NITROGEN SPECIES

Nitrogen is present in water samples in a variety of different forms including;

- Total Nitrogen
- Organic Nitrogen
- TKN (Total Kjeldahl Nitrogen)
- NH4N (Total ammoniacal nitrogen)
- NOxN (Total oxidised nitrogen)
- NO3N (Nitrate nitrogen)
- NO2N (Nitrite nitrogen)

Total Nitrogen

Total nitrogen is measured by combusting the sample in an oxygen atmosphere, then measuring the nitrogen dioxide produced. This gives the total elemental nitrogen present in the sample in both organic and inorganic forms, including cyanide. Combustion analysis requires specialised (and expensive) laboratory instrumentation which few laboratories are equipped with.

Total nitrogen can also be analysed using persulphate/UV or persulphate oxidative digestion followed by analysis of the nitrate-N formed. These methods are not suitable for samples with high organic loading (which will require dilution before digestion, raising the detection limit), or for some industrial organic nitrogen compounds.

Total nitrogen may be calculated (approximately) by adding TKN and NOxN. See comments for each of these tests. This is the default method currently used by Hill Labs. However, we recommend that for clean waters (low particulates) and saline waters, the TN by persulphate oxidative digestion method is used as the detection limit for this method is significantly lower than for the TN calculation method.

Organic Nitrogen

"Organically bound nitrogen in the trivalent state." It does not include all organic nitrogen compounds, but includes such natural materials as proteins and peptides, nucleic acids, urea, and many synthetic organic materials (eg quaternary ammonium compounds, nitrogen containing pesticides, polymers, etc).

Analytically, organic nitrogen and ammonium nitrogen are determined together as Total Kjeldahl Nitrogen (TKN), a term which reflects the method used rather than any specific chemical form of nitrogen.

The Organic Nitrogen is then calculated as
Norg = TKN - NH4N

Protein concentration may be approximated from;
Protein = Total Organic N x 6.25[1]
= [TKN – NH4N] x 6.25

TKN (Total Kjeldahl Nitrogen)

This form of nitrogen is defined by the test method used (i.e. a 'Kjeldahl' digestion) which determines nitrogen in the trivalent state. The method does not determine nitrogen present in azide, azine, azo, cyanide, hydrazone, nitroso, oxime, or

[1] United Nations Food and Agriculture Organization (FAO), FOOD AND NUTRITION PAPER 77, Chapter 2 (https://www.fao.org/3/Y5022E/y5022e03.htm).

semicarbazone forms, nor as nitrate or nitrite, and also does not recover nitrogen from some industrial chemicals (eg refractory tertiary amines).

TKN can be considered to comprise

- Ammonium-N (NH4N)
- Protein N
- Non-protein N eg urea, DNA, benzalkonium salts

Dissolved Kjeldahl nitrogen (DKN)

DKN is TKN analysed on a sample which has been filtered through a 0.45µm membrane filter. DKN thus represents the soluble portion of TKN.

Ammonia/Ammonium-N (NH4N)

In solution total ammoniacal nitrogen may be present as either Ammonia ("Free ammonia", NH3) or Ammonium ion depending on the pH and temperature.

$$NH_3 + H^+ \Leftrightarrow NH_4^+$$

"Free" ammonia is toxic to aquatic organisms.
Ammonia is a toxic gas which is very soluble in water.

At low pH (i.e. more H^+) the equilibrium is to the right and $NH4^+$ predominates. Below a pH of about 6.5 over 99.9% of the ammonia is in this form (see Table). At higher pH the un-ionised or 'free' form of ammonia predominates.

Environmentally, the term ammonia refers to two chemical species, which are in equilibrium in water (NH3, un-ionized and $NH4^+$, ionized). These species are present in the environment from the metabolism of nitrogen species by humans, bacteria, etc. More rarely, they may come from a spill or leak or liquefied ammonia gas, which is often used in large refrigeration units (e.g. freezing works) or fertilizer production.

The toxicity (e.g. to fish, insects) to ammonia in water is primarily attributable to the un-ionized form (NH3), as opposed to the ionized form ($NH4^+$). In general, more NH3 and greater toxicity exists at higher pH. However, limited data also indicate that less NH3 is needed at lower pH to produce its toxic effects.

High NH4N levels in water also stimulate the growth of aquatic plants and algae, sometimes leading to 'blooms' which may then die and produce anoxic conditions because of decomposition.

Tests for ammonia usually measure total ammonia (NH3 plus $NH4^+$) and the results reported as "Total ammoniacal nitrogen (NH4N)".

NH4N results are reported to allow easy comparison of nitrogen budgets – all nitrogen species are reported as the nitrogen content only e.g. NH4N, NO2N, NO3N, TKN.

To determine the amount of un-ionised ammonia it is necessary to

a. determine the percentage of un-ionised ammonia at a given pH and temperature from tables such as those in ANZECC Guidelines for Fresh and Marine Water Quality, then

Convert the ammonium-N result from N to NH3 (multiply by the molecular weight of NH3/atomic weight of N = 17/14 = 1.21. This value can then be compared with reference tables eg that below – check source for latest version.

b. Trigger values as total ammonia-N ($g.m^{-3}$) at different pH (Temperature is not taken into consideration)

[Adapted from ANZECC Freshwater Guidelines, 2000, Table 8.3.7]

pH	Freshwater Trigger Value (g.m^{-3} as NH4-N)	Marine Trigger Value (g.m^{-3} as NH4-N)	pH	Freshwater Trigger Value (g.m^{-3} as NH4-N)	Marine Trigger Value (g.m^{-3} as NH4-N)
6.0	2.57	5.96	7.6	1.47	1.85
6.1	2.56	5.87	7.7	1.32	1.56
6.2	2.54	5.76	7.8	1.18	1.32
6.3	2.52	5.63	7.9	1.03	1.10
6.4	2.49	5.47	8.0	0.90	0.91
6.5	2.46	5.29	8.1	0.78	0.75
6.6	2.43	5.07	8.2	0.66	0.62
6.7	2.38	4.83	8.3	0.56	0.51
6.8	2.33	4.55	8.4	0.48	0.42
6.9	2.26	4.24	8.5	0.40	0.35
7.0	2.18	3.91	8.6	0.34	0.29
7.1	2.09	3.56	8.7	0.29	0.24
7.2	1.99	3.20	8.8	0.24	0.20
7.3	1.88	2.84	8.9	0.21	0.17
7.4	1.75	2.49	9.0	0.18	0.14
7.5	1.61	2.15			

NOxN (Total oxidised nitrogen)

Total oxidised nitrogen is the sum of nitrate-N plus nitrite-N

Note that the abbreviation TON can be used to mean both Total Oxidised Nitrogen and Total Organic Nitrogen, so use of this abbreviation is discouraged.

- NO2N = nitrite N
- NO3N = nitrate N

There are several different methods for measuring oxidised nitrogen species in the laboratory.

1. Measure NOxN and NO2N (cadmium reduction, colourimetry), then calculate NO3N from the difference between these.
   NO3N = NOxN – NO2N
   This is our preferred method for environmental samples as it is the most robust method and gives the lowest detection limit.
2. Ion chromatography [Useful if other anions (e.g. Cl, SO4) are being analysed at the same time, though not if these are present in very high concentrations (e.g. seawater, trade wastes, leachates). Note that NO2N is not analysed by this method at Hill Labs, and the detection limit of NO3N is significantly higher than in the cadmium reduction and colourimetry method].
3. Specific colorimetric tests [Useful if the matrix is well characterised. Doesn't give low DL, and each method has specific interferences. Often used with portable instruments]

Calculated Nitrogen species

A number of 'derived' values can be calculated from the preceding tests.

Dissolved Organic Nitrogen	DON	DKN - NH4N
Total Dissolved Nitrogen	TDN	DKN + NOxN
Total Inorganic Nitrogen	TIN	NOxN + NH4N
Total Nitrogen	TN	TKN + NOxN
Total Organic Nitrogen	TON	TKN - NH4N

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