

## Dissolved organic nitrogen in drinking water supplies: a review

Paul Westerhoff and Heath Mash

### ABSTRACT

Dissolved organic nitrogen (DON) is an issue for the water field primarily due to the formation of disinfection by-products of health concern, and its potential role in membrane fouling. This article reviews the following DON issues: (1) analytical measurement, (2) occurrence, (3) structural composition, and (4) treatability during potable water treatment. There is no direct measurement for DON, rather DON is calculated by the difference between total dissolved nitrogen and inorganic nitrogen ions. DON concentrations range from <0.1 to >10 mg N/l with a median value of ~0.3 mg N/l in surface waters. DON sources include wastewater discharges, agricultural fertilizers, algae, forest litter and soils. DON is comprised of a broad spectrum of molecular weight compounds encompassing multiple N-containing functional groups. Carbon to nitrogen ratios (C/N or DOC/DON) range between 5 and 100 mg C/mg N (median ~15 mg C/mg N), and may be a good indicator of organic matter sources. During chlorination higher org-N content leads to (1) increasing chlorine demand, (2) production of di-HAA>tri-HAA, (3) production of HAA>THM, and (4) production of higher levels for halogenated (nitromethanes, HANS) and non-halogenated (NDMA) org-N DBPs. Information on DON removal during potable water treatment is lacking and should be a focus of future research.

**Key words** | dissolved organic nitrogen, natural organic matter, organic carbon

**Paul Westerhoff** (corresponding author)  
Department of Civil and Environmental  
Engineering,  
Arizona State University,  
Box 5306,  
Tempe,  
AZ 85287-5306,  
USA  
Tel: 480-965-2885  
Fax: 480-965-0557  
E-mail: [p.westerhoff@asu.edu](mailto:p.westerhoff@asu.edu)

**Heath Mash**  
National Center for Sustainable Water Supply,  
Department of Civil and Environmental  
Engineering,  
Arizona State University,  
Box 5306,  
Tempe,  
AZ 85287-5306,  
USA  
Tel: 480-965-2327  
Fax: 480-965-0557  
E-mail: [heath.mash@asu.edu](mailto:heath.mash@asu.edu)

### INTRODUCTION

Organic nitrogen (org-N) is commonly the dominant form of the total dissolved nitrogen (TDN) in pristine waters, but represents a lower percentage of TDN in human-impacted waters (e.g. nitrate inputs) (Perakis & Hedin 2002). Org-N comprises a relatively small portion (0.5% to 10% by weight) of the mass of natural organic matter (NOM). Dissolved organic carbon (DOC) has traditionally been used as a surrogate for NOM; NOM contains between roughly 30% and 50% carbon by weight. Conceptual models for NOM structures include aromatic and aliphatic carbon with carboxyl, hydroxyl or carbonyl function groups, but often lack significant inclusion of org-N structures (Larson & Weber 1994). While exclusion of org-N structures may be associated with its generally low elemental composition in NOM, other than amine functionality very little is known about org-N structures in NOM (e.g. pyroles, amides, nitriles, purines, pyrimidines and nitro compounds). Despite

considerable information on various aspects of DOC cycling in the environment or fate during water treatment, little is known about the nitrogenous fraction of NOM (Alberts & Takacs 1999). Org-N exerts oxidant/disinfectant demands (e.g. chlorine and ozone) and can be a disinfection by-product (DBP) precursor. Removal of particulate organic nitrogen (PON) poses treatment challenges (e.g. removal of algae cells), as does dissolved organic nitrogen (DON). Total organic nitrogen (TON) is the sum of DON plus PON. During water treatment, biological treatment processes can produce DON (e.g. soluble microbial products (SMPs)), and DON can affect the performance of other physico-chemical processes (e.g. contribution to membrane fouling). Very few direct measurements of DON are available from drinking water systems.

An abundance of information has been published on the occurrence of org-N in marine and freshwater

ecosystems. However, a detailed study of the occurrence and treatability of org-N during drinking water treatment is not readily available. Research needs workshops organized over the past decade by the American Water Works Association Research Foundation all identified org-N as a potentially critical issue for drinking water treatment (AWWARF 1993, 2001). The European Community directive has a maximum admissible concentration for org-N of 1 mg Kjeldhal-N/l (org-N plus ammonia) in finished waters, but the United States has no similar guideline related to org-N. Emerging concerns over the increased contribution of potentially elevated DON levels from upstream wastewater or agricultural sources in drinking water supplies, and identification of potent carcinogenic org-N disinfection by-products (e.g. nitrosamines and nitromethanes) increases the need to better understand the concentrations, structures and reactivity of the nitrogenous NOM in drinking water systems.

This article reviews the following DON issues: (1) analytical measurement, (2) occurrence, (3) structural composition, and (4) treatability during potable water treatment. Discussion of DON analytical methods is presented in the context of understanding its indirect quantification and reporting level limitations, as DON determination is a new concept for the drinking water field. Critical to understanding DON occurrence includes the identification of sources and hydrologic transport through watersheds. There are no published reports on the fate of DON through water treatment processes, so ancillary data are presented to identify potential DON removal mechanisms. Chlorination studies using model compounds and NOM fractions enriched in org-N are reviewed to provide a framework for the potential involvement of DON in halogenated and non-halogenated disinfection by-products. Finally, a summary and research needs are presented for the drinking water field.

## ORGANIC NITROGEN COMPOUNDS AND METHODS OF MEASUREMENT

Nitrogenous organic matter can be incorporated into aliphatic or cyclical structures, and can occur as amines,

General classifications	Structure
Primary amine	
Secondary amine	
Tertiary amine	
Amide	
Substituted amide	
Nitrile	
Nitro compounds	
Nitrosamine	
<b>Examples of polyamines</b>	
Putrescine	
Spermidine	
<b>Examples of N-heterocyclic compounds</b>	
Pyrimidine	
Imidazole	
Purine	

Figure 1 | Classification and structure of org-N compounds.

nitriles or nitro compounds (Figure 1). Free amino acids represent a major pool of low molecular weight org-N matter, and are categorised either as acids, bases or neutrals (Figure 2). Amino acids incorporated into larger NOM molecules (i.e. combined or hydrolysable amino acids), heterocyclic-N, polyamine peptides and bio-molecular material (i.e. RNA, DNA) represent the dominant pool of larger molecular weight org-N. The nitrogen atom in the nitrogenous matter has a lone pair of electrons and acts as a nucleophile, which is central to its chemical and biochemical reactivity. This section describes analytical methods for DON measurement and characterisation of DON structure.

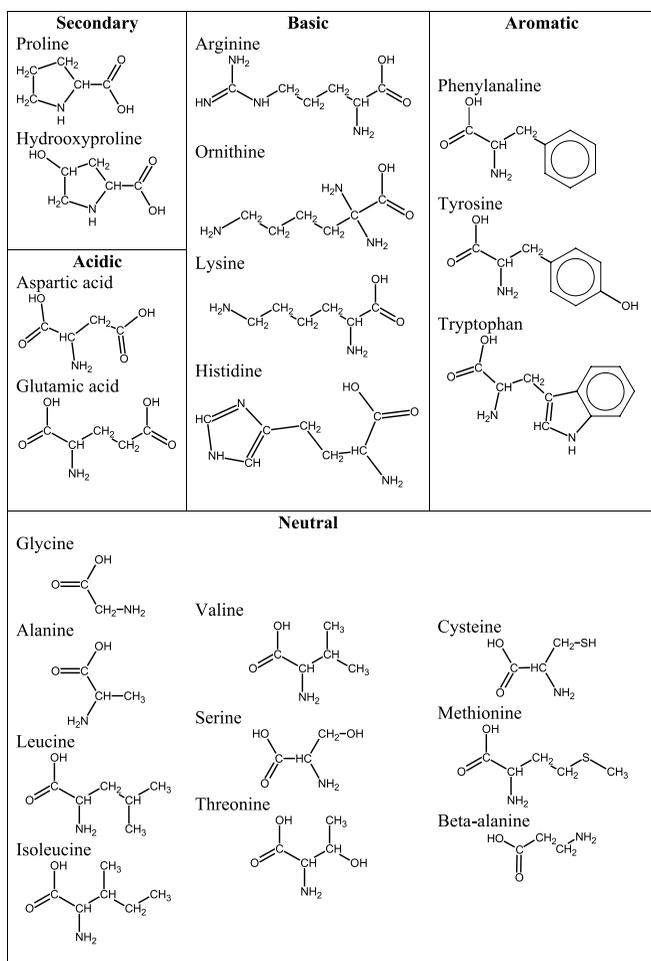


Figure 2 | Classification and structure of free amino acids.

## Analytical methods

DON is measured after a preparative step in which all the organic material is converted/digested (combusted or oxidised) to an inorganic nitrogen species ( $\text{NO}_2^-$ ,  $\text{NO}_3^-$  or  $\text{NH}_3/\text{NH}_4^+$ ) for measurement in solution or into gaseous nitrogen oxides ( $\text{NO}_x$ ). TDN is measured after conversion of all DON and DIN compounds to a single inorganic species (e.g.  $\text{NO}_3^-$  or  $\text{NO}$ ). Dissolved material is typically operationally defined as passing a  $0.45 \mu\text{m}$  filter, although many ecology-based studies employ a  $0.7 \mu\text{m}$  glass-fibre filter to minimise contamination. DON cannot be directly quantified in water due to the presence of  $\text{NH}_4^+$ ,  $\text{NO}_2^-$  and/or  $\text{NO}_3^-$  present in natural waters. Therefore,

DON is quantified by the difference between TDN minus DIN:

$$\text{DON} = \text{TDN} - \text{DIN} \quad (1)$$

$$\text{DON} = \text{TDN} - [\text{NO}_2^-] - [\text{NO}_3^-] - [\text{NH}_4^+] \quad (2)$$

The measurement of org-N commonly involves three sequential steps:

1. *Measurement of inorganic nitrogen species*—Table 1 summarises methods for detection of inorganic nitrogen species that are sufficiently sensitive to be relevant to measurement of org-N in natural waters. The methods must have low analytical error since inorganic nitrogen is subtracted from TDN (Equations 1 & 2) to determine org-N levels.
2. *Digestion of organic nitrogen to inorganic nitrogen*—Three general types of DON to DIN digestion methods have been developed: wet-oxidation, high temperature oxidation and photolytic oxidation (Table 2). Both wet and photolytic oxidations rely on subsequent measurement of inorganic nitrogen species in solution, while high temperature oxidation methods measure nitrogen oxide indirectly through ozonation and chemiluminescent detection. Comparison of the methods for model organic compounds yielded nitrogen recoveries of >90% to 110%. Extracted humic acids and natural water samples also had similar results among the methods, although photolytic oxidation methods show higher levels of variability with possible lower recoveries compared to the other methods (Jones & Daughton 1985; Walsh 1989; Takeda & Fujiwara 1996; Ammann *et al.* 2000; Bronk *et al.* 2000).
3. *Detection of DIN after DON digestion*—In some instances the detection method used in Step 1 (above) can be used to quantify the amount of oxidised org-N released into an aqueous (e.g.  $\text{NO}_3^-$ ) or gaseous ( $\text{NO}_x$ ) form (Table 1).

Combinations of DON digestion (Table 2) and DIN detection (Table 1) methods have been employed, and in the future may provide opportunities to lower DON

**Table 1** | Methods for analysis of inorganic nitrogen species (NA=not available)

Inorganic species	Method of analysis	Detection limit	Reference(s)
NO <sub>3</sub> <sup>-</sup>	Ion selective electrode	0.1 mg NO <sub>3</sub> <sup>-</sup> -N/l	Antisari & Sequi 1988; APHA <i>et al.</i> 1992
	Ion chromatography	0.1 mg NO <sub>3</sub> <sup>-</sup> -N/l	APHA <i>et al.</i> 1992; Takeda & Fujiwara 1996; USEPA 600/4-79-020
	(VIII) reduction to NO	2–3 ng NO <sub>3</sub> <sup>-</sup> -N/l	Braman & Hendrix 1989
	Spongy cadmium reduction to NO <sub>2</sub> <sup>-</sup>	Depends upon NO <sub>2</sub> <sup>-</sup> method	Bronk <i>et al.</i> 2000
	Cadmium reduction to NO <sub>2</sub> <sup>-</sup>	Depends upon NO <sub>2</sub> <sup>-</sup> method	Nydahl 1978; Walsh 1989; Takeda & Fujiwara 1996
	Hydrazine reduction to NO <sub>2</sub> <sup>-</sup>	Depends upon NO <sub>2</sub> <sup>-</sup> method	Johnes & Heathwaite 1992; Marriam <i>et al.</i> 1996; Olesky-Frenzel & Jekel 1996
	Reduction to NH <sub>4</sub> <sup>+</sup> with Devarda's alloy	Depends upon NH <sub>4</sub> <sup>+</sup> method	Lowry & Mancy 1978
	Titanous chloride reduction to NH <sub>4</sub> <sup>+</sup> method	Depends upon NH <sub>4</sub> <sup>+</sup> method	APHA <i>et al.</i> 1992
	UV/spectroscopy (210 nm)	Screening tool	APHA <i>et al.</i> 1992
NO <sub>2</sub> <sup>-</sup>	2nd derivative spectroscopy	< 5 µg NO <sub>3</sub> <sup>-</sup> -N/l	Simal <i>et al.</i> 1985; Crumpton <i>et al.</i> 1992; Ferree & Shannon 2000
	V(III) reduction to NO	2–3 ng NO <sub>3</sub> <sup>-</sup> -N/l	Braman & Hendrix 1989
	Colorimetric determination using sulfanilamide and N-(1-naphthyl)ethylenediamine	1.46–1.89 µg N/l	Walsh 1989; APHA <i>et al.</i> 1992; Johnes & Heathwaite 1992; Takeda & Fujiwara 1996; Bronk <i>et al.</i> 2000
	Ion chromatography with conductivity detection	0.1 mg NO <sub>3</sub> <sup>-</sup> -N/l	APHA <i>et al.</i> 1992; Takeda & Fujiwara 1996; USEPA 600/4-79-020
	Ion chromatography with conductivity and post-column UV detection	0.1 µg N/l	Salhi & von Gunten 1999
NH <sub>3</sub> /NH <sub>4</sub> <sup>+</sup>	Reduction to NH <sub>4</sub> <sup>+</sup> with Devarda's alloy	Depends upon NH <sub>4</sub> <sup>+</sup> method	Lowry & Mancy 1978
	Hypochlorite-phenol (indophenol) with colorimetric determination	0.05–2.0 mg N/l	Walsh 1989; APHA <i>et al.</i> 1992; Takeda & Fujiwara 1996
	Titration (methyl red/methylene blue endpoint)	1 mg N/l	Jones & Daughton 1985; ASTM 2001
	Ion selective electrode	0.02–0.08 mg N/l	Lowry & Mancy 1978; APHA <i>et al.</i> 1992; ASTM 2001
	Nessler reagent	0.02–5 mg N/l	APHA <i>et al.</i> 1992; ASTM 2001
Ninhydrin with adsorption detection	1–4 µg N/l	Fleck & Munro 1965	

Table 1 | Continued

Inorganic species	Method of analysis	Detection limit	Reference(s)
NO <sub>2</sub>	V(III) reduction to NO	0.09–200 mg N/l	Martin <i>et al.</i> 1995; Burdige & Zheng 1998
	UV/Vis absorption (545 nm)	0–180 mg N/l	Suzuki <i>et al.</i> 1985
NO	O <sub>3</sub> oxidation producing NO <sub>2</sub> and NO <sub>2</sub> <sup>*</sup> with chemiluminescent detection	0.07 mg N/l	Drushel 1977; Walsh 1989; Alvarez-Salgado & Miller 1998; Bronk <i>et al.</i> 2000; ASTM 2001

detection limits. Traditionally, wet chemical digestion methods and colorimetric determination of DIN species have been widely applied in the fields of limnology and marine sciences, and probably offer sufficient resolution for the drinking water industry (0.1–0.2 mg N/l). Several partially or fully automated digesters of inorganic and organic species and colorimetric in-line analysers are commercially available for these measurements (e.g. Bran & Luebbe TrAAcs 800 Autoanalyzer, Lachat Quick Chem 8000 Flow Injection Analyzer). The presence of elevated DIN in water (e.g. NO<sub>3</sub><sup>-</sup> >1 mg N/l) may significantly impact the accuracy and applicability of existing DON measurement techniques which rely upon calculation of DON by difference, and means to remove DIN should be investigated (Feuerstein *et al.* 1997). High temperature digestion systems with on-line detection are also commercially available (Shimadzu, Antek, Dohrmann), but such systems only quantify TDN. Advances in detection of DIN species with existing instrumentation (e.g. ion chromatography with post column reactions) that are becoming common in drinking water laboratories may be applicable for low level detection of DIN, and offer an economical means for monitoring DON concentrations during water treatment. Further research should investigate methods to completely (ion exchange, volatilization) or partially (dialysis) remove DIN prior to DON analysis to improve accuracy of the methods.

### Organic nitrogen characterisation

Numerous analytical techniques have been applied to characterise org-N structures in different matrices

(Table 3). Most techniques required pretreatment involving desalting and fractionation of NOM from inorganic matrices. Resin chromatography was the most common fractionation method (Hwang *et al.* 2001). In relationship to drinking water NOM the following approaches may hold the greatest potential:

1. *Free and hydrolysable amino acids* can be measured by HPLC (UV/Vis, fluorescence) or GC after derivatization. Total protein content of water has been measured by traditional techniques (Lowry Method) as well as emerging techniques (resonance light-scattering with tetra-substituted sulphonated aluminium phthalocyanine or 4-azochromotropic acid phenylfluorone) that potentially offer much lower concentration (ng/l) sensitivity (Guo & Shen 2000; Chen *et al.* 2001). Future work should analyse the fate of amino acids across water treatment plant unit processes.
2. *Pyrolysis (PY) GC-MS* is an analytical technique that characterises non-volatile, macromolecular NOM. Preconcentration (e.g. roto-evaporation) of NOM samples is generally required. Upon rapid heating at a high temperature, natural and synthetic biopolymers are degraded into low-molecular weight thermal decomposition products that are volatile enough to be separated and identified by GC/MS. Proteins yield pyroles, indoles and nitriles; toluene and styrene from the specific decomposition of phenylalanine; phenol and para(*p*)-cresol from tyrosine and indole; and methylindole from tryptophan. Peak heights or peak areas for different

**Table 2** | Methods for organic nitrogen digestion (conversion to inorganic N)

Method	Summary	Species measured	Limitations/comments
Kjeldahl nitrogen	Digestion (at 182–210°C) of sample in H <sub>2</sub> SO <sub>4</sub> in the presence of a catalyst (usually Hg) and a salt (typically K <sub>2</sub> SO <sub>4</sub> ); many modifications available	NH <sub>4</sub> <sup>+</sup> , HSO <sub>4</sub> <sup>-</sup>	Azide, azine, azo, hydrazone, nitrate, nitrite, nitrile, nitro, nitroso, oxime and semi-carbazone-compounds unreactive (Fleck & Munro 1965; APHA <i>et al.</i> 1992); pyrazolones (N-N linkages) and nitrophenols resistant (Jones & Daughton 1985)
Dumas method	Combustion in CO <sub>2</sub> , reduction of NO <sub>x</sub> followed by CO <sub>2</sub> removal	Volumetric quantity of N <sub>2</sub> produced	Dry, solid samples required (Fleck & Munro 1965)
Alkaline persulfate (peroxodisulphate) oxidation	Autoclave digestion under alkaline conditions in the presence of S <sub>2</sub> O <sub>8</sub> <sup>2-</sup>	NO <sub>3</sub> <sup>-</sup>	N = N bonds uncleaved; urea and some proteins problematic; antipyrine (HN = C) and some heterocyclic-N non-quantifiable (Delia <i>et al.</i> 1977; Nydahl 1978; Solorzano & Sharp 1980; Marriam <i>et al.</i> 1996; Dafner <i>et al.</i> 1999a)
	Microwave digestion under alkaline conditions in the presence of S <sub>2</sub> O <sub>8</sub> <sup>2-</sup>		Proteins problematic; antipyrine non-quantifiable (Johnes & Heathwaite 1992; Dafner <i>et al.</i> 1999a; 1999b)
	Boiling digestion under alkaline conditions in the presence of S <sub>2</sub> O <sub>8</sub> <sup>2-</sup>		EDTA and antipyrine not efficiently oxidized (Bronk <i>et al.</i> 2000)
	UV digestion under alkaline conditions in the presence of S <sub>2</sub> O <sub>8</sub> <sup>2-</sup>		Heterocyclic compounds show low recovery (Olesky-Frenzel & Jekel 1996)
Pyrolysis oxidation	High temperature oxidation at 1100°C	NO, possibly NO <sub>2</sub> and N <sub>2</sub>	Pyrazole and azoxy compounds recovery poor; poor recovery of azo, nitro and nitroso compounds (Drushel 1977; Jones & Daughton 1985; Walsh 1989; Bronk <i>et al.</i> 2000; ASTM 2001)
High temperature catalytic oxidation	High temperature oxidation at 680°C in the presence of a catalysis (typically Pt, CuO or CoO)	NO, possibly NO <sub>2</sub> and N <sub>2</sub>	Urea may be recalcitrant; methyl orange (N = N) and sulfathiazole (HC = N and heterocyclic S) may not be quantifiable; antipyrine (N-N) non-quantifiable (Suzuki <i>et al.</i> 1985; Marriam <i>et al.</i> 1996; Alvarez-Salgado & Miller 1998; Burdige & Zheng 1998; Ammann <i>et al.</i> 2000)
Photooxidative degradation	UV-oxidation, typically assisted by the presence of an oxidizing agent (S <sub>2</sub> O <sub>8</sub> <sup>2-</sup> or H <sub>2</sub> O <sub>2</sub> )	NO <sub>3</sub> <sup>-</sup>	Urea and EDTA refractory; compounds with N-N and N = N bonds poor recovery; includes some NO <sub>3</sub> <sup>-</sup> and NO <sub>2</sub> <sup>-</sup> when UV is used alone (Delia <i>et al.</i> 1977; Lowry & Mancy 1978; Walsh 1989; Bronk <i>et al.</i> 2000)
	UV-oxidation in the presence of TiO <sub>2</sub> or TiO <sub>2</sub> /Pt catalyst	NH <sub>4</sub> <sup>+</sup> , NO <sub>3</sub> <sup>-</sup> and NO <sub>2</sub> <sup>-</sup>	High salt content interference; antipyrine (N-N bond) not quantitatively recovered (Takeda & Fujiwara 1996)

compound classes are generally determined to characterise the relative amounts of polysaccharides, proteins and peptides, amino sugars and polyhydroxy aromatics (Bruchet *et al.* 1990; Gjessing *et al.* 1998). PY-GC-MS offers the potential to ‘fingerprint’ NOM in relation to its source (autochthonous or allochthonous) (Widrig *et al.* 1996). Chemolysis fragmentation, rather than pyrolysis, may lead to decomposition products more easily assigned to a parent compound.

- <sup>15</sup>N-NMR must be analysed in relatively large dry-masses of NOM due to the low content of org-N in NOM and the low (0.36%) occurrence of <sup>15</sup>N relative to <sup>14</sup>N. Alternatively <sup>15</sup>N enrichment studies (e.g. green algae grown from <sup>15</sup>N enriched nitrate) can be conducted. Solid-state <sup>15</sup>N-NMR can detect a range of nitrogen bonding arrangements: amide-nitrogen including peptides, indoles, lactams and carbazoles (220–285 ppm); amino-nitrogen of terminal amino acids or free aliphatic amino groups of peptides, amino sugars and amino acids (348 ppm); NH<sub>2</sub> or NR<sub>2</sub> groups of guanidines, aromatic amines or aniline (285–325 ppm); heterocyclic-nitrogen in purines, indoles, imidazoles, pyrrole-like compounds or proline-nitrogen in peptides (145–220 ppm); pyridine-nitrogen and imine structures (25–90 ppm). Analysis of soil organic matter indicated that >50% of the <sup>15</sup>N signal was attributed to amide structures (Clinton *et al.* 1995; Mahieu *et al.* 2000). Future work should employ <sup>15</sup>N-NMR on raw and finished less hydrophobic NOM fractions, but will require large material masses or long run times due to the low percentage of nitrogen, and specifically <sup>15</sup>N.
- X-ray techniques* have not been widely applied to NOM analysis, but can provide information on pyrolic, pyridinic, quaternary and aromatic amine nitrogen. Recent results suggest that amide nitrogen is the dominant type in humic substances, and pyridinic nitrogen may comprise 20%–30% of the total nitrogen (Vairavamurthy & Wang in press). These results contradict amino acid occurrence information (see below), offer direct measurement of

amide and pyridinic nitrogen, and should be investigated further.

- Fluorescence* analysis has been used to characterise aromatic amino acids (tryptophan, tyrosine) in water and differentiate between allochthonous and autochthonous NOM (Coble 1996; McKnight *et al.* 2001). Some amino acids absorb UV light near 220 nm, and although difficult to resolve from nitrate and other salts by UV spectroscopy, better resolution may be achieved by fluorescence. Fluorescence may be a surrogate for DON.

### Nitrogen characterization in NOM isolates

Org-N content and functional group composition affects NOM fractionation into hydrophobic and hydrophilic acid, neutral and base fractions. Amino acid and peptide functional groups tend to increase the hydrophilic character of NOM due to the potential of forming hydrogen bonds with surrounding water molecules. Aliphatic DON structures may contain basic, neutral and acidic functional groups (Figures 1 & 2), therefore org-N is not restricted to solely basic NOM fractions. Neutral and base NOM fractions tend to be nitrogen-enriched compared to acid fractions. More polar acid fractions are nitrogen-enriched relative to more hydrophobic acid fractions (e.g. fulvic acids). Nitrogen enriched NOM fractions have lower C/N ratios (Table 4). Base fractions represent a rather small portion of the NOM, but are enriched in org-N (e.g. polynuclear amines, amino sugars), and therefore may be of minor significance to the DON in bulk water. The neutral fractions have lower C/N ratios than acid fractions, and constitute a larger percentage of the DOC than base fractions. C/N ratios of NOM isolates span a larger range than DOC/DON ratios (Table 5, Figure 3B) because the bulk water measurements represent a suite of NOM fractions.

The specific UV absorbance at 254 nm (SUVA = UVA [m<sup>-1</sup>]/DOC [mg/l]) of hydrophobic base and neutral fractions from Forge Pond, MA (DOC = 6.6 mg/l) were lower than acid fractions (Table 4). However, the hydrophilic base and neutral fractions had SUVA values which were comparable to fulvic and weak hydrophobic

**Table 3** | Methods for analysis of org-N structures

Method of analysis	Brief description of method	Samples measured and representative nitrogen compound results
<sup>15</sup> N-NMR	CP/MAS—solid-state <sup>15</sup> N NMR at 30.4 MHz (300 MHz)	Soil humic material (labile humic acid and recalcitrant humate): heterocyclic, amide, amine and amino nitrogen (Mahieu <i>et al.</i> 2000)
	CP/MAS—Solid-state <sup>15</sup> N NMR at 40.55 MHz (400 MHz)	HF treated municipal solid waste (Pichler <i>et al.</i> 2000)
	2-D DCP MAS <sup>15</sup> N <sup>13</sup> C NMR	Biodegraded algae residues: amide-N in peptide-like structures comprised major portion of org-N (Knicker 2000)
	Broch decay MAS <sup>15</sup> N-NMR	Soils: Review article on how to follow evolution of N & C pools (Kogel-Knabner 1997)
Pyrolysis-GC	PY-GC-MS—flash pyrolysis followed by GC capillary column separation with mass spectroscopy detection	Norwegian freshwater NOM isolated by RO and evaporation, amino sugars based on the fragment acetamide Estimation of proteins based on pyrole derivatives Correlation between amino sugars + protein fractions and total org N (Christy <i>et al.</i> 1999)
	PY-GC-MS—direct pyrolysis field ionization MS	Hydrolysed residue from soil samples 30–45% of total N was volatilised and determined Heterocyclic and benzene derivative N-containing compounds and N-containing were identified (Schulten & Schnitzer 1997)
	PY-GC-MS	Particulate organic matter: aminosugars, nitrogenous compounds, aromatic hydrocarbons, polysaccharides, phenols (Cotrim da Cunha <i>et al.</i> 2000)
	Pyrolysis GC-FID	Sewage sludge: nitrogenated compounds (Barrio <i>et al.</i> 1996)
	Pyrolysis GC-NPD	Sewage sludge: pyroles, pyridines, aliphatic nitriles, aromatic nitriles, indoles, other N-containing compounds (Barrio <i>et al.</i> 1996)
	Curie-Point pyrolysis GC/ MS and NPD	Residue from soil samples: hydrolysed samples: heteroaromatic compounds, N-containing benzene derivatives, long-chain aliphatic nitriles Methylated hydrolysed samples: Methylated benzenamine (Schulten & Schnitzer 1997)
	PY-GC-MS—identification by PBM-algorithm	Wastewater: 133 probable nitrogen-containing compounds (Grohmann <i>et al.</i> 1998)
	Amino acid assay	Derivatization with Dansyl-Cl, Bansyl-Cl, and Mansyl-Cl with fluorescence detection
Derivatization with fluoescamine with fluorescence detection		Primary amino acids (Lindroth & Mopper 1979)
Derivatization with o-phthalaldehyde/2-mercaptoethanol reagent with fluorescence detection		Urine, clinical sample, and seawater: 25 amino acids (Lindroth & Mopper 1979) All common amino acids except cysteine (without prior oxidation), proline and hydroxyproline, inferior for amine-substituted amino acids (Roth 1971) Freshwater RO isolates: 21 amino acids, hydrolysable AA only detected (Munster 1999)

Table 3 | Continued

Method of analysis	Brief description of method	Samples measured and representative nitrogen compound results
Amino acid assay <i>continued</i>	Conventional ion-exchange amino acid analyser utilizing ninhydrin as locating agent using colorimetry	Clinical samples: 11 acid/neutral and basic amino acids (Lindroth & Mopper 1979)
	Derivatization with 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole reagent with fluorescence detection	Clinical samples: amendable with amino-substituted amino acids (Ghosh & Whitehouse 1968)
	Hantzsch condensation with acetylacetone and formaldehyde	Clinical samples: amendable with amino-containing amino acids (Sawicki & Carnes 1968)
	Oxidation by the enzymes D- and L-amino acid oxidase	Clinical samples: only applicable to amino acids attacked by these enzymes, including proline (Guilbault & Heiserman 1968)
	Hydrolysis followed by reduction and conversion to alditol acetates measured by GC	Soil humic acids and fungal melanins: amino sugars (glucosamine, galactosamine and mannosamine) (Coelho <i>et al.</i> 1997)
	Gas-phase hydrolysis followed by o-phthalaldehyde/2-mercaptoethanol reagent and measured by HPLC	Protein and peptide solutions: 16 amino acids (Meltzer <i>et al.</i> 1987)
FTIR	N-H stretching absorption measured at 3,460–3,480 $\text{cm}^{-1}$	Coal and coal-derived liquids: pyrrolic NH (Wallace <i>et al.</i> 1989)
	Absorption bands centred at 1,660–1,630 $\text{cm}^{-1}$ and 1,513–1,508 $\text{cm}^{-1}$	(Olk <i>et al.</i> 2000)
	1,550 $\text{cm}^{-1}$ (amide 2) and 1,670 $\text{cm}^{-1}$ (amide 1)	Wastewater and wetland treated water (Barber <i>et al.</i> 2001)
	Amide type I and II 1,650–1,550 $\text{cm}^{-1}$	Sediment humic and fulvic acids (Calace <i>et al.</i> 1999)
	FT-IR identification of proteins and amino sugars	Raw water and fouled membrane samples (Kaiya <i>et al.</i> 2000)

acids and may have been associated with aromatic amino acids (e.g. tryptophan) or conjugated substituents containing multiple bonds (e.g. nitriles) or non-bonding electrons (e.g. amines) (Reckhow *et al.* 1991). With the exception of the base fractions, decreasing SUVA generally correlates with decreasing C/N ratios (increasing org-N content).

For waters with autochthonous NOM, N-acetylamino sugars have been identified, through spectral characterisation, and dominate colloidal and hydrophobic neutral NOM fractions (Leenheer *et al.* 2001). The colloidal or hydrophobic neutral fraction constituted 32%

of the DOC of Siene River water and 40% in a reclaimed wastewater, respectively. Approximately 15% of the DOC was colloidal in the Mississippi River and characterised by a low C/N ratio that is typical of autochthonous NOM. The colloidal material was hypothesised to have micro-organism cell wall origins (e.g. aminosugars such as acetamide), rather than originating from soils or plant material (Rostad *et al.* 1997). The authors hypothesised that colloid NOM was readily biodegradable and as the size of NOM decreased the colloids transformed into nitrogen-rich humic materials. Therefore, the colloidal

**Table 4** | Characterization and reactivity of NOM fractions related to org-N content

Fraction	SUVA @ 254 nm (l/mg-m)	C/N ratio	Oxidant reactivity		Chlorine reactivity			Metal binding
			Ozone rate constant	Chlorine demand	THMFP/ DOC	TOXFP/ DOC	HANFP/ THMFP	
Forge Pond, MA <sup>1</sup>			(M <sup>-1</sup> s <sup>-1</sup> )		(µg/mg C)		(µg/µg)	
Hydrophobic:								
Humic acids	6.3		93		68		0.072	5.4
Fulvic acids	4.4		28		46		0.035	2.0
Weak acids	3.5		18		29		0.010	4.1
Bases	0.5		22		5			0.3
Neutrals	0.8		18		18		0.010	4.0
Hydrophilic:								
Acids	1.0		4		24		0.024	5.6
Bases	3.1		41		60		0.155	5.6
Neutrals	3.9		11		40		0.020	5.8
Hydrophobic acids: <sup>2</sup>								
			(s <sup>-1</sup> )	(mol/mol)	(mM/M-C)			
Lake Fryxell, Antarctica	1.9	20	0.0011	0.49	3.0			
Suwannee River, GA	4.2	90	0.0023	0.65	4.8			
Ohio River, Ohio	4.2	43	0.0013	0.58	3.6			
Suwannee River, GA <sup>3</sup>								
				(mg Cl <sub>2</sub> /mg C)	(µg/mg C)	(µg/mg C)		
Acids:								
Hydrophobic	4.6	70		1.4	55	268		80%
Transphilic	3.4	46		1.4	40	224		75%
Hydrophilic	2.7	34		0.8	36	171		74%
Neutrals:								
Hydrophobic	3.0	47		1.5	51	182		20%
Transphilic	2.9	30		1.2	40	204		—
Hydrophilic	0.7	15		0.8	23	117		38%
Bases:								
Hydrophilic	2.3	8		2.5	29	175		36%

Table 4 | Continued

Fraction	SUVA @ 254 nm (l/mg-m)	C/N ratio	Oxidant reactivity		Chlorine reactivity			Metal binding
			Ozone rate constant	Chlorine demand	THMFP/ DOC	TOXFP/ DOC	HANFP/ THMFP	
South Platte River, CO <sup>5</sup>				(mg Cl <sub>2</sub> /mg C)		(µg/mg C)		
Acids:								
Hydrophobic	2.9	44		0.95		122		48%
Transphilic	1.7	18		0.81		100		55%
Hydrophilic	1.7	15		0.86		98		56%
Neutrals:								
Hydrophobic	1.6	28		0.83		114		19%
Transphilic	0.7	4		2.3		66		53%
Hydrophilic	0.5	9		1.0		81		—

<sup>1</sup>Metal complexation represents the calcium complexed (meq/g C) at pH 5 in the presence of 1 mM free calcium (Reckhow *et al.* 1991; Bose 1994).

<sup>2</sup>Ozonation rate constants are in the presence of 2 mM t-butanol (s<sup>-1</sup>) (Debroux 1998; Westerhoff *et al.* 1999).

<sup>3</sup>Metal complexation represents percentage DOC removal during alum jar tests (1 mg Al/mg DOC) at pH 6.5 (Benjamin *et al.* 2000).

fraction may represent a significant portion of the org-N in surface waters. DON occurs as a continuum of molecular weight material, comprised predominantly of amino sugars and amino proteins.

## ORGANIC NITROGEN OCCURRENCE

### DON occurrence

Public data (<http://water.usgs.gov/nawqa/>) were obtained from the United States Geologic Survey (USGS) National Water Quality Assessment (NAWQA) programme for approximately 23,000 surface water and shallow or deep groundwater samples. DON was calculated from the data (DON = (DON + ammonia) – (ammonia)); land-use data were also available. Approximately 30% of the surface water sites consisted of measurements that were reported to be less than the detection limit for the parameter

‘DON + ammonia’ (0.2 mg-N/l), and are referred to as below detection limit (BDL). Seventy-six percent (76%) of the shallow groundwater samples, hydrologically linked to specific land uses (operationally defined as Shallow GW), and 81% of the deep groundwater aquifer (Deep GW) samples were characterised as BDL, supporting the observation that groundwaters typically contain very low DON concentrations (Nolan & Stoner 2000). DON mineralises during infiltration of surface water into groundwater. DON concentration percentile occurrence graphs are presented in Figure 3A, for samples where both DON + ammonia was greater than BDL and ammonia concentrations were reported. For data above BDL the median DON concentrations in surface, shallow and deep groundwaters were 0.37, 0.24, and 0.18 mg N/l, respectively. DON concentrations in surface waters are greater than in groundwater systems.

Additional data on DON occurrence in rivers and lakes are presented in Table 5. For rivers in the south-eastern US, DON constituted >50% of the TDN and the

**Table 5** | DON occurrence and relationship with TDN and DOC

Water source	DON (mg N/l)	Average DON/TDN	Average DOC/DON
Southeastern US (Alberts & Takacs 1999)			
Altamaha River	0.35 ± 0.16	50%	23
Black River	0.64 ± 0.28	85%	17
Edisto River	0.41 ± 0.22	71%	19
Ogeechee River (Eden)	0.52 ± 0.33	74%	17
Ogeechee River (Oliver)	0.39 ± 0.24	66%	22
Peedee River	0.46 ± 0.44	46%	11
Satilla River	0.75 ± 0.33	81%	23
Savannah River	0.34 ± 0.24	91%	46
St. Mary River	0.66 ± 0.26	85%	38
Scotland (28 catchments) (Chapman <i>et al.</i> 2001a)			
Winter	0.16 ± 0.12	27%	45
Summer	0.20 ± 0.14	43%	36
Sweden/Finland (20 forested watersheds) (Arheimer <i>et al.</i> 1996)	0.2 to 1.2	30% to > 80%	
Chile (31 undisturbed watersheds) (Hedin <i>et al.</i> 1995)	0.08 to 0.42	> 95%	39 to 49
Ontario, Canada (old growth forest) (Creed & Band 1998)	< 0.1 to 1.0		
Virginia, USA (Prairie Run stream) (Buffam <i>et al.</i> 2001)			
Average baseflow (maximum, <i>n</i> = 85)	0.024 (0.08)		36
Average stormflow (maximum, <i>n</i> = 67)	0.042 (0.11)		42
Florida, USA (Tuschall & Brezonik 1980)			
Lake Weir (hypereutrophic)	0.4		25
Lake Apopka (mesotrophic)	2.5		14
Algae culture	21		12
Lakes (Wetzel 1983)			
Fureso, Denmark	0.44–0.64		
Ysel, Netherlands	0.59–1.84		
Bodensee, Germany	0.05–0.15		

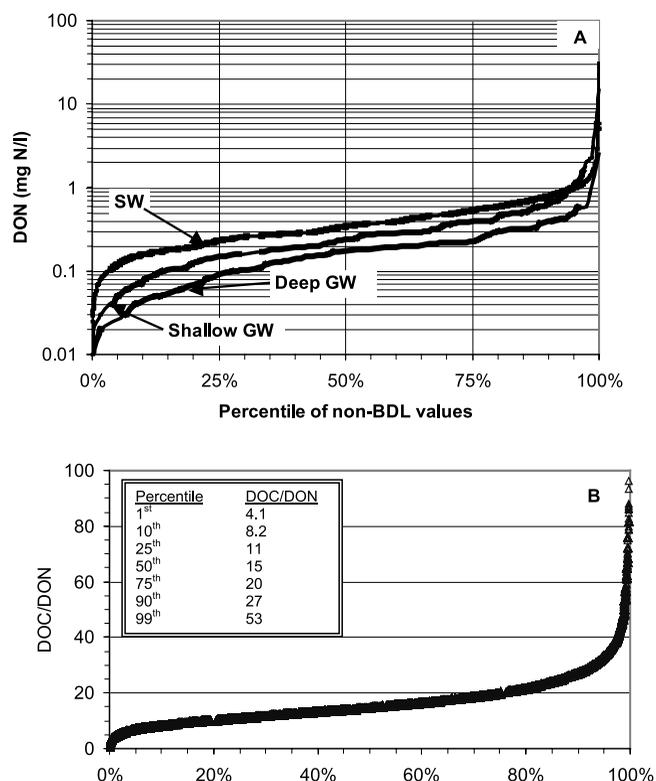
**Table 5** | DON occurrence and relationship with TDN and DOC

Water source	DON (mg N/l)	Average DON/TDN	Average DOC/DON
Lakes (Wetzel 1983) ( <i>continued</i> )			
Lucerne, Switzerland	0.08–0.18		
Rotsee, Switzerland	0.27–0.66		
Wintergreen, Michigan/USA	0.50–1.32		
Lawerance, Michigan/USA	0.08–0.24		
Kinneret, Israel			
Epilimnion	0.09–0.55		
Hypolimnion	0.04–0.34		
Miscellaneous (Frank <i>et al.</i> 2000; Meybeck 1982)			
Precipitation	0.225		
Precipitation	< 0.1 to 0.2		> 16
Tree throughfall	0.45		22
Riverine inflows to oceans	0.26		20 (range 8–41)

average DON concentrations were slightly higher than the median value of 0.37 mg N/l for surface waters from the USGS database analysis. Lakes have a wide range of DON concentrations, representing seasonal shifts in DON sources. More eutrophic conditions increases DON concentrations. Data in Table 5 and analysis of the USGS-NAWQA database provide an excellent baseline for DON occurrence in potential drinking water supplies.

The ratio of DOC/DON for a subset of the surface water samples where both datasets were available are presented in Figure 3B. A low DOC/DON is indicative of more autochthonous NOM sources, while NOM with higher DOC/DON ratios represents allochthonous NOM sources (Aiken & Cotsaris 1995). Algae and bacteria processes result in production of NOM containing nitrogen. DOC/DON ratios could serve as potential indicators of NOM origin.

Land-use data were available for samples from the USGS-NAWQA database, and were operationally defined into five categories (see legend in Figure 4). For surface water samples with high DON, a high proportion of the samples were categorised as agricultural (Figure 4). Sites with minimal human impact (termed 'Reference' sites) constituted a larger percentage of sites with low rather than high DON values. The mixed land-use category was not specifically delineated, but included wastewater treatment plants, which can have elevated DON levels (see below). Urban sites accounted for 10–20% of samples within each DON concentration range (Figure 4). Many of the high DON concentrations associated with urban sites occurred concurrent with elevated streamflow or runoff events (i.e. urban stormwater). Sites categorised as 'Other' included forests, mines, etc. and exhibited a bimodal pattern of either having low or high DON values. For

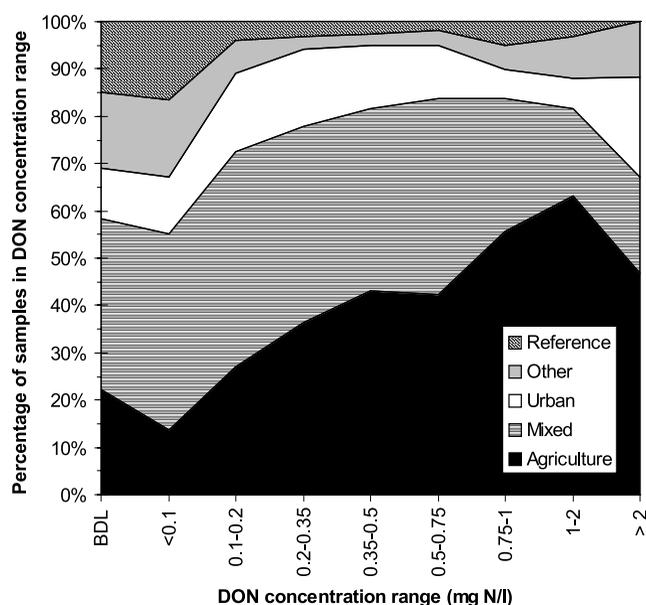


**Figure 3** | (A) DON occurrence in surface waters (SW;  $n=14,803$  samples; 30%<BDL) and shallow groundwater (GW) hydrologically linked to specific land uses ( $n=296$ ; 76%<BDL) and deep GW ( $n=120$ ; 81%<BDL) based upon data from the USGS-NAWQA database. Samples below the detection limit (BDL) of 0.2 mg-N/l for DON+NH<sub>3</sub>-N are not included in analysis. (B) Ratio of DOC/DON (mg C/mg N) percentile occurrence for a subset of SW data ( $n=9846$ ; DOC<30 mg/l)

shallow groundwater sites (not shown on Figure 4), those hydrologically linked to agricultural sites had higher DON concentrations than urban sites. A key factor for high DON levels (>1 mg N/l) appears to be a linkage with fertilization (e.g. biosolids, animal manure, urea, inorganic nitrogen) and poorly treated wastewater or runoff events.

### Amino acid occurrence

Amino acids constitute a significant portion of the DON pool, and are present predominantly as polypeptides and complex organic compounds (Wetzel 1983). Amino acids in rivers and lakes have concentrations ranging between



**Figure 4** | Distribution of land-use types associated with ranges of DON concentrations for surface waters based upon data from the USGS-NAWQA database.

50 and 1000  $\mu\text{g/l}$ , and may account for between 15% and 35% of the DON, and 2.6% of the DOC (Thurman 1985). The major amino acids include glutamic acid, glycine, serine and aspartic acid with lesser amounts of ornithine, histidine, lysine and cysteine.

Amino acids are present in the atmosphere, dew and rain, presumably with biogenic or anthropogenic emission sources (Frank *et al.* 2000; Scheller 2001). Dry deposition on water bodies could serve as a direct source of DON, or an indirect source after runoff events. Algae are considered a primary producer of amino acids during growth and succession. Humification/condensation and bacterial degradation have been hypothesised as processes responsible for decreasing amino acid concentrations over time in surface waters.

A study of export of free and combined amino acids from the Mackenzie River drainage basin (Canada) concluded that amino acids associated with suspended solid material constituted between 15 and 220  $\mu\text{g N/l}$  in the water while dissolved amino acids ranged from 11 to 79  $\mu\text{g N/l}$  and that combined acids were 10 times more abundant than free acids (Peake *et al.* 1972). On average the contribution of four amino acids (alanine, glycine,

aspartic acid, glutamine) accounted for nearly 60% of total amino acid concentration (16 measured). In several Swiss watersheds, amino acids accounted for 20% to >75% of the DON in catchment runoff, but constituted a larger percentage immediately after peak discharges and were lower later in the runoff event (Frank *et al.* 2000). In a study of humic-bound amino acids in river water (Williamson River, Oregon) the five most abundant amino acids in relative order were glycine>aspartic acid>alanine>serine≈glutamic acid (Lytle & Perdue 1981). Similar amino acids were dominant in two Florida lakes (Table 5—Lakes Weir and Apopka) where peptidic nitrogen (free and hydrolysable) accounted for between 20% and 30% of the org-N (Tuschall & Brezonik 1980). Higher concentrations of hydrolysable amino acids were observed from soil humic and fulvic acids than from stream acid, and were dominated by alanine, aspartic acid, glutamic acid and glycine (Malcolm 1990).

## SOURCES AND TRANSPORT OF DON IN WATERSHEDS

Based upon the above DON occurrence section, wastewater and agricultural impacted waters are likely to have the highest DON levels. While many watersheds and raw drinking water supplies are impacted by upstream wastewater or agricultural discharges, other sources (algae, forests, soils) of DON are important in watersheds with fewer wastewater impacts. It is unclear whether the apparent relationship between DON and agricultural land use is due to manure or urea fertilizers directly, or application of inorganic nitrogen fertilizers that stimulate plant roots and soil microbes to produce nitrogen-enriched organic products. Hydrologic watershed processes transport DON from the DON sources to raw drinking water intakes.

### Bacterial soluble microbial products

Autotrophic and heterotrophic bacteria can produce soluble microbial products (SMPs) that contain org-N.

Common cellular material (e.g. *E. coli*) contains 70% water, 15% proteins, 7% nucleic acids, 3% carbohydrates and 2% lipids by weight. Proteins (amino acids) and nucleic acids (pyrimidines, purines, d-ribose) contain org-N while the carbohydrates/polysaccharides (glucose) and lipids (glycerol, choline, palmitic) typically contain much lower percentages of org-N. Both biomass-associated and utilization-associated SMPs contain these building block (molecular weight: 100–350) or macromolecular (molecular weight:  $10^3$ – $10^9$ ) compounds that can be released into the water column as dissolved material ( $<0.45\ \mu\text{m}$ ) (Namkung & Rittmann 1986). For example, in the Chesapeake Bay, rates of autotrophic DON release were highest in May but heterotrophic DON release was more important in summer and fall. The DOC/DON ratio was low (3.4–4.5 mg C/mg N) indicating release of nitrogen-rich compounds (Bronk *et al.* 1998), very similar to the C/N ratio for simple proteins (3–4 mg C/mg N).

Analysis of the USGS-NAWQA database indicated that hydrologic linkage between a water body and wastewater treatment discharge leads to elevated DON levels. Untreated sewage contains high levels of proteinaceous materials. During wastewater treatment these materials are converted to soluble (nitrate, ammonia) and gaseous (nitrogen gas) inorganic forms, but a portion remains as DON. For example, secondary and tertiary treated wastewater contains 5–25 and  $<4\ \text{mg DON/l}$ , respectively, while higher DON concentrations can be present in industrial discharges (Hanson & Lee 1971; Gakstatter *et al.* 1978; Metcalf & Eddy 1979; Henze 1992; Cheng *et al.* 1996; Austermann-Haun *et al.* 1998; Baker & Westerhoff 2000; Sartoris *et al.* 2000). In a study of wastewater discharges into the South Platte River Basin, CO, median DON concentrations in the effluents ranged from 1.3 to 4.3 mg N/l, including secondary and tertiary treatment facilities (Pocernich & Litke 1997). Given that many raw drinking water supplies are impacted by upstream wastewater discharges, this contribution of DON may be significant.

The macromolecular proteins and amino acids, plus polysaccharides, were cited as important SMP constituents and were found to vary in concentration and characteristics primarily as a function of characteristics of primary substrate, biomass density and hydraulic

residence time during wastewater treatment (Barker & Stuckey 1999). The fractionation of SMP samples has shown that they contain basic (7.6%), neutral (28.5%) and acid (60.6%) groups (Hejzlar & Chudoba 1986). Investigation of hydrophobic and hydrophilic acid fractions of EfOM from a set of French wastewater treatment plants concluded that: (1) hydrophilic acids were nitrogen-enriched ( $C/N = 18 \pm 2$ ,  $n = 6$ ) compared to corresponding hydrophobic acids ( $C/N = 31 \pm 3$ ,  $n = 6$ ) (Debroux 1998). Activated sludge heterotrophic bacteria produced extracellular polymers in which 75% of the TOC was accounted for by total amino acids and only 5% from total sugars (Dignac *et al.* 1998). About 25% of those amino acids were negatively charged and about 24% exhibited hydrophobic properties. Pyrolysis GC-MS analysis of the bulk extracellular material also indicated a large portion of nitrogen-containing biopolymers. Dominant amino acids in wastewater effluents usually differ from natural waters, and include higher concentrations of proline, tyrosine and tryptophan (Dignac *et al.* 2000). Therefore, the presence of elevated levels of aromatic amino acids, relative to aliphatic amino acids, in raw drinking water may be an indicator of upstream wastewater contributions. Treated wastewaters also contain DNA (furfuryl alcohol) and amino sugars (acetamide). Non-proteinaceous org-N can constitute up to 90% of the total nitrogen in wastewater, and may contain amide groups that originated from refractory polymers and cell walls (peptidoglycans) or membranes (lipopolysaccharides) and or recondensation productions (melanoidin-like structures) (Dignac *et al.* 2000). Although wastewater effluent includes base and neutral fractions which contain amino acids, these fractions tend to readily biodegrade (Namour & Muller 1998; Dignac *et al.* 2000; Leenheer *et al.* 2001). For example, infiltration of tertiary treated wastewater decreased DON from 0.7 to 0.3–0.6 mg N/l over 10 m of unsaturated soil, but did not show any further decline in the saturated zone and the results were similar to other studies (DeSimone & Howes 1998; Sumner *et al.* 1998).

Bacteria in natural systems and wastewater treatment systems produce SMPs which do have an effect on drinking water sources. SMPs are characterised as follows: (1) comprised of nitrogen-enriched compounds (low C/N; predominantly amino acids); (2) comprised of less

hydrophobic or more basic fractions; (3) comprised of cell-wall decomposition products and other macromolecular biopolymers; (4) contain low molecular weight fractions that readily biodegrade.

### Algal sources of DON

Surface waters with algal-dominated sources of NOM can be characterised as having low C/N ratios and low aromatic carbon content. Based upon a synopsis of extracellular products from algae, nitrogenous substances are liberated from algae (Fogg 1966). Blue-green algae (nitrogen fixers) have received considerable attention since they can excrete up to 45% of the total fixed nitrogen as org-N. Green algae also excrete DON. Using PY-GC-MS analysis it was shown that extracellular organic material (EOM) of one green alga was dominated by nitrogen-containing compounds, mostly aliphatic in nature and of proteinaceous origin, as well as by long-chain carboxylic acids derived from lipids (Widrig *et al.* 1996). Algal and bacteria cell wall integrity emerges from cross-linked peptide chains of N-acetylglucosamine and N-acetylmuramic acid, for example, resulting in the presence of colloidal aminosugars. Some trace compounds produced by algae (e.g. microcystins, nodularians) contain org-N as well.

A positive correlation has been found between sustained high productivity of algal populations and average DON concentrations, with eutrophic lakes (DON~0.7–1.2 mg N/l) having higher levels than mesotrophic (DON~0.4–0.7 mg N/l), oligotrophic (DON~0.2–0.4 mg N/l) or ultra-oligotrophic (DON<0.2 mg N/l) lakes (Wetzel 1983). Hydrophilic neutrals were found to be the main constituent of NOM isolated from a eutrophic reservoir (Chang *et al.* 2000). This is consistent with the observation of low fulvic-acid content in algal-rich Antarctic ponds (McKnight *et al.* 1994). Submerged macrophytes are also significant sources of DOC (Otsuki & Wetzel 1974; Stepanauskas *et al.* 2000), and may be sources of org-N. Thus the degree of eutrophication, photoproductivity and abundance of different algal groups (e.g. blue-green dominance) will affect the amount of DON in a water body.

Eutrophication of surface waters leads to increased DOC concentrations, and evidence suggests increased DON levels (Thurman 1985). For example, an *Anabaena* alga culture had a DOC/DON ratio of 12, and 71% of the DON was comprised of peptidic nitrogen (Tuschall & Brezonik 1980). Further characterisation of this alga DON indicated that it was less than 10,000 daltons in molecular weight. Hellebust (1965) observed considerable quantities of amino acids and peptides as excretory products of algae, and quantified the amount of carbon uptake which was excreted as proteinaceous material (0.2 to 5.9%) (Hellebust 1965, 1980). NOM fractions isolated from Antarctic ponds where autochthonous processes dominate NOM production (i.e. minimal terrestrial vegetation or allochthonous processes) have lower C/N ratios (20) than watersheds with significant allochthonous inputs (e.g. Suwannee River fractions in Table 4) (Aiken *et al.* 1996). Overall, algal productivity in surface waters will increase both the DOC and DON concentrations.

Daily fluctuations of DOC generated by algae have been observed to increase by as much as 40% from the daily minimum in a Piedmont stream (Pennsylvania, USA), and net algal DOC excretion accounted for 20% of the total DOC exported from the watershed on days with high photoperiods (Kaplan & Bott 1982). The authors concluded that rapid bacterial DOC degradation occurred, controlling DOC levels. A large portion of the autochthonous org-N material (e.g. simple amino acids) can be easily biodegraded (Wetzel 1983; Sondergaard *et al.* 2000). Algae produce EOM of variable molecular size that can be utilised by heterotrophic bacteria and result in larger molecular weight compounds as by-products of bacterial respiration and other cellular processes (Nalewajk & Lean 1972; Wetzel 1983; Vallino *et al.* 1996).

### DON leached from soils

Watershed DON fluxes are controlled by hydrologic flow regimes through sources, and follow similar patterns as observed for DOC (Aiken & Cotlaris 1995). Proteinaceous materials and heterocyclic-N have been shown to be the major soil org-N components; proteinaceous material (proteins, peptides and amino acids) ~40%, amino sugars

~5%, heterocyclic org-N compounds (e.g. purines, pyrimidines) ~35%; ammonia ~20% (Schulten & Schnitzer 1997). Based upon a review of DON in agricultural soils, DON concentrations in soil pore waters are a function of soil org-N content that is leached during infiltration (Murphy *et al.* 2000).

Inorganic nitrogen in plant throughfall is efficiently retained probably by microbial uptake, but DON is released from the forest floor organic matter and constitutes a major flux of nitrogen from forested ecosystems (Yavitt & Fahey 1984; Qualls & Haines 1991). In contrast to DOC, considerably more DON is leached from the forest floor than can be accounted for as initially water-soluble nitrogen in litterfall (Qualls & Haines 1991, 1992). In a separate study, DON concentrations (range <0.01 to 1.8 mg N/l) were larger in leachates from soil cores with vegetation (mean = 0.93 mg N/l) than without vegetation (mean = 0.26 mg N/l) (Chapman *et al.* 2001b). They concluded that the majority of DON, similar to DOC, in the leachates from the vegetated cores were probably leached from foliage, plant residues in the root mat and/or exuded from roots.

The DOC/DON ratio of soil water solution from several soil horizons ranged between 20 and 40, with organic rich horizons (Oi and Oa horizons) having higher DOC/DON ratios than A, Bs, Bg or Cu horizons (Qualls & Haines 1991; Frank *et al.* 2000; Kalbitz *et al.* 2000). Therefore, the organic content of the soil horizons and hydrologic flowpaths will affect the DON leaching into surface and shallow ground waters (Michalzik *et al.* 2001). Water percolation through soil horizons can therefore leach org-N, and C/N ratios in such waters are typically less than 50 as this represents the more polar fraction of soil organic material (Pomes *et al.* 2000).

In a forest floor porewater (Massachusetts) DOC and DON production peaked in later summer and early fall. However, stream DON showed little seasonal variation and the authors concluded that abiotic factors such as sorption to mineral soil retained and regulated DON export (Goodale *et al.* 2000). Furthermore, a DON loss correlated with both DOC loss and variables associated with DOC production and retention in the forest ecosystem (i.e. vegetation and soil type). The rate of biodegradation of DON leached from soils occurred at a

similar rate as DOC degradation—results consistent with the idea that hydrolysis of DON is linked to mineralisation of DOC rather than occurring selectively in response to the biochemical need for nitrogen (Qualls & Haines 1992).

### Hydrologic processes affecting DON transport

Rainfall through forest canopy, surface runoff, and percolation through soils transport organic matter through ecosystems (McDowell & Likens 1988; Aiken & Cotlaris 1995; Boyer *et al.* 1995, 1996). In addition to land-use classification or other descriptions of organic biomass in the watershed (e.g. forest type, soil development), stream discharge and DOC appear to be good indicators for DON fluxes and concentrations from watersheds, respectively. The following studies provide insight into DON temporal and spatial occurrence:

- In one study, discharge was a strong predictor of DON catchment flux (kg N/ha/mo; linear with slope = 0.0020;  $R^2 = 0.71$ ) but not DON concentration (mg N/l) (Creed & Band 1998). The rationale was that the DON measurements represented a discrete sample under a specific hydrologic condition, the DON catchment flux intrinsically accounted for the influence of one hydrologic event to the next.
- DON export from several watersheds in Switzerland was ~6 kg N/ha/yr and accounted for 60% of the TN export (Frank *et al.* 2000). DOC and DON in weekly samples were positively correlated with streamflow discharge, suggesting terrestrial sources were similar and associated with leaching from topsoils during the descending limb of the hydrograph.
- Higher DON concentrations during increased streamflow were also observed for 20 small, forested catchments in Sweden and Finland in a 10-year dataset and in a small Appalachian USA stream catchment where DOC and DON concentrations approximately doubled during storm events (Arheimer *et al.* 1996; Buffam *et al.* 2001). Linkages were developed between DON concentrations to hydrologic events and DON ecosystem

compartments (throughfall, soil, groundwater, surface water).

- In an evaluation of patterns of DON export from sets of old-growth and historically (80–110 years) logged and burned watersheds in the northeastern USA, DON loss averaged  $0.7 \pm 0.2$  kg N/ha/yr and accounted for 28% to 87% of the TDN export, but did not vary as a function of forest successional status (Goodale *et al.* 2000). DON concentrations correlated with DOC, which varied inversely with hardwood forest cover.
- Stable isotopic composition for the  $^{15}\text{N}/^{14}\text{N}$  ratio of a sample relative to a standard ( $\delta^{15}\text{N}$ ) has been used to determine if org-N originated from atmospheric N or nitrate sources. For ultrafiltration NOM isolates  $\delta^{15}\text{N}$  values were  $-2.1\text{‰}$  for peat NOM,  $-1.2\text{‰}$  to  $5\text{‰}$  for Great Lakes (USA) NOM, and  $8\text{‰}$  for marine NOM (Benner *et al.* 1997; Feuerstein *et al.* 1997; Kracht & Gleixner 2000).
- Rainfall events leach org-N from the forest canopy and forest flow. Soil porewater can contain high levels of DON. However DOC is also solubilized in these systems, and microbial processes biodegrade both DOC and DON. Existing DOC mobilization models may be suitable for DON mobilization. For example, TOPMODEL conceptually represents a terrestrial (soil) reservoir in which DOC builds up during low flow periods and is flushed out when infiltrating waters cause the water table to rise into this “DOC reservoir” (Boyer *et al.* 1995, 1996).

On the Mississippi River DON concentrations may have at least doubled over the last century due to changes in land use (Goolsby & Battaglin 2001). Basins draining southern Minnesota, Iowa, Illinois, Indiana and Ohio had higher nitrogen fluxes than other basins. In conjunction with the NAWQA data that showed high DON concentrations associated with agricultural land use, the elevated fluxes may be attributable to increased agricultural development over the past century along the Mississippi River. In a separate study of eight major rivers in the southeastern US over a 20-year period (1974–1993) using USGS data, the authors found that DON levels ranged between 0.35 and 0.75 mg N/l, and concluded that DON

represented a majority of the TDN and that the DOC/DON ratio ranged from 11 to 46 (Table 5) (Alberts & Takacs 1999). Seasonal variation in DON concentration existed, but no consistent trend was noted. Therefore, long-term changes of DON concentrations may be linked to land-use changes.

In an attempt to study watersheds unaffected by human development or air pollution, 31 small stream watersheds in Chile (coastal, mid-elevation, high elevation forests) were studied for nitrogen transport (Hedin *et al.* 1995). Greater than 95% of the TDN exported from the watershed in the streams was DON (83 to 420  $\mu\text{g N/l}$ ), and the results suggested that while biologically available forms of nitrogen (ammonia and nitrate) were retained in the forest ecosystems, biologically unavailable and hydrologically mobile forms (DON) dominated the streamwater outputs. A relatively constant DOC/DON ratio (40 to 50 mg C/mg N) and anion/cation charge concentration discrepancy analysis concluded that hydrologic DON losses from the forested watersheds occur mainly due to the dissolution of highly refractory fulvic acids from soil organic pools. Variations in hydrological flowpaths through soils and dissolution kinetics of humic soil components were implicated as the controlling factors for DON mobilization, corresponding to conclusions from a number of studies (Watson *et al.* 2000; Hagedorn *et al.* 2001). Neural network modelling has been used for DOC and DON export from 15 river basins in Canada and observed that export was controlled largely by precipitation and temperature (Clair & Ehrman 1996). While temperature correlated with precipitation, increasing temperature was also hypothesised to increase primary productivity that supplied organic matter pools for the release of DOC and DON. Overall, DOC and DON transport processes from forested watersheds are similar. The presence of biologically degradable DOC mediates DON biodegradation.

## ORGANIC NITROGEN DBP FORMATION

Chlorination of org-N can result in the formation of nitrogenous DBPs of health and regulatory concern

(e.g. nitrosamines, haloacetonitriles (HAN), halonitromethanes, cyanogen halides) (Klotz & Pyrch 1999; Richardson *et al.* 1999; Krasner *et al.* 2001). Using comparative genotoxicity and cytotoxicity assays, it was concluded that nitromethanes, especially di- and tri-brominated nitromethanes, pose high potential human toxicity (Plewa *et al.* 2000). While the occurrence of HANs and nitromethanes, based upon the limited available data, indicate they occur at quite low levels, exposure assessments for DBPs that contain org-N have been limited (Singer *et al.* 1995; Diehl *et al.* 2000; Krasner *et al.* 2001). While the drinking water industry has recognised the significance of DOC and bromide as critical precursors for DBP formation, additional recognition, quantification and understanding for the role of DON as critical precursor material is necessary. The potential role of DON in oxidant demand, halogenated DBP formation (with and without org-N incorporation) and non-halogenated org-N DBP formation is discussed below.

### Oxidant reactivity with DON

Amino acids react rapidly with aqueous chlorine to form organic N-chloramines (Weil & Morris 1974; Alouini & Seux 1987). These reactions exert an oxidant/disinfectant demand and produce organic N-chloramines, which are poor bactericides. N-chloramines also interfere with the accurate measurement of combined residual chlorine using the iodimetric measurements (Conyers & Scully 1997; Keefe *et al.* 1997). Differentiation between inorganic and organic chloramines can be measured via HPLC methods (Yoon & Jensen 1993; Scully *et al.* 1996; Fox *et al.* 1997). Nitrogenous NOM may exert a chlorine demand without TOX formation, producing free amino acids as by-products that would in turn consume additional chlorine. The NOM fraction with the highest chlorine demand is typically the base fraction, which includes amino acids (e.g. Table 4), however this fraction usually represents a small percentage of the NOM (<10%) (Reckhow *et al.* 1991; Hwang *et al.* 2001). For a wide range of NOM fractions from four water sources, lower C/N ratios (N-enriched) lead to higher chlorine demand (Hwang *et al.* 2001). The corresponding rank order of NOM

fractions from highest to lowest chlorine demand was: base < hydrophilic acid + neutral > hydrophobic acid ≈ colloids. N-acetyl amides which comprise a large percentage of org-N in colloids are relatively non-reactive with chlorine. Overall, higher org-N content will lead to: (1) increasing chlorine demand which decreases the effectiveness of chlorine disinfection, (2) possible production of organic N-chloramines which have low disinfectant capability and interfere with DPD-colorimetric chlorine measurements, and (3) lead to DBP formation (see below).

Ozone reacts quite selectively with electron-rich organic compounds in general and DON may be influential in ozone demand during water treatment. When the nitrogen atom is deactivated, the rate of reaction with ozone is slower (Hureiki *et al.* 1998). Amino acid side chains seem to be the main sites that are responsible for the high ozone reactivity in polypeptide structures (Hureiki *et al.* 1998). Protonated amino groups were found not to react with ozone (Hoigne & Bader 1983). The increasing presence of methyl, alkyl or thio groups increased the rate of reactivity of ozone with the amino acids. Amino nitrogen and sulphur or activated aromatic rings were also identified as the main oxidant consumers and TOX precursor sites (Hureiki *et al.* 1994). Ozonation of a hydrophobic base NOM fraction was found to increase the hydrophilic base fraction by 58%, indicating the reactivity of basic NOM fraction sites (Marhaba & Lippincott 2000). The reactivity of the base fraction from Forge Pond, MA, was also found to be rapid with ozone. Elevated DON levels may be a significant factor in instantaneous ozone demand of a water. Furthermore, ozone may react with DON and release ammonia or organic amines, and the fate of these by-products may affect other water treatment processes.

### Role of DON in THM/HAA formation

Chlorination of amino acids (e.g. aspartic acid, tryptophan) can produce chloroform (Larson & Weber 1994). Rank order production of chloroform upon chlorination of amino acids has been observed (higher to lower reactivity order): tryptophan > tyrosine > histidine > aspartic acid > threonine > lysine > alanine > serine (Hureiki *et al.*

1994). The amino acids were found to be low precursors for chloroform, but characterised by high TOX formation potential. Yields of chloroform from tryptophan increased from <20% at pH 5.5 to 50% at pH 9, and nearly 100% at pH 11, while overall chlorine demand also increased with pH (Morris & Baum 1978). It was hypothesised that chlorination of amino acids to intermediate products occurred at acidic or neutral pH levels, with chloroform being liberated by hydrolysis at higher pH levels.

Chlorine reactions with amino acids form the unstable intermediate dichloroacetonitrile (DCAN), which can continue to react with chlorine to form chloroform, di- and tri-chlorinated acetic acid, trichloroacetamide and carboxylic acids (Trehy & Bieber 1981; Ueno *et al.* 1996; Glezer *et al.* 1999; Reckhow *et al.* 2001). As an intermediate DCAN produces more dihalogenated acetic acids (di-HAAs) than chloroform, and therefore DCAN may be more closely correlated with HAAs than THMs (Peters *et al.* 1990). Therefore, the presence of DON in water supplies may affect the relative distribution of THMs and HAAs.

Org-N compounds (i.e. proteins and amino sugars) are an important class of di-HAA precursor sites, perhaps as a consequence of the high chlorine demands (Benjamin *et al.* 2000; Hwang *et al.* 2001). NOM fractions isolated from the South Platte River (CO) were characterised and chlorinated. The South Platte transphilic neutral and Suwannee River hydrophilic base fractions were largely proteinaceous, while the South Platte hydrophilic neutral fraction had a stronger carbohydrate and amino sugar character. Decreasing hydrophobicity increased org-N content (decreasing C/N values—Table 4) generally leading to lower THMFP. The only exception was for the hydrophilic base fraction, which presumably contained amino acids and was moderately reactive in forming THMs (Table 4). During chlorination tests more tri-HAA was formed in all fractions except the hydrophilic base fraction from the Suwannee River. In the South Platte River fractions more tri-HAA was formed than di-HAA in the acid fractions, but the opposite trend was observed for the transphilic and hydrophilic neutral fractions. For all fractions SUVA was a good predictor for DBP formation, except for di-HAA formation where the role of proteinaceous material was presumed to affect the relationship

through direct contribution to di-HAA formation (Benjamin *et al.* 2000; Chang *et al.* 2000). In similar studies the neutral and base fractions of NOM were equally reactive in forming HAAs, as was the hydrophobic acid fraction (Sinha *et al.* 1997). As part of an examination of polar NOM, the nitrogen-enriched ( $C/N < 15$ ) hydrophilic acid and neutral fraction was characterised as containing carbohydrates, alcohols, and aminosugars and upon chlorination had higher di-HAA than tri-HAA and THM yields, and more than hydrophobic acid or colloidal fractions (Hwang *et al.* 2001).

In a comparison between laboratory and published literature on THM and HAA formation upon chlorination of algal cells, algal extracted organic matter and fulvic acids, it was concluded that cellular material contributed to nearly 70% of the DBP precursor pool (Plummer & Edzwald 2001). The reactivity of soluble algal material with chlorine ranged between 50 and 100% of the reactivity (in terms of  $\mu\text{g DBP}/\text{mg DOC}$ ) shown by hydrophilic acids. Chlorination of intact or lysed algal cells will form THMs and HAAs (Hoehn *et al.* 1980; Plummer & Edzwald 1998). An apparent contradiction exists between studies that imply cellular material as the precursor for DBPs and colloidal NOM fractions that are comprised primarily of decomposed cellular debris and dominated by N-acetylammides with low DBP formation potential. The difference may be related to the presence of amino acids associated with non-decomposed cell walls.

THM formation from model amino acids has been used to link changes in protein concentrations in natural waters with increases in THM formation that occur shortly after an algal bloom that has been treated by copper sulphate (protein material presumably released) (Scully *et al.* 1988). EOM, lysed due to the biocidal activity of copper, resulted in an increase in the protein concentration to nearly 1 mg N/l, four times its concentration earlier in the year, with a resultant large increase in THM formation. Four different molecular weight fractions of the NOM contained proteins, and all produced THMs upon chlorination. The majority of the proteins were present in either the <500 or 500–5 K molecular weight range, with lower protein concentration in the larger fractions. The authors estimated that roughly 10% of the THMs were

formed via the protein fractions with the remainder derived from humic substance precursors.

Org-N can affect the dominance of HAAs relative to THMs, and di-HAA relative to tri-HAA. Org-N reactions with chlorine form DCAN, an intermediate for di-HAA formation. The lone pair of electrons located on an amine functional group can interact with adjacent double-bonded or aromatic carbons making them more susceptible to halogen (chlorine, bromine, iodine) oxidation or substitution (i.e. ring activation) (Reckhow *et al.* 1990; Galapate *et al.* 1999). Therefore, it is important to consider org-N for accurate prediction of THM or HAA formation.

### Halogenated organic nitrogen DBPS

HAN, cyanogen chloride and nitromethanes (e.g. chloropicrin) form during chlorination (Richardson *et al.* 1999). Halogen substitution usually initiates with the substitution of the hydrogen on the nitrogen atom with the halogen followed by the formation of a double bond between the nitrogen atom and the adjacent carbon atom (Larson & Weber 1994). Hypochlorous acid converted, by oxidative decarboxylation, several amino acids into a mixture of nitriles (major product) and aldehydes (minor product) (Pereira *et al.* 1973). The amide nitrogen bond of several dipeptides was found to be resistant to HOCl, while chlorine substituted only into terminal amine functional groups of dipeptide (Pereira *et al.* 1973). Amides and many peptides were found not to be major TOX precursors (Hureiki *et al.* 1994).

The formation of halogenated acetonitriles (HANs) and cyanogen chloride (CNCl) is probably due to reactions with amino acids such as aspartic acid, tryptophan and tryptone (Larson & Weber 1994). Cyanogen chloride was reported as the dominant org-N DBP formed during chlorination of six model organic-N compounds, although other possible chlorinated by-products (N-chloroformamide, dichloroacetonitrile, chloroform, N,N-dichloroaminoacetonitrile and N-chloroiminoacetonitrile) and oxygenated by-products (acetone) were also observed in some samples (Shang *et al.* 2000). While cyanogen chloride is widely considered a chloramination rather than free-chlorine byproduct, it was shown that the

nitrogen atom of cyanogen chloride formed from leucine originated from the leucine and not from the chloramines (Hirose *et al.* 1988; Krasner *et al.* 1989). Amino acids (e.g. aspartic acid) in general, and kynurenine, a metabolite of tryptophan, specifically, were found to be suitable model DCAN precursor compounds (Trehly & Bieber 1981; Ueno *et al.* 1996). DCAN chlorination formation potential for kynurenine was five to ten times larger than the formation potential for the three most reactive amino acids (histidine, glutamic acid and phenylalanine). Trichloro-nitromethane (i.e. chloropicrin) also forms during chlorination, and has been associated with substitution reactions of nitrophenols (Thibaud *et al.* 1988). Amino acids, formed during ozonation oxidation of DON, can also form chloropicrin (Hoigne 1998).

Chlorination of two blue-green algae, which had high nitrogen content, produced more di-HAN upon chlorination than green algae. It was also noted that hydrophilic bases were by far the most reactive in forming HANs and this was attributed to the abundant amines that comprise base fractions (Table 4) (Reckhow *et al.* 1991). Chlorination of NOM fractions from the Colorado River indicated hydrophilic acids plus neutrals, and bases, were more reactive in forming HANs and nitromethanes than hydrophobic acids or transphilic acids, and colloidal material, which had the lowest DBP yields (Hwang *et al.* 2001).

DON precursor materials in water from Lake Kinneret (Table 4) were implicated in cyanogen bromide formation upon addition of chlorine or chloramine, subsequent to bromide oxidation (Heller-Grossman *et al.* 1999). Higher org-N content of isolated fulvic acids yielded nitrogen-rich DBPs constituting a major fraction of the volatile DBPs. Based upon a brief review, the authors concluded that org-N in aliphatic amino acids and in heterocyclic compounds was the source for the nitrogen in cyanogen chloride, and presumably cyanogen bromide. Peters *et al.* (1990) determined that, on average, dihalo-acetonitrile concentrations were approximately 5% of the average TTHM concentration ( $\mu\text{g/l}$  basis), whereas Oliver (1983) observed dihalogenated HAN concentrations were about 10% (molar basis) of the average molar TTHM concentration. Chlorination of a fulvic acid resulted in 1.3% conversion of org-N to dihalogenated HANs (Oliver 1983).

## Non-halogenated organic nitrogen DBPS

While chlorination of amino acids can form non-halogenated DBPs such as aldehydes (Alouini & Seux 1987; Larson & Weber 1994), formation of nitrosamines potentially pose a greater health risk. N-nitrosodimethylamine (NDMA),  $(\text{CH}_3)_2\text{NNO}$ , has received the most attention recently since the USEPA estimated its  $10^{-6}$  cancer risk level in drinking water at 0.7 ng/l (Najm & Trussell 2001). NDMA can form during nitrosation, an acid catalysed reaction between org-N precursors (e.g. primary or secondary amines) and nitrite, or nitrate (Williams 1988). A non-nitrosation mechanism for NDMA formation could involve chlorine and ammonia, and has been illustrated with dimethylamine (DMA) as a precursor (Choi & Valentine 2001). NDMA is only one of a series of nitro compounds (e.g. N-nitrosodipiperidine, N-nitroso-N-methylurea), and DMA one of a large spectrum of potential DON precursors (e.g. piperidine, methylurea). DMA could be present in drinking water as DON, or be produced during oxidation of DON. Other sources of trace-level DON may also contribute to NDMA precursor material. Strong-base anion exchange resin, which contains DON, has been shown to produce NDMA (Kimoto *et al.* 1980; Najm & Trussell 2001). The role of other chemicals used in water treatment (copper-trien complexes, nonionic/anionic/cation polyamide or polyamine polymers) which contain DON, remains an unanswered question as to their potential to form nitrogenous DBPs. In bench-scale studies the presence of chlorine and coagulant polymers together in a natural water lead to NDMA production, whereas NDMA production was not observed in chlorinated water without the polymer (Barrett 2001).

## ORGANIC NITROGEN TREATABILITY

### DON interactions with metals

Traditionally coagulation studies have focused primarily on organic *acid* interactions with charged cations or positive surfaces, since the acidic NOM fraction typically represents the majority of DOC and is DBP precursor

material (Edwards 1997). SUVA, which is correlated with aromatic carbon and hydrophobic acid content, has also been used as a surrogate for the ability of metal coagulants to remove NOM (Edzwald *et al.* 1985). More polar acidic fractions, which tend to be nitrogen enriched, are removed during coagulation with similar efficiency to the hydrophobic fractions (Table 4). This implies that DON removal may accompany removal of acidic fractions. The fate of non-acidic fractions during water treatment has received limited attention, and these constitute fractions with higher org-N content (e.g. Table 4). In general, basic and neutral fractions are more poorly removed during coagulation than acid fractions (Hoehn *et al.* 1984; Gehr *et al.* 1993; Dennett *et al.* 1996; Marhaba & Lippincott 2000). For example, on the Suwannee River and South Platte River, acidic NOM fractions were better removed during coagulation (Table 4) than the neutral or base fractions (Benjamin *et al.* 2000). The only exception was the South Platte River transphilic neutral fraction, which was considered proteinaceous, where the removal efficiency was as high as the acid fraction. It was concluded that non-isolated neutral and base fractions from NOM would also have been only slightly removed by coagulation. SUVA was able to predict DOC removal of acid fractions, but not neutral NOM fractions.

All amino acids contain carboxylic functional groups (Figure 2) that can complex with cationic metal coagulants. For example, one conditional stability constant ( $K \sim 10^{10}$ ) is reported for ferrous iron complexation with aliphatic primary amino acids and is probably associated with the carboxylic group (Martell & Smith 1974). In contrast, the aromatic amino acids (phenylalanine and tryptophan) have three reported conditional stability constants ( $10^{10}$ ,  $10^{19}$ ,  $10^{26}$ ) but only one carboxylic group, implying that nitrogen groups may be involved in metal binding. Aromatic amino acids are probably more readily removed than aliphatic amino acids. Heterocyclic-N complexes with metal cations as well, probably involving complexation with the lone pair of electrons on the nitrogen atom. Lone electron pairs on org-N have been shown to be strong cationic complexing sites, and could be significant in weak ionic strength waters where cation complexing competition is extremely low (Wu & Tanoue 2001).

Studies on DON removal during coagulation are lacking in the literature. However, a compilation of data is summarised below, suggesting (or not suggesting) interactions between DON and metals or metal hydroxides:

- Adsorption of NOM onto iron oxide coated sand in two full-scale WTPs was studied in Washington, USA (Korshin *et al.* 1997). Adsorption preferentially removed acidic NOM fractions, did not remove neutral fractions, and resulted in a shift in NOM fractionation. Similar results were observed at two other full-scale alum coagulation facilities (Marhaba & Lippincott 2000; Marhaba *et al.* 2000). Since NOM acid fractions contain lower org-N content, overall the NOM remaining after such treatment may have relatively more nitrogen on a DOC/DON basis.
- Using fluorescence spectroscopy, strong metal (copper) binding ligands were associated with NOM that exhibited protein-like fluorescence, which could be correlated with the presence of aromatic amino acids (tryptophan, tyrosine, phenylalanine) (Wu & Tanoue 2001).
- Metal (copper) was complexed by a base-fraction of DOC isolated from an *Anabaena* sp. alga culture, and was associated with higher molecular weight DON fraction (Tuschall & Brezonik 1980).
- Base fraction of NOM may participate in cation exchange or can adsorb onto negatively charged surfaces (especially silica) (Thurman 1985).
- Hydrophilic neutrals were a better ligand for calcium than fulvic acids (Table 5) (Bose 1994), and would imply potential involvement of org-N functional groups on metal binding.
- In jar tests with the NOM fractions the hydrophobic neutrals had the lowest removal (~10%), while the hydrophobic bases (~30%), hydrophilic bases (~35%), and hydrophilic neutrals (~50%) had improved removals but were still lower than observed removals for fulvic (70%) or humic (80%) acids (Bose 1994). Overall DOC removal was judged to be more dependent on molecular size than the NOM fraction, a result that tends to de-emphasize the importance of org-N content.

- Alum coagulation with water from an Australian river achieved moderate removal of hydrophobic acid fractions (~50% removal), hydrophilic acid fractions (64%), and unfractionated material (~64%) but zero percentage removal of the neutral fraction (Carroll *et al.* 2000). Based upon the NOM fractionation scheme and authors comments, proteinaceous material would have been part of the neutral and unfractionated material. Thus, the results could suggest that portions of DON may be readily removed during coagulation (DON associated with acidic fractions) or difficult to remove (DON associated with neutrals).
- Based on PY-GC-MS analysis, neither aluminium nor iron-based coagulants removed the protein or amino-sugar biopolymer fractions of NOM from the Siene River which had NOM containing ~40% amino sugars and <5% protein biopolymer fractions (Vilge-Ritter *et al.* 1999).
- Unpublished data by the authors have indicated slightly higher DON removal (30% to 40%) than DOC removal (20% to 30%) for two conventional water treatment plants in Arizona using low alum doses (5 to 10 ppm) treating a low SUVA (~2.0 (mg/l)<sup>-1</sup> m<sup>-1</sup>) water (pH~8; alkalinity ~150 ppm as CaCO<sub>3</sub>). Approximately 60% of the influent DON was characterised as having a molecular weight >1000 daltons by ultrafiltration.

Research related to algae which produce NOM enriched with DON, also provides potentially useful information related to potential DON interactions with, and removal by, metals. Widrig *et al.* (1996) concluded algal EOM was difficult to remove by coagulation. While coagulant demand by cellular algal material has been investigated (e.g. charge neutralization), less information is available on interactions between coagulants and dissolved algal organic material that may be enriched in org-N (Van Benschoten & Edzwald 1990; Karr *et al.* 1997). Algal EOM can be a more significant source of DOC in some cases than whole cells or cell fragments, and is generally characterised as containing hydrophobic neutral and acid material, with less base material (Nalewajk & Lean 1972; Hoehn *et al.* 1984; Widrig *et al.* 1996). While algal EOM

has been shown to increase coagulant demand it has been the large molecular weight polysaccharide fractions that have been primarily implicated (Paralkar & Edzwald 1996). Using PY-GC-MS analysis it was shown that EOM from a green alga was dominated by nitrogen-containing compounds, mostly of an aliphatic nature and proteinaceous origin, and by long-chain carboxylic acids derived from lipids (Widrig *et al.* 1996). A different green alga produced pyrimidine, an aromatic heterocyclic nitrogen compound with very little carboxylic acid material detected. Coagulation was found to poorly remove the org-N fragments monitored by PY-GC-MS. Preozonation converted aromatic nitrogen to aliphatic nitrogen that was preferentially removed during coagulation, possibly by increasing its polarity (e.g. carboxylic acid functionality) and ability to bind with metals.

#### Role of biofiltration on DON

Biofiltration could potentially remove or produce DON. Amino acids can be bacterially degraded. During bacterial degradation of DOC, SMPs will include fulvic-like material, polysaccharides, proteins, enzymes, nucleic acids, amino acids and steroids (Rittmann *et al.* 1987). Based upon biofilter modelling and field measurements of SMPs, and the fairly low percentage of DOC removed by most drinking water biofiltration plants (10–20% DOC removal), SMPs constitute a very small fraction (1–5%) of the DOC in the biofilter effluent water, having concentrations in the range of 20 to 100 µg DOC/l (Carlson 1996; Carlson & Amy 2000). If this material were enriched in nitrogen similar to algal-dominated systems (DOC/DON~12 mg C/mg N), then DON contribution from SMPs would be in the order of 2 to 10 µg N/l. Biofiltration systems that degrade large concentrations of DOC (e.g. methane supplied denitrification systems) could produce more significant quantities of SMPs (see previous section on SMPs), and hence lower DOC/DON ratios (Barker & Stuckey 1999).

#### Membrane biofouling by DON

Foulant material includes NOM, colloids, cellular material, chemical coagulants, inorganic precipitates and

homogenous inorganic-NOM precipitates (DiGiano *et al.* 2000; Her *et al.* 2000). Protein-related membrane (MF, UF, NF, RO) fouling mechanisms have been found to be important, but can be overcome through modifications to membrane surfaces (increased hydrophilicity, surfactant-modified membranes, simulated non-adhesive biological surfaces) (Kontturi & Vuoristo 1996; Wilbert *et al.* 1998; Roudman & DiGiano 2000; Lee *et al.* 2001; Lee & Lueptow 2001). Neutral and base NOM fractions generally have been considered to cause reduced permeate fluxes to a greater degree than humic acids (Nilson & DiGiano 1996; Schafer *et al.* 1998; Speth *et al.* 1998; Amy & Cho 1999; Carroll *et al.* 2000; Cho *et al.* 2000). Overall, higher rejection is observed for hydrophobic material than for hydrophilic material. Addition of powdered activated carbon prior to membrane treatment was found to remove N-acetylaminosugars, which may contribute to membrane fouling (Hwang *et al.* 2001).

The presence of cations (e.g. calcium) in conjunction with NOM has been implicated as a major cause for irreversible membrane fouling (Schafer *et al.* 1998; Her *et al.* 2000). FTIR analysis of fouled membrane surfaces showed significant contribution of amide-functional groups and polysaccharide-like substances on the membrane surface implicating them as a major source in NOM membrane fouling (Her *et al.* 2000). Other studies have also shown the importance of polysaccharide contributions to membrane fouling (Amy & Cho 1999). Coagulation prior to MF treatment removed significant amounts of acidic NOM fractions, but the poor removal of low UV-adsorbing neutral NOM fractions implicated them as membrane foulant material (e.g. polysaccharides); base fractions were not explicitly studied. One study showed that amino acids were poorly removed from drinking water during NF treatment, and their presence in the NF permeate constituted nearly all the chlorine demand (Agbekodo *et al.* 1996). In an attempt to physically separate DIN from DON in seawater, a 100 dalton molecular weight dialysis system was used, and the results suggested that very little DON passed the membrane (Feuerstein *et al.* 1997). Therefore, DON may contain higher molecular weight components capable of membrane fouling (e.g. polymeric materials), while low molecular weight material has a negligible influence on fouling.

Molecular weight DON characterisation of an *Anabaena* sp. alga culture (NOM isolated on cationic resin) indicated significant interaction of the charged solute with Sephadex gel or ultrafilters (Tuschall & Brezonik 1980). The foulant material was visually brown, insoluble, contained 50% of the hydrolysable material and 21% of the total DOC and was presumed to be the condensation product of compounds containing carboxylic acid groups with proteinaceous material (i.e. the so-called browning reaction). Ultrafiltration of activated sludges concluded that extracellular and metabolic compounds produced by microorganisms changed the molecular weight of proteins and sugars, and were the cause for permeate flux declines (Mukai *et al.* 2000). The specific role of DON on membrane fouling remains unresolved, but evidence suggests that proteinaceous material, including colloidal amino-sugars (e.g. microorganism cell walls), may be important NOM fractions associated with irreversible fouling.

## SUMMARY AND FUTURE ORGANIC NITROGEN RESEARCH NEEDS

DON occurrence studies have been conducted by ecologists, commonly undertaken in pristine or low human-impacted watersheds, and have focused more on nitrogen fluxes than composition of DON. Commonly DON constitutes ~50% of the TDN. Analytical methods have been developed for DON, and currently have detection limits of ~0.1 mg N/l. The presence of elevated DIN concentration (e.g. >1 mg N/l) complicates analytical quantification of DON, mainly because DON is calculated as TDN after digestion minus DIN prior to digestion. Furthermore, there is no direct analytical method for DON quantification, and research efforts should address these inadequacies in current methods.

Based upon analysis of USGS-NAWQA databases and a review of the literature, median DON concentrations for surface waters were ~0.3 mg N/l, but ranged by three orders of magnitude from <0.01 to 10 mg N/l. DON concentrations are higher in surface waters than most groundwaters. Primary sources of DON include agricultural fertilizers (manure, urea, DIN), wastewater

discharges, and eutrophic bodies of water. Site-specific variation of DON concentrations in lakes was associated with seasonal eutrophication, and in rivers was associated with urban runoff events or stream discharge. Algae produce protein-rich material (i.e. DON) and may cause seasonal or rapid changes in DON concentrations in drinking water supplies. The eutrophication status of a reservoir affects DON levels. Bacteria readily biodegrade proteinaceous and carbohydrate material released by algae, but in turn release SMPs that can become humified and more refractory. DON spatial and temporal fluxes and concentrations trend those of DOC. Ratios of DOC/DON range from <10 in systems with high algae productivity, wastewater discharge impacts, or agricultural activity to >40 from forested watersheds or those with well developed soils in temperate climate zones. DOC/DON ratios, or surrogates for DON (e.g. fluorescence), may be useful in monitoring sources of DON in drinking water sources. Understanding present-day DOC/DON ratios at a utility may allow retrospective evaluation of DON at the utility if DOC data are available.

The precise structural composition of org-N is still debatable, primarily due to analytical limitations and its incorporation into a wide range of molecular weights. Amino acids appear to constitute between 30% and 50% of the DON, with less than 5% present as free amino acids. In natural systems glutamic acid, glycine, serine and aspartic acid are the dominant amino acids, while in wastewater- or manure fertilizer-impacted systems, aromatic amino acids may also be present. Fluorescence spectroscopy detects these aromatic amino acids, and may serve as an indicator for DON sources. Cell walls (peptidoglycans) or membranes (lipopolysaccharides) and recondensation products (melanoidin-like) are amides and constitute approximately 20% of the DON. Heterocyclic-N compounds (e.g. pyroles, pyrrolidines, pyridines, pyrimidines and pyrazoles) may account for 25% of the org-N. Identification of specific compounds within amide and heterocyclic-N or the remaining org-N material (e.g. nitriles, nitrosamines) has not been well characterised. Base and neutral NOM fractions are N-enriched compared to acid fractions, and more polar acidic fractions are N-enriched relative to hydrophobic acid fractions. Colloidal NOM fractions are also N-enriched

and consist of decomposed biological cellular material (e.g. N-acetylaminosugars). Future work should employ  $^{15}\text{N}$ -NMR on raw and finished drinking waters, but will require large material masses or long run times due to the low percentage of nitrogen, and specifically low  $^{15}\text{N}$  content.

There are no direct reports of DON removal during water treatment, and only limited information using PY-GC-MS on the treatability and reactivity of org-N under drinking water conditions. Based on PY-GC-MS analysis neither aluminium nor iron-based coagulants removed the protein or amino-sugar biopolymer fractions of NOM. However, unpublished data by the authors have indicated slightly higher DON removal (30% to 40%) than DOC removal (20% to 30%) for two conventional water treatment plants. Drawing conclusions on the effect of org-N from treatment studies with NOM isolates that contain variable C/N ratios may also be difficult since the isolates differ significantly in terms of other variables (e.g. carboxylic and alcohol functionality, aromaticity) for which nitrogen bonds may contribute a minor role. Powdered activated carbon can remove some org-N compounds (e.g. N-acetylaminosugars). The specific role of DON in membrane fouling remains unresolved, but evidence suggests that proteinaceous material, including colloidal amino-sugars (e.g. microorganism cell walls), may be important NOM fractions associated with irreversible fouling. Research is needed to monitor and optimise DON removal across WTPs, and to understand which org-N components (amino acids, amides, heterocyclic-N, low vs. high molecular weight) are removed or transformed.

While the drinking water industry has recognized the significance of DOC and bromide as critical precursors for DBP formation, additional recognition, quantification and understanding for the role of DON as a critical DBP precursor is necessary. Chlorine reactions with amino acids form the unstable intermediate dichloroacetonitrile (DCAN), which can continue to react with chlorine to form chloroform, di- and tri-chlorinated acetic acid, trichloroacetamide and carboxylic acids. The presence of DON in water supplies affects the relative distribution of THMs and HAAs, and DON should be considered in prediction of THM and HAA formation. Higher org-N

content will lead to: (1) increasing chlorine demand which decreases the effectiveness of chlorine disinfection, (2) possible production of organic N-chloramines which have low disinfectant capability and interfere with DPD-colorimetric chlorine measurements, (3) production of di-HAA>tri-HAA, (4) production of HAA>THM, and (5) production of higher levels of halogenated (nitromethanes, HANs) and non-halogenated (NDMA) org-N DBPs. Use of  $^{15}\text{N}$ -enriched NOM could be used to better quantify the involvement of DON generally, and classes of org-N bonds specifically, on DBP formation.

Complex water supply systems, dense urban development and intensive agricultural and livestock development have already been associated with increasing DON concentrations over time in watersheds. Many WTPs are impacted by upstream wastewater discharges or agricultural activity, and thus have a risk of elevated DON levels. Continuation of development within watersheds or changes in regional climate will continue to change DON fluxes and concentrations. Org-N will affect disinfectant demand, DBP formation, and possibly the biological stability of drinking water. The water industry should develop capabilities to monitor and characterise DON in watersheds, treatment facilities (water and wastewater) and distribution systems.

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## LIST OF ABBREVIATIONS

AWWARF American Water Works Association  
Research Foundation

BDL	Below detection limit
C/N	Carbon to nitrogen ratio
CNCL	Cyanogen chloride
CO	Colorado, USA
DBP	Disinfection by-product
DCAN	Dichloroacetonitrile
Di-HAA	Dihalogenated acetic acid
DIN	Dissolved inorganic nitrogen
DMA	Dimethylamine
DOC	Dissolved organic carbon
DON	Dissolved organic nitrogen
EOM	Extracellular organic matter
FTIR	Fourier transfer infrared resonance spectroscopy
GA	Georgia, USA
GW	Groundwater
HAA	Haloacetic acid
HAN	Haloacetonitrile
HOCL	Hypochlorous acid
HPLC	High performance liquid chromatography
MA	Massachusetts, USA
MF	Microfiltration
NAWQA	National water quality assessment
NDMA	N-nitrosodimethylamine
NF	Nanofiltration
$\text{NH}_4^+$	Ammonia
NMR	Nuclear magnetic resonance
$\text{NO}_2^-$	Nitrite
$\text{NO}_3^-$	Nitrate
$\text{NO}_x$	Gaseous nitrogen oxides
NOM	Natural organic matter
Org-N	Organic nitrogen
PON	Particulate organic nitrogen
PY-GC-MS	Pyrolysis gas-chromatography mass-spectrometry
RO	Reverse osmosis
SMPs	Soluble microbial products
SUVA	Specific ultraviolet absorbance
TDN	Total dissolved nitrogen
THM	Trihalomethane
THMFP	Trihalomethane formation potential
TOX	Total organic halide
TTHM	Total trihalomethane
UF	Ultrafiltration

USA	Unites States of America
USGS	United States Geological Survey
UV	Ultraviolet
UVA	Ultraviolet absorbance
WTP	Water treatment plant

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