

# WHITEPAPER

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Analytical and Measuring Instruments

Eliminate TKN  
Digestions and Obtain  
Better Nutrient Data



## ■ Introduction

On August 27, 2009 the State-EPA Nutrient Innovations Task Group issued an urgent call to action to EPA Administrator Lisa Jackson<sup>1</sup>. The task group documented excessive levels of nutrients in our nation's waterways. Current, and past, efforts to control these pollutants have been inadequate on the national and statewide scale. Efforts to date have been predominantly "regulation at the pipe" and have not addressed the non-point sources that are the root cause of elevated concentrations of nutrients in ground and surface waters.

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Nutrient pollution significantly impacts all of us. It impacts our drinking water, our recreational water, such as beaches and rivers and lakes, and it affects, and even kills, aquatic life.

## ■ What are Nutrients?

Nutrients are elements that are essential to life. Being essential to life they are also essential components of the molecules that make up living tissue. Decaying organic matter and human and animal waste are significant sources of nutrient pollution in ground and surface water. The chemicals we manufacture and use in day-to-day life may also contain nutrients, as well as the fertilizers that we spread on our yards and farms. It is the broad application of fertilizers, the animal feeding lots, the widespread use of septic tanks, and runoff water from rainfall that are responsible for the immeasurable amounts of nutrients that find their way into the watershed. These are the non-point sources that cannot be easily regulated.

For over 30 years the Clean Water Act<sup>2</sup> has regulated industrial and municipal effluents. Maximum allowed discharge concentrations are getting lower<sup>3</sup>. It is easy to take a sample at the end of a pipe. It's easy to know where the sample comes from and who is responsible for them if pollutants are too high. Unfortunately, over 30 years of regulation of industry have not significantly reduced the problem. Obviously, there are other sources.

And the sources are us. The source is our everyday activities of washing cars, fertilizing and watering our lawns, irrigating farms, and even desiring green golf courses. The essential nutrients in fertilizers and detergents are entering the groundwater, not by an industrial effluent but down the gutter and into the storm drain. Rainfall is washing nutrients into creeks and rivers and carrying them into basins such as the Gulf of Mexico or Chesapeake Bay. Hypoxic zones (Figure 1<sup>4</sup>), also known as dead zones, are forming where excess nutrients are deposited.

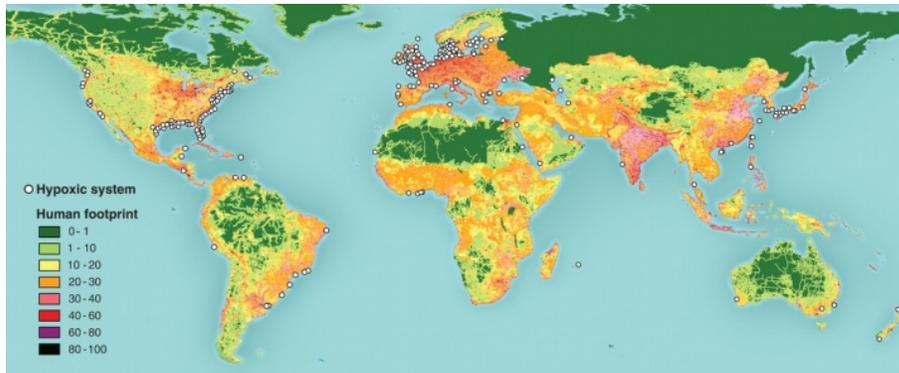


Figure 1: Hypoxic Zones

The excess nutrients cause a rapid growth of algae. The algae grow so rapidly that they cut off their own light and die. This is a normal, geologic process, occurring more rapidly than normal. As the algae dies it decays and the decay process consumes oxygen. Without oxygen, aquatic life cannot breathe.

### ■ What is Nutrient Pollution?

Nitrogen and Phosphorus are the principal elements referred to when discussing nutrient pollution. Organic nitrogen and organic phosphorus are associated with the organic matter we measure and report as Total Organic Carbon (TOC).

During a storm event or during irrigation, the nitrate in soil is easily leached into the runoff.

Nitrogen and phosphorus are essential elements in DNA, RNA, and nitrogen is a major component of protein and urine. Nitrogen and phosphorus occur as both water-soluble and water insoluble species. Unfortunately, both soluble and insoluble become bioavailable. Looking at a periodic table (Figure 2), we see that nitrogen and phosphorus are somewhat similar chemically; for instance, the plus 5 ions known as nitrate and phosphate are very stable and highly water-soluble.

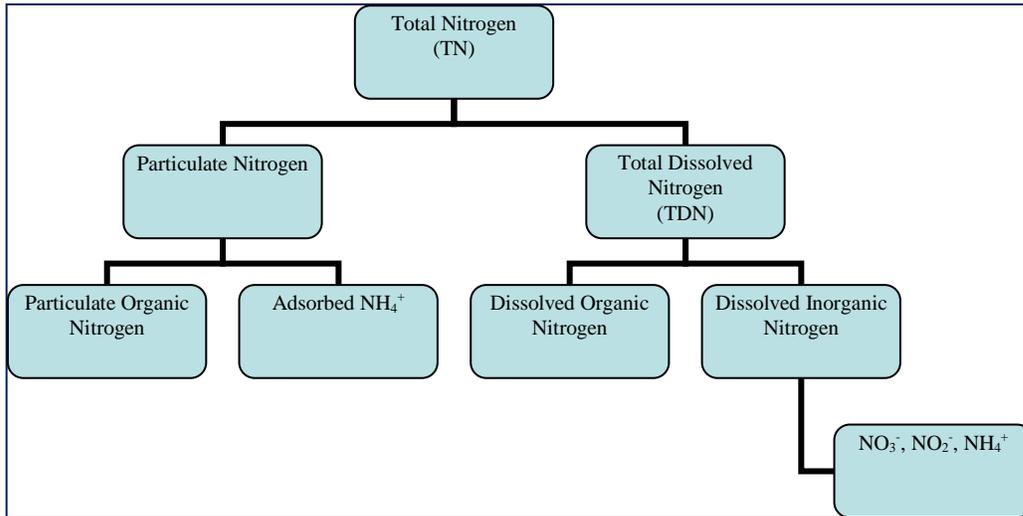
5 <b>B</b> Boron 10.811	6 <b>C</b> Carbon 12.011	7 <b>N</b> Nitrogen 14.0067	8 <b>O</b> Oxygen 15.9994	9 <b>F</b> Fluorine 18.998	2 <b>He</b> Helium 4.0026
3 <b>Li</b> Lithium 6.941	2.4 14 <b>Si</b> Silicon 28.086	2.5 15 <b>P</b> Phosphorus 30.9738	2.6 16 <b>S</b> Sulfur 32.064	2.7 17 <b>Cl</b> Chlorine 35.453	2 10 <b>Ne</b> Neon 20.183
2.3 3 <b>Al</b> Aluminum 26.9815	2.8.4 32 <b>C</b> Carbon 12.011	2.8.5 33 <b>As</b> Arsenic 74.9216	2.8 16 <b>S</b> Sulfur 32.064	2.8 18 <b>Ar</b> Argon 39.948	2.8 18 <b>Ar</b> Argon 39.948

Figure 2: Periodic Table Showing Nitrogen and Phosphorus

### ■ Nitrogen

Nitrogen is an essential nutrient for plant growth, ranking only behind carbon, hydrogen and oxygen in total quantity needed. The nitrogen gas making up about 80% of the atmosphere is largely inert and unavailable to life directly. Lightning will convert small amounts of nitrogen to nitrate. Nitrogen in fertilizers largely comes from the chemical reaction between nitrogen and hydrogen gas to form ammonia. The ammonia can then be oxidized to form nitrate. Fertilizers will often contain ammonia, nitrate, and/or urea as the sources of nitrogen. Organic matter, referred to as humic matter, manure, and mulch, contains about 5% nitrogen. This nitrogen is slowly converted to ammonia by bacteria and is eventually oxidized to nitrite then nitrate. During a storm event or during irrigation, the nitrate in soil is easily leached into the runoff<sup>5</sup>.

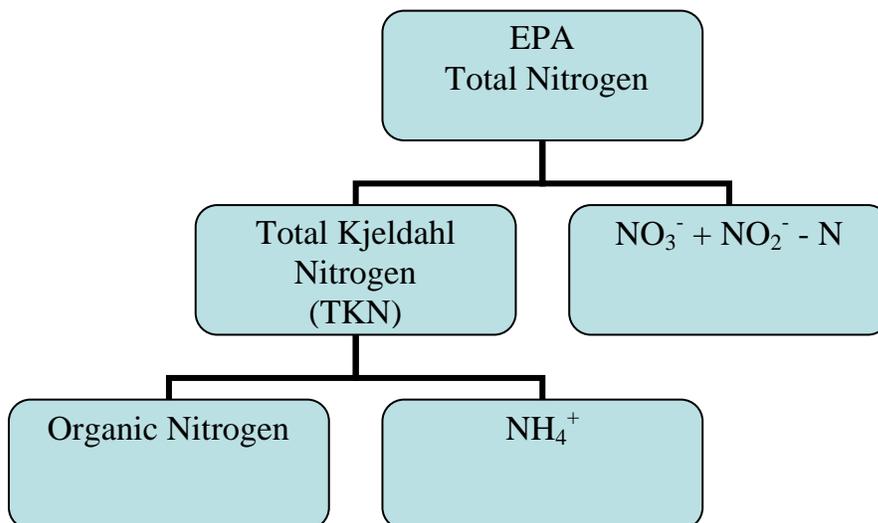
Dissolved inorganic nitrogen includes nitrate ( $\text{NO}_3^-$ ), nitrite ( $\text{NO}_2^-$ ), and the ammonium ion ( $\text{NH}_4^+$ ). Dissolved organic nitrogen includes water-soluble proteins, amines, amides, and so forth. Total organic nitrogen is the sum of dissolved organic nitrogen and particulate organic nitrogen. Particulate organic nitrogen is an integral part of the insoluble organic compounds, or organic matter, suspended in water. Total dissolved nitrogen (TDN) is the sum of the dissolved organic nitrogen plus the dissolved inorganic nitrogen, and total nitrogen (TN) includes all of the above (Figure 3).



**Figure 3:** Total Nitrogen Hierarchy

### ■ Chemical Analysis of Nitrogen Compounds

Total Kjeldahl Nitrogen, or TKN, is the classical analysis for total organic nitrogen. TKN plus nitrate-nitrite nitrogen is the USEPA regulated parameter for Total Nitrogen (Figure 4).



**Figure 4:** EPA Definition of Total Nitrogen

The TKN method does not measure nitrate or nitrite nitrogen; TKN measures organic nitrogen and ammonia. TKN is sufficient in POTW or municipal influents because these samples rarely have nitrate; however, sewage treatment and industrial plant effluents consist predominantly of inorganic

nitrogen (nitrate) with only traces of organic nitrogen because the treatment process is designed to completely oxidize dissolved organic nitrogen and ammonia to nitrate.

Since nitrate nitrogen interferes with the TKN analysis, the TKN test is not sufficient for the measurement of traces of organic nitrogen in samples that contain nitrate<sup>6,7</sup>.

Alternative methods used for the determination of Total Nitrogen measure all nitrogen compounds and report them as a single result. The methods rely on alkaline persulfate digestion followed by colorimetric detection<sup>8,9</sup>, ion chromatography detection<sup>10</sup>, or by high-temperature catalytic combustion with chemiluminescence detection<sup>11</sup>.

Nydahl studied the persulfate oxidation method extensively and determined the results of 10 sewage treatment plant effluents were equivalent to the results obtained by the TKN method<sup>12</sup>. Nitrate was measured colorimetrically on a visible spectrophotometer similar to the Shimadzu UV-1800 (Figure 6). Kroon compared TKN with an automated UV persulfate digestion method and found no significant differences in surface waters or wastewaters<sup>13</sup>. Bronk, and others, compared the persulfate oxidation method with the high-temperature catalytic combustion method and found that they provide reproducible results that are consistent with each other<sup>14</sup>.

Daughton, in testing the applicability of the high-temperature catalytic combustion with the chemiluminescence detection method as a substitute for TKN in oil shale retort waters, found that for 12 oil shale wastewaters the results did not differ significantly. Daughton also reported that some heterocyclic nitrogen compounds that are not quantitatively recovered by TKN are recovered by the high-temperature combustion method<sup>15</sup>.

The Shimadzu TOC/TN Analyzer shown in Figure 5 analyzes for total nitrogen by the high-temperature catalytic combustion with chemiluminescence detection method. The method is capable of accurate and complete oxidation and measurement of TN in sewage treatment plant influents, industrial wastewaters, oxidized effluents and ambient water containing organic and inorganic nitrogen compounds.

The inorganic nitrogen fraction comprised of nitrate nitrogen, nitrite nitrogen, and ammonia nitrogen is analyzed on a filtered sample by wet chemistry using a spectrophotometer, such as the Shimadzu UV-1800.



**Figure 5:** Shimadzu TOC Analyzer with TN Module

## ■ Phosphorus

Phosphorus is an essential nutrient found in living organisms as part of DNA among other important molecules. Phosphorus is always found in nature bound to other atoms and usually as the inorganic polyatomic anion called phosphate. Phosphate is available to plants and used as a fertilizer, and has the potential to cause algal blooms. Measurement of total phosphorus is important because it can, with time, convert to bioavailable soluble phosphate.

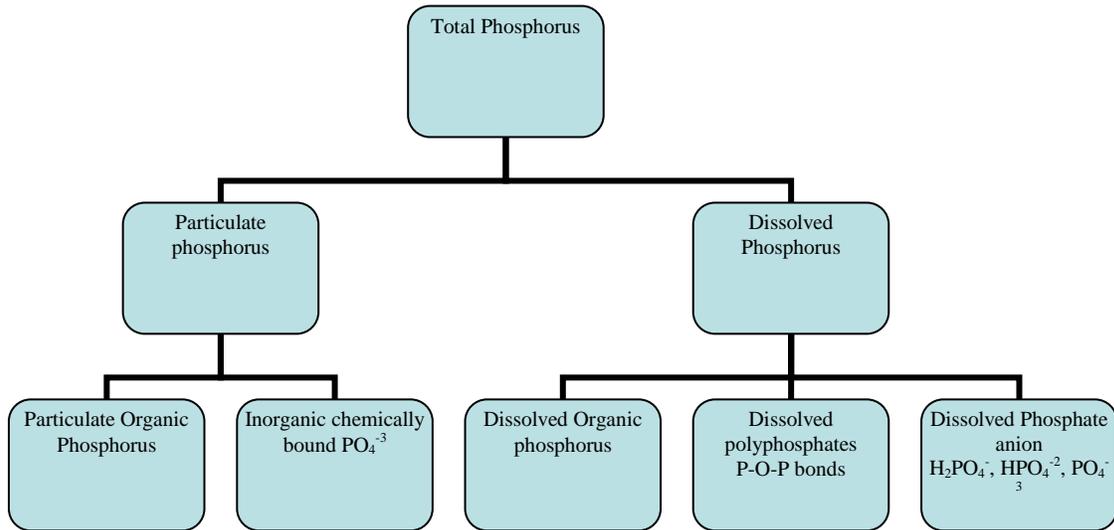
Dishwashing, laundry, and many hand detergents and/or soap contain phosphorus. The phosphorus content in detergent can be as high as 8.7%. Phosphate is a very effective way to improve soap quality, especially in waters that contain high amounts of calcium and magnesium. Unfortunately, the phosphates in these detergents find their way into the environment. Although there is not a federal ban on phosphate in soap, many states are taking action.

Measurement of total phosphorus is important because it can, with time, convert to bioavailable soluble phosphate.

For example, Washington State has limited the amount of phosphate in dishwasher detergent to 0.5%<sup>16</sup>.

### ■ Chemical Analysis of Phosphorus

Phosphorus can exist in water suspended as insoluble inorganic minerals or organic compounds, and as soluble organic compounds, polymerized polyphosphate chains, as pyrophosphates, or as the polyatomic phosphate anion (Figure 6).



**Figure 6:** Total Phosphorus Hierarchy

Phosphorus species are distinguished from each other empirically by filtration, and a series of digestions that selectively convert phosphorus to phosphate. After the digestion phosphate is measured. Thus, to analyze organic phosphorus only, one digests for total phosphorus in one sample aliquot, and hydrolysable phosphorus in another aliquot. Phosphate is determined in each digest and organic phosphorus is calculated by difference<sup>17</sup>.

Instrumental methods require that samples be filtered, or the turbidity and/or solids will interfere. The portion for dissolved phosphorus is filtered at the time of sampling and the portion for the total phosphorus analysis is filtered after digestion. If organic carbon or particulate concentrations (TOC and TSS) are high, the total phosphorus results can be biased low since the organic carbon and/or particulates also consume oxidant<sup>18</sup> during sample digestion. Total phosphorus is batch digested to convert all phosphorus compounds to phosphate. Phosphate is measured in the digested solution, on a visible spectrometer like the Shimadzu UV-1240 or UV-1800 (Figure 7) using the molybdenum blue method<sup>19</sup>; however, ICP-AES, such as the Shimadzu ICPE-9000 (Figure 8), is also allowed<sup>20</sup> at 40 CFR Part 136 for wastewater reporting.



**Figure 7:** Shimadzu UV-1800 Spectrophotometer



**Figure 8:** Shimadzu ICPE-9000 ICP-AES

As discussed previously, TKN is the classical digestion for total organic nitrogen. The TKN digestion can also be used to analyze for total phosphorus<sup>21</sup> and this determination is sometimes called TKP. The advantage of a TKP analysis is the ability for the laboratory to test for TN and TP in a single digest. The perceived benefit is time and labor savings; however, because of the higher acid and salt concentration of the resulting TKP digest compared to the acid digests used in colorimetric or ICP tests, the TKP detection limits are usually higher.

Determinations made with a high-resolution visible spectrophotometer or ICP, such as the Shimadzu UV-1800<sup>22</sup> and the ICPE-9000<sup>23</sup>, can achieve total phosphorus detection limits in the low single digit parts per billion.

## ■ Conclusion

The Clean Water Act was signed amid stories of massive fish kills and rivers catching on fire<sup>24</sup>. While similar environmental catastrophes rarely happen in the US anymore, algal blooms caused by excessive nutrients are common.

Clean Water Act regulations that only monitor priority pollutants discharged at the end of a pipe have been ineffective in controlling algal blooms. New regulations and methods that define total nitrogen should be approved so that compliance with ambient water criteria can be accurately assessed. Methods based on newer, more accurate techniques, such as high temperature combustion with chemiluminescence detection and updated methods validated for low level total phosphorus are needed.

Developing these methods requires collaboration between industry and regulatory agencies, and an ongoing commitment from instrumentation vendors to develop the tools needed for accurate analysis. Guided by its core management principle, For the Well-being of both Mankind and the Earth, Shimadzu has always been at the forefront of engineering systems to address environmental concerns. Now, answering the State-EPA Nutrient Innovations Task Group's call to action, Shimadzu is actively working with consensus standard

**New regulations that establish maximum allowable levels for nutrients in effluents and in ambient water are needed.**

organizations, such as ASTM and Standard Methods, to develop and gain USEPA approval for newer, more accurate and cost-effective methods for the analysis of nutrients in water.

## ■ References

1. [http://water.epa.gov/scitech/swguidance/standards/criteria/nutrients/upload/2009\\_08\\_27\\_criteria\\_nutrient\\_nitreport.pdf](http://water.epa.gov/scitech/swguidance/standards/criteria/nutrients/upload/2009_08_27_criteria_nutrient_nitreport.pdf), accessed July 1, 2014
2. <http://www2.epa.gov/laws-regulations/summary-clean-water-act>, accessed July 1, 2014
3. <http://www.waterworld.com/articles/print/volume-23/issue-3/editorial-feature/wastewater-industry-moving-toward-enhanced-nutrient-removal-standards.html>, accessed July 1, 2014
4. <http://water.epa.gov/type/watersheds/named/msbasin/hypoxia101.cfm>, accessed June 30, 2014
5. <http://www2.epa.gov/nutrientpollution/sources-and-solutions>, accessed June 30, 2014
6. Schlueter, A.; *Nitrate Interference in Total Kjeldahl Nitrogen Determinations and its Removal by Ion Exchange*; EPA 600/7-77-017, February 1977
7. [http://pubs.usgs.gov/sir/2012/5281/sir12\\_5281.pdf](http://pubs.usgs.gov/sir/2012/5281/sir12_5281.pdf), accessed July 16, 2014
8. <http://nwql.usgs.gov/pubs/WRIR/WRIR-03-4174.pdf>, accessed July 16, 2014
9. Method 4500-P J, Persulfate Method for Simultaneous Determination of Total Nitrogen and Total Phosphorus, Standard Methods for the Examination of Water and Wastewater 22 ed, 2012
10. <http://www.astm.org/DATABASE.CART/WORKITEMS/WK44300.htm>, accessed July 16, 2014
11. <http://www.astm.org/DATABASE.CART/WORKITEMS/WK46665.htm>, accessed July 16, 2014
12. Nydahl, F. On the Peroxodisulfate oxidation of Total Nitrogen in Waters to Nitrate, *Water Research* Vol 12, 1123-1130, 1978
13. Kroon H., Determination of Nitrogen in Water; comparison of a continuous-flow method with on-line UV digestion with the Original TKN method, *Analytica Chimica Acta*, 276, 287-293, 1993
14. Bronk D.A, Lomas W.L, Gilbert P.M, Schukert K.J, Sanderson M.P;
15. Doughton C.G, Jones B.M, Chemiluminescence vs. Kjeldahl Determination of Nitrogen in Shale Retort Waters and Organonitrogen Compounds, *Anal. Chem.* 57, 2320-2325, 1985
16. [http://www.issa.com/data/File/regulatory/phosphate\\_survey2013.pdf](http://www.issa.com/data/File/regulatory/phosphate_survey2013.pdf), accessed July 1, 2014
17. American Public Health Association (APHA). 2012. Standard Methods of Water and Wastewater. 22nd ed. American Public Health Association, American Water Works Association, Water Environment Federation publication. APHA, Washington D.C
18. <http://nwql.usgs.gov/Public/pubs/WRIR03-4174/WRIR03-4174.pdf>, accessed July 1, 2014
19. Method 4500-P E, Ascorbic Acid Method, Standard Methods for the Examination of Water and Wastewater 22 ed, 2012
20. [http://water.epa.gov/scitech/methods/cwa/bioindicators/upload/2007\\_07\\_10\\_methods\\_method\\_200\\_7.pdf](http://water.epa.gov/scitech/methods/cwa/bioindicators/upload/2007_07_10_methods_method_200_7.pdf), accessed July 16, 2014
21. <http://www.epa.gov/grtlakes/lmmb/methods/methd230.pdf>, accessed July 16, 2014
22. [http://oewri.missouristate.edu/assets/OEWRI/3010R02\\_Total\\_P.pdf](http://oewri.missouristate.edu/assets/OEWRI/3010R02_Total_P.pdf), accessed July 1, 2014
23. Shimadzu Publication C113-528, ICPE-9000 Detection Limits
24. Budde W.L.; *Analytical Mass Spectrometry, Strategies for Environmental and Related Applications*; Oxford University Press; 2001



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