Review Paper

Physical and chemical fractionation of dissolved organic matter and trihalomethane precursors: A review

Alex T. Chow, Suduan Gao and Randy A. Dahlgren

ABSTRACT

High levels of dissolved organic matter (DOM) and its potential to form disinfection by-products (DBPs), such as trihalomethanes (THMs), during drinking water treatment raise challenges to management of source waters worldwide. Not all DOM is equally reactive in DBP formation during water disinfection and various physical and chemical fractionation techniques have been applied to identify the major reactive DBP components in DOM. In this review paper, we evaluate three commonly used fractionation techniques used in THM precursor research: XAD fractionation, ultrafiltration and size exclusion chromatography. Research findings from different source waters worldwide are summarized in order to understand the role of various DOM fractions in THM formation and to identify knowledge gaps. The hydrophobic fraction and the fraction with an apparent molecular weight of 1–10 kDa have been implicated as the primary source of THM precursors, but exceptions have been observed. Differences in isolation procedures, chlorination methodologies, origins of DOM and nomenclature used to describe DOM fractions may contribute to discrepancies in results among some studies. Advancement of fractionation techniques in conjunction with sensitive spectroscopic techniques (e.g. UV absorbance and fluorescence) is essential for effectively evaluating DOM quality and quantity in source waters and optimizing water treatment processes.

Key words | disinfection by-products, dissolved organic carbon, drinking water quality, size exclusion chromatography, ultrafiltration, XAD fractionation

Alex T. Chow (corresponding author)
Randy A. Dahlgren
Department of Land, Air, and Water Resources,
University of California,
Davis, CA 95616,
USA
Tel: (530) 752-0630
Fax: (530) 752-5262
E-mail: atchow@ucdavis.edu;
    radahlgren@ucdavis.edu

Suduan Gao
USDA-ARS, Water Management Research
    Laboratory,
9611 S Riverbend Ave Parlier,
CA 93648,
USA
Tel: (559) 596-2870
Fax: (559) 596-2851
E-mail: sgao@fresno.ars.usda.gov

INTRODUCTION

The presence of trihalomethanes (THMs) and other disinfection by-products (DBPs) in finished drinking water have raised public health concern (Richardson *et al.* 2002). A variety of DBPs are formed when dissolved organic matter (DOM) in water reacts with disinfectants during drinking water treatment (Krasner 1999; Xie 2004). Over 500 DBPs have been identified and major DBPs include trihalomethanes (THMs), haloacetic acids (HAAs), 3-chloro-4-(dichloromethyl)-5-hydroxy-2[5H]-furanone (MX), cyanogen halides, aldehyde, haloacetonitriles, haloketones, halopyrrole and halonitromethane (Richardson 1998). THMs and HAAs are the two largest classes of DBPs on a weight basis in chlorinated drinking waters (Krasner *et al.* 1989). THMs and DBPs in drinking waters are of concern because of their carcinogenic and mutagenic properties (Bull *et al.* 2001). Among identified DBPs, iodoacetic acid, bromoacetic acid, and MX appear to have higher cytotoxic and mutagenic potencies than other DBPs (Kargalioglu *et al.* 2002; Plewa *et al.* 2002, 2004). The levels of DBPs in drinking water have been the subject of regulatory activity by the US Environmental Protection Agency (USEPA) for over 20 years (Pontius 1999). The major provisions of the Stage 1 disinfectant/disinfection by-product (D/DBP) rule include maximum contaminant level (MCL) monitoring, precursor

removal, and technology improvement from the results of Information Collection Rule (ICR) monitoring and supplemental research. The current MCL, as determined for a distribution system-wide running annual average, for finished drinking water in the United States is 80 ppb THMs and 60 ppb HAAs (USEPA 1996); however, values of 40 ppb THMs and 30 ppb HAAs and a new computation method – locational running annual average for MCL – have been proposed (USEPA 2004). Thus, water utilities using source waters with high levels of DOM may face an even greater challenge in meeting drinking water standards if these lower MCLs are adopted.

DOM derived from different sources, such as vegetation, soil, wastewater and agricultural return, may have distinctive chemical characteristics associated with its origins. For example, DOM derived from aquatic algae has a relatively large nitrogen content and low aromatic carbon and phenolic contents; in contrast, terrestrially derived DOM has relatively low nitrogen content but large amounts of aromatic carbon and phenolic compounds (Aiken *et al.* 1996). The reactivity of DOM in forming THMs could be source dependent because the aromatic carbon content, which is believed to be the major reactive component forming THMs (Boyce & Hornig 1983; Norwood *et al.* 1987), varies with different sources. Not all aromatic carbons are equally reactive with disinfectants, depending on the positions of hydroxyl and carboxylate functional groups and other functional moieties. Particularly, 2, 6-dihydroxybenzoic acid produced high yields of THMs during chlorination (Norwood *et al.* 1980; Ichihashi *et al.* 1999; Leenheer *et al.* 2001). To further complicate the issue, the contribution of each carbon source is potentially seasonally dependent. The hydrological and biogeochemical processes involved in physical mixing and carbon cycling can alter the chemical compositions and physical structures of DOM (Bano *et al.* 1997, 1998; Hood *et al.* 2003). In sum, DOM is a heterogeneous mixture and is composed of a variety of organic carbon compounds that have a wide range of reactivity in THM formation.

DOM, which is operationally defined as that portion passing through a 0.45 μm pore-size filter, is the fraction of natural organic matter that is of greatest interest to water industry research (Zsolnay 2003). The size fraction greater than 0.45 μm, such as particulate organic matter, can be removed effectively with conventional treatment methods such as coagulation and filtration processes (Edzwald 1993; Owen *et al.* 1993; Chow *et al.* 2005a). Thus, research on the characteristics of THM and DBP precursors is focused on organic matter that is smaller than 0.45 μm.

Different physical and chemical fractionation techniques, such as co-precipitation, freeze concentration, liquid extraction, cation and anion exchange resins, reverse osmosis, flow field-flow fractionation, ultrafiltration (UF), size exclusion chromatography (SEC), and XAD fractionation (nonionic macroporous sorbents), have been applied to characterize and isolate DOM from source waters (Aiken 1985; Pelekani *et al.* 1999; Leenheer & Croue 2003). Nevertheless, DOM is such a complex matrix that no fractionation scheme is able to extract all DOM from all types of source water. Furthermore, no method has been developed for specifically characterizing or isolating THM precursors. DOM has been well known as the primary precursor for THM formation under chlorination since the 1970s (Rook 1977); thus, various organic carbon fractions isolated by different procedures have been tested to determine their reactivity in THM formation. With a large set of data regarding organic carbon reactivity in forming THMs available in the literature, analysis of these data provides an opportunity to reveal some systematic compositional features of THM precursors and provide assistance for further study of DOM fractionation and isolation techniques in THM precursor studies.

Three commonly used fractionation techniques, XAD resin adsorption, UF and SEC in THM research, are the focus of this review paper. XAD fractionation is primarily based on chemical characteristics of DOM, whereas UF and SEC techniques are based on the physical size of DOM. In the following sections, we provide information about the basic principles, findings, and the advantages and disadvantages of these techniques in characterizing THM precursors, and summarize DOM composition and reactivity in THM formation from various water sources.

In particular, we evaluate the efficacy of these DOM fractionation techniques in characterizing THM precursors based on three considerations. (1) Abundance of reactive carbon in each fraction: Does any fraction isolated by a fractionation scheme contain a high density of reactive carbon contributing to THM formation? Does the separation mechanism involved in fractionation isolate the

reactive carbon fraction in DOM? (2) Consistency among DOM sources: Do the same fractions from different sources have the same characteristics or similar reactivity in THM formation? (3) Implications for characterizing a water system: Does any water system, such as river, lake or groundwater, have a unique combination of chemical and/or physical fractions? Does any system contain higher amounts of THM precursors than others?

Other THM precursors, such as bromide and dissolved organic nitrogen, other DBPs, such as HAAs and MX, and the physical and chemical structures of DBP precursors, are not included in this review because these topics by themselves are complex issues and would require extensive discussion. Information about these topics can be found in Singer *et al.* (2002), Croue *et al.* (2000) and Westerhoff & Mash (2002).

DETERMINATION OF TRIHALOMETHANE FORMATION POTENTIAL

Three laboratory approaches have been commonly used to assess the formation of THMs or DBPs during chlorination in drinking water treatment: the formation potential (FP) test, the simulated distribution system (SDS) test, and the uniform formation condition (UFC) test (Krasner 1999; Xie 2004). In the FP test, waters are spiked with high doses of chlorine at pH 7–8.3 and incubated at 20 or 25°C for a long incubation time (usually 7 days). Standard methods for FP tests include US Environmental Protection Agency (USEPA) method 510.1 (USEPA 1983, 1995) and Standard Method 5710B (Standard Methods 1998). In the SDS test, waters are chlorinated at a specific dosage with specific time, temperature and pH such that it reflects the conditions of a water distribution system. Conditions are customized for each system to be evaluated. The details of this method are described in Standard Method 5710C: Simulated Distribution System Trihalomethane (SDS-THM) (Standard Methods 1998). In the UFC test, waters are chlorinated at a pH of 8.0 ± 0.2, a temperature of 20.0 ± 1.0°C, and an incubation time of 24 ± 1 hour, with a residual chlorine concentration of 1.0 ± 0.1 mg l^{-1} (Summers *et al.* 1996). Furthermore, these standard methods have been modified among laboratories to fit the scope of their

research or tests. For example, modified versions of the EPA methods have been used by the US Geological Survey Sacramento Laboratory and California Department of Water Resources Bryte Laboratory (Agee 1992; Crepeau *et al.* 2004). Each test method has been optimized to yield specific information and choice of the test method is subject to the purpose of the research. Also, readers should recognize that these laboratory testing methods are used to examine the reactivity of DOM in water and to predict the potential DBPs in finished waters, but their results do not represent the actual concentration of DBP measured in tap water.

Each approach to assess the formation of THMs or DBPs has its own advantages and disadvantages. The conditions in the FP test maximize THM formation and allow comparisons of results from different sources. The primary purpose of the FP test is to determine the potential to form THMs based on the amount of precursors in waters. However, the FP test does not represent conditions in a treatment plant or distribution system; also, its high chlorine dosages and long incubation times result in more chlorine-substituted DBP formation (Stevens & Symons 1977; Symons *et al.* 1993). In contrast to the FP test, the conditions in the SDS test simulate the conditions in an actual distribution system and the concentrations and speciation of DBPs formed in SDS tests were found to parallel those formed in finished waters (Symons *et al.* 1993). It is used to predict the concentration and types of DBP that would form in an actual distribution system (Koch *et al.* 1991). Unfortunately, SDS conditions are site specific and the inherent variations in SDS tests complicate DBP comparisons among studies. The development of the UFC test allows direct comparisons of DBP formation in different waters using conditions representative of many US distribution systems. Particularly, the pH, temperature and residual chlorine were chosen to reflect the influence of the lead and copper rule on distribution system pH, the average temperature used in the SDS testing, and chlorine level commonly found in distribution systems (Krasner 1999).

Each method specifies different conditions of pH, contact time, temperature and residual Cl_2. Such large variation in reaction conditions adds difficulties in data interpretation from different studies because the yields of

DBP formation from chlorination, especially THMs and HAAs, are highly dependent on solution pH, chlorine dosage, DOM concentration, temperature and reaction time (Summers *et al.* 1996; Krasner 1999; Xie 2004). Oliver and Visser (1980) determined THMFP with $1 \, mg \, l^{-1}$ total organic carbon and $15 \, mg \, l^{-1} \, Cl_2$ at pH 11 for 72 h; Amy *et al.* (1990) measured THMFP with a 3:1, Cl_2 to dissolved organic carbon ratio at pH 7 for 168 h. The pH affects chlorine species and reaction mechanisms in THM formation. HOCl predominates at pH values below 7.5, while OCl^- is the predominant species at pH above 7.5. The disinfecting ability of HOCl is generally regarded to be far greater than that of OCl^- (Snoeyink & Jenkins 1980). The impact of these two disinfecting species on THM yields and species is not certain. THM formation was more evident at pH values above 8, in accordance with the increased formation of phenoxide ions (Rook 1976). The high production of THMs at high pH may be due to the instability of DBP intermediates. These intermediate by-products can undergo hydrolysis reactions to form THMs (Xie 2004). Also, THM precursors can be divided into fast and slowly reacting fractions and the formation of THM can be described by second-order kinetics (Gallard & Von Gunten 2002b). Resorcinol-type structures could be responsible for the fast reacting THM precursors, whereas slowly reacting THM precursors may consist of phenolic compounds (Gallard & von Gunten 2002a). The $\delta^{13}C$ of THMs formed from DOM extracted from two plants was 12‰ lower than DOM extracts, indicating particular chemical structures or C in DOM responsible for THM formation (Bergamaschi *et al.* 1999b). Few studies report the ionic strength or inorganic solute content of water samples. At high ionic strength and lower pH, the configuration of organic macromolecules is coiled (small hydrodynamic radius) such that some THM reactive sites may be sterically hindered. It is unknown whether these different protocols for determining THMFP have added difficulties for comparing results among various studies. Furthermore, these testing methods do not account for DOM removal and/or DOM fractionation during water treatment. Thus, comparing results among various studies should be conducted with appropriate caution.

In addition to the differences in protocols, inconsistency in the reporting units of THM formation makes data comparison among studies difficult. Most studies used mass units to express THMFP (i.e. $\mu g \, l^{-1}$ in THMFP and μg-THM mg-C^{-1} in specific THMFP). Using mass units for THMFP introduces problems from bromide species. Bromine has twice the atomic mass of chlorine; thus, water samples containing bromide appear to have much higher THMFP if mass units are used (Symons *et al.* 1993). Recent studies often express specific THMFP in molar units, representing the reactivity of organic carbon in THM formation (i.e. μmol-THM mol-C^{-1} or μmol-THM mg-C^{-1}). However, it does not completely resolve the problem. At drinking water pH levels, bromide was often converted to aqueous bromine (HOBr/OBr^-) in the presence of chlorine and ozone: $Br^- + O_3 \rightarrow OBr^- + O_2$ and $Br^- + HOCl \rightarrow HOBr + Cl^-$ (Symons *et al.* 1987; Westerhoff *et al.* 1998). Notably, aqueous bromine is more efficient in substitution reactions than chlorine (Westerhoff *et al.* 2004). Not only does its existence yield a higher portion of brominated THM species, but it also causes more THM formation from previously unreactive DOM (Singer *et al.* 2002; Liang & Singer 2003). In other words, DOM becomes more reactive to form THMs or there is more THM formation during chlorination when bromide exists. To determine the reactivity of organic carbon and the impact of bromide in THM formation, THMFP should ideally be expressed in molar units. However, THMFP expressed in mass units has an advantage in practical terms, particularly for regulators, engineers and water utilities, because the MCL stated in the D/DBP rule for THMs and HAAs is in mass units (ppb or $\mu g \, l^{-1}$). In this review, the carbon normalized THMFP, termed specific THMFP, are expressed in μg-THM mg-C^{-1}. Studies reporting their results in molar units are also summarized in this paper but the results are converted to mass units by assuming chloroform is the only species in THM formation. The molar units are also listed in parentheses for these references.

Although the existence of bromide in water may influence the THMFP test for whole water samples, the bromide effect on most organic carbon fractions collected by XAD fractionation, UF and SEC should be minimal. XAD resins are nonionic, macroporous resins that do not retain bromide during fractionation. Similarly, the pore size in UF and SEC is larger than the diameter of the bromide ion, allowing bromide to pass through. Thus, the analytical

procedures for isolating the chemical and physical fractions result in almost no bromide, except for the fraction that is not retained by either XAD resin or the filter medium. Nearly all of the THMs formed from chlorination of the hydrophobic, transphilic, and high molecular weight fractions would be $CHCl_3$. However, THMs formed from chlorination of the original water samples and the hydrophilic or the lowest molecular weight fractions could include all four THM species ($CHCl_3$, $CHCl_2Br$, $CHClBr_2$, and $CHBr_3$) if bromide existed in the water samples. The relative abundance of the four THM species varies with Br concentration. Particularly, $CHCl_3$ may not be formed if water contains high levels of bromide. Readers should be aware of a higher THMFP for waters containing bromide and the fact that fractions isolated by other techniques such as reverse osmosis may contain bromide.

SPECIFIC ULTRAVIOLET ABSORBANCE

Ultraviolet/visible (UV/VIS) absorbance has been widely used in the water industry as a surrogate parameter to estimate concentrations of dissolved organic carbon and THM precursors (Dobbs *et al.* 1972; Edzwald *et al.* 1985; Korshin *et al.* 1999; McDonald *et al.* 2004). Absorption in the UV (200–400 nm) and visible (400–800 nm) spectrum is caused by atomic and electrometric vibrations, and involves elevation of electrons in σ-, π-, η-orbitals from the ground state to higher energy levels. DOM containing unbonded electrons on oxygen and sulfur atoms and conjugated C = C double bonds are capable of showing absorption (Stevenson 1994; Perdue & Ritchie 2004). The 254 nm wavelength has been chosen because it coincides with one of the sharp spectral lines of the low-pressure mercury lamp, and at this wavelength the absorbance of DOM is normally high enough to be measured reliably (Dobbs *et al.* 1972). Other wavelengths such as 260, 272 and 280 have also been used in THM precursor studies and DOM characterization (Galapate *et al.* 1999; Wu *et al.* 2000; Imai *et al.* 2003). Both UV absorbance at 254 and 272 nm correlate well with the aromatic carbon content of DOM, which is a special type of conjugated C = C double bond (Traina *et al.* 1990; Novak *et al.* 1992; Wu *et al.* 2000). Also, UV absorbance at 280 nm and the width of the electron transfer absorbance band is found to be proportional to the molecular weight and gyration radii of the humic

molecules, respectively (Chin *et al.* 1994; Korshin *et al.* 1999). The UV/VIS spectra can be used in THM research not only because of its positive correlation with the dissolved oganic carbon concentrations, but also because colour centres in the molecule, probably in both phenol groups and conjugated double bonds, were the loci for chlorine attack and subsequent THM formation (Oliver & Thurman 1983; Norwood *et al.* 1987). Reckhow *et al.* (1990) showed that THM formation was related to the phenolic or activated aromatic content, and phenolic ring rupture is possibly one major reaction step in the THM formation mechanism (Norwood *et al.* 1987). Thus, determination of UV absorbance provides insight into the characteristics of DOM studied and its propensity in THM formation.

Not all aromatic carbon is equally reactive in THM formation. Thus, carbon normalized UV absorbance at 254 nm, termed specific ultraviolet absorbance ($SUVA_{254}$), which is defined as UV absorbance at 254 nm divided by the concentration of dissolved organic carbon, is a widely used surrogate for indicating the concentration of THM precursors in source waters (Korshin *et al.* 1997; Weishaar *et al.* 2003; Chow *et al.* 2004). Standard procedure for the determination of UV absorbance is described in Standard Method 5910B: Ultraviolet Absorption Method (Standard Methods 1998). Particularly, the UV absorbance should be only determined at neutral pH, between 4 and 10, and dilution should be made if the absorbance is above $0.900 \, cm^{-1}$. The units of UV absorbance and $SUVA_{254}$ are commonly expressed in cm^{-1} and $l \, mg\text{-}C^{-1} \, cm^{-1}$, respectively. However, the numerical value of $SUVA_{254}$ in this unit is usually in the range of a few hundredths and it is preferably expressed in $l \, mg\text{-}C^{-1} \, m^{-1}$. Also, readers should be aware that some studies express $SUVA_{254}$ in $l \, g\text{-}C^{-1} cm^{-1}$. In this review paper, $SUVA_{254}$ is expressed in $l \, mg\text{-}C^{-1} \, m^{-1}$.

CHEMICAL FRACTIONATION

XAD-8/XAD-4 fractionation

XAD fractionation is based on differences in DOM sorption efficiency on XAD resins under acid or base conditions. A hierarchical fractionation procedure developed by the US Geological Survey has been widely used to fractionate DOM (Leenheer 1981; Thurman & Malcolm 1981; Aiken

et al. 1992). Different fractionation schemes based on these protocols have been further proposed and developed to improve the recovery efficiency and purity of DOM fractions (Croue *et al.* 2000; Leenheer & Croue 2003). In these fractionation schemes DOM is fractionated into three major operationally defined fractions: hydrophobic, transphilic and hydrophilic.

Hydrophobic fractions (HPO) can be isolated by using a column of Amberlite XAD-8 resin (Figure 1). HPO is that portion of DOM that sorbs to XAD-8 resin at pH 2 with a capacity factor (k′) of 50, where k′ is defined as the time a solute spends in the stationary phase (XAD resin) divided by the time the solute spends in the mobile phase (Aiken *et al.* 1992). HPO is also referred to as the humic fraction. The portion of DOM that does not sorb to XAD-8 at pH 2 is termed the non-humic fraction. The non-humic fraction can be further fractionated into transphilic (TPH) and hydrophilic fractions (HPI) by using XAD-4 resin (Croue *et al.* 2000; Leenheer & Croue 2003). TPH is the portion of DOM that sorbs on XAD-4 resin at pH 2 with a k′ of 50. HPI is the portion of DOM passing though both the XAD-8 and XAD-4 resins at pH 2. In addition, the two fractions that sorb on XAD-8 and XAD-4 columns, respectively, can be further fractionated into acidic and neutral fractions by using different eluting solvents. Hydrophobic acid (HPOA) and transphilic acid (TPHA) are the portions of DOM that are eluted at pH 13 with NaOH from XAD-8 and XAD-4 resins, respectively. The hydrophobic neutral (HPON) and transphilic neutral (TPHN) fractions are those compounds that adsorb onto the XAD-8 and XAD-4 resins, respectively, do not dissolve during elution with NaOH, but can be extracted by organic solvents such as acetonitrile (Aiken *et al.* 1992). HPOA may be further fractionated into humic acid and fulvic acid by differences in solubility at pH 1. Humic acid is precipitated, whereas fulvic acid is soluble at pH 1 (Thurman 1985) (Figure 1). For collecting humic acid, a concentration step such as freeze-drying is usually required because of its low concentration in source waters.

HPOA mainly contains aliphatic carboxylic acids, aromatic acids and phenols (Aiken *et al.* 1992). This fraction contains materials derived from organic compounds in terrestrial plants and soils, such as lignin, tannins and pigments that give rise to highly coloured humic and fulvic acids with high polyphenol content. Particularly, both

pyrolysis-gas chromatography/mass spectroscopy (GC/MS) and tetramethylammonium hydroxide-GC/MS suggested that humic acid contains large portions of lignin and cuticular materials in its structure (Chefetz *et al.* 2002). TPHA contains less aromatic character but a higher content of oxygen, nitrogen and sulfur. It is composed of strong acids, possibly polyfunctional organic acids and aliphatic acids with five or fewer C atoms (Aiken *et al.* 1992; Croue *et al.* 2000). TPHA is probably produced by autochthonous processes, such as phytoplankton, bacteria, and microbial degradation. This fraction has been shown to change seasonally (Bourbonniere 1989; Croue *et al.* 2000). HPON consists of hydrocarbon-like materials and amorphous polymers with little peripheral functionality. Pronounced aliphatic hydrocarbon character was shown in this fraction (Croue *et al.* 2000). This fraction should be relatively stable and unreactive. TPHN isolated from several surface waters appeared nearly invariant with regard to sample type (i.e. bog, lake and river), but exhibits a strong seasonal trend and should be diagenetically close to the original biochemical source (Bourbonniere 1989). TPHN isolated from river samples appears to be almost entirely proteinaceous (Croue *et al.* 2000). Moreover, the C:H, C:O and C:N ratios of whole DOM in freshwaters were in the range 0.5–1.2, 1.1–2.1, 9.6–167, respectively (Perdue & Ritchie 2004). An analysis of DOM from an autochthonous-dominated river and an allochthonous-dominated river showed that the neutral fractions have higher C:O ratios than the acidic fractions; meanwhile, C:N and C:H ratios of the neutral fractions were generally lower than those of the acidic fractions (Croue *et al.* 2000). These results reinforce the concept that neutral molecules have greater aliphatic character. With any acidic or neutral fraction, the higher the hydrophilic character, the higher the proportions of oxygen, nitrogen and hydrogen in the DOM; that is, the more aliphatic it is. This is indicated by the C:O, C:N and C:H ratios, which generally decrease with increasing hydrophilic character for both acidic and neutral fractions (Croue *et al.* 2000; Perdue & Ritchie 2004).

Chemical fractionation of THM precursors in various water sources

Thurman (1985) and Perdue & Ritchie (2004) indicated that HPO, or humic substances, account for more than 50% of
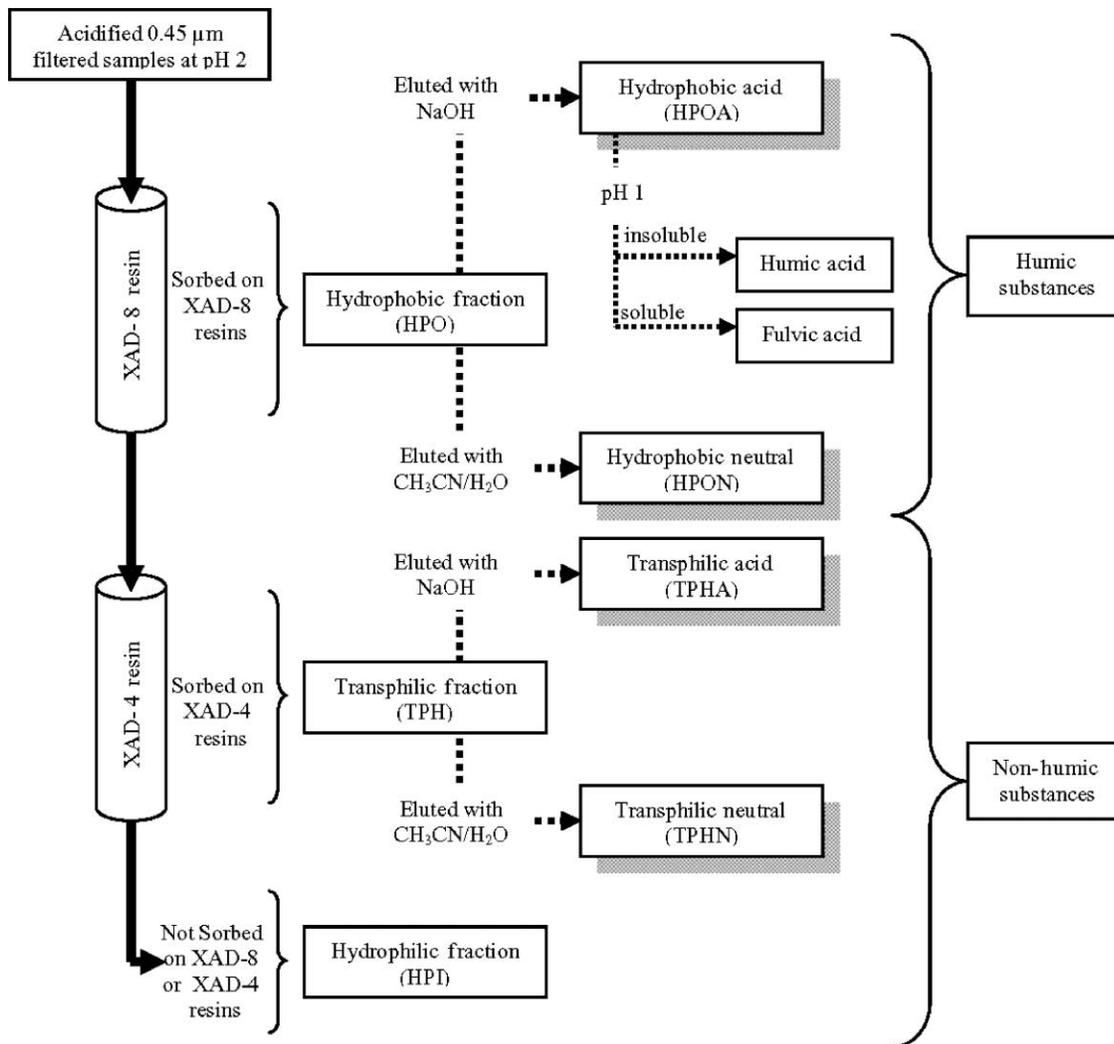
Figure 1 | XAD-8/XAD-4 fractionation scheme adapted and modified from Aiken *et al.* (1992) and Croue *et al.* (1999).

DOM in most source waters. We have summarized the results of some major DOM fractionation studies conducted after 1985 in Table 1. HPO (humic fraction), TPH and HPI (non-humic fraction) for different freshwater sources including rivers, lakes, groundwater and swamps were found to be in the range of 23–78%, 7–33% and 8–70%, respectively. Although there are a few exceptions, the results are consistent with other studies in that HPO is usually the largest fraction of the DOM pool, and TPH and HPI are minor fractions. HPO is particularly important in water systems for which terrestrial materials such as soil and agricultural return are the major source of DOM. In some extreme cases, almost 80% of DOM is HPO, such as the Suwannee River in Georgia and

Okefenokee Swamp in Florida. The DOM in these waters is primarily derived from terrestrial plants (allochthonous) (Croue *et al.* 2000). In contrast, low humic content or HPO has been observed in the South Platte River in Colorado and Clinton Lake in Kansas, composing 34 and 33% of the DOM, respectively. A substantial portion of the DOM in these waters is derived from its own aquatic biota, such as algae, bacteria and macrophytes (autochthonous) (Croue *et al.* 2000; Pomes *et al.* 2000). Results show that the source of DOM is one of the major factors controlling DOM composition in different water sources.

Each water system can have multiple sources of DOM. Changes in the distribution of organic carbon fractions were

Table 1 | Chemical fractionation data of DOM from various water sources. Numbers located in the middle of TPH and HPI columns indicate the sum of the two fractions, which is equivalent to non-humic fraction. HPO and TPH are the carbon fractions that adsorbed on the XAD-8 and XAD-4 resins, respectively. HPI is the carbon fraction passing through both the XAD-8 and XAD-4 resins

Sources	DOC (mg l⁻¹)	SUVA$_{254}$ (l mg⁻¹ m⁻¹)	(HPO) (%) Humic fraction	(TPH) (%) Non-humic fraction		(HPI) (%)	References
Rivers							
Charente River, France	2.8	2.0	50	27		23	(Martin-Mousset *et al.* 1997)
Colorado River, Nevada	3.0	1.5	35		65		(Collins *et al.* 1986)
Colorado River, Nevada	2.5	1.2–1.7	42–44	15–17		13–14	(Hwang *et al.* 2000)
Colorado River, Nevada	3.0	1.3	42		58		(Owen *et al.* 1993)
Grasse River, New York	7.7	4.1	57		43		(Collins *et al.* 1986)
Kanto Plain, Japan	2.8	2.1^c	47		53		(Imai *et al.* 2001)
Loire River, France	3.9	2.2	41	33		26	(Martin-Mousset *et al.* 1997)
Mayenne River, France	4.2	3.3	48	27		25	(Martin-Mousset *et al.* 1997)
Mississippi River, Illinois	5.1	3.3	43		57		(Singer *et al.* 2002)
Ohio River, Ohio	2.5–2.8	2.2–2.6	52–60		40–48		(Owen *et al.* 1993)
Sacramento River, California	2.1–3.1	2.0–2.8	38		62		(Amy *et al.* 1990)
San Joaquin River, California	3.5–3.7	1.7–2.8	44		56		(Amy *et al.* 1990)
Sevre Nantaise River, France	5.3	2.8	49	26		25	(Martin-Mousset *et al.* 1997)
South Fork Tolt River, Washington	1.1	4.8	56		44		(Singer *et al.* 2002)
South Platte River, Colorado	2.6	2.4	34	26		40	(Croue *et al.* 2000)
Suwannee River, Georgia	47	NA	78	11		8	(Croue *et al.* 2000)
Suwannee River, Georgia	58	NA	58	25		17	(Aiken *et al.* 1992)
Wakarusa River, Kansas	5.4	NA	46		54		(Pomes *et al.* 2000)
White River, Indiana	3.4	3.1	47		53		(Singer *et al.* 2002)
Yakima River, Washington	1.6–2.4	NA	23–26	8–12		65–66	(Aiken *et al.* 1992)

Table 1 | (continued)

Sources	DOC (mg l⁻¹)	SUVA$_{254}$ (l mg⁻¹ m⁻¹)	(HPO) (%) Humic fraction	(TPH) (%) Non-humic fraction		(HPI) (%)	References
Reservoirs & Lakes							
Apremont Reservoir, France	7.3–11.5	2.6–3.7	51–60	19–24		21–25	(Croue et al. 1999)
Apremont Reservoir, France	7.1	3.7	56	27		17	(Martin-Mousset et al. 1997)
Bartlett Lake, Arizona	NA	2.1–2.4	40[d]	13[d]		27[d]	(Mash et al. 2004)
Chamboux Reservoir, France	7.2–7.8	3.6–3.7	51–62	24–28		14–21	(Martin-Mousset et al. 1997)
Clinton Lake, Kansas	4.1	NA	33		67		(Pomes et al. 2000)
Cobble Mtn Reservoir, Massachusetts	2.5	2.6	49		51		(Collins et al. 1986)
Fryxell Lake, Antarctica	5.2	NA	23	7		70	(Aiken et al. 1992)
Harwood Mill Reservoir, Virginia	4.1–5.6	1.9–2.6	46–54		46–54		(Owen et al. 1993)
Hoare Lake, Antarctica	2.0	NA	23	9		68	(Aiken et al. 1992)
Lake Kasumigaura, Japan	2.9–4.4	1.5–2.1	40		60		(Imai et al. 2003)
Lake Pleasant, Arizona	NA	1.3–1.5	25	11		28	(Mash et al. 2004)
Laurel Reservoir, Connecticut	2.9–3.0	2.6	35–45		55–65		(Owen et al. 1993)
Manatee Reservoir, Florida	8.7	4.2	54		46		(Singer et al. 2002)
Mervent Reservoir, France	6.8	3.2	55	27		18	(Martin-Mousset et al. 1997)
Poquonnock Reservoir, Connecticut	3.6	3.5	44		56		(Singer et al. 2002)
Saguaro Lake, Arizona	NA	1.6–1.7	22[d]	10[d]		31[d]	(Mash et al. 2004)
Tomhannock Reservoir, New York	3.3	2.1	36		64		(Kitis et al. 2002)
Groundwater							
Bemidji, Minnesota	16	NA	42	22		36	(Aiken et al. 1992)
Biscayne Aquifer, Florida	5.3–1.5	2.5–2.6	50–61		39–50		(Owen et al. 1993)
Floridan Aquifer, Florida	8.3	2.6	58		42		(Collins et al. 1986)

Table 1 | (continued)

Sources	DOC (mg l⁻¹)	SUVA$_{254}$ (l mg⁻¹ m⁻¹)	(HPO) (%) Humic fraction	(TPH) (%) Non-humic fraction	(HPI) (%)	References
Mesa, Arizona	1.0	0.51[b]	37	64		(Drewes & Fox 1999)
Wetlands						
Everglades Swamp, Florida	30–37	2.2–3.8	52–54	18–27	19–30	(Croue et al. 1999)
Okefenokee Swamp, Florida	53–56	ND	64–78	22–36		(Bano et al. 1997)
Soil						
Kingile-Ryde Complex, California	4.8–5.5[a]	6.1	74	23		(Chow et al. 2005b)
Surface Rindge Muck, California	6.6–14.7[a]	5.8–6.2	71–72	28–29		(Chow et al. 2005b)
Surface Rindge Muck, California	14.0[a]	3.0–6.0	56	23	21	(Chow et al. 2003)
Subsurface Rindge Muck, California	7.9[a]	1.8–5.8	38	30	32	(Chow et al. 2003)
Surface Rindge Muck, California	42.8–139	4.2–11.6	41–50	21–24	18–34	(Fujii et al. 1998)
Subsurface Rindge Muck, California	5.8–172	4.1–6.7	50–57	22–27	11–15	(Fujii et al. 1998)
Agricultural drainage						
Empire Tract, California	18.7–22.3	5.9	51–60	40–49		(Amy et al. 1990)
Grand Island, California	6.4–7.2	3.9	48–62	38–52		(Amy et al. 1990)
Paddy Field, Japan	7.3	2.2	32	68		(Imai et al. 2001)
Twitchell Island, California	10.0–52.6	3.6–10.7	50–54	21–25	23–25	(Fujii et al. 1998)
Tyler Island, California	7.7–10.4	3.9–6.0	58	42		(Amy et al. 1990)
Upper Jones Tract, California	6.4–10.0	3.8–4.0	40	60		(Amy et al. 1990)
Wastewaters						
Reclaimed water, Arizona	2.2–5.7	2.1–3.3[b]	50–66	34–50		(Drewes & Fox 2000)
Domestic sewage, Japan	3.5–6.3	1.2–1.9[c]	19–30	60–81		(Imai et al. 2002)
Human waste, Japan	2.3–31.6	0.9–2.5[c]	4–25	75–96		(Imai et al. 2002)

Table 1 | (continued)

Sources	DOC (mg l^{-1})	SUVA$_{254}$ (l mg^{-1} m^{-1})	(HPO) (%) Humic fraction	(TPH) (%) (HPI) (%) Non-humic fraction	References
Others					
Banks Pumping Plant, California	3.4–4.1	2.5–3.1	55	45	(Amy *et al.* 1990)
Forest stream, Japan	0.47	2.3[c]	65	35	(Imai *et al.* 2001)
Myrtle Beach, South Carolina	20	4.7	66	34	(Kitis *et al.* 2002)
Salt River Project, Arizona	2.8–5.1	1.8–2.7	40–47	53–60	(Owen *et al.* 1993)
State Project Water, California	3.0	2.7	47	53	(Owen *et al.* 1993)

[a]DOC was collected by mixing 10 g of dry soil into 1 l deionized water.
[b]SUVA of hydrophobic acid.
[c]260 nm was used.
[d]Only acid fractions are included and neutral fractions are not included.
NA: Not available.

shown to be associated with seasonal processes (Aitken-head-Peterson *et al.* 2003). Physical processes such as snowmelt and storm events can change the hydrology and deliver various sources of organic matter to water bodies (Boyer *et al.* 2000; Hood *et al.* 2003; Inamdar *et al.* 2004; Avery *et al.* 2004). Chemical processes such as solar UV radiation break down photosensitive organic compounds. Ninety-six per cent of chromophoric dissolved organic carbon and 41% of DOM can be decomposed by solar UV radiation in about two months (Vahatalo & Wetzel 2004). Also, biological processes such as microbial degradation and algal blooms can alter the concentration and composition of DOM in waters (Bano *et al.* 1997, 1998). For example, bacteria can consume up to 6% of HPO (humic substances) and 11% of TPH and HPI fractions (non-humic substances) in a few days (Bano *et al.* 1998). In-stream primary production has also been shown to account for 40–80% of total carbon inputs in a riverine system (Bertilsson & Jones 2003). Each water system has unique water chemistry such as pH, alkalinity, salinity, nutrients and other constituents, and variable physical and hydrological factors such as temperature, water depth, turbidity and water flow rate. All these factors can affect the rate of DOM production and degradation and thus the overall composition of DOM. In other words, the concentration and composition of each organic carbon fraction are dynamic variables (spatially and temporally), depending on seasonal and environmental factors. Therefore, there is no obvious trend in chemical fractionation associated with the type of water system (i.e. rivers, lakes or groundwater), but a water system with terrestrial materials as a major DOM input tends to have a higher HPO content.

$SUVA_{254}$ was usually found to be in the range of 2.0–4.0 $l mg^{-1}$ m^{-1} in most water sources, with some exceptional cases where it was lower than 1.5 or higher than 10.0 (Table 1). Among all water sources, a higher $SUVA_{254}$ was usually found in leachates and agricultural returns from terrestrial environments with a high content of soil organic matter. Soil fulvic acid, a portion of HPOA, contains a higher aromatic content and fewer carboxylated molecules, whereas aquatic fulvic acid contains molecules with less aromaticity and a higher carboxylate content (Reemtsma & These 2005). Therefore, waters with high HPO content derived from terrestrial environments usually have a high

$SUVA_{254}$ and a high reactivity in THM formation during chlorination. Indeed, HPO from source waters or treated industrial wastewaters was found to be more reactive in forming THMs than HPI (Galapate *et al.* 1999; Croue *et al.* 2000; Kitis *et al.* 2002) (Table 2). For example, the HPO of the South Fork Tolt River in Washington and Poquonnock Reservoir in Connecticut was 30% more reactive in forming THM precursors than the TPH and HPI fractions. The Grasse River in New York, Cobble Mountain Reservoir in Massachusetts, and Floridan Aquifer in Florida contained HPO that was 60% more reactive in THM formation than the TPH and HPI fractions. However, HPO is not always the primary THM precursor. A study using waters with low HPO or humic content from the Colorado River showed that the HPI had the highest yield of THMs among all fractions (Hwang *et al.* 2000). Also, humic (HPO) and non-humic (TPH + HPI) fractions from Harwood Mill Reservoir in Virginia had similar reactivity in THM formation (Owen *et al.* 1993). Martin-Mousset *et al.* (1997) showed that there was no correlation between HPO and THM formation in eight source water samples. As shown in Table 2, the specific THMFP of humic (HPO) and non-humic fractions (TPH + HPI) from various waters ranged from 7.1 to 114 and 1.9 to 82 μg-THM mg-C^{-1}, respectively. Such a wide range of specific THMFP indicates that the reactive carbon in each fraction is source dependent, and this fractionation technique does not effectively isolate THM precursors from the bulk DOM.

Several factors may contribute to the inconclusive results between DOM fractions and THMFP. Fractions from various sources may contain distinct chemical characteristics that are inherited from the organic matter source. For example, allochthonous organic material or terrestrial plants contains higher N content and less aromatic C than autochthonous organic matter, such as planktonic algae (Hedges *et al.* 1988). Organic carbon fractions that are isolated by the same procedure become less aromatic and have a higher N content as the contribution of autochthonous organic material increases. As discussed, aromatic carbon is believed to be the major reactive site for THM formation (Norwood *et al.* 1980, 1987; Boyce & Hornig 1983; Reckhow *et al.* 1990). Thus, changes in chemical properties and aromatic carbon content would be expected to influence DBP production. However, results indicate that

Table 2 | Specific THMFP of whole water, HPO, TPH and HPI fractions from various water sources. Numbers located in the middle of TPH and HPI columns indicate the specific THMFP of non-humic fraction. HPO is the carbon fraction that adsorbed on the XAD-8 resin and is also refernced to as the humic fraction. TPH and HPI are the carbon fractions that passed the XAD–8 resin and are referenced to as the non–humic fraction

Sources	Whole water	Humic fraction (HPO)	Non-humic fraction		References
			(TPH)	(HPI)	
			(μg-THM mg-C^{-1})		
Rivers					
Colorado River, Nevada[a]	39	46	54		(Owen et al. 1993)
Colorado River, Nevada[b]	55	69	48		(Collins et al. 1986)
Colorado River, Nevada[c]	14 (0.15)*	10 (0.10)*	13 (0.13)*	19 (0.19)*	(Hwang et al. 2000)
Middle River, California[d]	–	92 (9.2)*	69 (6.9)*		(Bergamaschi et al. 1999)
Mayenne River, France[c]	–	41–79[†]	28[†]	–	(Croue et al. 1999)
Mississippi River, Illinois[f]	73 (0.39)*	35 (0.29)*	33 (0.28)*		(Singer et al. 2002)
Grasse River, New York[b]	93	114	65		(Collins et al. 1986)
Sacramento River, California[d]	–	96 (9.7)*	–		(Bergamaschi et al. 1999)
San Joaquin River, California[d]	–	90 (9.0)*	80 (8.0)*	–	(Bergamaschi et al. 1999)
San Joaquin River, California[b]	68	123	24		(Amy et al. 1990)
Suwannee River, Georgia[g]	–	51–55[†]	40[†]	23–41[†]	(Croue et al. 2000)
South Platte River, Colorado[g]	36[†]	29–46[†]	25–39[†]	28–35[†]	(Croue et al. 2000)
South Fork Tolt River, Washington[f]	4 (0.62)*	97 (0.81)*	74 (0.62)*		(Singer et al. 2002)
Old River, California[d]	–	92 (9.2)*	82 (8.2)*		(Bergamaschi et al. 1999)
Ohio River, Ohio[a]	35–42	33–45	20–59		(Owen et al. 1993)
White River, Indiana[e]	46 (0.38)*	42 (0.35)*	29 (0.24)*		(Singer et al. 2002)
Reservoirs & Lakes					
Apremont Reservoir, France[e]	–	74–82[†]	56[†]	–	(Croue et al. 1999)

Table 2 | (continued)

Sources	Whole water	Humic fraction (HPO)	Non-humic fraction		References
			(TPH)	(HPI)	
		(μg-THM mg-C^{-1})			
Cobble Mtn Reservoir, Massachusetts[b]	78	95	58		(Collins *et al.* 1986)
Harwood Mill Reservoir, Virginia[a]	22–28	25–36	23–38		(Owen *et al.* 1993)
Hellurude Lake, Norway[c]	–	91–93[†]	82[†]	–	(Croue *et al.* 1999)
Lake Kasumigaura, Japan[j]	23 (0.19)*	24 (0.20)*	22 (0.18)*		(Imai *et al.* 2003)
Manatee Reservoir, Florida[f]	51 (0.43)*	53 (0.44)*	33 (0.28)*		(Singer *et al.* 2002)
Poquonnock Reservoir, Connecticut[f]	53 (0.44)*	71 (0.59)*	51 (0.43)*		(Singer *et al.* 2002)
Tomhannock Reservoir, New York[c]	52	86	44–48		(Kitis *et al.* 2002)
Groundwater					
Biscayne Aquifer, Florida[a]	13–27	13–20	10–27		(Owen *et al.* 1993)
Floridan Aquifer, Florida[b]	63	75	45		(Collins *et al.* 1986)
Soils					
Kingile-Ryde Complex, California[h]	98–111	141–166	101–110		(Chow *et al.* 2005*b*)
Surface Rindge Muck, California[h]	87–94	108–124	79–81		(Chow *et al.* 2005*b*)
Surface Rindge Muck, California[i]	75	92	54	38	(Chow *et al.* 2003)
Surface Rindge Muck, California[j]	41–64	41	37	–	(Fujii *et al.* 1998)
Subsurface Rindge Muck, California[i]	68	44	49	45	(Chow *et al.* 2003)
Subsurface Rindge Muck, California[i]	30–70	38–50	36–45	–	(Fujii *et al.* 1998)
Agricultural drainage					
Empire Tract, California[b]	111–121	122–147	83–99		(Amy *et al.* 1990)
Grand Island, California[b]	37–40	28–48	27–48		(Amy *et al.* 1990)

Table 2 | (continued)

Sources	Whole water	Humic fraction (HPO)	Non-humic fraction		References
			(TPH)	(HPI)	
			(μg-THM mg-C^{-1})		
Twitchell Island, California[d]	–	85 (8.5)*	69 (6.9)*	–	(Bergamaschi et al. 1999a)
Twitchell Island, California[i]	46–70	44–54	42–45	–	(Fujii et al. 1998)
Tyler Island, California[b]	60–62	46–81	35–77		(Amy et al. 1990)
Upper Jones Tract, California[b]	64	102	38		(Amy et al. 1990)
Others					
Myrtle Beach, South Carolina[c]	72	76	48–64		(Kitis et al. 2002)
Treated Industry Wastewaters, Japan[j]	1.3–23	7.1–142	1.9–29		(Galapate et al. 1999)
Salt River Project, Arizona[a]	37–53	35–47	14–67		(Owen et al. 1993)
State Water Project, California[a]	57	39	–		(Owen et al. 1993)
Banks Pumping Plant, California[b]	55	86	17		(Amy et al. 1990)
Delta Export Pumping Plant, California[d]	–	85 (8.6)*	74 (7.4)*	–	(Bergamaschi et al. 1999a)

[a] 20°C, pH = 7, Cl$_2$/DOC = 3, and contact time = 96 h.
[b] 20°C, pH = 7, Cl$_2$/DOC = 3, and contact time = 168 h.
[c] 20°C, pH = 8, DOC = 3 mg l^{-1} with 1.0 mg l^{-1} residual chlorine after 24 h, and contact time = 24 h.
[d] 25°C, pH = 8.3, Cl$_2$/DOC = 3–4, and contact time = 168 h.
[e] 20°C, pH = 7.5, Cl$_2$/DOC = 4, and contact time = 72 h.
[f] 20°C, pH = 8, Cl$_2$/DOC = 1–2 with 1.0 mg l^{-1} residual chlorine after 24 h, and contact time = 72 h.
[g] 20°C, pH = 8, Cl$_2$/DOC = 4, and contact time = 72 h.
[h] 20°C, pH = 8.3, Cl$_2$ = 120 mg l^{-1}, DOC < 10 mg l^{-1}, and contact time = 168 h.
[i] 20°C, pH = 8.3, Cl$_2$ = (3[DOC]) + (7.6[NH$_3$]), and contact time = 168 h.
[j] 20°C, pH = 7, DOC = 2 mg l^{-1} with 1–2 mg l^{-1} residual chlorine after 24 h, and contact time = 24 h.
*Data in the literature are given as μmol-THM/mg-C (shown in parenthesis). Speciation of THM4 is not given. It is converted μg-THM mg-C^{-1} based on the MW of chloroform (MW = 119.5).
†Only chloroform was determined.

the mechanism involved in fractionation does not clearly associate with THM-reactive carbon in DOM.

Different constituents in waters (e.g. Br) and modified THMFP protocols and fractionation schemes may alter the composition of isolated fractions, affect the reaction between DOM and disinfectants, and change the species of DBPs formed. For example, when high levels of Br were present in water, it was more reactive with TPH to form brominated THM species and resulted in an increase of TPH as THM precursors (Kitis *et al.* 2002). High dissolved organic nitrogen in waters also shifted the speciation of THMs and increased the production of other DBPs, such as dichloroacetic acid and haloacetonitriles (Hwang *et al.* 2000; Westerhoff & Mash 2002). Various constituents in source waters can generally alter the reactivity of HPO, TPH, HPI in forming THMs. Therefore, THMFP of waters are unique to their sources and the results from one study cannot be directly applied to others.

Advantages and disadvantages of XAD-8 / XAD-4 fractionation techniques

The primary use of XAD fractionation has always been the isolation of DOM from the water matrix. Many advanced analytical methods, such as ^{13}C-NMR, for DOM characterization, detect the chemistry of the ^{13}C isotope, rather than the major isotope ^{12}C. The natural abundance of ^{13}C is only 1.11% (Clark & Fritz 1997) and DOM concentrations in source waters are usually in the range of a few parts per million (Thurman 1985; Aitkenhead-Peterson *et al.* 2003). Therefore, many advanced analytical methods cannot be used on water samples directly and fractionation to concentrate samples prior to analysis is required (Wershaw 1985; Wilson 1987). There is no effective technique to extract all DOM from water; therefore all isolation methods are also fractionation methods. Furthermore, the fractionation can be carried out on the original sample without the need for a pre-concentration step, thereby maintaining fractionation consistency and comparability between samples.

However, there are several potential drawbacks in DOM fractionation using XAD resins. First, the fractions isolated by XAD-8/XAD-4 fractionation may not fully represent the actual chemical properties of the DOM mixture because the use of strong acid and base potentially alters the chemical

characteristics of DOM, such as ester hydrolysis (Aiken 1987; Mace *et al.* 1998; Maurice *et al.* 2002; Gadmar *et al.* 2005). Mace and co-workers (Mace *et al.* 1998) demonstrated that the polarity of DOM increased, as indicated by the decrease in SUVA$_{254}$, when DOM was chemically manipulated to mimic the XAD-fractionation. A lower heteroaliphatic carbon and ester content was measured in XAD-8 isolates than in reverse osmosis isolates (Mace *et al.* 1998). Importantly, studies have shown that reactivity in forming THMs from DOM extracted by alkaline agents was different from DOM extracted by deionized water (Ishikawa *et al.* 1984; Chow *et al.* 2005c). Thus, DOM isolated by XAD-fractionation may have a lower THM reactivity than the actual DOM in source waters.

Second, the distribution of each fraction can be affected by sample preparation. Storage methods and filter type may contribute to variations in fractionation (Christ & David 1994; Gadmar *et al.* 2005). DOM extracted from frozen samples was relatively enriched in TPHN and the increases in this fraction indicated that the storage method might have disrupted microbial biomass (e.g. cell lysis) (Guggenberger *et al.* 1994). In addition, hydrophobic membranes, such as nylon, can remove more HPOA from samples.

Third, fractions of organic matter obtained using these isolation methods are not sharply defined and thus overlapping and operational variations can occur (Gadmar *et al.* 2005). There is a continuum of DOM. Some of the organic matter could be retained by XAD-8 resins in certain water environments (e.g. low SUVA$_{254}$ water), whereas the same material could pass through the XAD-8 column in different water environments (e.g. high SUVA$_{254}$ water).

Fourth, chemical fractionation procedures have not been standardized although the same chromatographic conditions, such as capacity factor, are used. The fractionation procedure is modified and is subject to the preferences of different research groups. For example, humic acid is sometimes precipitated and isolated from the bulk sample before samples are run through XAD-8 resin (Ma *et al.* 2001; Chow *et al.* 2005c). In such cases, only fulvic acid is contained in the HPO. Although humic acid is a relatively minor fraction compared with fulvic acid in most source waters, it is a highly reactive fraction in forming DBPs and its presence can significantly change the reactivity of the HPO.

Fifth, the manufacture of the most popular non-ionic adsorbent Amberlite® XAD-8 resin was discontinued several

years ago and Supelite™ DAX-8 has been substituted by some research teams (Ma et al. 2001; Kitis et al. 2002). The DAX-8 and XAD-8 resins appear to isolate HPO (humic substances) almost equally in quantity but the composition of the fractions varies slightly (Peuravuori et al. 2001, 2002). Although it is unknown how much variation this change has made to DOM fractions that contain THM precursors, we speculate that the difference in DOM fractions between XAD-8 and DAX-8 is relatively minor, compared with the variations in DOM isolates using different pore size filters.

Apparently, the XAD-8 and XAD-4 fractionation scheme cannot fully isolate or fractionate THM precursors from DOM in natural systems (Croue et al. 2000; Leenheer & Croue 2003; Gadmar et al. 2005). Notably, each isolated fraction does not have unique chemical structures. The amount of reactive carbon or THM precursors in the same isolated fractions from different water sources can be different. Thus, fractions resulting from this scheme may have distinguishable chemical characteristics but the correlations between these fractions and their reactivity to form THMs are inconsistent. The THMFP and XAD fractionation data are most useful for comparing samples from similar sources or treatment studies.

Particular attention has to be paid to chemical fractions reported in the literature because different terminologies have been used. For example, the portion that sorbs on a column of XAD-4 resin is sometimes referred to as the HPI, and the portion that passes through both XAD-8 and XAD-4 is sometimes referred to as the low molecular weight hydrophilic fraction or non-adsorbed hydrophilic fraction (Fujii et al. 1998; Chow et al. 2003). Thus, interpreting and comparing fractionation data from the literature should be done with appropriate caution.

PHYSICAL FRACTIONATION

Ultrafiltration and size exclusion chromatography

DOM is a heterogeneous mixture and contains various sizes of organic molecules. Traditionally, a pore size filter of $0.45\,\mu m$ has been used to operationally define particulate and dissolved organic matter and to isolate DOM from bulk mixtures (Zsolnay 2003). However, the choice of filter

pore size is arbitrary and different pore size filters, such as 0.22, 0.1 and $0.025\,\mu m$, have been used to isolate and define DOM. Natural organic matter can be further separated into particulate organic matter, colloidal organic matter, and DOM by using different pore size filters in tandem (Owen et al. 1993; Guo et al. 2003; Chow et al. 2005a). Regardless of filter pore size and operational definitions, all studies agree that particulate organic matter ($>0.45\,\mu m$), such as zooplankton, algae and detritus organic matter, is usually a small fraction of total natural organic matter in drinking water sources (Thurman 1985; Owen et al. 1993; Perdue & Ritchie 2004; Chow et al. 2005a). Owen et al. (1993) estimated that this portion accounts for 17% or less of organic matter in source waters. Moreover, particulate organic matter can usually be removed by conventional treatment methods, such as coagulation, filtration and pH adjustment (Edzwald 1993; Farahbakhsh et al. 2004; Xie 2004). Thus, its impact on water disinfection and THM formation should be minimal. However, a recent study showed that particulate organic matter from soils and algae cells probably serves as an important pool of THM precursors (Plummer & Edzwald 2002, 2001; Li et al. 2003). This portion should not be ignored, particularly for water utilities that have pre-chlorination treatment or source water that does not require enhanced coagulation or enhanced softening (if source water contains total organic carbon of $4.0\,mg\,l^{-1}$ or less and alkalinity of $60\,mg\,l^{-1}$ or greater, according to the Stage I D/DBP rule).

DOM ($<0.45\,\mu m$), which corresponds to the apparent molecular weight (AMW) fraction $<10^8$ Da, is predominant and consists of more than 80% of the DOM in most source waters (Thurman 1985; Owen et al. 1993). DOM contains a wide variety of organic compounds, including humic substances, viruses and identifiable organic compounds, such as amino acids, carbohydrates, fatty acids and hydrocarbons. HPO (humic substances) generally accounts for more than 50% of the DOM and is the major class of organic compounds in source waters. Thus, most studies of molecular weight fractionation focus on HPO. The average molecular size of HPO from aquatic environments varies from about 0.47 to $3.3\,nm$ in the radius of gyration, corresponding to an AMW range of 500 Da to 10 kDa (Thurman et al. 1982).

Molecular weight fractionation in THM precursor research is commonly achieved by ultrafiltration (UF) and

size exclusion chromatography (SEC) (also called gel permeation chromatography). More detailed information about these two techniques of DOM fractionation can be found in Amy *et al.* (1987) and Swift (1985). Only a brief overview will be presented here. UF is a pressure-driven process by which colloids, particulates and high-molecular-mass soluble species are rejected by an UF membrane, which is a porous structure that retains components by a sieving mechanism (Amy *et al.* 1987; Van der Bruggen *et al.* 2003). Separation depends on the pore size of the membrane and the size of the organic components. UF modules can operate in either cross-flow mode or dead-end mode. Most full-scale UF systems for drinking water treatments and treatability studies are operated in cross-flow mode, in which the feed is pumped across or tangentially to the membrane surface. In contrast, UF systems for DOM characterizations are operated in the dead-end mode and the feed is pumped directly towards the filter. Cross-flow is advantageous in that it limits the build-up of solids on the membrane surface. UF has been widely used in fractionation because it has a small pore size (2–100 nm) for better fractionation at the molecular level (500 Da–100 kDa). Nevertheless, fractions isolated by this technique contain a range of AMW, instead of an exact number. The pore size of the membrane is not isotropic, meaning that it is not uniform throughout the body of the membrane. The extent of the pore size distribution depends on the composition of the membrane. The concept of molecular weight cutoff (MWCO) is often used. MWCO refers to the size for which greater than 90% of the particles are retained (Amy *et al.* 1987; Cheryan 1998). The range of MWCO membranes commercially available is 500 Da to 300 kDa (e.g. 500 Da, 1, 3, 10, 30, 100 and 300 kDa) (Swift 1985; Cheryan 1998). The actual size of the material retained by the membrane may be different from the nominal pore size of a given membrane. The exclusion of molecules of varying size depends upon the shape of the molecules, presence of other solutes, ionic strength, membrane material and configuration, operating parameters, and even lot-to-lot variability (Yuan & Zydney 2000; Guo *et al.* 2001; Howe & Clark 2002).

SEC uses a porous gel that characterizes a unique AMW range over which molecules can be fractionated. The size of the pores in a gel is determined by the degree of polymer cross-linking (Amy *et al.* 1987). AMW determination by the SEC method is based on the key presumption that the size-exclusion effect is solely responsible for the fractionation (i.e. no chemical interactions). The passage of small molecules is hindered by penetration into the micropores of the stationary phase and thus their pathway is longer, resulting in a longer retention time or greater elution volume. In contrast, large molecules are excluded from the micropores and the surface functional groups (steric interactions) resulting in shorter retention time or a smaller elution volume (Janos 2003; Winzor 2003). Thus, the gel pores act as a chromatographic medium giving separations based on differences in hydrodynamic size or effective diameter. This method can be used to separate organic molecules from 500 Da to 1,000 kDa. In addition, SEC can be equipped with sequential on-line detectors consisting of UV, fluorescence and quantitative dissolved organic carbon measurement for investigating the composition of DOM fractions and monitoring DOM removal (Her *et al.* 2002, 2003; Allpike *et al.* 2005; Gadmar *et al.* 2005).

Similar to the problems described for UF, separation by SEC is based on differences in molecular size rather than differences in actual molecular weight (Janos 2003). SEC is not an absolute molecular weight fractionation method and conditions can change the molecular size of DOM through aggregation processes. Thus, the term AMW is usually used to describe the organic carbon fractions isolated by this method. Importantly, both UF and SEC fractionation are operationally defined because the fractions collected by these two methods depend on their operating conditions.

Physical fractionation of THM precursors in various water sources

DOM is typically fractionated into four major AMW ranges: <1, $1–10$, $10–30$ and >30 kDa. In most source waters, DOM with AMW of 10 kDa or less is the dominant fraction and comprises more than 50% of total DOM (Table 3). The larger fractions, such as $10–30$ and >30 kDa, are relatively minor and usually compose less than 30% of DOM. In some cases, such as the Colorado River, Iowa River and Cobble Mountain Reservoir, over 95% of DOM was smaller than 10 kDa. Her *et al.* (2003) examined groundwater, lake water

and wastewater with this technique and showed that the DOM fraction in the high AMW range (>10 kDa) consisted of polysaccharide-like/protein-like substances and the DOM in the range of 1–5 kDa had higher aromaticity than other fractions. A study also indicated that the molecular size distribution of DOM is constrained within a relatively narrow range (<3 kDa) (Chin *et al.* 1994). HPO (humic substances) generally have an AMW range of 500 Da to 10 kDa. The fulvic acid fraction contains substances with molecular weights ranging from 500 Da to 2 kDa and is monodispersed, whereas the humic acid fraction contains substances with molecular weights ranging from 1 to 10 kDa and is generally polydispersed (Thurman *et al.* 1982). HPO with an AMW above 10 kDa is uncommon. This coherence in AMW suggests that the <1 and 1–10 kDa fractions in most source waters are mainly composed of HPO (humic substances), although TPH and HPI fractions (non-humic substances), and identifiable organic compounds are also part of these fractions. Particularly, attention should be paid to the <1 kDa fraction because the 1–10, 10–30 and >30 kDa fractions can be effectively removed by coagulation and ozonation during water treatment (Owen *et al.* 1993). The non-coagulated/non-removable fraction represents 20–50% of DOM in most source waters with some rare exceptions (Table 3). The <1 kDa fraction in rivers, reservoirs, groundwater and other source waters was in the range 14–70%, 25–51%, 20–44% and 23–46%, respectively. With such large variations in AMW distribution, the fractionation data do not provide any definitive link between the composition of DOM and the type of source water.

A wide range of THM formation potentials results from chlorination of the various AMW fractions (Table 4). In general, there is no clear correlation between AMW and THMFP for the range of DOM studied. For example, Owen *et al.* (1993) established correlations of THMFP and different AMW fractions for raw and treated waters, and they concluded that the <1 kDa DOM was more reactive in forming THMs than other fractions. Via & Dietrich (1996) also pointed out that the <1 kDa fraction had the greatest DBP formation potentials, including THM, haloacetonitriles and haloketones. The low AMW fraction also appears to be more reactive with Br to form brominated THMs

(Kitis *et al.* 2002). In contrast, Schnoor *et al.* (1979) found that the fraction <3 kDa contributed 75% of THMFP, compared with 25% for the fraction <1 kDa. Joyce *et al.* (1984) showed that DOM with AMW less than 1 kDa had very low THMFP and suggested that this organic fraction was not the major THM precursor. Further, Oliver & Visser (1980) showed that chloroform concentrations per unit weight of humic material reached a maximum for the AMW of 20–30 kDa, but Rest *et al.* (1983) and Amy *et al.* (1990) found the AMW range of 1–15 kDa was the major fraction for THM yields. The DOM fraction in the range of 1–5 kDa consisted of higher aromatic carbon content than other fractions (Her *et al.* 2003). Oliver & Thurman (1983) and Collins *et al.* (1985) also concluded that THM yields increased with AMW, but Kitis *et al.* (2002) found no clear trend between THM yield and AMW. In sum, there is no unifying conclusion on the most reactive AMW fraction, but many studies indicated that maximum THMFP generally occurs for AMW size classes between 1 and 10 kDa.

The AMW distribution and the chemical composition of each AMW fraction most likely vary from source to source, and even from time to time because the chemical composition may be altered by seasonal factors (Owen *et al.* 1993). In other words, fractions isolated by these methods can have different properties and reactivity in THM formation, and fractionation data do not provide any information about the sources of DOM. The range of results for physical size fractionation of DOM vs. THM formation may also be attributable to the different procedures and operationally defined conditions used in various studies. DOM fractions obtained from UF and SEC depend on operational conditions, such as initial DOM concentration, pH and ionic strength because these parameters can affect molecular configuration of DOM (Ghosh & Schnitzer 1980). Kitis *et al.* (2002) adjusted their water samples to an ionic strength of 0.01 M (as NaCl) and pH 7 for all samples prior to ultrafiltration. Oliver and Visser (1980) adjusted their water samples to an ionic strength of 1.5 M and pH 8.4 with TRIS buffer. Amy *et al.* (1990) fractionated their samples consisting of 0.45 μm filtrates without any adjustment.

Scientific data remain inconclusive as to which physical size fraction of DOM contains the most THM precursors and some research suggests that the chemical composition of DOM components is a more important predicator of

Table 3 | Physical fractionation data and AMW ranges of DOM from various water sources. Numbers located in the middle of columns indicate the mean of the corresponding data.

Sources	DOC (mg l⁻¹)	SUVA₂₅₄ (l mg⁻¹ m⁻¹)	<1k	1–10k	10–30k	>30k	References
Rivers				(%)			
Colorado River, Nevada	3.0	1.3	46	49	4	1	(Owen *et al.* 1993)
Colorado River, Nevada	3.0	1.5	70	24	1	<1	(Collins *et al.* 1986)
Grasse River, New York	7.7	4.1	30	66	2	2	(Collins *et al.* 1986)
Grasse River, New York	6.6	4.4	28[c]	11[c]	34[c]	27[c]	(Chadik & Amy 1987)
Iowa River, Iowa	61	NA	14	84	1	1	(Schnoor *et al.* 1979)
Ohio River, Ohio	2.5–2.8	2.2–2.6	34–53	46–47	1–20	0	(Owen *et al.* 1993)
Pearl River, Mississippi	5.6	2.4	44[c]	12[c]	34[c]	10[c]	(Chadik & Amy 1987)
Quabbin Tributaries, Massachusetts	1.0–8.7	1.7–3.9	22	37	30	11	(Garvey & Tobiason 2003)
Sacramento River, California	2.1–3.1	1.7–2.8	58[d]	35[d]	8[d]		(Amy *et al.* 1990)
San Joaquin River, California	3.5–3.7	2.6–2.8	52[d]	35[d]	13[d]		(Amy *et al.* 1990)
Reservoirs & lakes							
Barr Lake, Colorado	10	1.5	76[e]		24[e]		(Her *et al.* 2003)
Bradford Lake, Florida	11	NA	40[b]	42[b]		20[b]	(Marley *et al.* 1992)
Cobble Mtn Reservoir, Massachusetts	2.5	2.6	39	58	1	2	(Collins *et al.* 1986)
Harwood Mill Reservoir, Virginia	4.1–5.6	1.9–2.6	25–28	39–40	19–22	10–16	(Owen *et al.* 1993)
Kaw Reservoir, Oklahoma	5.2	2.9	40[c]	14[c]	32[c]	14[c]	(Chadik & Amy 1987)
Quabbin Reservoir, Massachusetts	1.1–3.0	1.1–1.8	35	26	18	20	(Garvey & Tobiason 2003)
Tombannock Reservoir New York	3.3	2.1	51[a]	38[a]	11[a]		(Kitis *et al.* 2002)
Groundwater							
Biscayne Aquifer, Florida	4.0	4.7	36	63	<1	<1	(Logan & Qing 1990)

Table 3 | (continued)

Sources	DOC (mg l⁻¹)	SUVA₂₅₄ (l mg⁻¹ m⁻¹)	<1k	1–10k	10–30k	>30k	References
				(%)			
Biscayne Aquifer, Florida	5.3–15	2.5–2.6	20–27	61–66	8–15	0–4	(Owen *et al.* 1993)
Irvine Ranch, California	12	6.6	87		13		(Her *et al.* 2003)
Floridan Aquifer, Florida	8.3	2.6	44	54	<1	<1	(Collins *et al.* 1986)
Wanneroo, Australia	5.7	5.7	6	81	7		(Allpike *et al.* 2005)
Soils							
Amherst Soil, Massachusetts	NA	NA	46	48	6		(Joyce *et al.* 1984)
Middleton Soil, Massachusetts	NA	NA	90		10		(Joyce *et al.* 1984)
Agricultural drainage							
Empire Tract, California	19–22	4.7–5.9	16[d]	73[d]	12[d]		(Amy *et al.* 1990)
Wetlands							
Volo Bog, Illinois	25	NA	27[b]	44[b]		30	(Marley *et al.* 1992)
Wastewaters							
Wastewater, Arizona	14–16	NA	51–64	7–18	18–42		(Logan & Qing 1990)
Wastewater effluent, Hawaii	13	2.2	81[e]		19[c]		(Her *et al.* 2003)
Others							
Banks Pumping Plant, California	3.4–4.1	2.5–3.1	53[d]	38[d]	8[d]		(Amy *et al.* 1990)
Mesa Consolidated, California	3.3	5.6	18	35	38	9	(Amy *et al.* 1992)
Myrtle Beach, South Carolina	20	4.7	44	40	2	14	(Kitis *et al.* 2002)
Orange County, California	NA	NA	40	48	1	12	(Logan & Qing 1990)
Orange County, California	3.4	4.8	17	34	19	30	(Amy *et al.* 1992)
Saganashkee Slough, Illinois	13	NA	23	55[b]		22	(Marley *et al.* 1992)

Table 3 | (*continued*)

Sources	DOC (mg l⁻¹)	SUVA254 (l mg⁻¹ m⁻¹)	<1k (%)	1–10k	10–30k	>30k	References
Salt River Project, Arizona	2.8–5.1	1.8–2.7	35–46	44–51	5–13	3–8	(Owen *et al.* 1993)
State Water Project, California	3.0	2.7	30	58	9	3	(Owen *et al.* 1993)

[a]The values represent percentage of DOC in the MW fractions of <1kDa, 1–5kDa, and >5kDa.
[b]The values represent percentage of DOC in the MW fractions of <3kDa, 3–30kDa, and >30kDa.
[c]The values represent percentage of DOC in the MW fractions of <5kDa, 5–10kDa, 10–22kDa, and >22kDa.
[d]The values represent percentage of DOC in the MW fractions of <1kDa, 1–5kDa, and >10kDa.
[e]The values represent percentage of DOC in the MW fractions of <10kDa and >10kDa.
NA: Not Available.

THM formation than physical size (Kitis *et al.* 2002). However, size fractionation techniques provide a tool to collect homogeneous fractions without altering the chemical structure of DOM. They can distinguish large molecules from small molecules whether they have similar or differing chemical properties. While molecular size fractionation alone may not be able to provide useful information about the THM precursor, data from chemical fractionation is also inconclusive for THM formation potentials. To compensate for weaknesses and limitations in each technique, the two fractionation techniques should be applied in tandem to isolate and characterize DOM. Particularly, molecular size fractionation can collect homogeneous size fractions for subsequent chemical fractionation or analytical measurements in identifying dominant THM reactive sites or THM precursors.

Advantage and disadvantage of ultrafiltration and size exclusion chromatography

Unlike XAD-8 and XAD-4 fractionation, UF or SEC does not require acids, bases or any other harsh chemicals for fractionating DOM. The alteration of DOM characteristics is minimal and the chemical nature of DOM is preserved. In addition, UF has been widely used as a process in water treatment facilities and the results from these studies can be directly applied to removal of DBP precursors. There are some drawbacks, however, in DOM fractionation using UF or SEC. SEC and UF provide only AMW because neither technique provides precise separation of various molecular weight fractions. The MWCO is a specification used by membrane manufacturers to describe the retention capabilities of a membrane, and it refers to the molecular mass of a macro-solute for which the membrane has a retention capability greater than 90%. Thus, the separation is not absolute. Moreover, the volume of water used can significantly affect the AMW distributions. The error in the nominal MWCO is inversely proportional to the volume of collected filtrate and the magnitude of the permeation coefficient (Logan & Qing 1990). Reproducibility of AMW fractionation may depend on the particular membrane, volume of water filtered, and flow rates used in the determination (Amy *et al.* 1987). Different membrane materials with the same nominal MWCO will appear to give different solute rejection.

Table 4 | Specific THMFP of different AMW fractions of DOM from various water sources. Numbers located in the middle of columns indicate the mean of the corresponding data.

Sources	Whole water	<1k	1–10k	10–30k	>30k	References
			(μg-THM mg-C^{-1})			
Rivers						
Chicoutimi River, Canada[a*] (fulvic acid)	–	42	105	115–138	30–75	(Oliver & Visser 1980)
Chicoutimi River, Canada[a*] (humic acid)	–	–	160	130–142	90–132	(Oliver & Visser 1980)
Colorado River, Nevada[b]	39	56	28	0	0	(Owen et al. 1993)
Grasse River, New York[c]	72	30[†,I]	12[†,I]	30[†,I]	28[†,I]	(Chadik & Amy 1987)
Iowa River, Iowa[d]	1.2[*]	3.6[*]	1.3–5.8[*]	3.1–7.7[*]	2.8[*]	(Schnoor et al. 1979)
Ohio River, Ohio[b]	33–42	33–52	16–38	23	–	(Owen et al. 1993)
Pearl River, Mississippi[c]	50	50[†,I]	11[†,I]	21[†,I]	18[†,I]	(Chadik & Amy 1987)
Sacramento River, California[c]	52	45[†,p]	46[†,p]	9[†,p]		(Amy et al. 1990)
San Joaquin River, California[c]	68–74	70[†,p]	36[†,p]	4[†,p]		(Amy et al. 1990)
Reservoirs & Lake						
Harwood Mill Reservoir, Virginia[b]	22–28	28–40	25–28	3–21	0–30	(Owen et al. 1993)
Harwood Mill Reservoir, Virginia[b]	–	93	64	63	51	(Via & Dietrich 1996)
Kaw Reservoir, Oklahoma[c]	60	30[†,I]	20[†,I]	22[†,I]	28[†,I]	(Chadik & Amy 1987)
Picauba Lake, Canada[a*] (fulvic acid)	–	100	150	138	15–30	(Oliver & Visser 1980)
Picauba Lake, Canada[a*] (humic acid)	–	–	120	165–170	38–130	(Oliver & Visser 1980)
Tomhannock Reservoir, New York[c]	52	59	20–66	–	–	(Kitis et al. 2002)
Groundwater						
Biscayne Aquifer, Florida[b]	13–27	45	7–23	0–23	0	(Owen et al. 1993)
Agricultural drainage						
Empire Tract, California[c]	111–121	18[†,p]	65[†,p]	17[†,p]		(Amy et al. 1990)

Table 4 | (continued)

Sources	Whole water	<1k	1–10k	10–30k	>30k	References
			(μg-THM mg-C^{-1})			
Others						
Banks Pumping Plant, California[c]	55	49[†,p]	48[†,p]	3[†,p]		(Amy et al. 1990)
Mesa consolidation, California[g]	59	99	30–58	36	100	(Amy et al. 1992)
Myrtle Beach, South Carolina[e]	72	52	60–94	77	86–92	(Kitis et al. 2002)
Orange County, California[g]	42	54	56	36	23	(Amy et al. 1992)
Salt River Project, Arizona[b]	37–53	33–63	40–48	28–44	19–36	(Owen et al. 1993)
State Water Project, California[b]	57	85	26	20	–	(Owen et al. 1993)

[a]20°C, pH = 11, DOC = 1.0 mg l^{-1}, Cl$_2$ dosage = 15 mg l^{-1}, and contact time = 72 h.
[b]20°C, pH = 7, Cl$_2$/DOC = 3, and contact time = 96 h.
[c]20°C, pH = 7, Cl$_2$/DOC = 3, and contact time = 168 h.
[d]3°C, pH = 10.8, Cl$_2$ dosage = 6 mg l^{-1}, and contact time = 10 h.
[e]20°C, pH = 8, DOC = 3.0 mg l^{-1}, with 1.0 mg l^{-1} residual chlorine after 24 h incubation, contact time = 24 h.
[g]20°C, pH = 7, Cl$_2$/DOC = 5, and contact time = 168 h.
[*]Only chloroform was determined.
[†]The values represent corresponding percentage in forming THM of the bulk samples. They are not THMFP.
[t]The values represent the MW fractions of <5 kDa, 5–10 kDa, 10–22 kDa, and >22 kDa.
[p]The values represent the MW fractions of <1 kDa, 1–10 kDa, >10 kDa.

Compared with polysulfone membranes, membranes made of cellulose acetate or regenerated cellulose had broader pore size distributions and higher rejections (Cheryan 1998). Manufacturers establish their own MWCO values and calibrate membranes by measuring membrane rejection of macromolecules having known AMW, often spherical proteins. However, these macromolecules often have structural characteristics significantly different from aquatic DOM. Thus, results may not be fully comparable when using membranes from different suppliers.

Molecular weight fractionation depends greatly on interactions between organic solutes and other constituents in solution. Ionic strength, types of cation and pH can influence the shape and hydrodynamic radius of macromolecules, and change the ability of DOM to pass through or be retained on the membrane (Cheryan 1998; Schafer *et al.* 2000; Yuan & Zydney 2000). Basically, humic substances are flexible linear colloids at low sample concentrations, provided hydrogen ion and salt concentrations are not too high. However, the molecules become spherocolloids when sample concentrations are high, pH of the medium is very low, or when appreciable amounts of neutral electrolytes are present (Ghosh & Schnitzer 1980). Importantly, a linear molecule has a greater probability of passing through the pore than a globular molecule of the same molecular weight (Cheryan 1998). The difference in molecular weight between two fractions does not mean the same degree of difference in molecular size; that is, fractions that are different in AMW by $10 \times$ may differ in size by $3 \times$ when in the globular or folded form. In addition, the rate and extent of DOM membrane fouling increases at low pH, high ionic strength, and in the presence of calcium due to changes in intermolecular electrostatic interactions (Hong & Elimelech 1997; Yuan & Zydney 2000). Therefore, the organic carbon fractions collected using the same membrane under different pH or ionic strength conditions can be different even when the same DOM source is used.

LIMITATION OF FRACTIONATION TECHNIQUES IN THM PRECURSOR RESEARCH

If chemical and physical fractionation of DOM are considered as analytical methods, the fractionation of DOM provides little information about the distribution of THM precursors in DOM. These fractionation techniques were not developed for THM characterization and the operationally defined fractions should not necessarily have any relationship to THM precursors. Importantly, the XAD fractions are compositionally different in different samples and XAD fractionation data are not a discrete parameter. For example, the aromatic carbon content, as measured by ^{13}C-NMR or $SUVA_{254}$, is usually greater in the HPOA isolate than in the TPHA isolate from surface water samples (Aiken *et al.* 1992). However, the aromatic carbon content of a TPHA isolate from a peat soil extract will probably be greater than the aromatic carbon content of a HPOA isolate from algal-dominated lake water (Aiken *et al.* 1992). Therefore, THM reactivity among XAD isolated DOM fractions is not a discrete property of the water sample and cannot be treated the same way as DOM concentration, cation/anion concentrations or specific conductivity. However, fractionation data are useful for comparing samples along some sort of gradient. For example, XAD fractionation data provided useful information when comparing samples from rivers (Croue *et al.* 2000) and when comparing extracts from different soils (Chow *et al.* 2003). However, comparing the river DOM to the soil extracts using XAD fractionation data provides much less information because the compositional nature of the DOM from those two sample types is so different.

Fractions isolated by XAD from various sources have been examined for THM formation potential and it is suggested that THM precursors may be reactive sites on complex organic molecules rather than discrete molecules. The range of specific THMFP of DOM is generally within $3–20$ mmol-THM mol-C^{-1}, which is equivalent to $30–199\,\mu g$-THM mg-C^{-1} (assuming chloroform is the only species). In other words, only $3–20$ carbon atoms per 1,000 carbon atoms in the DOM react to form a THM molecule; less than 2% of the carbon in DOM is reactive in THM formation. Fractionation data from SEC and UF suggest that most DOM molecules in source waters are $0.5–3$ kDa (Thurman *et al.* 1982; Chin *et al.* 1994). Elemental analysis of several surface waters indicated that the DOM contains 41–57% carbon (Croue *et al.* 2000; Perdue & Ritchie 2004). Thus, DOM molecules

comprise approximately 20–125 carbon atoms. Moreover, chlorination is an oxidation–reduction process and a carbon fragment in DOM is oxidized when chlorine is attached and substituted with other hydrogen or carbon atoms. The average oxidation state of carbon fragments in a DOM molecule is different (Perdue & Ritchie 2004). Thus, reduced carbon fragments or carbon fragments with lower oxidation state should be more reactive with chlorine (an oxidizing agent). Indeed, chlorine incorporation into DOM is not a completely even process during chlorination (Zhang *et al.* 2005). The chlorine to carbon atomic ratios of some DBPs can be higher than others. In particular the high AMW DBPs (AMW > 500) have a roughly constant chlorine to carbon ratio of 0.025 (Zhang & Minear 2002). This suggests that some fragments of DOM are more reactive with chlorine to form THMs, not the whole molecule. If this hypothesis is true, THM precursors can be isolated if properties that control sorption/desorption from XAD resins are related to the reactive sites of DOM molecules. The lack of a stronger relationship between XAD isolated fractions and THM reactivity suggests that the separation mechanism in XAD fractionation does not interact directly with these reactive sites.

FURTHER APPROACH

The enhanced coagulation process is defined as an optimized coagulation process for removing DOM, or DBP precursors, and it is one of the best available technologies for DBP control under the Stage 1 D/DBP Rule (USEPA 1996). However, the diversity of sources, temporal and spatial variation, and the complexity of natural processes continually and unpredictably alter the quantity and quality of DOM and DBP precursors in source waters. To effectively detect and identify reactive fractions of DOM in such dynamic environments and to apply suitable technology to remove DBP precursors add many challenges for water utilities. Developing economical and rapid detection methods for determining the abundance of DBP precursors in source waters is essential for optimizing coagulation processes during water treatment. Many sensitive analytical techniques, such as UV absorbance and

fluorescence emission, have been identified as reliable surrogates for DBP formation in treated waters (Edzwald *et al.* 1985; Korshin *et al.* 1997; Her *et al.* 2003; Uyguner *et al.* 2004). However, bulk water consists of heterogeneous components that can affect the turbidity of water and reduce the accuracy of these spectroscopic measurements (Chow *et al.* 2005*a*). Determining the UV absorbance and fluorescence emission of homogeneous samples can provide more reliable information about the characteristics of DOM and its tendency to form DBPs during water treatment. Thus, fractionation techniques in conjunction with sensitive spectroscopic techniques enable an effective monitoring of quantitative and qualitative DOM properties in source waters. As discussed, the HPO and 1–10 kDa fractions, which are usually considered as major THM and DBP precursors, have high $SUVA_{254}$. A combination of fractionation techniques and analytical methods should increase the sensitivity and improve the reliability. Several studies using this approach, such as enhanced high performance liquid chromatography-size exclusion chromatography with multiple on-line detectors, consisting of UV, fluorescence and quantitative DOM measurement, showed a good outcome in water and wastewater treatment (Her *et al.* 2002, 2003; Allpike *et al.* 2005; Gadmar *et al.* 2005).

Several studies mentioned that $SUVA_{254}$ is good for representing the nature of organic matter, but not for predicting its reactivity in chemical reactions (Fram *et al.* 1999; Weishaar *et al.* 2003). We have to recognize that $SUVA_{254}$ measured before disinfection may only indicate the possible amount (viewed as potential) of activated aromatic rings in a particular organic matrix which will form DBP; it does not represent the actual estimation of activated aromatic ring destruction which leads to DBP formation (viewed as reactivity). Aromatic carbon substituted by oxygen, nitrogen or other moieties may not be reactive in DBP formation. Model studies showed that the location of functional groups, such as hydroxyl and carboxylate groups, can significantly alter their reactivity in chemical reaction (Norwood *et al.* 1980; Ichihashi *et al.* 1999). Because of their similar structures, simple spectroscopy may not be able to detect their differences. The abundance of these UV-active but non-DBP-reactive aromatic carbons depends on its sources. Thus, empirical relationship of DBPFP and UV absorbance is often

site-specific and different DBP species may have different correlations with UV absorbance.

Despite the inconsistent correlation of DBPFP and $SUVA_{254}$ for DOM from different sources, $SUVA_{254}$ is still the most common surrogate for DOM and DBP precursors. UV/VIS analysis is inexpensive and it can be performed on aqueous samples with less than one $mg\,l^{-1}$ of humic material. It is non-destructive and requires only a short measurement time. To overcome the deficiencies of $SUVA_{254}$, differential UV absorbance at 272 nm (ΔUVA_{272}) may be a possible solution. ΔUVA_{272} is defined as the difference in UV absorbance at 272 nm before and after chlorination. Based on an assumption that ring cleavage is the main reaction mechanism of DBP formation (Reckhow *et al.* 1990) and unreactive sites will remain intact during chlorination (Korshin *et al.* 1999; Li *et al.* 2000), the magnitude of ΔUVA_{272} represents the extent of the destruction of activated aromatic rings and DBP formation (Korshin *et al.* 1997; Korshin *et al.* 1999). A strong correlation between ΔUVA and THMFP has been observed (Li *et al.* 1998). Further study on these applications in conjunction with fractionation techniques will provide an effective way to optimize water treatment processes and monitor DBP formation in source waters and treated waters.

SUMMARY

The literature on isolation and characterization of organic THM precursors was reviewed with a focus on the most commonly used chemical (XAD-8/XAD-4) and physical (UF and SEC) methodologies. Large variations were found for correlations of organic isolates and chemical properties with their reactivity to form THMs. However, fractionation techniques are particularly useful for providing homogeneous size fractions for subsequent chemical fractionation or analytical measurements for identifying dominant THM reactive sites or THM precursors. Several main points are summarized as follows:

XAD-8/XAD-4 fractionation

- XAD-fractionation was not developed for isolating THM precursors and the separation mechanism does not

directly interact with the THM reactive carbon fraction. The fractions resulting from this technique, such as hydrophobic, transphilic and hydrophilic fractions, have distinguishable chemical characteristics, but the correlations between these fractions and their reactivity in THM formation from different sources can be different. However, HPO from most source waters generally has a higher THMFP than other fractions and it is considered as the major THM precursor.

- XAD-fractionation data are not a discrete chemical parameter and the chemical constituents of each fraction vary with source. Thus, XAD fractionation data are most useful for comparing samples from similar sources or treatment studies. The information from a particular water source should not be simply applied to different water sources, and only carbon normalized THMFP with the same testing procedures or protocols should be used. Also, water systems do not have a unique combination of DOM chemical fractions, although water from terrestrial environments, particularly from soils with high organic content, have a higher content of HPO.

- This method generally extracts 50–90% of DOM from source waters. The application of acid/base and lack of complete recovery of DOM implies that it is difficult to completely characterize the bulk DOM fraction of source waters. This method was developed mainly for isolating or fractionating aquatic DOM such that high concentrations of homogeneous fractions were obtained for characterization by advanced analytical methods, such as ^{13}C-NMR.

Ultrafiltration/size exclusion chromatography

- Physical size fractionation methods using UF and SEC isolate DOM into fractions with different molecular weight or size ranges. The sieving mechanism on the molecular scale does not show any distinct relationship with the THM-reactive carbon in DOM. The fractionation data vary among sources and it remains inconclusive as to which physical size fraction of DOM contains the most THM precursors. However, DOM in

the $<1-10$ kDa range was generally the major fraction contributing to THM yields in most source waters.

- These fractionation techniques are operationally defined and the resulting data are not a discrete parameter. Also, water systems do not have a unique combination of different chemical fractions,
- UF or SEC does not require acids, bases or any other harsh chemicals for fractionating DOM. The alteration of DOM characteristics is minimal and the chemical nature of DOM is preserved. This fractionation technique in conjunction with sensitive spectroscopic techniques allows effective monitoring of quantitative and qualitative DOM properties in source waters.

LIST OF ABBREVIATIONS

AMW	apparent molecular weight
Da	Dalton
DBP	disinfection by-product
D/DBP	disinfectants/disinfection by-product
DOM	dissolved organic matter
FP	formation potential
GPC	gel permeation chromatography
HAA	haloacetic acid
HPI	hydrophilic fraction
HPOA	hydrophobic acid
HPO	hydrophobic fraction
HPON	hydrophobic neutral
ICR	information collection rule
MCL	maximum contaminant level
MWCO	molecular weight cutoff
MX	3-chloro-4-(dichloromethyl)-5-hydroxy-2[5H]-furanone
NMR	nuclear magnetic resonance
SDS	simulated distribution system
SEC	size exclusion chromatography
SUVA$_{254}$	specific ultraviolet absorbance at 254 nm
TPHA	transphilic acid
TPH	transphilic fraction
TPHN	transphilic neutral
THM	trihalomethane
THMFP	trihalomethane formation potential
UF	ultrafiltration
UV/VIS	ultraviolet/visible
UFC	uniform formation condition
USEPA	US Environmental Protection Agency
ΔUVA$_{272}$	differential ultraviolet absorbance at 272 nm

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