

Get Your Lab Ready for HABs

WEBINAR

Dr. Stephanie A. Smith

Product Segment Manager, Xylem Laboratory Solutions

- Over 20 years in technology development and management
- [Dr. Smith's page](#)
- @WaterWoman,
<https://www.linkedin.com/in/ysiwaterwoman/>
- [HAB HUB](#) and [HAB PAGE](#)
- Missouri State University Biology (B.S., M.S.), The Ohio State University Microbiology (Ph.D.).



Acknowledgments

- Tim Smith, FS3700 Automated Chemistry Analyzer Expert, OI Analytical
- Cynthia Elmore, GC Expert, OI Analytical
- Developed and Validated the methods we will talk about

Agenda

- HAB Monitoring
- The Methods
 - Total Phosphorus (TP) by USEPA Method 365.4
 - Total Kjeldahl Nitrogen (TKN) by USEPA Method 351.2
 - Purge and Trap with GC/MS, guided by USEPA Method 524.3 for volatile organics



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HAB Monitoring

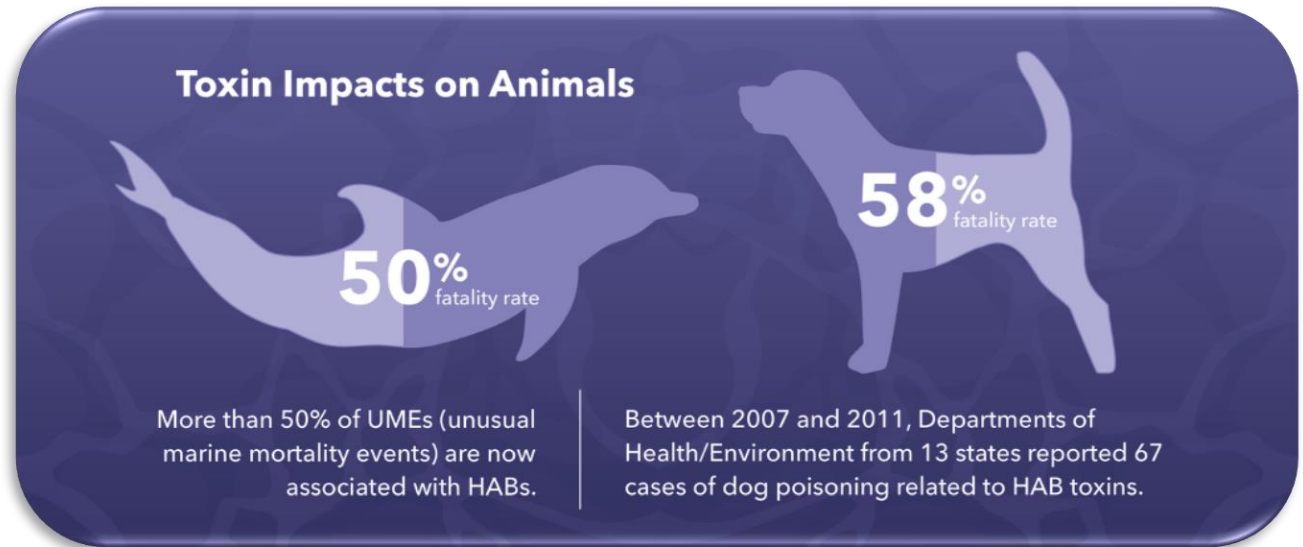




Eutrophication:
78% of US Coastlines
65% of Europe's Coastlines
Globally?

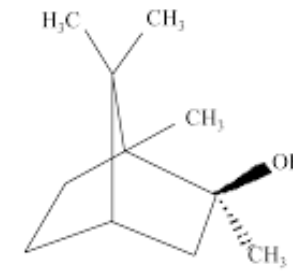
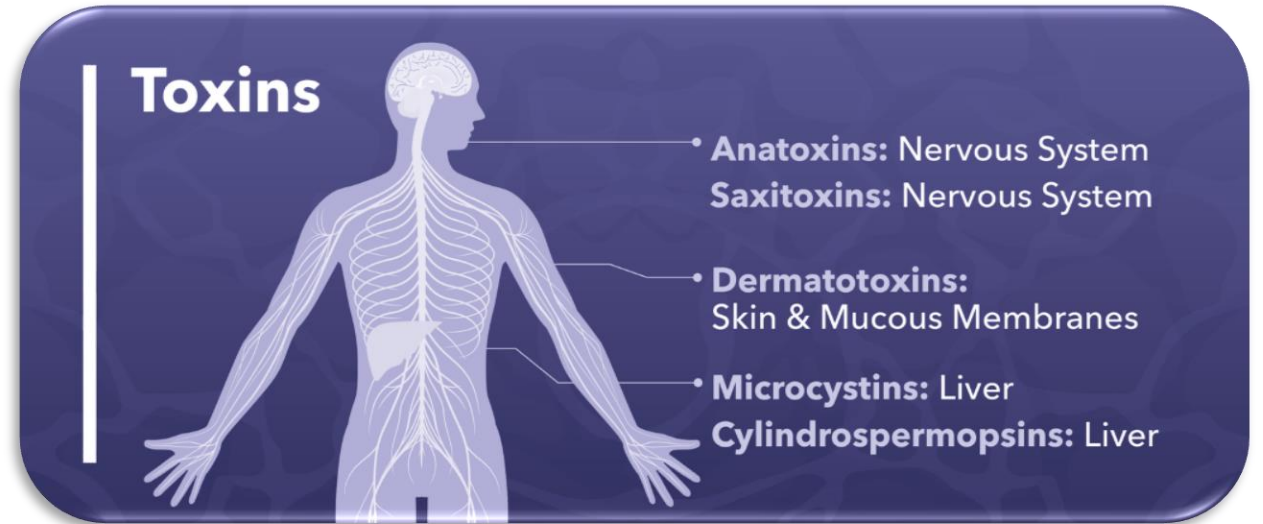
HAB Impacts

- Environmental Impacts
 - Reduced DO (hypoxia/anoxia)
 - Toxins and wildlife

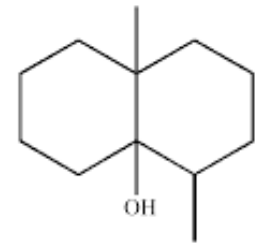


HAB Impacts

- Environmental Impacts
 - Reduced dissolved oxygen
 - Toxins
- Drinking Water
 - Toxins
 - Taste & Odor



2 Methylisobornel (MIB)



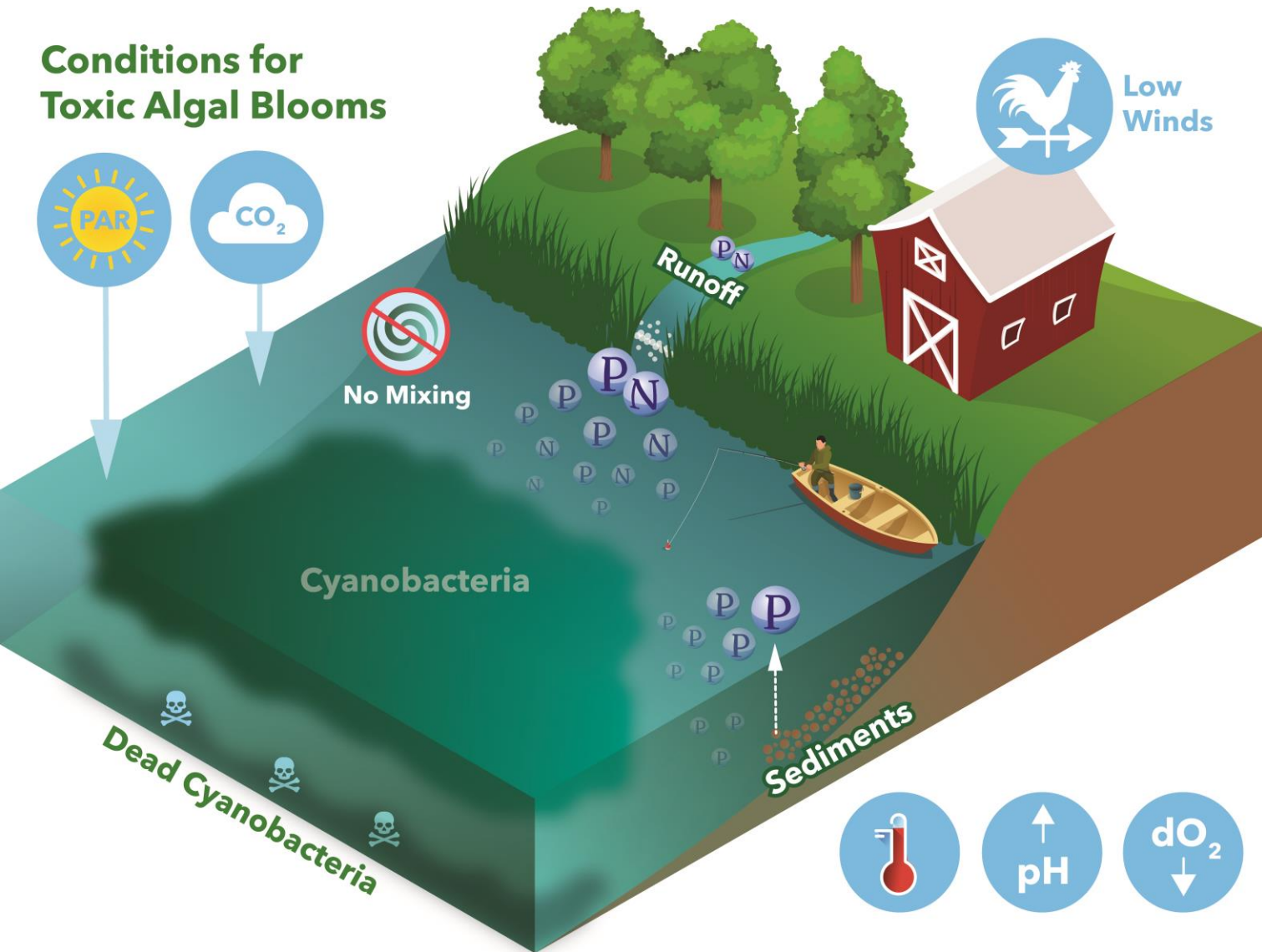
Geosmin

HAB Impacts

- Environmental Impacts
 - Reduced dissolved oxygen
 - Toxins
- Drinking Water
 - Toxins
 - Taste & Odor
- Economic
 - Water treatment
 - Environmental cleanup
 - Recreational/Tourism impacts



Conditions for Toxic Algal Blooms



Water Quality & HABs

- Drivers

- \uparrow Nutrients (*eutrophication*)
- \uparrow Temperature
- \downarrow Flow/mixing

- Responses

- \uparrow pH
- \downarrow DO
- \uparrow Pigments (growth indicator)

HAB Monitoring

What Sondes *Can Do*

- Continuous data acquisition in the field
- High-precision measurements of DO, temp, pH
- Trending measurements of chlorophyll, phycocyanin, phycoerythrin, **nitrate**, turbidity, fDOM



EXO NitraLED™

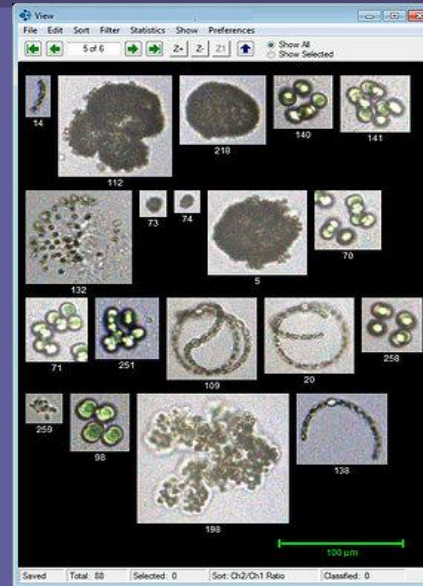
Select Your HAB System

HAB Monitoring

What Sondes *Can* Do

What Sondes *Can't* Do

- Algae Speciation
- Toxin measurements
- Phosphorus or orthophosphate
- *High precision* measurements of chlorophyll, phycocyanin, phycoerythrin, nitrate, turbidity, fDOM, geosmin and MIB



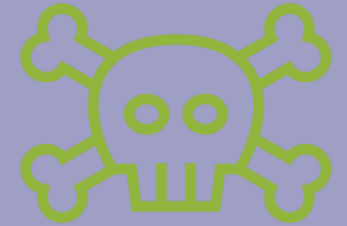
HAB Monitoring

What Sondes *Can* Do

What Sondes *Can't* Do

Get Your Lab Ready For HABs

- Toxins (ELISA)
- Chlorophyll extractions
- Algae speciation (microscopy)
- Total Phosphorus
- Total Nitrogen (via TKN)
- Geosmin & MIB



Sample Guy Blog

HAB Monitoring

What Sondes *Can* Do

What Sondes *Can't* Do

Get Your Lab Ready For HABs

OI Analytical Solutions: FS3700 Automated Chemistry Analyzer (ACA) for Nutrients

- High-throughput
- ML600 Autodilutor
 - High accuracy and precision
 - Redilutes overrange hits
 - Repeats followers
- Preconfigured “cartridges” with all of the reactants



[FS3700 Brochure](#)

HAB Monitoring

What Sondes *Can* Do

What Sondes *Can't* Do

Key Lab Methods for HABs

OI Analytical Solutions: GC Autosampler with Purge & Trap

- 4100 Autosampler
- 4760 Eclipse Purge & Trap
 - #7 Trap
- Agilent 7890A/5975C GC/MS



P&T Info

HAB Monitoring Summary

- The management and mitigation of HABs requires water quality monitoring
- The most important parameters to understand for HAB management are nutrient inputs
- One of the biggest challenges for drinking water reservoirs is management of taste and odor (geosmin and MIB)
- Nutrients and taste and odor compounds are best measured in the lab
 - In support or in place of field monitoring

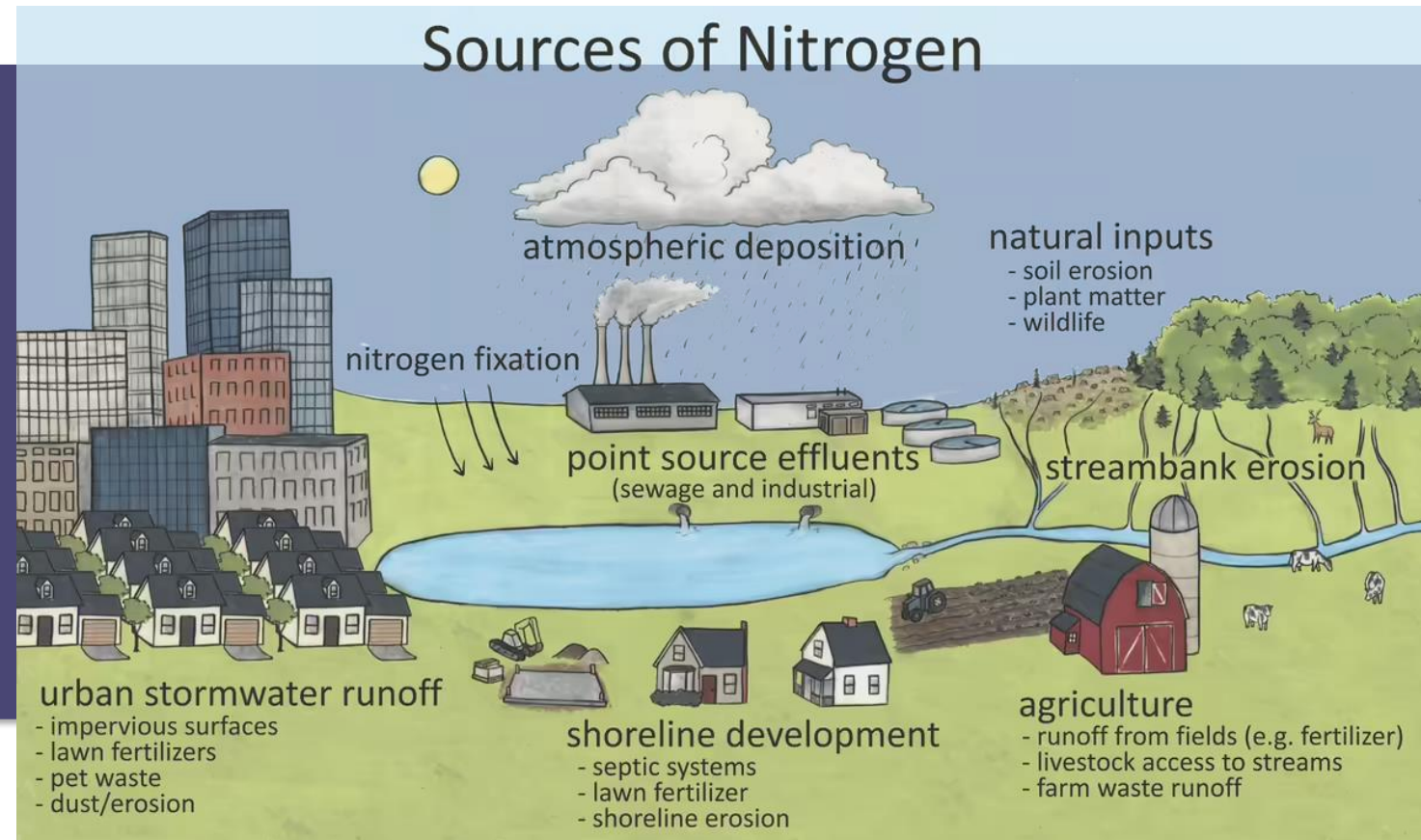
2

Total Kjeldal Nitrogen EPA Method 351.2

EPA Method 351.2 Total Kjeldahl Nitrogen

What Is TKN?

- TKN = organic nitrogen + ammonia + ammonium
- High concentrations indicate animal-originating runoff
- TN = TKN + Nitrite + Nitrate



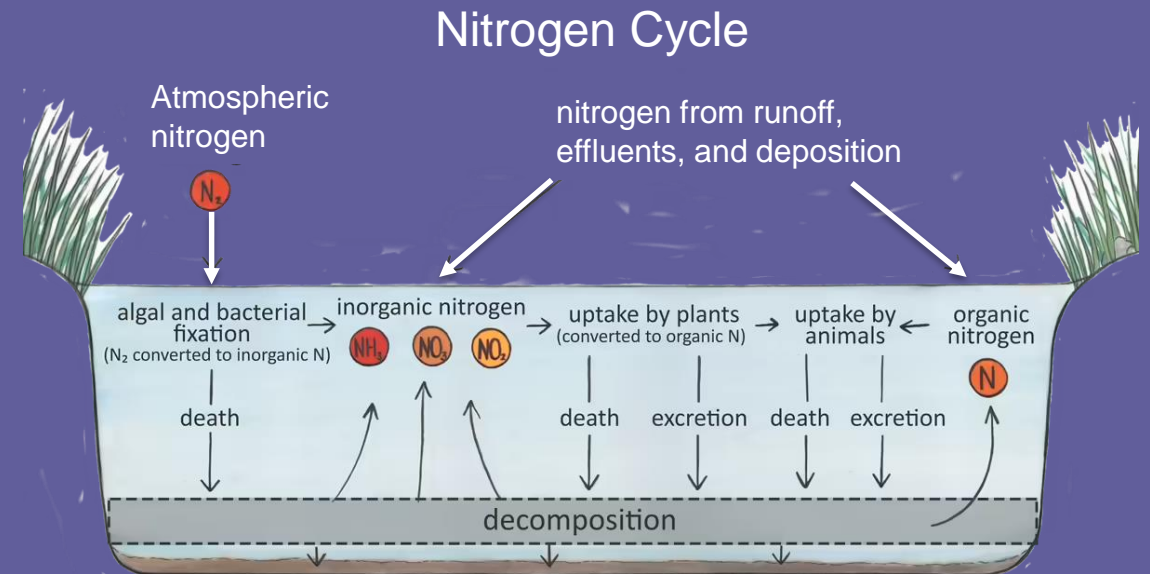
<https://datastream.org/en/guide/phosphorus>, June 2022

EPA Method 351.2 Total Kjeldahl Nitrogen

What Is TKN?

TKN Relevance For HABs

- Algae may be N_2 fixing, or non- N_2 fixing
- TKN represents highly assimilable N forms
- Ammonium may increase toxicity¹
- Ammonium and urea may lead to increased *Microcystis* biomass and toxin production²



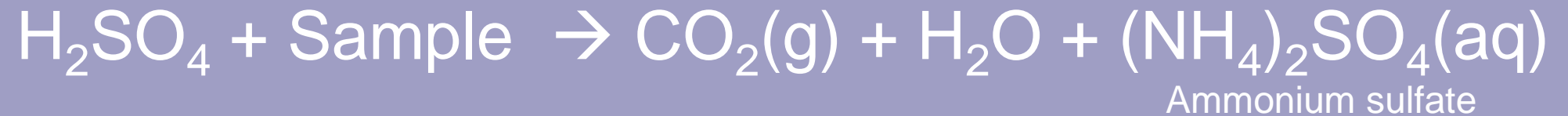
1-Davis et al. 2010, 2015; Chaffin et al. 2018
2-Harke et al. 2016

EPA Method 351.2 Total Kjeldahl Nitrogen

What Is TKN?

TKN Relevance For HABs

Chemistry Of 351.2: Kjeldahl Digestion



CuSO₄ – catalyst to speed up the oxidation rate added to make digestion

K₂SO₄ - salt used to raise the temperature of the digestion

351.2 Method Abstract

EPA Method 351.2 Total Kjeldahl Nitrogen

What Is TKN?

TKN Relevance For HABs

Chemistry Of 351.2: Ammonium Gas Generation and Diffusion



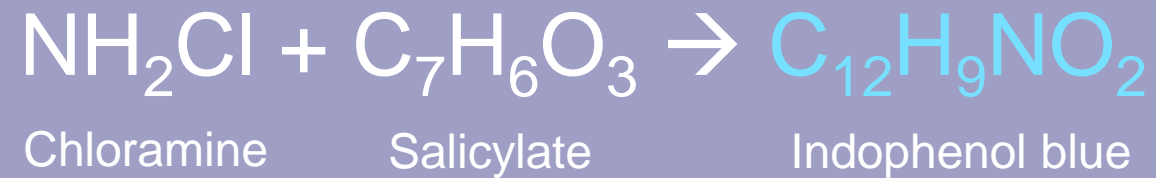
Sodium hypochlorite

EPA Method 351.2 Total Kjeldahl Nitrogen

What Is TKN?

TKN Relevance For HABs

Chemistry Of 351.2: Berthelot Reaction



EPA Method 351.2 Total Kjeldahl Nitrogen

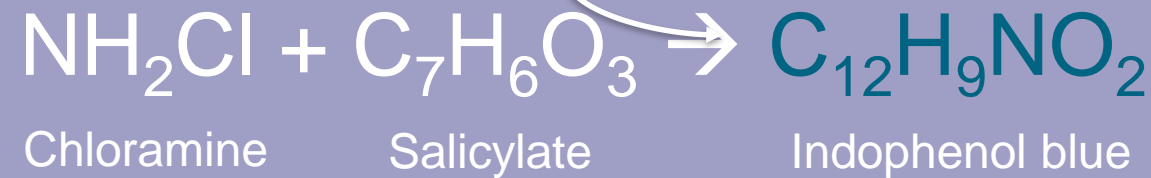
What Is TKN?

TKN Relevance For HABs

Chemistry Of 351.2: Berthelot Reaction



Sodium nitroferricyanide - intensifies blue color



EPA Method 351.2 Total Kjeldahl Nitrogen

What Is TKN?

TKN Relevance For HABs

Chemistry Of 351.2

FS3700 Cartridge 330094CT for Method 351.2

- Drinking water, surface water, wastewater
- Segmented Flow Analysis
- Can also be used for EPA 350.1 (Ammonia Nitrogen)
- EPA Total Nitrogen Requires Nitrate/Nitrite, too
 - $TN = TKN + NO_3 + NO_2$

[351.2 Method Abstract](#)

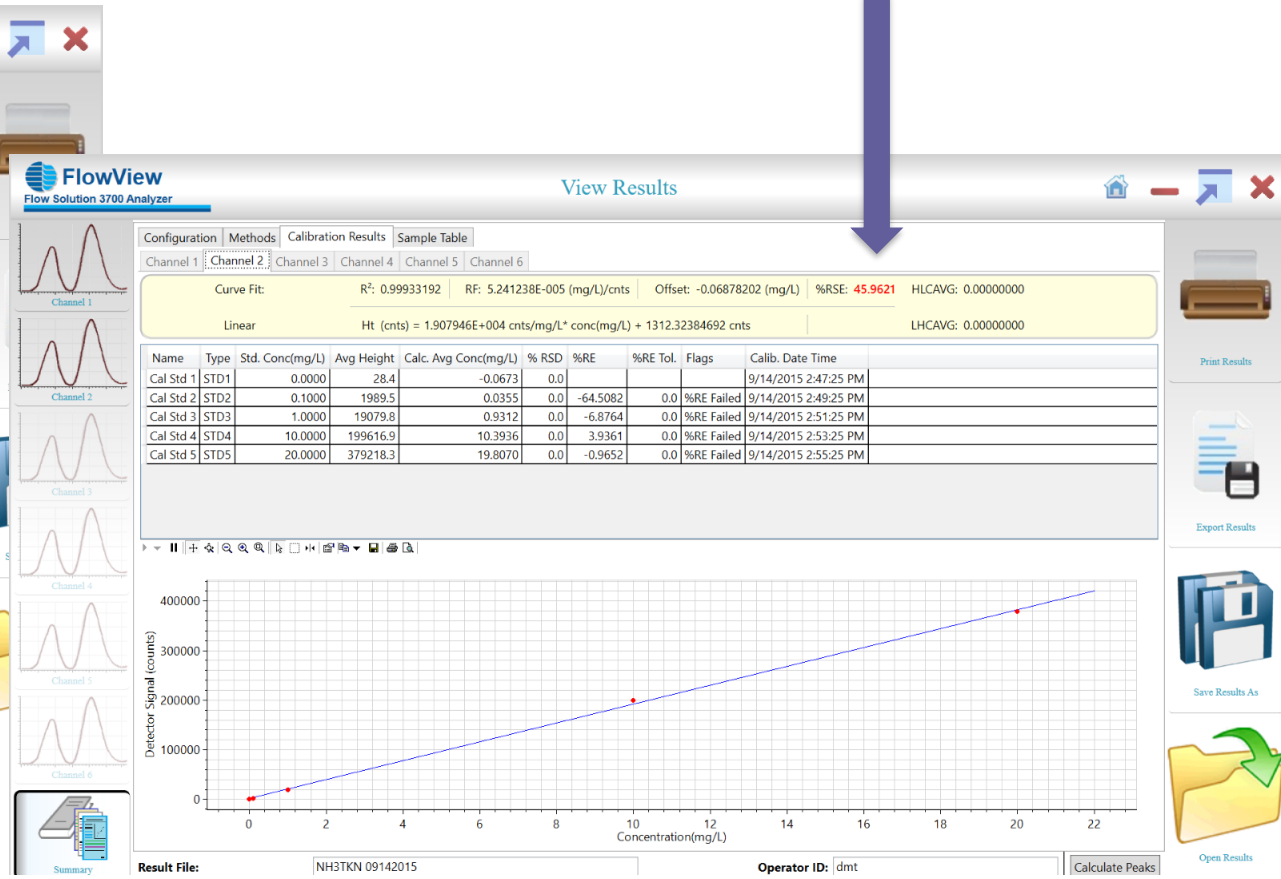
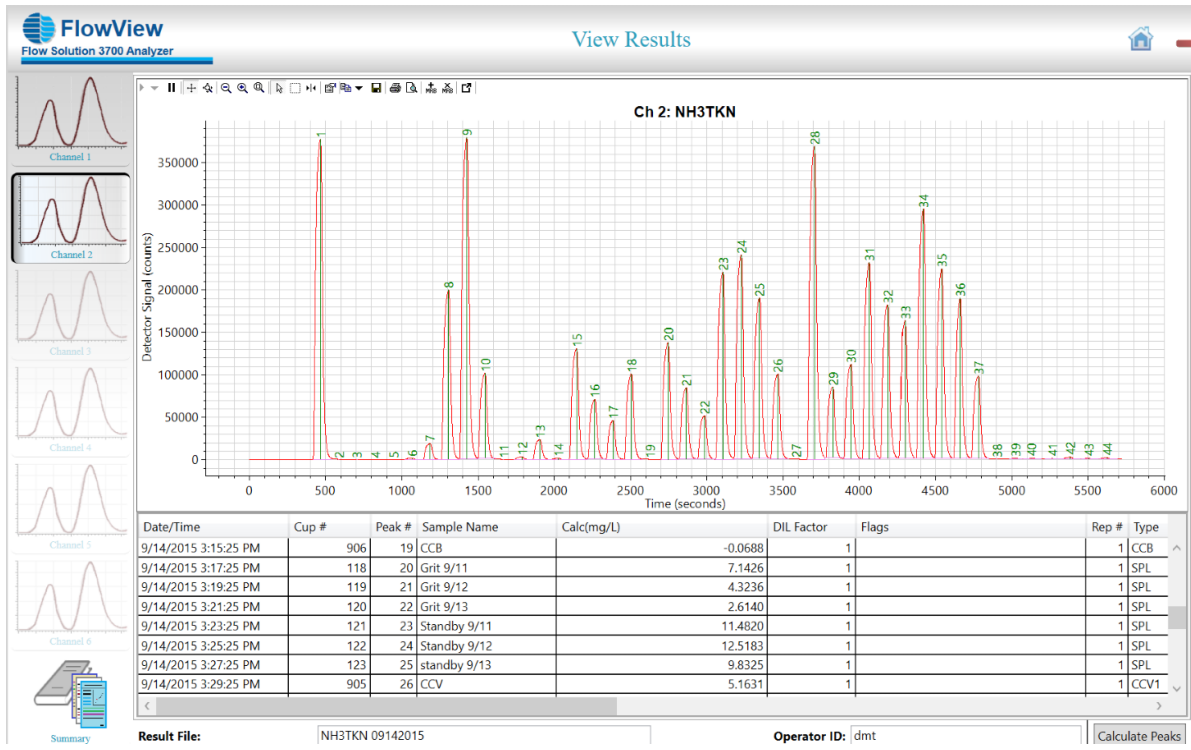
[NO₃/NO₂ Method Abstract](#)

EPA Method 351.2 on the FS3700

WWTP Samples

Check peak markers, the software does the calcs

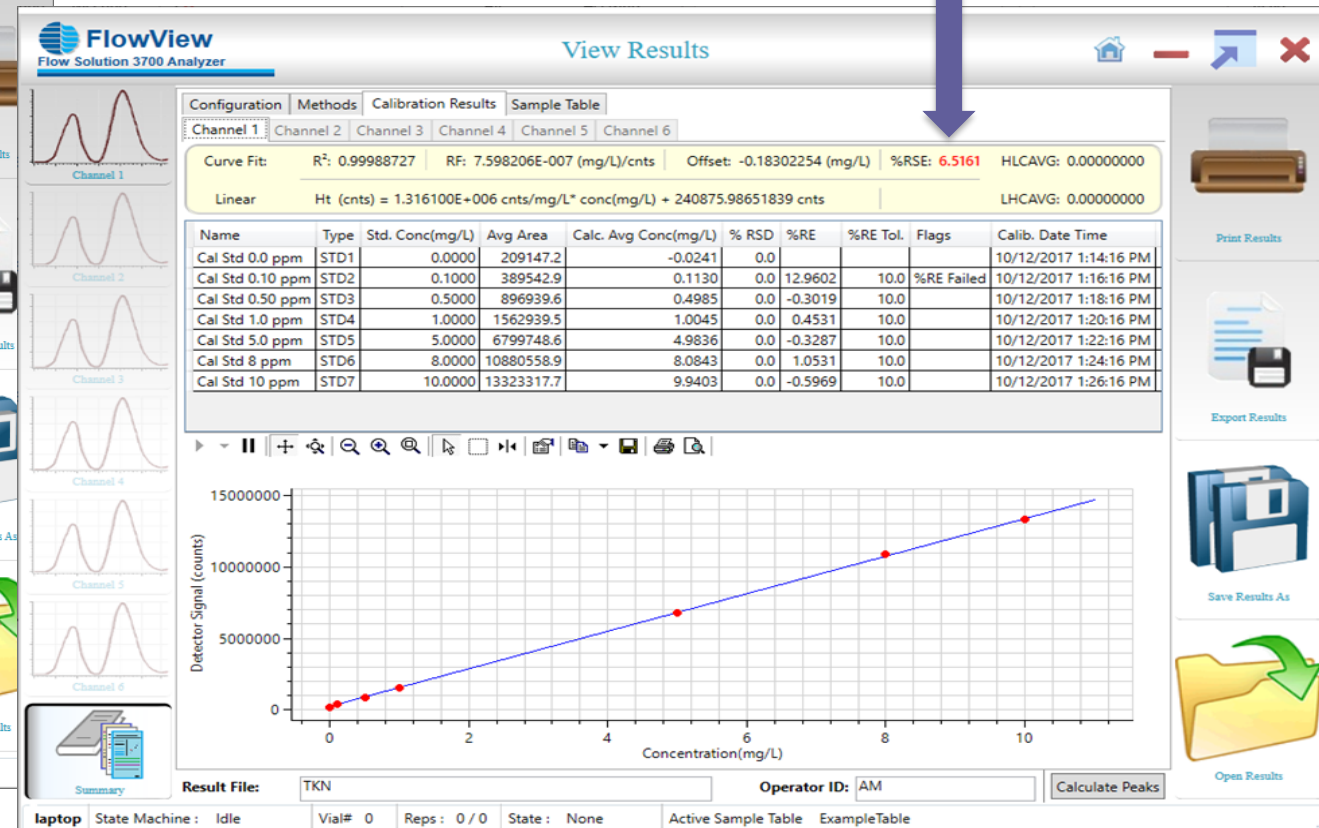
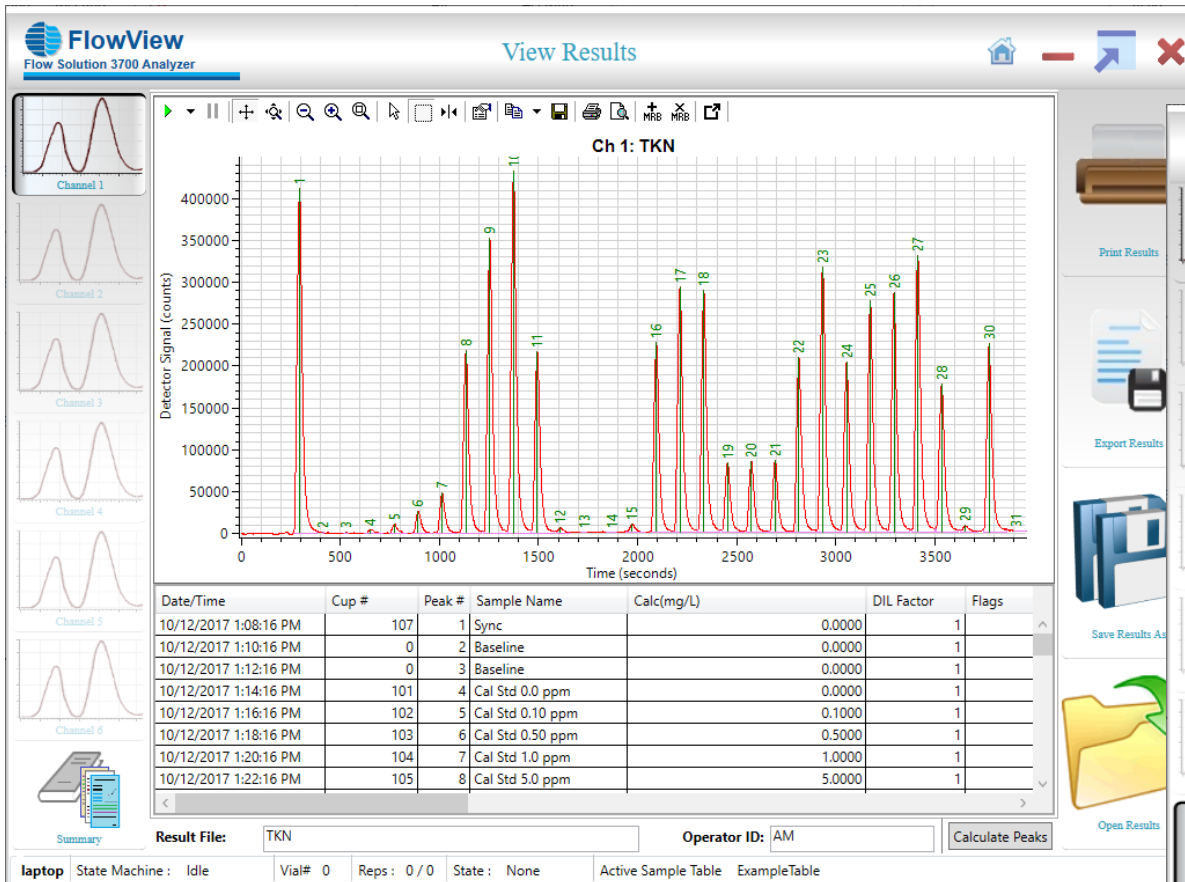
Calibration Using Ammonium Sulfate
Check curve fit, %RSE with manual dilution*



EPA Method 351.2 on the FS3700

Calibration Using Ammonium Sulfate
Check curve fit, %RSE

Calibration Using Ammonium Sulfate
Check curve fit, %RSE with ML600 autodilutor



TKN Method Performance & Quality Control

Method Performance

Range	0.010– 20 mg/L N
Rate	40 samples/hour
Precision	≤ 2 % RSD at mid-range
MDL	0.0012 mg/L P

- You can adjust your calibration range to your expected concentrations
- Published method has MDL, accuracy and precision study for reference

Sample Considerations

- Eliminate suspended particulates and ionic interferences by selectively diffusing ammonia through hydrophobic, microporous, polypropylene membrane.
- Filter turbid samples and digests prior to analysis.
- Heavy metals in the sample and digest matrices may interfere by precipitating.
 - Remove these interferences through complexation with sodium citrate.
- Occasional issues with residual chlorine.



Total Kjeldahl Nitrogen Summary

- TKN is a water quality parameter to aid in the management of eutrophication that leads to HABs
- TKN cannot be measured in the field—it requires a lab-based colorimetric method
- The FS3700 platform has pre-configured chemistry cartridges, autodilution, and methods to support TKN
- Paired with FS3700 nitrate and nitrate method, one can derive Total Nitrogen (TN)

3

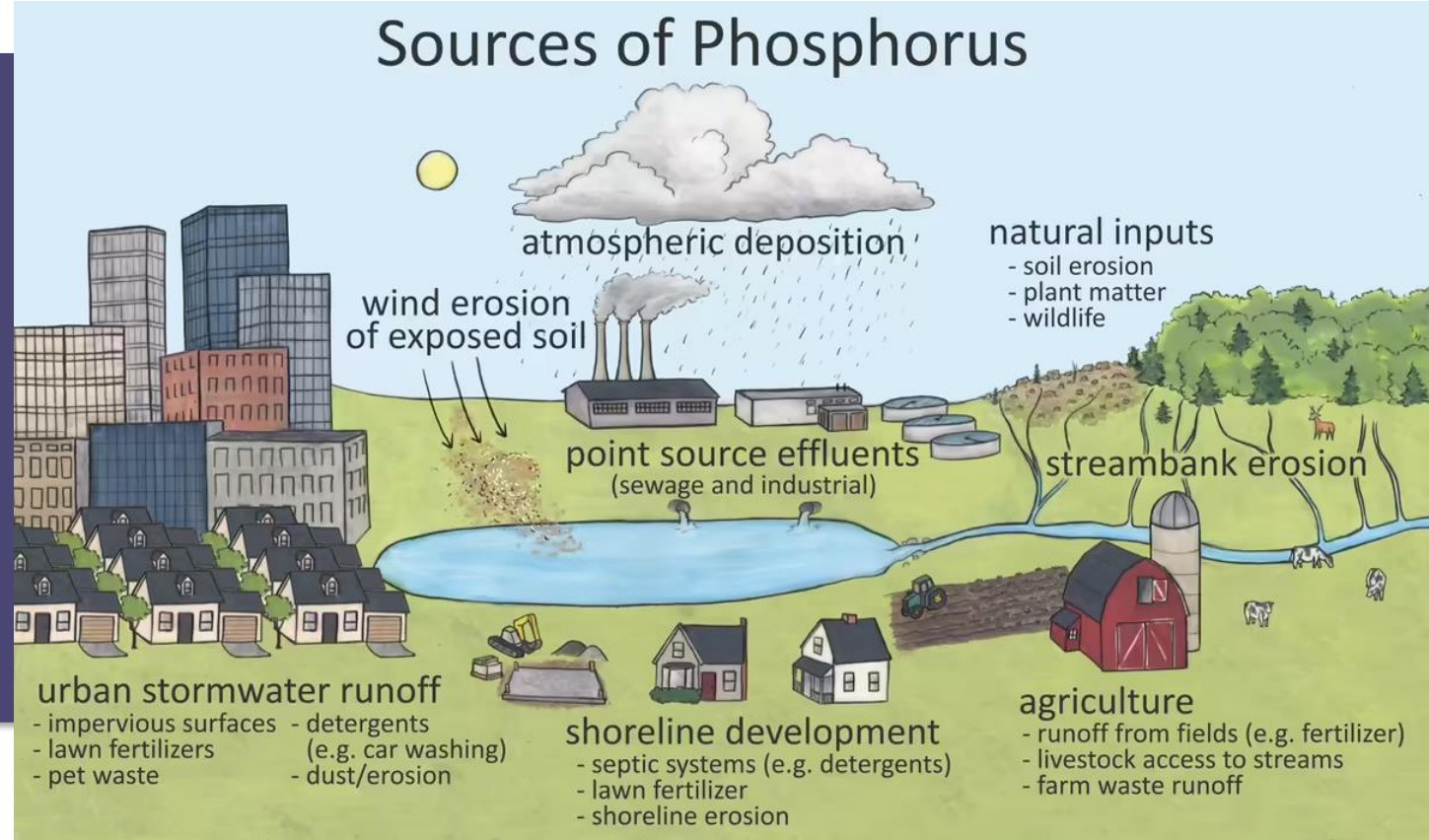
Total Phosphorus EPA Method 365.4



EPA Method 365.4 Total Phosphorus

What Is TP?

- All particulate and soluble forms of phosphorus
- Multiple inorganic and organic types, but orthophosphate is considered the most biologically relevant



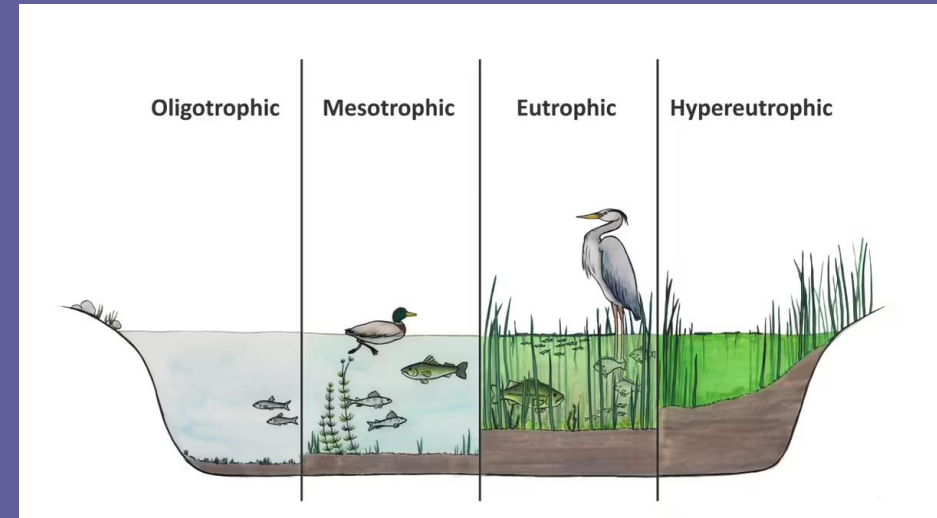
<https://datastream.org/en/guide/phosphorus>, June 2022

EPA Method 365.4 Total Phosphorus

What Is TP?

How TP Affects HABs

- P is the limiting nutrient for algae in most freshwaters.
- Eutrophic and hypereutrophic conditions lead to HABs
- N:P ratio is an indicator of HAB risk in a system



<https://datastream.org/en/guide/phosphorus>, June 2022

EPA Method 365.4 Total Phosphorus

What Is TP?

How TP Affects HABs

Chemistry Of 365.4 : Kjeldahl Digestion



Total P by SFA (365.4)

EPA Method 365.4 Total Phosphorus

What Is TP?

How TP Affects HABs

Chemistry Of 365.4 : Orthophosphate Complex



potassium antimony added to accelerate the color-forming reaction

Total P by SFA (365.4)

EPA Method 365.4 Total Phosphorus

What Is TP?

How TP Affects HABs

Chemistry Of 365.4 : Ascorbic acid reaction



Total P by SFA (365.4)

EPA Method 365.4 Total Phosphorus

What Is TP?

How TP Affects HABs

Chemistry Of 365.4

FS3700 Configuration for Method 365.4

- Segmented Flow Analysis
- Drinking water, surface water, wastewater
- Flow Injection Analysis Methods from OI Analytical
 - Low P via 365.1, SM4500-P-I and ISO 15681-1
 - P (All Forms) via 365.1, SM4500-P-I and ISO 15681-1
 - Total P via UV/Persulfate for SM4500-P-I and ISO 15681-1

Total P by SFA (365.4)

Low P by FIA

P (All Forms) by FIA

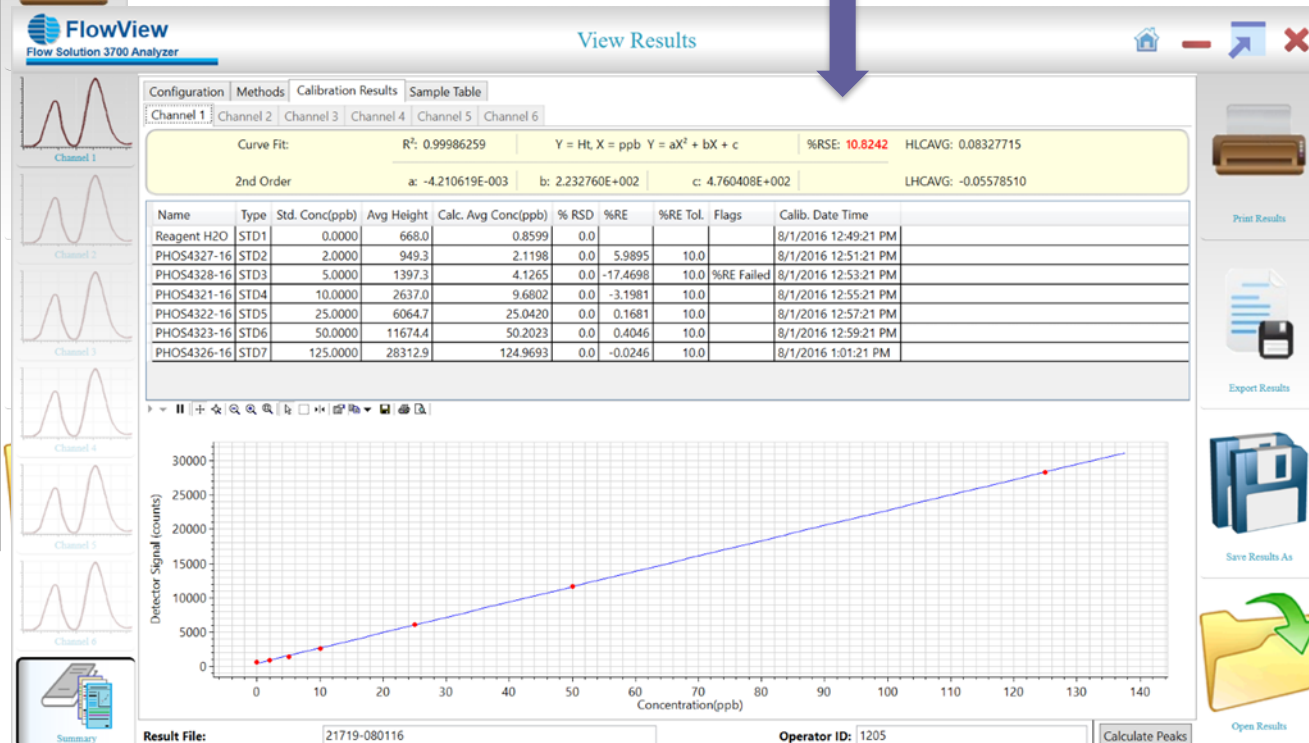
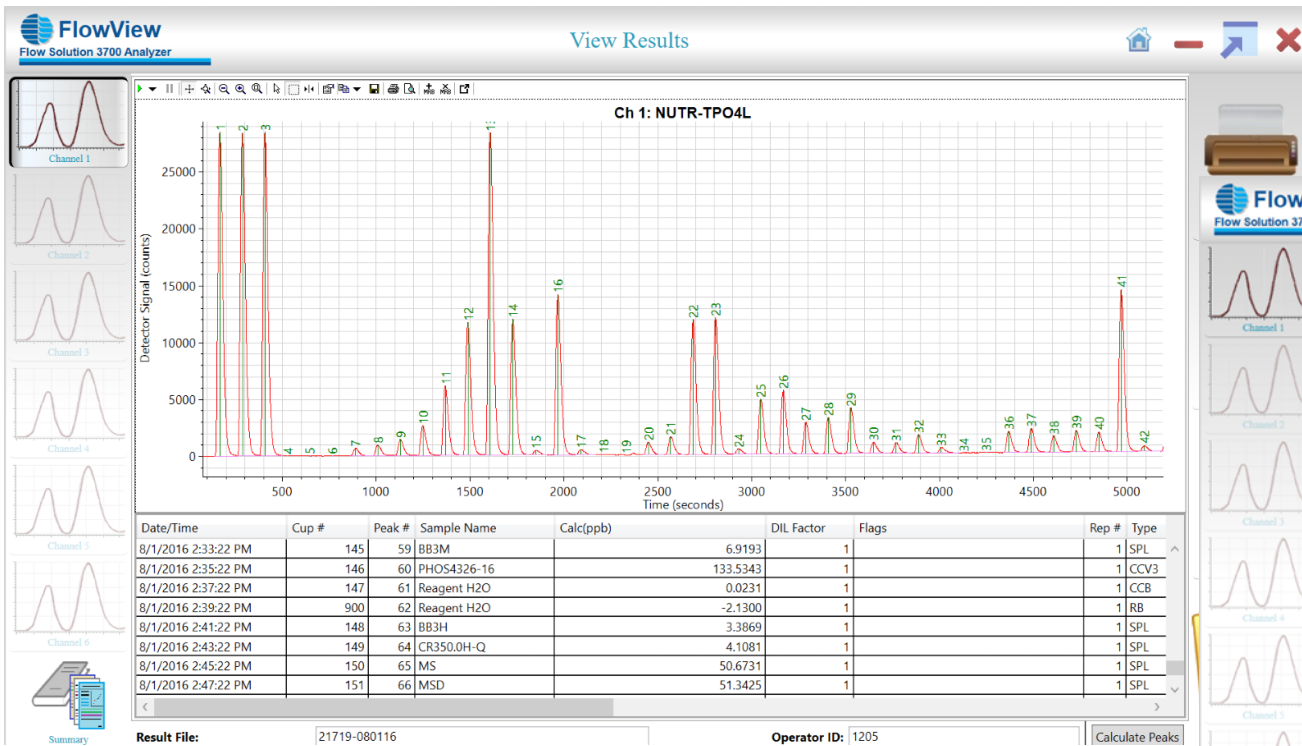
Total P UV/Persulfate by FIA

EPA Method 365.4 on the FS3700

WWTP Samples

Check peak markers, the software does the calcs

Calibration Using Ammonium Sulfate
10.8% RSE with manual dilution*



Sample Considerations

- Filter turbid samples
- Samples with background absorbance
- Residual reacted phosphate
 - Wash the system and glassware with 0.1-1 N HCl
- Other compatible procedures for removing or suppressing interferences
- Take care to keep all such items free of contaminants.



Total Phos Method Performance & Quality Control

Method Performance	
Range	0.010 – 20 mg/L P
Rate	60 samples/hour
Precision	≤ 2 % RSD at mid-range
MDL	0.0012 mg/L P

- You can adjust your calibration range to your expected concentrations
- Published method has MDL, accuracy and precision study for reference

Total Phosphorus Summary

- TP is a water quality parameter to aid in the management of eutrophication that leads to HABs
- The most accurate TP measurements are made in the lab
- The FS3700 platform has pre-configured chemistry cartridges, autodilution, and methods to support TP

4

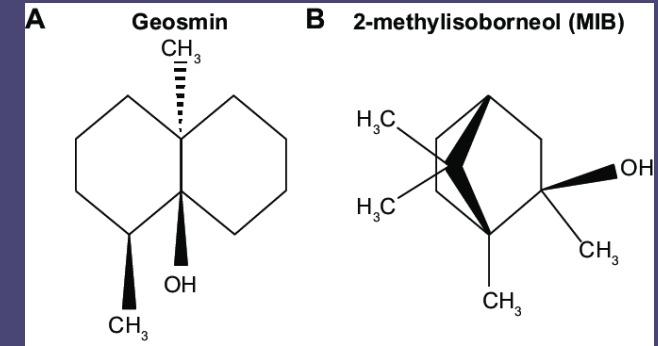
Geosmin & MIB EPA Method 524.3



Why EPA 524.3?

What are Geosmin & MIB?

- Earthy/musty taste and odor
 - Humans can detect as low as 5 ppt!
- Made by microorganisms, especially in soils
- Volatile organic compounds (VOCs)
- Non-toxic



Geosmin & MIB via EPA 524.3

What are Geosmin & MIB?

HABs and Geosmin/MIB

- Made by some blue-green algae (but not all and not all the time)
- May be an “early warning sign” of an HAB
- Co-occurred with toxins $\geq 90\%$ of the time in one study*
- Can be removed via Advanced Oxidation Processes (AOP), Carbon, etc.



AOP Article in WWD

*Graham JL, et al. Environ Sci Technol. 2010;44(19):7361–7368.
doi: 10.1021/es1008938.

Geosmin & MIB via EPA 524.3

What are Geosmin & MIB?

HABs and Geosmin/MIB

Geosmin/MIB Methods*

- ≤ 1 ppt in finished water is the target
- GC/MS with selective ion monitoring (SIM)
 - Sample concentration is necessary

*Bristow, R.L., et al. TrAC. 2019;110:233-248 <https://doi.org/10.1016/j.trac.2018.10.032>.

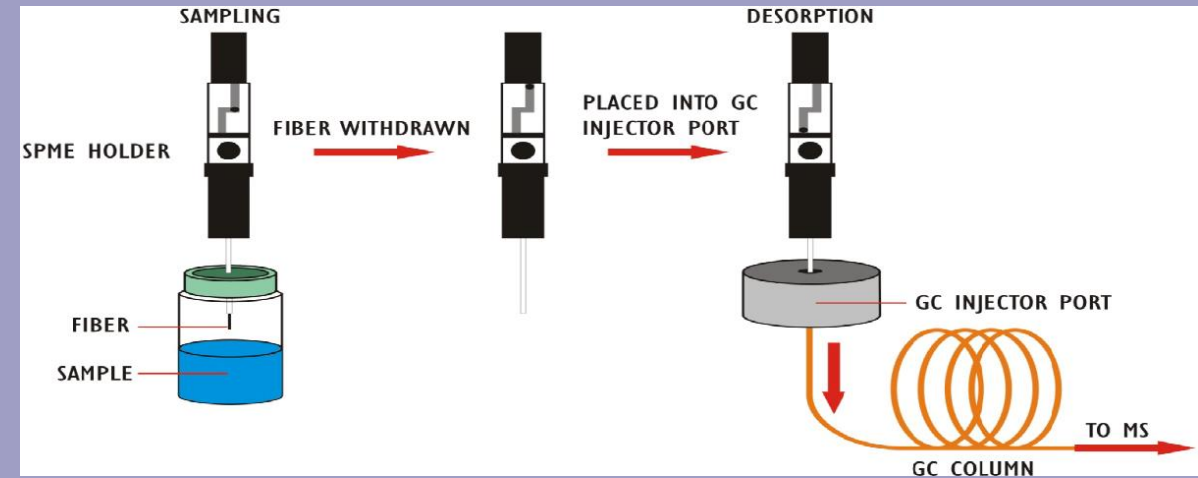
Geosmin & MIB via EPA 524.3

What are Geosmin & MIB?

HABs and Geosmin/MIB

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- ≤ 1 ppt in finished water is the target
- GC/MS with selective ion monitoring (SIM)
 - Sample concentration is necessary
 - Solid phase microextraction (SPME)



**See ref.

*Bristow, R.L., et al. TrAC. 2019;110:233-248 <https://doi.org/10.1016/j.trac.2018.10.032>.

**Schmidt and Podmore.. J. Mol. Biomark. Diagn. 2015;6(6).
<http://dx.doi.org/10.4172/2155-9929.1000253>.

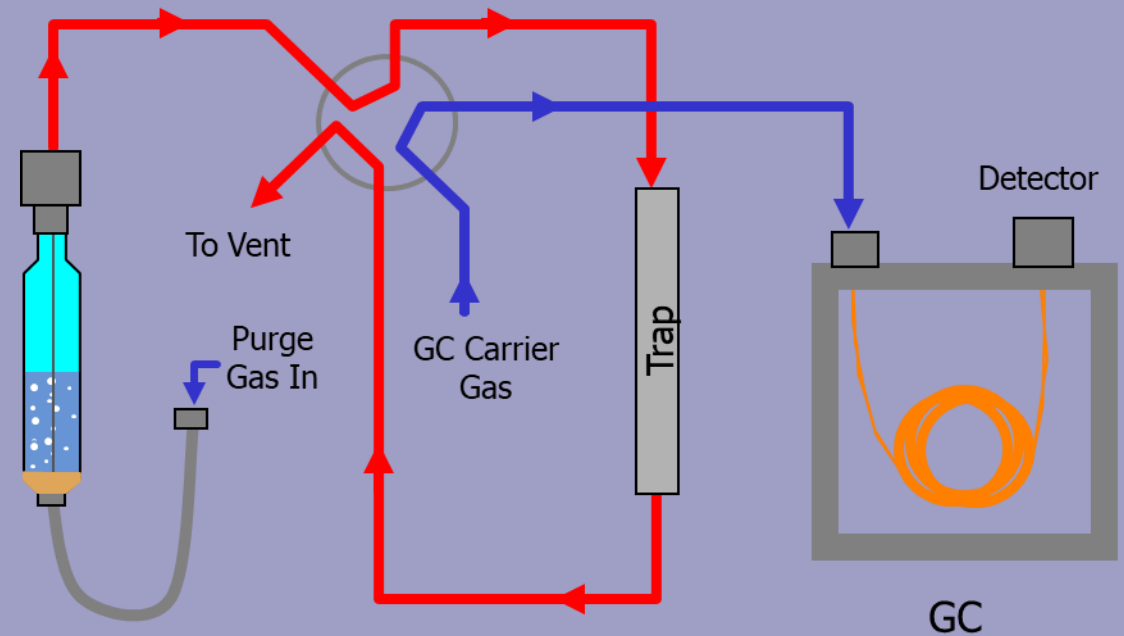
Geosmin & MIB via EPA 524.3

What are Geosmin & MIB?

HABs and Geosmin/MIB

Geosmin/MIB Methods*

- ≤ 1 ppt in finished water is the target
- GC/MS with selective ion monitoring (SIM)
 - Sample concentration is necessary
 - Solid phase microextraction (SPME)
 - Purge & Trap (PT)
 - Extraction, concentration, sample into all in one step



*Bristow, R.L., et al. TrAC. 2019;110:233-248 <https://doi.org/10.1016/j.trac.2018.10.032>.

Geosmin & MIB via EPA 524.3

What are Geosmin & MIB?

HABs and Geosmin/MIB

Measuring Geosmin/MIB

OI Analytical Method via EPA 524.3

- 4100 Autosampler
- 4760 Eclipse Purge & Trap
 - #7 Trap
- Agilent 7890A/5975C GC/MS



Geosmin/MIB Method

Geosmin & MIB via EPA 524.3

What are Geosmin & MIB?

HABs and Geosmin/MIB

Measuring Geosmin/MIB

OI Analytical Method via EPA 524.3: Method Goals

- *High sensitivity, hi reproducibility, using a validated method that labs are already familiar with*
- No sample salting
- Lower volume (10 mL vs. 25 mL in some methods)
- Lower temp purge (60° C vs. 80° C)



Geosmin/MIB Method

Geosmin & MIB via EPA 524.3: Calibration Acceptance & Validation

Compound	1 ppt		100 ppt	
	Std	%Rec	Std	%Rec
1,2-DCB-d4 (SS)	9.81	98.1	10.16	102
2-MIB	1.03	103	97.2	97.2
Geosmin	1.13	113	98.7	98.7

- 1,2-Dichlorobenzene-d4 is the surrogate standard, at 10 ppt
- Concentrations: 1, 2, 5, 10, 25, 50, 75 and 100 ppt
- Low level %Rec must be +/- 50%, all other levels must be +/- 30%

Geosmin & MIB via EPA 524.3: Minimum Reporting Level (MRL)

Compound	Mean	Std. Dev.	HR _{PIR}	Upper PIR Limit (%)	Lower PIR Limit (%)
1,2-DCB-d4 (SS)	9.69	0.137	0.54	102	91.5
2-MIB	1.13	0.060	0.24	137	89.8
Geosmin	1.05	0.061	0.24	129	80.9

- HR – Half Range Prediction of Interval Results
- Upper PIR limit must be $\leq 150\%$ recovery (true value is 1 ppt)
- Lower PIR Limit must be $\geq 50\%$ recovery (true value is 1 ppt)

Geosmin & MIB via EPA 524.3: Initial Demonstration of Capability

Compound	Precision (%RSD)	Accuracy (%)
1,2-DCB-d4 (SS)	1.92	98.4
2-MIB	3.73	109
Geosmin	3.46	106

- The 1,2-DCB-d4 surrogate standard was at 10 ppt
- Target compounds at 25 ppt
- %RSD must be $\leq 20\%$ for precision for all
- Accuracy (%) must be $\pm 20\%$
 - (80-120% recovery)

Geosmin & MIB via EPA 524.3: Considerations

- Method 524.3 is for finished drinking water
 - Haven't tested this method with source water
- Clean sample pathway, clean MS source
- Recommend minimum of three vials for sample testing
- Use same care you would use for all VOC sample collection



Geosmin & MIB Summary

- Taste and odor in drinking water are among the most insidious problems caused by HABs
- Ideal method should have
 - ≤ 1 ppt detection limit
 - No pre-treatment of samples
 - Minimal reconfiguration of instruments
- This method for the 4760 Purge & Trap achieves ≤ 1 ppt MRL, employing EPA 524.3 and a common setup found in many environmental labs

Get Your Lab Ready For HABs!

 Contact Us:
info@ysi.com

 Chat With Us:
[YSI.com](https://ysi.com)

 Learn More:
[YSI.com/HABs](https://ysi.com/HABs)

