Aquatic humic substances react with chlorine to produce numerous disinfection byproducts (DBPs) during chlorination of drinking water. Although low molecular weight (MW) chlorinated DBPs have been intensively studied over the past several decades, relatively little is known about high MW chlorinated DBPs (above 500 Da) that may be associated with adverse health implications. In this work, carrier-free radioactive $^3{}_{26}Cl$ was introduced into a Suwannee River fulvic acid sample to label the chlorine-containing DBPs. By combining the fractionation techniques of ultrafiltration (UF) and size exclusion chromatography (SEC) with the detection of $^3{}_{35}Cl$, UV, and dissolved organic carbon (DOC), the high MW region in the SEC–$^3{}_{35}Cl$ profiles of the chlorinated sample with and without UF was defined. SEC–UV and SEC–DOC profiles were found to be approximately indicative of SEC–$^3{}_{35}Cl$ profiles for the high MW region. The MW distribution shows that the high MW chlorinated DBPs were highly dispersed with an average MW around 2000 Da based on calibration with polystyrene sulfonate standards. The C1/C atomic ratios of the high MW DBPs were roughly constant (0.025), which is much lower than those of the common known chlorinated DBPs.

**Introduction**

Aquatic humic substances (HS), ubiquitous in raw waters, react with chlorine during disinfection to produce a number of halogenated disinfection byproducts (DBPs) in drinking water. A collective parameter for all the halogenated organic compounds is total organic halogen (TOX). It has been shown that common known DBPs only constitute a part of TOX (1–4). By simulating a typical drinking water disinfection condition, the authors demonstrated that common known DBPs including trihalomethanes (THMs), haloacetic acids (HAAs), haloacetonitriles, haloketones, chloropicrin, etc. only account for roughly 50% of TOX during chlorination (4). There are several reasons for the uncounted part of TOX. First, only about 20 or fewer of the common known halogenated DBPs were quantified in the previous work. Most of DBPs identified to date (about 260 halogenated species; 5) were not counted. This is mainly because (a) almost all the identified halogenated DBPs were determined by gas chromatography/mass spectrometry (GC/MS), but no commercial or synthetic standards were available for calibration; (b) levels of some identified DBPs were one to several orders of magnitude lower than the common known DBPs. This increases the difficulty in quantification and often results in reported values of “below detection limits”. Second, some unknown/unidentified DBPs may be excluded because of the method limitation of GC/MS. All the early targeted DBPs or their derivatized species were thermally stable, volatile or semi-volatile, neutral or hydrophobic. These compounds or their derivatives can be easily isolated from the aquatic matrix by purge-and-trap, liquid–liquid extraction, or solid-phase extraction and then identified by GC/MS (6). But GC/MS is not amenable to identification of the highly polar/hydrophilic/nonvolatile compounds and the polar/hydrophilic/nonvolatile derivatives and especially is not amenable to the identification of compounds with high molecular weights, which may constitute a significant part of TOX.

A general reaction taking place during water chlorination can be expressed as

$$\text{HS} + \text{Br}^- + \text{HOCI/OCI}^- \rightarrow \text{hs} + \text{X-hs} + \text{Cl}^-$$

where HS indicates humic substance precursor, hs indicates nonhalogenated byproducts, and X-hs indicates halogenated byproducts. Generally, three major types of reactions occur simultaneously during disinfection: oxidation, addition, and substitution. Oxidation reactions can lead to the decomposition of precursors to form low MW compounds, but addition and substitution reactions increase the MW of precursors slightly. The high MW and polydisperse nature of natural water organics is well-known and reaffirmed by Wagoner et al. (7). With precursors of high MW, it is most likely that some halogenated DBPs with high MW will be produced during disinfection.

Previous ultrafiltration (UF) experiments seem to demonstrate the existence of high MW DBPs. Kopfer et al. (8) fractionated chlorinated solutions of humic material by using an Amicon stirrer/UF cell equipped with Nucleapore UF membranes having nominal MW cutoffs of 500, 5000, and 50 000 Da. The results for the fractionation (expressed as percentage of TOX) were as follows: 500 Da, 47%; 500–5000 Da, 14%; 5000–50 000 Da, 3%; > 50 000 Da, 9%. Khiai et al. (9) used a UF cell with membranes of different MW cutoffs. When a chlorinated drinking water passed through the UF cell, retentate and permeate were collected for TOX analysis, and the following results (expressed as percentage of TOX) were obtained: < 500 Da, 48.4%; 500–3000 Da, 29.6%; 3000–10 000 Da, 13.6%; > 10 000 Da, 8.4%.

High MW DBPs may have health implications. Kopfer et al. (8) also reported that UF fractions of chlorinated humic substances were tested for mutagenicity using the Salmonella histidine reverse mutation assay. The assays were performed with S. typhimurium his strains TA98 and TA100. For the TA98 mutagenesis, only 36% of revertants was attributed to the fraction with nominal MW less than 500 Da; For the TA100 mutagenesis, 59% of revertants was attributed to the fraction with nominal MW less than 500 Da. The results indicate that a significant portion of the mutagenicity was represented by the fraction with nominal MW above 500 Da. On the other hand, it has been preassumed that chemicals of MW higher than 5000 Da may not be associated with toxicological risks (10, 11). The reason is that, for a chemical to produce an adverse health effect in humans following exposure via drinking water, it must be able to be absorbed into the body. Molecular size as well as physicochemical properties play an important role in determining whether a
molecule can be absorbed across the gastrointestinal tract membranes and be taken up into the circulation such that it might produce biological interactions. Thus, some toxicologists proposed an upper limit of 5000 Da for toxicological significance. There are two MW boundaries for consideration, 500 and 5000 Da. For DBPs with MW below 500 Da, a significant portion of the major species have likely been identified by GC/MS. For DBPs with MW above 5000 Da, these species are dismissed as being of little toxicological significance. Therefore, DBPs with MW in the range of 500–5000 Da would seem to warrant more attention.

To characterize high MW chlorinated DBPs, a key step was to select appropriate methods for separation and detection. UF can provide preliminary fractionation of chlorinated HS as mentioned above. Size exclusion chromatography (SEC) is an effective method for size distribution determination of macromolecules and can provide useful and reliable MW distributions of natural organic matter or HS with appropriate calibration standards (12–14). Little has been reported about the characterization of chlorinated HS with SEC. UV detection is sensitive to unsaturated and aromatic compounds but not to those that contain C–C or C–Cl bonds only. On-line/off-line dissolved organic carbon (DOC) detection is responsive to all the organic compounds and not selective. A probe specific to chlorine-containing compounds was needed accordingly. TOX analysis would be a logical choice, but it is very time-consuming, not sensitive (with a detection limit of around 500 μg/L for a 2-mL fraction sample), and has significant measurement errors. Therefore, the radiisotope 36Cl, a 0.714 MeV pure β-emitter with a half-life of 3.1 × 105 yr, was used to label chlorine-containing DBPs in this study.

The specific objectives of this work were (i) to separate fractionate chlorinated HS and better define the chlorinated high MW DBPs with UF and SEC techniques with a combination detection of 35Cl, UV, and DOC and (ii) to roughly estimate MW distribution and Cl/ C atomic ratios of high MW chlorinated DBPs from chlorinated HS. Since this work represents a part of an ongoing study into the MW separation and HPLC/MS/MS characterization of previous unidentified drinking water DBPs (funded by the U.S. EPA), the experimental design for separation/fractionation was performed as part of a broader objective, future MS/MS analysis.

Experimental Methods

Preparation of HO36Cl from Na36Cl. Stock chlorine solution was prepared based on the method reported by Ghanbari et al. (15) via the reaction of Na36Cl with KMnO4 and H2SO4:

\[
2\text{KMnO}_4 + 10\text{NaCl} + 8\text{H}_2\text{SO}_4 \rightarrow 2\text{MnSO}_4 + 2\text{K}_2\text{SO}_4 + 5\text{Na}_2\text{SO}_4 + 8\text{H}_2\text{O} + 5\text{Cl}_2^\dagger
\]

Modifications were made to improve gas-collecting efficiency and eliminate possible radioactive contamination of the vacuum system by adding three gas bubbling diffusers and two more scrubber bottles (16). Before using radioactive Na36Cl, nonradioactive NaCl was used instead to ensure that the system was gastight and to obtain optimum conditions for generating Cl2. Also, since this was a reduced pressure system, great care was taken to prevent back flow to the reactor or surging into the vacuum system when the reaction was stopped. A total of 100 μCi of Na36Cl was purchased from ICN in a volume of 0.103 mL, a radionuclue purity of 99+%, a specific activity of 16.0 μCi/mg Cl. The 1 + 1 mL of Milli-Q water supplied by a Millipore MRS purifier system was added to the vial containing Na36Cl, most of which was transferred to a two-neck flask. Then 100 mg of KMnO4 was put into the flask. After the flask was capped with a separatory funnel, 16 mL of Milli-Q water and 4 mL of concentrated H2SO4 were added to the funnel. Twenty milliliters of Milli-Q water was put into theCollector tube, and 400 mL of 0.02 N NaOH in Milli-Q water was put into each of the scrubber bottles in advance. After the vacuum system was started for several minutes, H2SO4 was added dropwise to the flask. The process was considered complete when bubbles stopped forming. The concentration of Cl2 in the collector tube was measured by the iodometric method (17).

Preparation of Chlorinated HS Samples. Suwannee River Fulvic Acid (SRFA) from the International Humic Substances Society (IHSS) was employed as a representative of HS. Ultrahigh purity (UHP) chlorine gas (99.97%) was purchased from Scott Specialty Gases. HOCl stock solution was prepared by the absorption of the UHP chlorine gas with Milli-Q water and was standardized by the iodometric method. Two samples were prepared under the same conditions except for the chlorinating agent, one with HO36Cl and the other with HOCI. Initial concentrations of SRFA and HO36Cl/HOCI were 100 mg/L as C and 50 mg/L as Cl2, respectively, which is 10–30 times higher than the average level of NOM and Cl2 in drinking water treatment, to amplify and observe the possible reactions and phenomena. For this experiment, Br− was not added for the sake of simplifying the reaction mixture. The reaction was controlled at pH 7.5 with phosphate buffer and was quenched with NaAsO2 after a contact time of 24 h. The same sample as above without adding HO36Cl/HOCI was prepared as a control.

UF. UF was conducted in a continuous manner with series 400- and 10-mL Millipore stirred cells as shown in Figure 1. A membrane made of cellulose acetate with nominal MW cutoff of 500 Da was used in the 10-mL cell only. The 400-mL cell was used to continuously replenish Milli-Q water to the 10-mL UF cell. The silicone tubing connecting these two cells was wrapped with Teflon tape and properly sealed on both ends to endure relatively high pressure. Two milliliters of the chlorinated SRFA sample was put into the 10-mL UF cell. The total amount of Milli-Q water used for flushing was 550 mL (with which 99.4% of Cl2 was found to be flushed out of the 10-mL cell). UHP nitrogen gas supplied to the 400-mL cell was kept at 45–50 psi. The solution in the 10-mL cell was continuously mixed with a magnetic stir bar throughout the process. The UF was stopped when 2 mL of retentate remained.

SEC. HPLC was conducted with a Beckman Gold system, consisting of two model 110B solvent delivery pumps, a model 166 programmable UV detector, and a data-collecting computer. The column used was a 25 × 200 mm BIAx (Chrom, Germany) with a column packing of Toyopearl HW 50S resin (Japan). The resin is semi-rigid spherical beads synthesized by copolymerization of ethylene glycol and methacrylate
polymers. The SEC column has a nominal fractionation range (per poly(ethylene glycol) calibration by the supplier) of 100–20000 g/mol. Particle size was 30 μm. The eluent was 0.03 M NH₄HCO₃ with a flow rate of 0.80 mL/min. The injection loop was 500 μL. One fraction of 1.6 mL was collected every 2 min with a fraction collector. NH₄HCO₃ was used as eluent because it can be readily removed from fractions by either freeze-drying or nitrogen-sparging. This was considered to be very important for future MS/MS analysis by reducing adduct formation and simplifying mass spectra.

The column void volume was determined using Blue Dextran with a MW of 2 million Da. A series of aliphatic and aromatic compounds, most of which are common known halogenated DBPs, were used as low MW markers. Polystyrene sulfonates (PSS) with MW of 18K, 8K, 4.6K, and 1.8K (Polysciences Inc., MA) were used as high MW markers. All markers were prepared in the eluent, and their retention times were determined by UV absorption. The retention time of chloride ion was determined by the following procedure: 500 μL of 2 g/L KCl in the eluent was injected into the HPLC system. For each fraction collected, 1 mL of 0.1 M AgNO₃ was added and shaken till yellow precipitate (Ag₂CO₃) was observed, then 5 drops of 1:1 nitric acid was added and shaken vigorously. The precipitate disappeared in all fractions except those with retention time between 66 and 70 min, in which the white precipitate of AgCl was left.

To obtain the MW distribution of high MW chlorinated DBPs, a semilog-linear calibration curve defined by PSS 18K, 8K, 4.6K, and acetone (12) were used to determine the MW of an analyte, Mi, at some eluted volume i. For a mixture of molecules, the MW distribution is represented by number average MW (Mn), weight average MW (Mw), and polydispersity (ρ):

\[
M_n = \frac{\sum_{i=1}^{N} h_i M_i}{\sum_{i=1}^{N} h_i}, \quad M_w = \frac{\sum_{i=1}^{N} h_i M_i}{\sum_{i=1}^{N} h_i} \quad \rho = \frac{M_w}{M_n}
\]

where hi is the height of the sample SEC curve eluted at volume i, and Mi is the MW at eluted volume i determined from the calibration curve (18).

**Liquid Scintillation Counting.** Two milliliters of ScintiVerse II (Fisher) was added to each SEC fraction from the fraction collector and shaken for thorough mixing. Liquid Scintillation Counting was performed with a Beckman LS 6000IC. The counting time for each fraction was set at 30 min to guarantee low relative standard deviations (RSD). The following RSDs resulted: for 36Cl activity > 10 000 cpm, RSD < 0.5%; for 36Cl activity > 1000 cpm, RSD < 1.5%; for 36Cl activity > 100 cpm, RSD < 5.0% for 36Cl activity > background, RSD < 8.0%. A blank fraction of 1.6 mL of 0.03 M NH₄HCO₃ was used for background correction. Counting efficiency and possible quenching effects of each fraction were determined by adding standard spikes of 36Cl activity to the same fraction collected from injection of the nonradioactive sample.

**DOC Analysis.** DOC concentrations in SEC fractions from injection of the nonradioactive sample were not measured directly because of the high level of inorganic carbon (360 mg/L as C from the eluent of 0.03 M NH₄HCO₃) and the low levels of organic carbon (from 0 to several mg/L as C). Our method for removing inorganic carbon in each fraction was to add 0.10 mL of 1 N HCl and sparge with UHP nitrogen gas for 10 min and then repeat the HCl addition and sparge for another 10 min. By using this procedure, up to 99.97% of the inorganic carbon was removed and did not interfere with the detection of organic carbon. After the inorganic carbon was removed, DOC was determined with a TOC-500 analyzer coupled with an ASI-502 autosample injector (Shimadzu). Newly changed high-sensitivity catalyst and halogen scrubber were used. During the measurement of a series of fractions, the autosampling device attached to the injector was always used to prevent carbon dioxide in the air from diffusing into the fractions.

**Results and Discussion**

**Elution Behavior of the SEC Column.** It is well-known that size exclusion fractionations are not based on size alone because other factors such as charge, molecular structure, steric effects, and hydrophobicity can influence the results (19). The SEC column used here is no exception: two mechanisms determine the whole separation, one is size exclusion, and the other is solute–gel interaction. For high MW compounds, size exclusion appears to be dominant; but for specific low MW compounds, solute–gel interaction appears to be dominant. In the study of MW distribution of NOM, researchers generally discount the latter interaction. These two mechanisms, however, can play important roles in separating high MW compounds from low MW compounds and possibly separating low MW compounds from one another. Table 1 shows that the SEC column used was such an effective and promising tool: High MW markers appeared with retention times of 29–68 min, but low MW markers eluted from 65 through 1630 min.

For low MW compounds, chlorine incorporation generally increased the retention time significantly, e.g., monochloroacetic acid (MCAA, 68.2 min) < acetic acid (AA, 65.5 min); 4-chlorobenzoic acid (136.6 min) < benzoic acid (82.8 min); chlorobenzene (973 min) < benzene (350 min); 4-chlorophenol (1447 min) < phenol (424 min). In addition, the more the chlorine incorporated, the longer the retention time (e.g., trichloroacetic acid (TCAA, 101.9 min) < dichloroacetic acid (DCAA, 82.1 min) < MCAA (68.2 min)). But for chlorine-substituted phenols, 2,4-dichlorophenol (1623 min) > 4-chlorophenol (1447 min) > 2,4,6-trichlorophenol (1053 min). This is mainly due to the low pKₐ value (6.80) of 2,4,6-trichlorophenol, which was readily ionized in the 0.03 M NH₄HCO₃ eluent. Frimmel’s group (20, 21) systematically studied the solute–gel interactions with a SEC column of the same packing material (Toyopearl HW 50S resin). They tried to

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Wavelength/Method</th>
<th>Retention Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blue Dextran</td>
<td>260 nm</td>
<td>29.83 ± 0.03</td>
</tr>
<tr>
<td>PSS MW18000</td>
<td>263 nm</td>
<td>31.86 ± 0.06</td>
</tr>
<tr>
<td>PSS MW8000</td>
<td>263 nm</td>
<td>36.39 ± 0.05</td>
</tr>
<tr>
<td>PSS MW4600</td>
<td>263 nm</td>
<td>40.44 ± 0.02</td>
</tr>
<tr>
<td>PSS MW1800</td>
<td>263 nm</td>
<td>40–68</td>
</tr>
<tr>
<td>acetone</td>
<td>275 nm</td>
<td>88.1 ± 0.1</td>
</tr>
<tr>
<td>phenol</td>
<td>256 nm</td>
<td>424 ± 2</td>
</tr>
<tr>
<td>4-chlorophenol</td>
<td>276 nm</td>
<td>1447 ± 4</td>
</tr>
<tr>
<td>2,4-dichlorophenol</td>
<td>285 nm</td>
<td>1623 ± 10</td>
</tr>
<tr>
<td>2,4,6-trichlorophenol</td>
<td>310 nm</td>
<td>1053 ± 7</td>
</tr>
<tr>
<td>benzoic acid</td>
<td>254 nm</td>
<td>82.8 ± 0.2</td>
</tr>
<tr>
<td>4-chlorobenzoic acid</td>
<td>254 nm</td>
<td>136.6 ± 0.8</td>
</tr>
<tr>
<td>benzene</td>
<td>254 nm</td>
<td>350.5 ± 0.5</td>
</tr>
<tr>
<td>chlorobenzene</td>
<td>264 nm</td>
<td>973 ± 2</td>
</tr>
<tr>
<td>acetic acid</td>
<td>210 nm</td>
<td>65.53 ± 0.03</td>
</tr>
<tr>
<td>chloroacetic acid</td>
<td>215 nm</td>
<td>68.24 ± 0.04</td>
</tr>
<tr>
<td>dichloroacetic acid</td>
<td>240 nm</td>
<td>82.1 ± 0.1</td>
</tr>
<tr>
<td>trichloroacetic acid</td>
<td>230 nm</td>
<td>101.9 ± 0.1</td>
</tr>
<tr>
<td>chloroform</td>
<td>204 nm</td>
<td>303.5 ± 0.1</td>
</tr>
<tr>
<td>chloride ion</td>
<td>AgNO₃ titration</td>
<td>66–70</td>
</tr>
</tbody>
</table>

* Eluent 0.03 M NH₄HCO₃; flow rate 0.80 mL/min.
correlate properties of organic compounds (e.g., elemental ratios, C/H, C/O, distribution of O among phenolic and carbonyl groups, N and S content) with their ratios, C/H, C/O, distribution of O among phenolic and correlating properties of organic compounds (e.g., elemental proportions). FIGURE 2. Comparison of SEC-UV profiles of the chlorinated SRFA sample with and without UF (panels a–c are for the same data with different scaling factors). The closed triangles correspond to individual compounds, and the number next to each compound is its MW.

SEC-36Cl Profile. SEC-36Cl profiles of the chlorinated SRFA sample with and without UF and some low MW compounds are shown in Figure 2a–c. These three panels are for the same data but with different scaling factors. Figure 2a displays a huge peak for the sample without UF, which corresponds to Cl-Cl. By integrating this peak, the total amount of Cl-Cl was determined to be more than 80% of initial chlorine dose, which means that oxidation was the dominant reaction as compared to chlorine incorporation during chlorination. This has been addressed elsewhere in detail (16). After UF, the Cl-Cl peak appears to be mostly removed. If Figure 2a is enlarged by expanding both the x-axis and the y-axis, the SEC-36Cl profile of the sample without UF shows three major peaks within retention times of 0–120 min (Figure 2b) and a number of minor peaks within retention times of 120–600 min (Figure 2c). Accordingly, the SEC-36Cl profile is very informative and indicative of whether a SEC fraction contains chlorinated DBPs for purposes of isolating and concentrating samples for MS/MS analysis.

For the three major peaks contained in Figure 2b, the peaks with retention times of 65–74 and 98–110 min mainly correspond to Cl-Cl and TCAA, respectively. The peak with retention time of 40–65 min is regarded primarily as a high MW DBP peak given the following considerations. First, most of the common known halogenated DBPs (HAAs, THMs, chlorophenols, etc.) with low MW had much longer retention times than this peak. According to the elution behavior of the SEC column, a chlorine-containing compound tends to have longer retention time than its analogues. A good explanation for this peak that contained chlorine and had a short retention time is that it primarily comprised chlorinated DBPs with high MW. Second, this peak has a strong UV absorption at 254 nm (Figure 3), which is representative of mainly aromatic chromophores in SRFA (22). Since all the known single-ring aromatic DBPs such as chlorobenzoic acid, chlorobenzene, 4-chlorophenol, 2,4-dichlorophenol, and 2,4,6-trichlorophenol eluted with much longer retention times, it is very likely that this peak consisted of chlorinated, multiple benzene ring-containing, aromatic DBPs. Third, comparison with high MW markers such as polyethylene sulfonates (PSS) shows that this peak partly overlapped with those of PSS 8K, PSS 4.6K, and PSS 1.8K. Fourth, after UF with a 500-Da membrane, almost all the low MW chlorinated DBPs and Cl-Cl were flushed out, but most of this peak remained (Figure 2b,c).

The decrease in this peak after UF could be due to adsorptive loss to the membrane or the removal of the low MW, highly charged DBPs possibly contained in that peak, which are eluted early due to charge-charge exclusion. The activity left on the membrane (3970 dpm) was about 1.6% of the total activity of the ultrafiltered sample (244 800 dpm), which excluded membrane adsorption as the primary reason for reduction in this region. Therefore, the peak with a retention time of 40–65 min in the SEC-36Cl profile of the chlorinated SRFA sample with UF is ascribed to high MW chlorinated DBPs.

SEC–UV Profile. The SEC–UV (254 nm) profile of the chlorinated SRFA sample without UF looks very simple: only one major peak with a little hump on the shoulder (Figure 3). With retention time above 75 min, the UV absorption curve decreased rapidly to the background level. It is of interest that the major peak in the SEC–UV profile showed up with a retention time of 40–65 min, which is quite consistent with the high MW DBP peak in the SEC–36Cl profile. This is not a coincidence because those high MW chlorinated DBPs still retained some of the aromatic chromophores in SRFA. This can be seen by the comparison of SEC–UV profiles of chlorinated SRFA sample and SRFA sample control (Figure 3). Consequently, a SEC–UV profile can be roughly indicative of SEC–36Cl profile for the high MW region. Since the acquisition of a SEC–36Cl profile is
very time-consuming, expensive, and requires special skills and instrumentation for the radioactive material treatment and measurement, a SEC–UV profile is advantageous to the study of high MW chlorinated DBPs. After UF with a 500-Da membrane, the SEC–UV profile decreased slightly along the right side of the peak, with the disappearance of the little hump. This also suggests that some relatively low MW DBPs contained in that peak were flushed out after UF.

**SEC–DOC Profile.** The SEC–DOC profile of the chlorinated SRFA sample without UF contained one major peak and a number of minor peaks, which eluted within 120 min (Figure 4). With longer retention time, the peaks became too small to be differentiated from background. After UF with a 500-Da membrane, the SEC–DOC profile decreased slightly along the right side of the major peak with the disappearance of most of the minor peaks, which indicates that some relatively low MW DBPs contained in that peak were flushed out after UF. As expected, the major peak in the SEC–DOC profile showed up with the same retention time range as the high MW DBP peak in the SEC–36Cl profile. Accordingly, the SEC–DOC profile can also be approximately indicative of the SEC–36Cl profile in the study of high MW DBPs. Most interestingly, the overlapping of the SEC–36Cl and SEC–DOC profiles in the high MW region means that Cl/C ratios of high MW DBPs were roughly constant. This point will be addressed later.

**MW Distribution of High MW DBPs.** To obtain a realistic MW distribution, the calibration compounds should ideally have similar structures and solution behavior to chlorinated high MW DBPs (chlorine-incorporated HS). Chin et al. (12) used SEC to determine the MW distributions of a variety of aquatic HS. PSS standards were used for calibration with good agreement obtained between the SEC MW distributions and those obtained using techniques such as vapor pressure osmometry and small-angle X-ray scattering. PSS molecules were assumed to have a similar configuration to aquatic HS. PSS standards have also been used for the calibration of SEC by other research groups (13, 21). Given that Cl/C ratio in high MW DBPs was very low (which is demonstrated below), chlorine incorporation to HS may not affect the structures and properties of those HS significantly. Therefore, PSS standards were chosen here for calibrating high MW chlorinated DBPs.

Figure 5 shows the comparison of the SEC–36Cl profile of the ultrafiltered chlorinated SRFA sample with PSS standards. The SEC–36Cl profile of the ultrafiltered sample partly overlapped with PSS 18K, 8K, and 4.6K and mainly overlapped with PSS 1.8K, which extended from 40 to 68 min. This demonstrates that the ultrafiltered chlorinated SRFA sample mainly comprised high MW DBPs. On the basis of these data, the MW distribution of the ultrafiltered chlorinated SRFA sample was constructed as depicted in Figure 6. Since the standard substances that are used for the mass calibration of the SEC column are not suited for all fractions equally, a calculation of the mass distribution of the DBPs is highly tentative.

![FIGURE 4. Comparison of SEC–DOC profiles of the chlorinated SRFA sample with and without UF.](image)

![FIGURE 5. Comparison of SEC–36Cl profile of the ultrafiltered chlorinated SRFA sample with calibration standards.](image)

![FIGURE 6. Molecular weight distribution of the ultrafiltered chlorinated SRFA sample.](image)

![FIGURE 7. Relationship between SEC–36Cl/DOC profiles and Cl/C ratios of the ultrafiltered chlorinated SRFA sample.](image)
be obtained, and the SEC–Cl/C profile is also shown in Figure 7. For most of the high MW region (40–55 min), the SEC–36Cl profile was very close to the SEC–DOC profile (almost overlapped), which means that Cl/C ratio was close to a constant and suggests that the chlorine incorporation to some HS precursors was approximately an even process (i.e., the higher the MW, the more the chlorine incorporated, if their MWs were high enough (well above 1500 Da)). The SEC–Cl/C profile gave a value that was roughly of constant, 0.025, which is much lower than the common known halogenated DBPs. This means that for a DBP with MW of around 1000 Da (containing about 40 carbon atoms estimated by the carbon content in SRFA), it may contain only one chlorine atom per molecule on average. To explain such an approximately even chlorine incorporation process for high MW DBPs, the possible reaction sites in SRFA should be considered. Given that the content of phenolic functionality in SRFA is 2.89 mol/kg DOC, the weight content of carbon considered. Given that the content of phenolic functionality per molecule of SRFA can be calculated as 0.73, which is much lower than the common known halogenated DBPs. This means that for a DBP with MW of around 1000 Da (containing about 40 carbon atoms estimated by the carbon content in SRFA), it may contain only one chlorine atom per molecule on average. To explain such an approximately even chlorine incorporation process for high MW DBPs, the possible reaction sites in SRFA should be considered. Given that the content of phenolic functionality in SRFA is 2.89 mol/kg DOC, the weight content of carbon

Acknowledgments
The authors acknowledge funding for the research from the United States Environmental Protection Agency (Grant R828344-01). The authors are grateful to the four anonymous referees for constructive critique and useful suggestions.

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