraction cartridges, and eluted with a 40% methanol/60% deionized water mixture. The $\alpha$-amino groups of amino acids were derivatized with 6-aminoquinoline-*N*-hydroxy-succinimidyl ester (AQC) to increase their hydrophobicity to facilitate separation by high-performance liquid chromatography (HPLC). Derivatized tyrosines were quantified by HPLC with mass spectrometry (LC/MS) using electrospray ionization in the multiple-reaction monitoring (MRM) negative ion mode. Figure S1 interprets the MRM transitions used for chlorotyrosines.

Simpson et al.[39] and Text S1 provide analytical details for total oleic acid and oleic acid chlorohydrins. Gas chromatography/mass spectrometry (GC/MS) was used after experiments indicated that LC/MS was insufficiently sensitive. Total chlorohydrins are reported because GC/MS could not separate the 9-chloro-10-hydroxy versus 9-hydroxy-10-chloro isomers (Figure 1). Acid digestion of lipids was pursued instead of base-catalyzed hydrolysis, because other experiments indicated that chlorohydrins were unstable under basic conditions. Briefly, ∼50 $\mu$L of DMSO extracts was extracted with MtBE and treated with a 90% methanol/10% sulfuric acid mixture to digest lipids and methylate the liberated oleic acid. The MtBE was evaporated. The residue was treated with *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSTFA) to silylate the chlorohydrin hydroxyl group. Samples were analyzed by GC/MS in the electron impact mode. Figure S2 interprets the GC/MS spectra.

To differentiate free versus peptide-bound chlorotyrosines, a second sample of chloramine-disinfected facility A wastewater effluent (sample A2) was analyzed in triplicate without and with acid digestion. 3-Chlorotyrosine was detected with digestion (standard deviation of 1 ± 0.32 nM), but not without digestion (<0.37 nM), indicating that 3-chlorotyrosine was predominantly peptide-bound; these results concur with research indicating that peptide-bound amino acids are ∼10-fold more prevalent than free amino acids in drinking waters and wastewaters.[25−27] 3,5-Dichlorotyrosine was not detected with or without digestion (<0.64 nM). A similar analysis for chlorohydrins was not possible, because the acid−methanol


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procedure simultaneously liberates lipid-bound fatty acids and methylates both the initially free and liberated fatty acids for analysis.

The average free monomer recoveries for 1 $\mu$g/L spikes into duplicate 10 L creek or deionized water samples were 77% for oleic acid, 58% for 9,10-chlorohydrins, 59% for tyrosine, and 52% for 3-chlorotyrosine. We estimated average solid-phase extraction efficiencies of 52% for peptide-bound 3-chlorotyrosine and 99% for lipid-bound oleic acid from spikes of 20 $\mu$M *N*-acetyl-isoleucine-3-chlorotyrosine-alanine-valine and 47 $\mu$M 1-oleoyl-*rac*-glycerol (Figure S3) into triplicate aliquots of chloramine-disinfected wastewater sample A2. The greater recovery observed for lipid-bound versus free oleic acid reflects its neutral charge. The tyrosine liberation efficiency by acid digestion from *N*-acetyl-glycine-tyrosine-glutamic acid-alanine-glutamic acid-histidine (Figure S3) was 78%, determined by comparison against direct derivatization and analysis of free tyrosine. The oleic acid liberation efficiency from five model lipids (Figure S3) was 66% when measured against free oleic acid processed by the acid−methanol derivatization procedure.

Overall recovery reflects solid-phase extraction efficiency for free amino acids or fatty acids, but both the extraction and digestion efficiencies for peptide- or lipid-bound species. Because our analyses of total tyrosine, oleic acid, and their byproducts did not distinguish free versus peptide- or lipid-bound species, we corrected concentrations for recoveries using the highest overall recoveries to be conservative (i.e., minimizing reported concentrations). Measured total (including liberated) amino acid concentrations were corrected using the 52% recovery for free 3-chlorotyrosine. Measured total fatty acid concentrations were not corrected for recovery because the 66% overall recovery associated with lipid-bound oleic acid was close to 70%. Concentrations measured in disinfected waters were corrected for any concentrations detected in duplicate 10 L deionized water blanks. On the basis of repeated analyses of native analyte concentrations in authentic water sample extracts, method detection limits (MDLs) were 3.7 nM for oleic acid, 0.13 nM for oleic acid 9,10-chlorohydrins, 1.4 nM for tyrosine, 0.37 nM for 3-chlorotyrosine, and 0.64 nM for 3,5-dichlorotyrosine. Text S1 provides additional details.

## RESULTS AND DISCUSSION

**Drinking Waters.** Table 1 presents concentrations of total oleic acid, oleic acid 9,10-chlorohydrins, tyrosine, 3-chlorotyrosine, and 3,5-dichlorotyrosine measured in samples from five potable reuse trains and drinking waters serving the same catchments. Oleic acid was detected in one of seven drinking waters above the 3.7 nM (1.0 $\mu$g/L) MDL. This water was a surface water disinfected with chlorine followed by chloramines (chlorine/chloramines). Oleic acid was not detected in groundwater-derived waters (facility A sample 1 and facilities B and C) or in chlorine-disinfected samples (facilities B and C). Total tyrosine concentrations in drinking waters were not detectable (<1.4 nM or 0.26 $\mu$g/L), except the concentration of 2.0 nM (0.37 $\mu$g/L) detected in facility C sample 1, a chlorinated groundwater.

Oleic acid 9,10-chlorohydrins were detected at 0.5 nM (0.17 $\mu$g/L) in one of seven drinking waters (facility D), a surface water disinfected with chlorine/chloramines. 3-Chlorotyrosine was detected only at the 0.37 nM (0.08 $\mu$g/L) MDL in one sample (facility A sample 2), a surface water disinfected with chlorine/chloramines. 3,5-Dichlorotyrosine was detected in only one sample (facility C sample 1) at 1 nM (0.25 $\mu$g/L). This sample was a chlorine-disinfected groundwater and was the only sample with measurable tyrosine.

To compare chlorine versus chloramine reactivity with biopolymers, 100 $\mu$M *N*-acetyl-glycine-tyrosine-glutamic acid-alanine-glutamic acid-histidine was treated with 200 $\mu$M free chlorine or chloramines at pH 7. LC/MS analysis indicated <1% of the parent peptide ($m/z$ 745) remained after chlorination, but 4.2% (3.8−4.6% range of experimental duplicates) after chloramination. Peptide products featuring one chlorine ($m/z$ 779; M + 34) and two chlorines ($m/z$ 813; M + 68) were observed at an abundance ratio of 8.5:1 during chloramination (Figure S4). During chlorination, only the $m/z$ 813 product was observed, with an abundance similar to that of the $m/z$ 779 product during chloramination. These findings align with expectations that, as a more reactive oxidant, chlorine should more rapidly convert peptides to chlorinated products.

**Potable Reuse Waters.** We expected that lipid-bound oleic acid, peptide-bound tyrosine, and their byproduct concentrations would be higher in secondary effluents than in drinking waters due to biomolecules being shed from bacteria during wastewater treatment. Secondary effluent samples typically were analyzed before and after addition of preformed chloramines (facilities A and B) or chlorine/chloramines (facilities C−E). Exceptions were facility A, where a sample prior to disinfectant addition was unavailable, and facility E after disinfection, where sufficient volume remained for only tyrosine analysis. Oleic acid was not detected in facility C samples, similar to its drinking waters. Oleic acid was detected at all other facilities and at higher concentrations in secondary effluent samples than in drinking waters, at concentrations reaching 91 nM (26 $\mu$g/L) at facility B. Addition of a disinfectant to secondary effluent reduced the levels of oleic acid at facilities B and D. Disinfected samples from potable reuse processes indicated that RO treatment at facility B reduced the level of oleic acid by at least 94%, while the non-RO-based processes at facilities D (e.g., AOP effluent) and E (e.g., GAC effluent) were less effective. Why the level of oleic acid was higher in facility E GAC effluent than secondary effluent is unclear; lipids shed from the BAC unit upstream of GAC may have contributed precursors.

Tyrosine was detected more frequently and at higher concentrations in secondary effluents than in drinking waters, concurring with research indicating that the concentrations of total amino acids decreased in the following order: secondary effluents (~15−20 $\mu$M)[27] > wastewater-impacted drinking water supplies (2.3 $\mu$M)[26] > pristine surface waters (0.3 $\mu$M).[25] However, tyrosine concentrations were lower than oleic acid concentrations. Disinfectant-treated samples from reuse processes indicated that RO-based and non-RO-based trains reduced the concentration of tyrosine to close to or below the 1.4 nM MDL.

Oleic acid 9,10-chlorohydrins, 3-chlorotyrosine, and 3,5-dichlorotyrosine were detected at higher levels in secondary effluents (with or without disinfection) than in drinking waters. Chlorohydrins were detected in two of six secondary effluents but in one of seven drinking waters. 3-Chlorotyrosine was detected in four of six secondary effluents but one of seven drinking waters. 3,5-Dichlorotyrosine was detected in three of six secondary effluents but one of seven drinking waters. The maximum DBP concentrations were also higher in secondary effluents than in drinking waters.



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Table 2. CAT Values for Chlorohydrins, Chlorotyrosines, and One- to Two-Carbon DBPs[a]

| | | conventional DWs | | secondary effluents | | |
|---|---|---|---|---|---|---|
| DBP class | DBP | median | maximum | median | 75th percentile | maximum |
| fatty acid chlorohydrins | oleic acid 9,10-chlorohydrin | $<1.23 \times 10^{-6}$ | $4.89 \times 10^{-6}$ | $<1.23 \times 10^{-6}$ | $3.90 \times 10^{-6}$ | $1.27 \times 10^{-5}$ |
| chlorotyrosines | 3-chlorotyrosine | $<1.17 \times 10^{-7}$ | $1.18 \times 10^{-7}$ | $1.35 \times 10^{-7}$ | $5.27 \times 10^{-7}$ | $1.04 \times 10^{-6}$ |
| | 3,5-dichlorotyrosine | $<9.01 \times 10^{-7}$ | $1.42 \times 10^{-6}$ | $<9.01 \times 10^{-7}$ | $1.92 \times 10^{-6}$ | $2.92 \times 10^{-6}$ |
| THMs | chloroform | $5.73 \times 10^{-6}$ | $2.92 \times 10^{-5}$ | $2.41 \times 10^{-5}$ | $7.16 \times 10^{-5}$ | $9.72 \times 10^{-5}$ |
| HAAs | chloroacetic acid<br>dichloroacetic acid<br>trichloroacetic acid | $1.76 \times 10^{-5}$ | $5.39 \times 10^{-5}$ | $7.53 \times 10^{-5}$ | $1.97 \times 10^{-4}$ | $3.07 \times 10^{-4}$ |
| HAMs | dichloroacetamide<br>trichloroacetamide | $7.82 \times 10^{-10}$ | $2.42 \times 10^{-5}$ | $3.77 \times 10^{-9}$ | $7.30 \times 10^{-9}$ | $1.45 \times 10^{-8}$ |
| HNMs | chloropicrin | $<2.28 \times 10^{-6}$ | $<2.28 \times 10^{-6}$ | $5.22 \times 10^{-6}$ | $6.14 \times 10^{-6}$ | $1.18 \times 10^{-5}$ |
| HALs | chloral hydrate | $4.84 \times 10^{-6}$ | $2.30 \times 10^{-5}$ | $4.71 \times 10^{-6}$ | $5.13 \times 10^{-5}$ | $1.98 \times 10^{-4}$ |
| HANs | dichloroacetonitrile<br>trichloroacetonitrile | $8.22 \times 10^{-5}$ | $1.91 \times 10^{-4}$ | $1.70 \times 10^{-4}$ | $3.40 \times 10^{-4}$ | $3.86 \times 10^{-4}$ |

[a]Where concentrations are < MDL, CAT values indicate < MDL/$LC_{50}$.

Figure S5 depicts the three byproduct concentrations for the facility B potable reuse train. Both chlorotyrosines were detected in secondary effluent prior to disinfection; although chlorohydrins were not detected in facility B secondary effluent prior to disinfection, they were detected in facility E secondary effluent prior to disinfection. These detections potentially arise from formation during drinking water disinfection or use of bleach within households (e.g., laundry). However, the application of chloramine to facility B secondary effluent increased the levels of all three byproducts. The concentrations of all three DBPs were below the MDLs in chloramine-treated RO effluent.

**Implications.** With research indicating that one- to two-carbon DBPs account for a minor fraction of cytotoxicity,[11] there is a need to identify toxicity drivers within the pool of DBPs with more than two carbons. Using oleic acid and tyrosine as exemplars, our results demonstrate that a portion of monomers retain their structures within the partially degraded biopolymer fraction of DOM in water supplies and wastewater effluents. Our findings illustrate the targeted analysis of byproducts predicted to form from these intact monomers as one approach to characterizing DBPs with more than two carbons. While biopolymer-bound fatty acid chlorohydrins[37] and halotyrosines[30−33] have been detected in disinfected waters, quantifying their total concentrations is difficult due to the range of biopolymers in which they could reside; this study illustrates their quantification by digestion to liberate monomers.

Total oleic acid 9,10-chlorohydrins and chlorotyrosines were quantified in drinking waters and potable reuse waters for the first time to the best of our knowledge. The concentrations of one- to two-carbon DBPs in these samples had been measured previously,[11] including THMs, HAAs, haloacetamides (HAMs), chloropicrin [a halonitromethane (HNM)], haloacetaldehydes (HALs), and haloacetonitriles (HANs). To indicate their contribution to cytotoxicity,[10] CAT values (eq 1) associated with chlorohydrin and chlorotyrosine concentrations (Table 1) were calculated using CHO cell cytotoxicity $LC_{50}$ values previously determined for oleic acid 9,10-chlorohydrins (0.106 mM),[39] 3-chlorotyrosine (3.17 mM), and 3,5-dichlorotyrosine (0.71 mM).[38] While these $LC_{50}$ values are for free chlorotyrosines and oleic acid chlorohydrins, these values are relevant for human ingestion of peptide-bound chlorotyrosines and lipid-bound oleic acid chlorohydrins, which are liberated via exposure to strong acid and digestive enzymes (e.g., proteases and lipases) in the human digestive system. Table 2 provides a percentile analysis for CAT values in the drinking water and secondary effluent samples for the chlorohydrins, chlorotyrosines, and one- to two-carbon DBPs containing only chlorine. Brominated and iodinated analogues of one- to two-carbon DBPs[4] and halotyrosines[40] typically are more cytotoxic than their chlorinated analogues. Because the brominated and iodinated halohydrin and halotyrosine concentrations were not available, only the chlorinated analogues were compared. For one- to two-carbon DBP classes containing several chlorinated constituents (e.g., HAAs), Table 2 provides the sum of the individual CAT values. Because chlorohydrins and chlorotyrosines were less frequently detected than one- to two-carbon DBPs, CAT calculations indicate that they did not contribute on a median basis to the cytotoxicity of drinking waters. On a maximum basis, the chlorohydrin contribution was greater than that of HNMs in drinking waters, but below that of other one- to two-carbon DBP classes. Chlorotyrosines contributed less to cytotoxicity. While chlorohydrins and chlorotyrosines were detected more frequently in secondary effluents than in drinking waters, their detection frequencies were still lower than for one- to two-carbon DBPs. Again, chlorotyrosines contributed less to cytotoxicity than the chlorohydrins. On a maximum or a 75th percentile basis, the contribution of chlorohydrins was greater than that of HAMs, comparable to that of HNMs, but less than those of other one- to two-carbon DBPs.

Although not the toxicity drivers, oleic acid and tyrosine served as exemplars for predicting chlorination byproducts formed from a wider array of chlorine-reactive biopolymers. Research has indicated that 18-carbon fatty acids featuring two to four alkenes are ∼30-fold more prevalent than oleic acid (a monoalkene) within cyanobacterial cultures.[41] The greater prevalence of polyunsaturated fatty acids suggests that oleic acid 9,10-chlorohydrins may represent a minor component of a larger chlorohydrin pool. While this predictive approach provides one path for characterizing high-molecular weight DBPs, it only complements the need for nontargeted analytical approaches that identify partially degraded biomolecular components.[12−24]



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## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.estlett.3c00143.

Details of sample collection, extraction, and analysis; volumes of extracts used; mass spectra of the derivatized chlorohydrin and chlorotyrosine byproducts; biomolecule structures; comparison of chlorine versus chloramine reactivity with a model peptide; and a profile of DBP concentrations at facility B (PDF)

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### Author Contributions

§M.-J.S. and M.M.H. contributed equally to this work.


### Notes

The authors declare no competing financial interest.

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## ■ REFERENCES


(1) Villanueva, C. M.; Cantor, K. P.; Cordier, S.; Jaakkola, J. J. K.; King, W. D.; Lynch, C. F.; Porru, S.; Kogevinas, M. Byproducts and bladder cancer: a pooled analysis. *Epidemiology* **2004**, *15*, 357−367.

(2) Richardson, S. D. Disinfection by-products: formation and occurrence in drinking water. In *Encyclopedia of Environmental Health*; Nriagu, J. O., Ed.; Elsevier, Inc., 2011; pp 110−136.

(3) Li, X.-F.; Mitch, W. A. Drinking water disinfection byproducts (DBPs) and human health effects: Multidisciplinary challenges and opportunities. *Environ. Sci. Technol.* **2018**, *52*, 1681−1689.

(4) Wagner, E. D.; Plewa, M. J. CHO cytotoxicity and genotoxicity analyses of disinfection by-products: an updated review. *J. Environ. Sci.* **2017**, *58*, 64−76.

(5) Zeng, T.; Plewa, M. J.; Mitch, W. A. N-Nitrosamines and halogenated disinfection byproducts in U.S. full advanced treatment trains for potable reuse. *Water Res.* **2016**, *101*, 176−186.

(6) Szczuka, A.; Parker, K. M.; Harvey, C.; Hayes, E.; Vengosh, A.; Mitch, W. A. Regulated and unregulated halogenated disinfection byproduct formation from chlorination of saline groundwater. *Water Res.* **2017**, *122*, 633−644.

(7) Plewa, M. J.; Wagner, E. D.; Richardson, S. D. TIC-Tox: a preliminary discussion on identifying the forcing agents of DBP-mediated toxicity of disinfected water. *J. Environ. Sci.* **2017**, *58*, 208−216.

(8) Chuang, Y. H.; Szczuka, A.; Mitch, W. A. Comparison of toxicity-weighted disinfection byproduct concentrations in potable reuse waters to conventional drinking waters as a new approach to assess the quality of advanced treatment train waters. *Environ. Sci. Technol.* **2019**, *53*, 3729−3738.

(9) Allen, J. A.; Plewa, M. J.; Wagner, E. D.; Wei, X.; Bokenkamp, K.; Hur, K.; Jia, A.; Liberatore, H. K.; Lee, C.-F. T.; Shirkhani, R.; Krasner, S. K.; Richardson, S. D. Disinfection By-Product Drivers of Cytotoxicity in U.S. Drinking Water: Should Other DBPs Be Considered for Regulation? *Environ. Sci. Technol.* **2022**, *56*, 392−402.

(10) Lau, S. S.; Wei, X.; Bokenkamp, K.; Wagner, E. D.; Plewa, M. J.; Mitch, W. A. Assessing additivity of cytotoxicity associated with disinfection byproducts in potable reuse and conventional drinking waters. *Environ. Sci. Technol.* **2020**, *54*, 5729−5736.

(11) Lau, S. S.; Bokenkamp, K.; Tecza, A.; Wagner, E. D.; Plewa, M. J.; Mitch, W. A. Toxicological comparison of potable reuse water versus conventional drinking water. *Nat. Sustainability* **2023**, *6*, 39−46.

(12) Zhang, H. F.; Zhang, Y. H.; Shi, Q.; Ren, S. Y.; Yu, J. W.; Ji, F.; Luo, W. B.; Yang, M. Characterization of low molecular weight dissolved natural organic matter along the treatment train of a waterworks using Fourier transform ion cyclotron resonance mass spectrometry. *Water Res.* **2012**, *46*, 5197−5204.

(13) Zhang, H. F.; Zhang, Y. H.; Shi, Q.; Hu, J. Y.; Chu, M. Q.; Yu, J. W.; Yang, M. Study on Transformation of Natural Organic Matter in Source Water during Chlorination and Its Chlorinated Products using Ultrahigh Resolution Mass Spectrometry. *Environ. Sci. Technol.* **2012**, *46*, 4396−4402.

(14) Lavonen, E. E.; Gonsior, M.; Tranvik, L. J.; Schmitt-Kopplin, P.; Köhler, S. J. Selective Chlorination of Natural Organic Matter: Identification of Previously Unknown Disinfection Byproducts. *Environ. Sci. Technol.* **2013**, *47*, 2264−2271.

(15) Zhang, H.; Zhang, Y.; Shi, Q.; Zheng, H.; Yang, M. Characterization of Unknown Brominated Disinfection Byproducts during Chlorination Using Ultrahigh Resolution Mass Spectrometry. *Environ. Sci. Technol.* **2014**, *48*, 3112−3119.

(16) Gonsior, M.; Schmitt-Kopplin, P.; Stavklint, H.; Richardson, S. D.; Hertkorn, N.; Bastviken, D. Changes in Dissolved Organic Matter during the Treatment Processes of a Drinking Water Plant in Sweden and Formation of Previously Unknown Disinfection Byproducts. *Environ. Sci. Technol.* **2014**, *48*, 12714−12722.

(17) Andersson, A.; Lavonen, E.; Harir, M.; Gonsior, M.; Hertkorn, N.; Schmitt-Kopplin, P.; Kylin, H.; Bastviken, D. Selective removal of natural organic matter during drinking water production changes the composition of disinfection by-products. *Environ. Sci.: Water Res. Technol.* **2020**, *6*, 779−794.

(18) Andersson, A.; Gonsior, M.; Harir, M.; Hertkorn, N.; Schmitt-Kopplin, P.; Powers, L.; Kylin, H.; Hellstrom, D.; Nilsson, K.; Pettersson, A.; Stavklint, H.; Bastviken, D. Molecular changes among non-volatile disinfection by-products between drinking water treatment and consumer taps. *Environ. Sci.: Water Res. Technol.* **2021**, *7*, 2335−2345.

(19) Zhang, X.; Minear, R. A.; Barrett, S. E. Characterization of high molecular weight disinfection byproducts from chlorination of humic substances with/without coagulation pretreatment using UF-SEC-ESI/MS/MS. *Environ. Sci. Technol.* **2005**, *39*, 963−972.

(20) Zhang, X.; Talley, J. W.; Boggess, B.; Ding, G.; Birdsell, D. Fast selective detection of polar brominated disinfection byproducts in

drinking water using precursor ion scans. *Environ. Sci. Technol.* **2008**, *42*, 6598−6603.
(21) Ding, G.; Zhang, X. A picture of polar iodinated disinfection byproducts in drinking water by (UPLC/)ESI-tqMS. *Environ. Sci. Technol.* **2009**, *43*, 9287−9293.
(22) Zhai, H.; Zhang, X. Formation and Decomposition of New and Unknown Polar Brominated Disinfection Byproducts during Chlorination. *Environ. Sci. Technol.* **2011**, *45*, 2194−2201.
(23) Gong, T.; Zhang, X. Detection, identification and formation of new iodinated disinfection byproducts in chlorinated saline wastewater effluents. *Water Res.* **2015**, *68*, 77−86.
(24) Yang, M.; Zhang, X.; Liang, Q.; Yang, B. Application of (LC/)MS/MS precursor ion scan for evaluating the occurrence, formation and control of polar halogenated DBPs in disinfected waters: A review. *Water Res.* **2019**, *158*, 322−337.
(25) Peake, E.; Baker, B. L.; Hodgson, G. W. Hydrogeochemistry of the surface waters of the MacKenzie River drainage basin, Canada - II. The contribution of amino acids, hydrocarbons and chlorins to the Beaufort Sea by the MacKenzie River system. *Geochim. Cosmochim. Acta* **1972**, *36*, 867−883.
(26) Dotson, A.; Westerhoff, P. Occurrence and removal of amino acids during drinking water treatment. *J. Am. Water Works Assoc.* **2009**, *101*, 101−115.
(27) Pehlivanoglu-Mantas, E.; Sedlak, D. L. Measurement of dissolved organic nitrogen forms in wastewater effluents: concentrations, size distribution and NDMA formation potential. *Water Res.* **2008**, *42*, 3890−3898.
(28) Pattison, D. I.; Davies, M. J. Absolute rate constants for the reaction of hypochlorous acid with protein side chains and peptide bonds. *Chem. Res. Toxicol.* **2001**, *14*, 1453−1464.
(29) Choe, J. K.; Richards, D. H.; Wilson, C. J.; Mitch, W. A. Degradation of Amino Acids and Structure in Model Proteins and Bacteriophage MS2 by Chlorine, Bromine and Ozone. *Environ. Sci. Technol.* **2015**, *49*, 13331−13339.
(30) Huang, G.; Jiang, P.; Li, X.-F. Mass spectrometry identification of N-chlorinated dipeptides in drinking water. *Anal. Chem.* **2017**, *89*, 4204−4209.
(31) Huang, G.; Jiang, P.; Jmaiff Blackstock, L. K.; Tian, D.; Li, X.-F. Formation and occurrence of iodinated tyrosyl dipeptides in disinfected drinking water. *Environ. Sci. Technol.* **2018**, *52*, 4218−4226.
(32) Huang, G.; Jmaiff Blackstock, L. K.; Jiang, P.; Liu, Z.; Lu, X.; Li, X.-F. Formation, identification, and occurrence of new bromo- and mixed halo-tyrosyl dipeptides in chloraminated water. *Environ. Sci. Technol.* **2019**, *53*, 3672−3680.
(33) Jiang, P.; Huang, G.; Jmaiff Blackstock, L. K.; Zhang, J.; Li, X.-F. Ascorbic acid assisted high performance liquid chromatography mass spectrometry differentiation of isomeric C-chloro- and N-chlorotyrosyl peptides in water. *Anal. Chem.* **2017**, *89*, 13642−13650.
(34) Burleson, J. L.; Peyton, G. R.; Glaze, W. H. Gas-chromatographic/mass-spectrometric analysis of derivatized amino acids in municipal wastewater products. *Environ. Sci. Technol.* **1980**, *14*, 1354−1359.
(35) Gibson, T. M.; Haley, J.; Righton, M.; Watts, C. D. Chlorination of fatty acids during water treatment disinfection: reactivity and product identification. *Environ. Technol. Lett.* **1986**, *7*, 365−372.
(36) Winterbourn, C. C.; van den Berg, J. J. M.; Roitman, E.; Kuypers, F. A. Chlorohydrin formation from unsaturated fatty acids reacted with hypochlorous acid. *Arch. Biochem. Biophys.* **1992**, *296*, 547−555.
(37) Gonsior, M.; Powers, L. C.; Williams, E.; Place, A.; Chen, F.; Ruf, A.; Hertkorn, N.; Schmitt-Kopplin, P. The chemodiversity of algal dissolved organic matter from lysed Microcystis Aeruginosa cells and its ability to form disinfection by-products during chlorination. *Water Res.* **2019**, *155*, 300−309.
(38) Komaki, Y.; Simpson, A. M.-A.; Choe, J. K.; Plewa, M. J.; Mitch, W. A. Chlorotyrosines versus volatile byproducts from chlorine disinfection during washing of spinach and lettuce. *Environ. Sci. Technol.* **2018**, *52*, 9361−9369.
(39) Simpson, A. M.-A.; Suh, M.-J.; Plewa, M. J.; Mitch, W. A. Formation of oleic acid chlorohydrins in vegetables during post-harvest chlorine disinfection. *Environ. Sci. Technol.* **2022**, *56*, 1233−1243.
(40) Tian, D.; Moe, B.; Huang, G.; Jiang, P.; Ling, Z. C.; Li, X.-F. Cytotoxicity of halogenated tyrosyl compounds, an emerging class of disinfection byproducts. *Chem. Res. Toxicol.* **2020**, *33*, 1028−1035.
(41) Hayakawa, K.; Tsujimura, S.; Napolitano, G. E.; Nakano, S.; Kumagai, M.; Nakajima, T.; Jiao, C. Fatty acid composition as an indicator of physiological condition of the cyanobacterium Microcystis aeruginosa. *Limnology* **2002**, *3*, 29−35.




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