



## LC-MS Library Development and Strategy for Identifying Harmful Organics in Drinking Water

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- US EPA Office of Pesticide Programs Biological and Economic Analysis Division Analytical Chemistry Branch, Ft. Meade, MD
  - Pat Schermerhorn, Adrian Burns, Diane Rains, Paul Golden
- Waters Corporation, Milford, MA
  - Jim Krol, Joe Romano

Mention of Vendor Names Does Not Constitute Product Endorsement

## Cooperative Research and Development Agreement (CRADA)

- US EPA Region 5 Central Regional Laboratory (CRL) and Waters Corporation are working together to produce a searchable LC/MS library system relevant to drinking water safety.
- The same instrument type from Waters Corporation was used by all laboratories.
- LC/MS Laboratory Spectra were created at CRL.
- Data collected at different laboratories successfully matched this library.



## Rationale

*What are we looking for and why??*

- We are undertaking an initiative to develop a robust screening method for drinking water using LC/MS and LC/MS/MS technologies.
- This method will provide detection and identification of deleterious organic compounds not amenable to GC/MS.
- Organic analytes amenable to analysis via LC/MS and LC/MS/MS include:
  - Agrochemicals: pesticides and insecticides
  - Controlled substances (LSD, methamphetamine, ecstasy)
  - Pharmaceuticals

## LC/MS Library System

- To date no transferable LC/MS libraries exist.
- GC/MS libraries (Wiley, NIST ...) are in widespread use.
- This system would provide laboratories with the tool needed to tentatively identify deleterious organic contaminants in drinking water.

## Round One

- 55 Compounds in our Second Library Proof of Concept
  - Neutrals
  - Bases
  - Acids
- Spectra Generated by Different Laboratories Matched to Libraries at CRL.

### System to Tentatively Identify a Compound Using an LC/MS Library

- Standards were infused in order to create full scan (MS) and product ion scan library spectra (MS/MS).
- These spectra were designed to contain characteristic fragmentation while maintaining the integrity of the molecular ion at approximately 10% relative intensity to insure proper tentative identification.



## Instrumentation

- **HPLC**
  - Waters Alliance 2695 HPLC unit
- **MS/MS**
  - Micromass Quattro *micro* API mass spectrometer (capable of single and triple quadrupole usage)
- **Software**
  - MassLynx version 4.0 SP4



## Experimental Conditions

### Columns

- Atlantis C<sub>18</sub> 2.1 X 150mm column, 3 μm particle diameter
- Xterra C<sub>18</sub> 2.1 X 150 mm column, 3 μm particle diameter

### • Mobile Phase

- Acetonitrile/Water

### • Modifiers

- Neutrals: 5 mmolar NH<sub>4</sub>Ac
- Acids: 0.1% Acetic Acid
- Bases: 5 mmolar NH<sub>4</sub>HCO<sub>3</sub> adjusted to Ph 10 with NH<sub>4</sub>OH

### • Flow

- Gradient
- Flow Rate: 0.300 mL/min

### • Injection

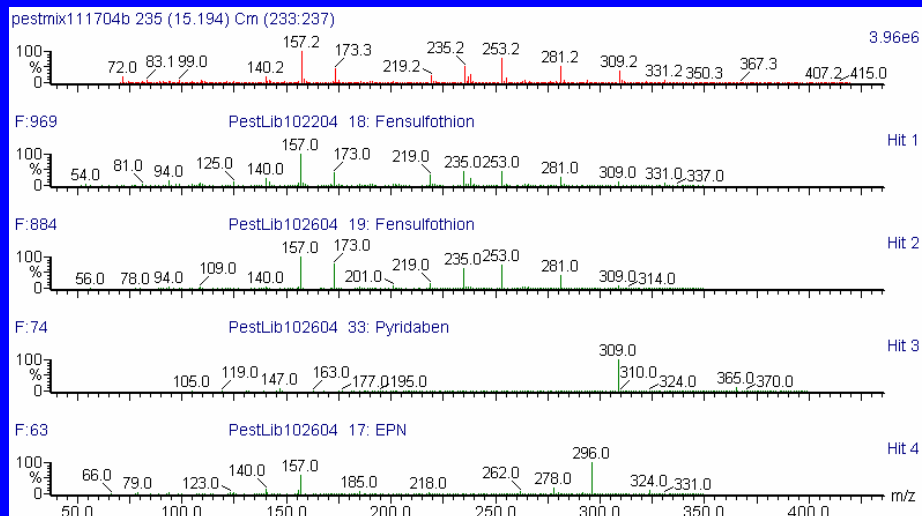
- Injection volume: Variable up to 100 μL

## Unknown Identification Scenario

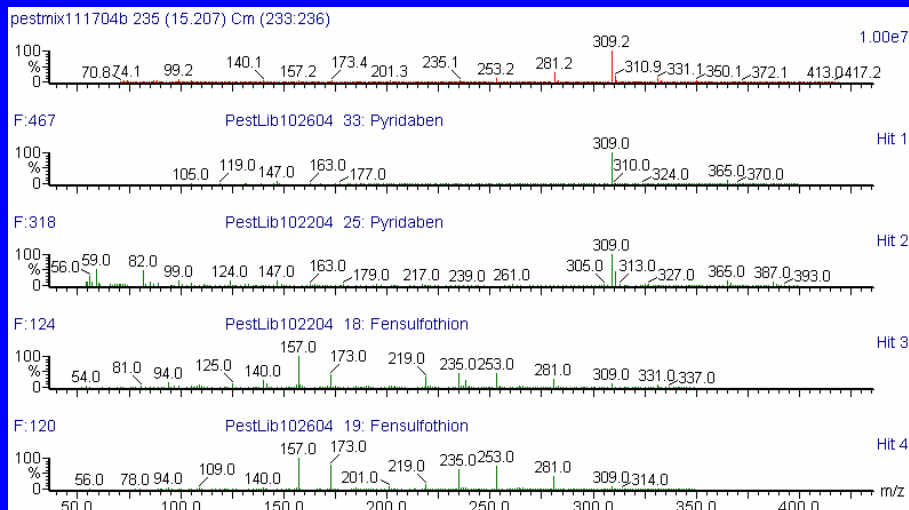
- Perform LC/MS on the water sample using full scan detection at a variety of cone voltages.
- This gives retention time data and full scan spectra which can be matched to MS libraries
- A TIC gives presumptive identification which can be further confirmed by generating its product ion spectrum under set MS/MS conditions which can be matched to MS libraries.

## Library Search of Spectrum

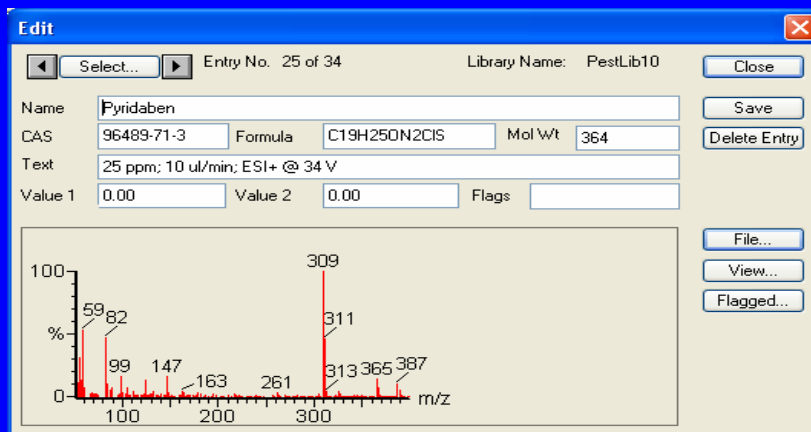
19.19 minutes @ 60 Volts



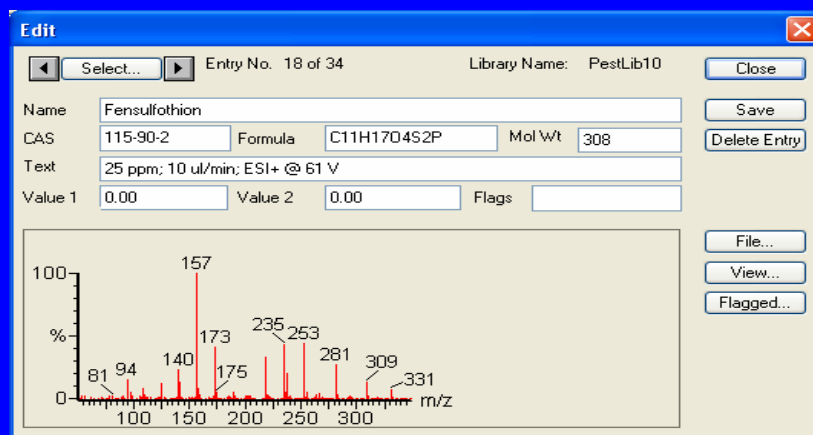
## Library Search of Spectrum 19.19 minutes @ 40 Volts



## Pyridaben Full Scan Library Spectrum



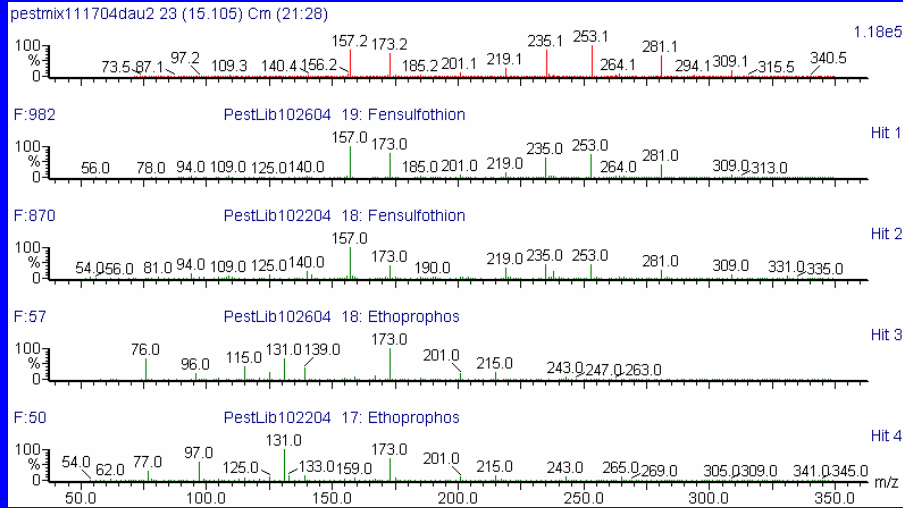
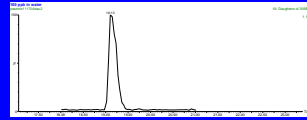
## Fensulfothion Full Scan Library Spectrum



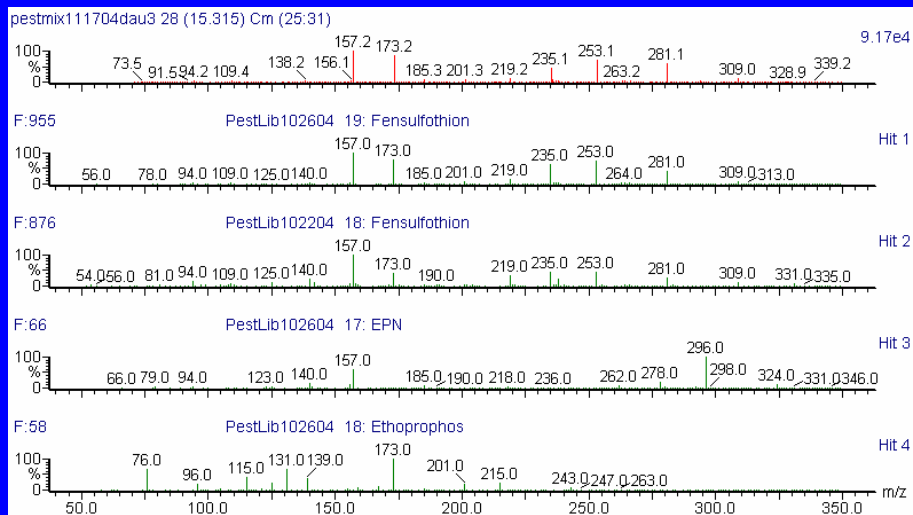
## Further Tentatively Identified Compound Confirmation using LC/MS/MS

- MS/MS Method set to acquire a product ion spectrum of 309 around the retention time at 19.19 minutes at a cone voltage of 33 and a collision voltage of 19
- The MS/MS library was acquired utilizing these same conditions
- The spectrum generated is then compared to the MS and MS/MS libraries for added confirmation
- Fensulfothion is tentatively identified at this point by matching of the Full scan spectra and daughter spectrum to our libraries.

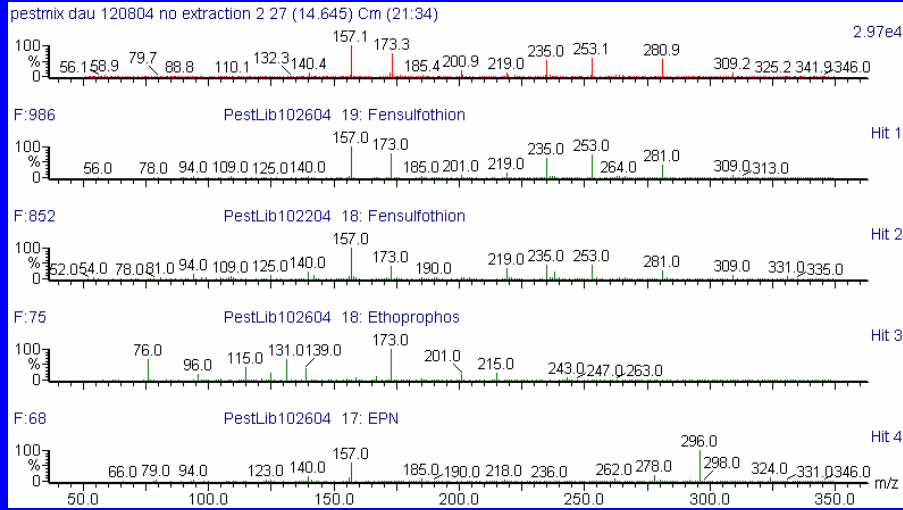
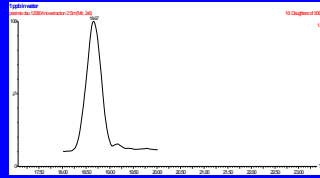
## Product Ion Spectrum of 100 ppb Fensulfothion in Water (no extraction) matched to both libraries



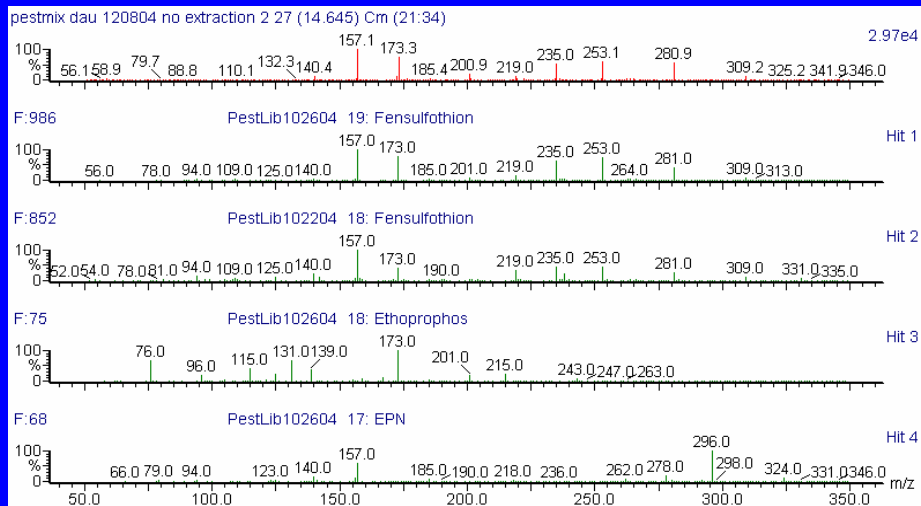
## Product Ion Spectrum of 25 ppb Fensulfothion in Water (no extraction) Matches to Both Libraries



Product Ion Spectrum of 1 ppb  
Fensulfothion in Water  
(no extraction) matched to both  
libraries

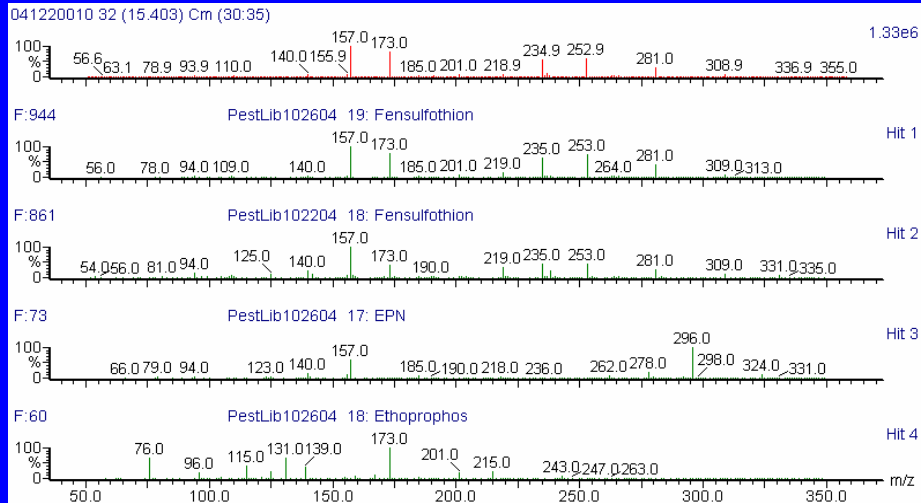


Fensulfothion Full Scan Matched  
to Both Libraries  
Analyzed at US EPA OPP Lab in Ft. Meade, MD



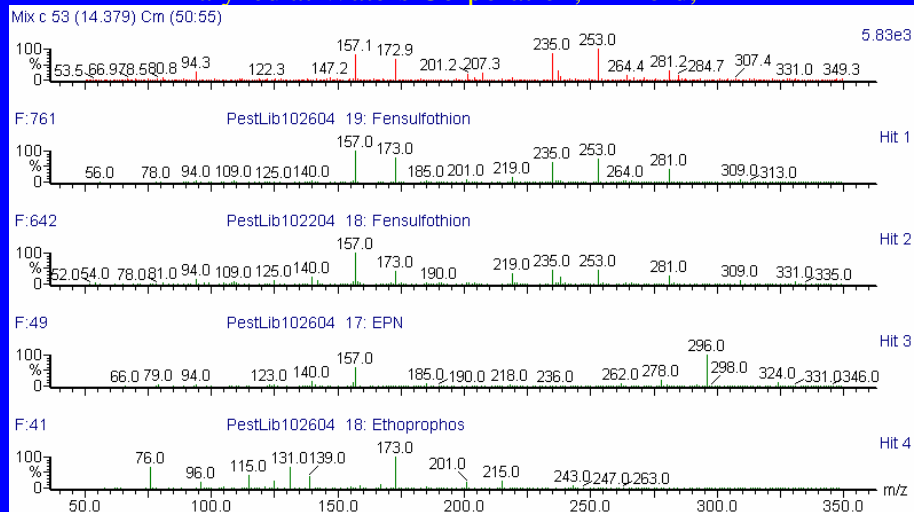
# Fensulfothion Product Ion Spectrum Matched to Both Libraries

Analyzed at US EPA OPP Lab at Ft. Meade, MD



# Fensulfothion at 1 ppb Product Ion Spectrum Matched to Both Libraries

Analyzed at Waters Corporation, Milford, MA



- Fensulfothion is a good example because it demonstrates both the reproducibility of a complicated fragmentation pattern as well as the ability to differentiate between compounds with a common ion (309).

### Compound Libraries Verified

- US EPA Office of Pesticide Programs  
Ft. Meade, MD
- Waters Corporation, Milford, MA
- Waters Corporation, Wooddale, IL

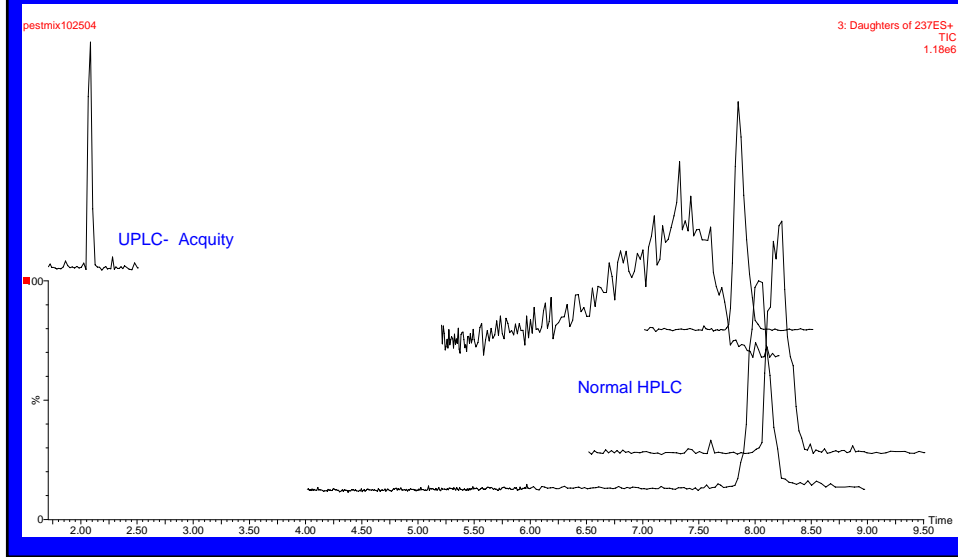
## Conclusions

- Sensitivity is Compound Dependent
- All of the compounds are successfully searchable at 100 ppb full scan or product ion scan.
- Many are successfully searchable at 1 ppb product ion scan.

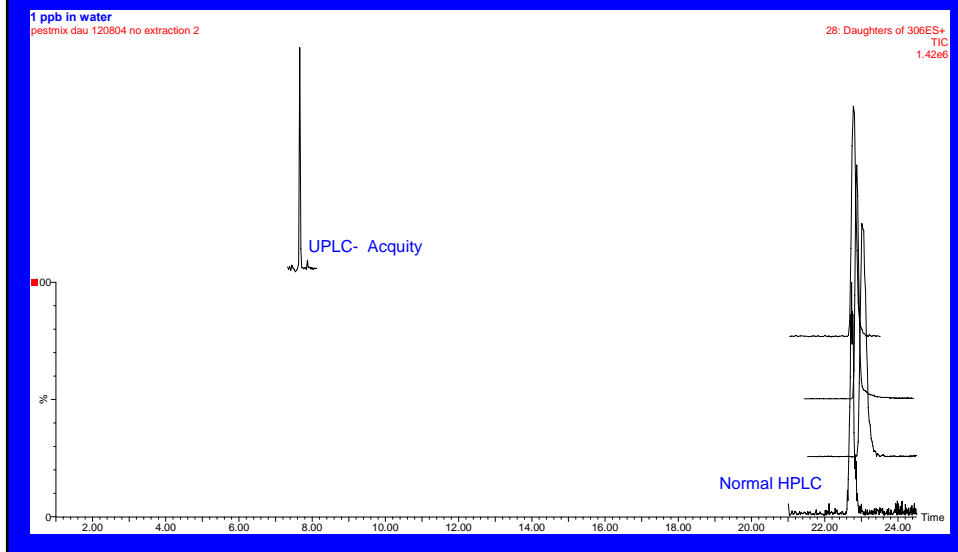
## Ultra Performance Liquid Chromatography (UPLC)

- Decrease in Stationary Phase Particle size allows for an Increase in Theoretical Plates which allows for better separation of Analytes
- Requires higher pressure LC System and Faster Acquiring Mass Spectrometer than “Normal” HPLC
- Decrease in Peak width results in an increase in signal sensitivity

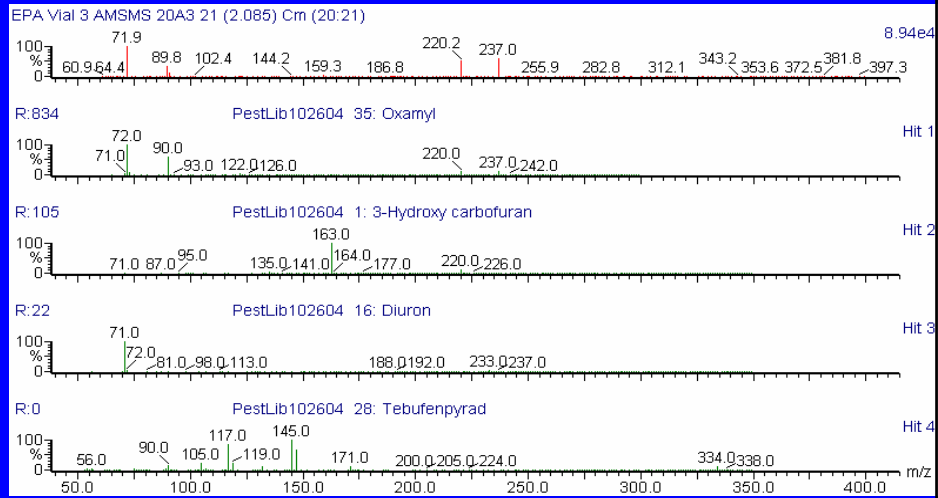
# OxamyI UPLC vs. HPLC



# Buprofezin UPLC vs. HPLC



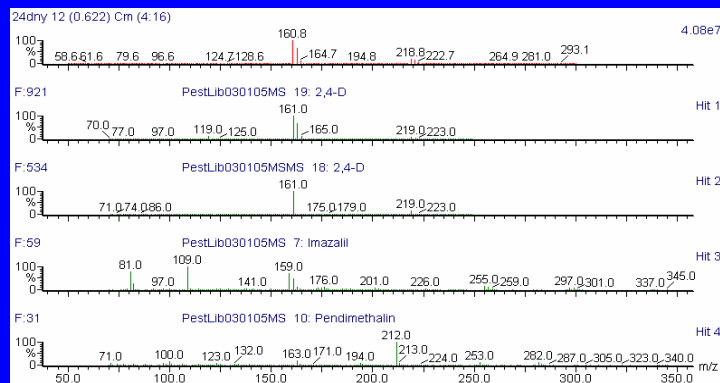
# Oxamyl Spectrum UPLC-MS/MS 2 Scans, Interscan Delay 0.05 Sec



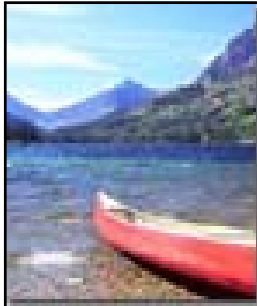
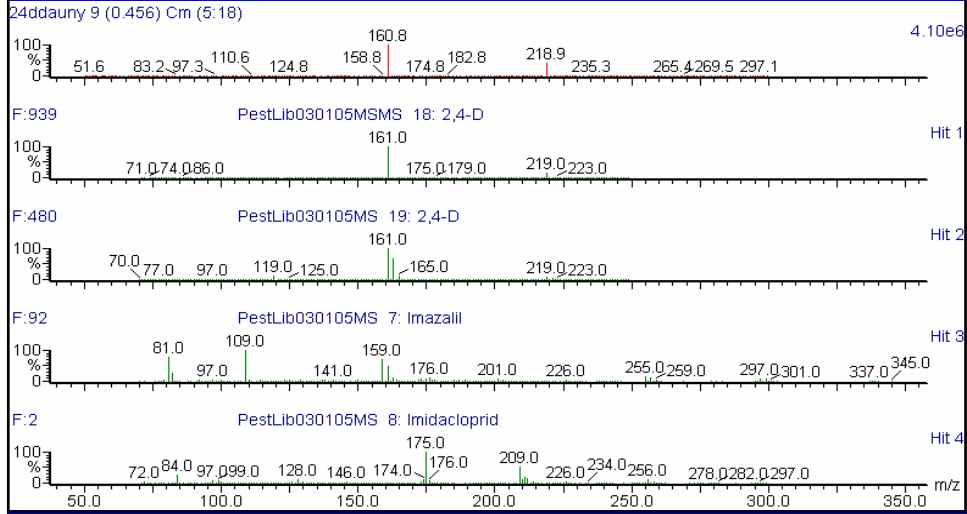
# Full Scan Premier Data from The Empire State



Lucy Deshjardins, NYS Dept. of Ag and Markets



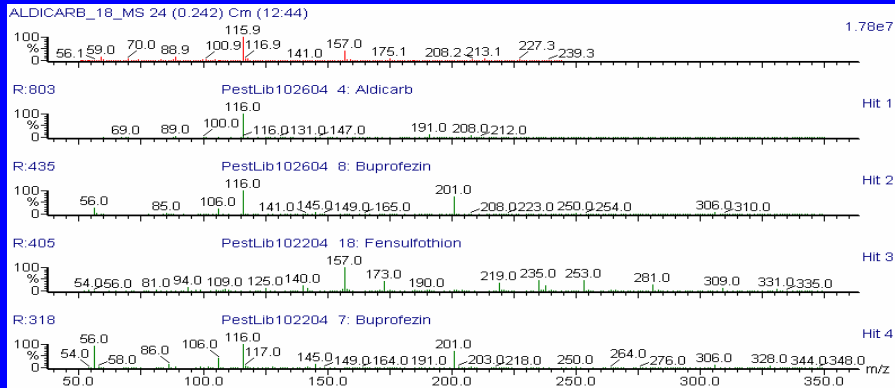
# Product Ion Scan Premier Data from The Empire State



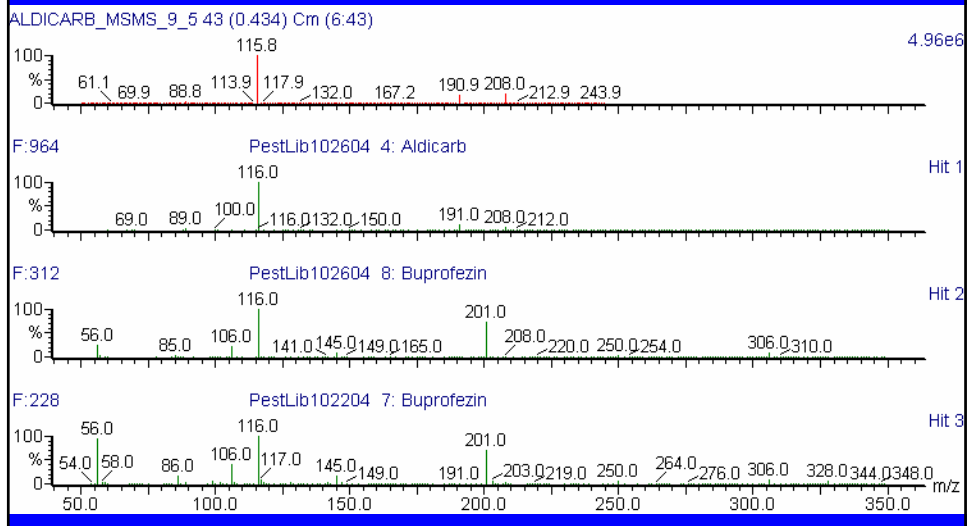
# Data from The Big Sky State

Angie Schaner  
Montana Dept. of Agriculture

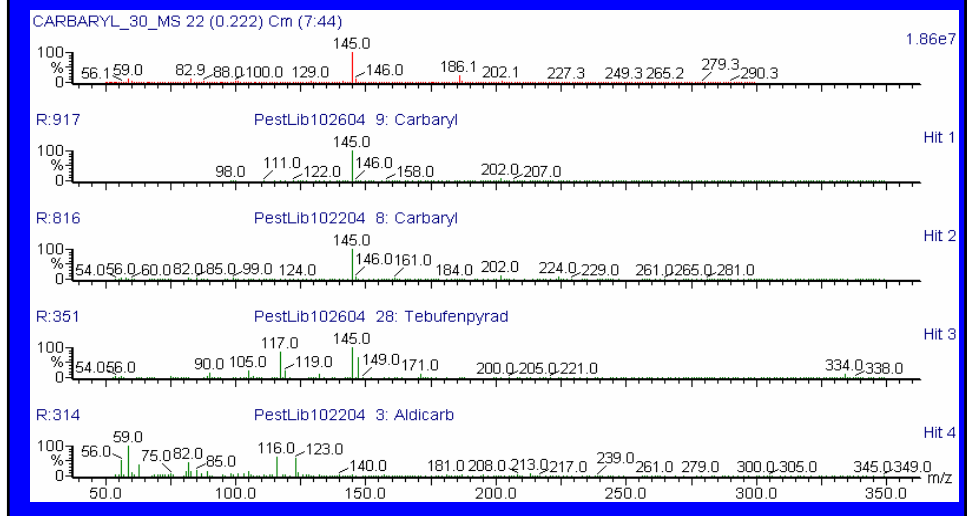
## Aldicarb Full Scan Spectrum



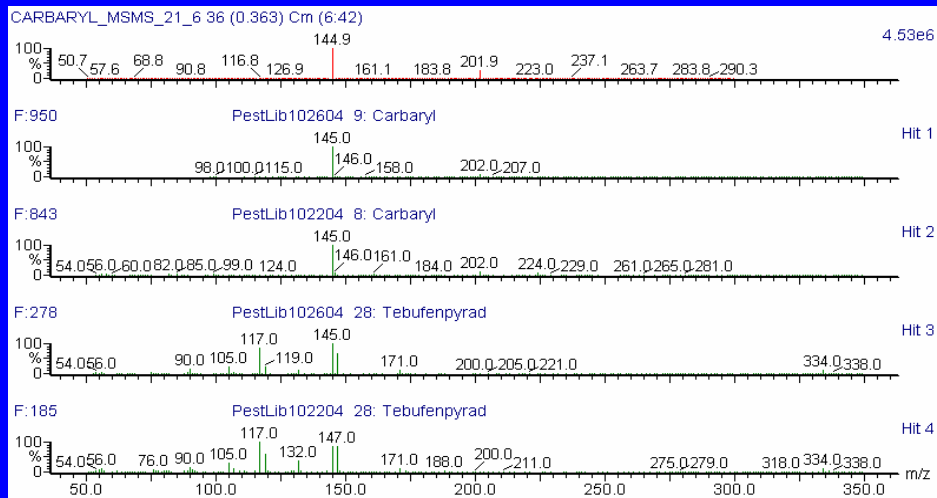
## Aldicarb Product Ion Spectrum of 208



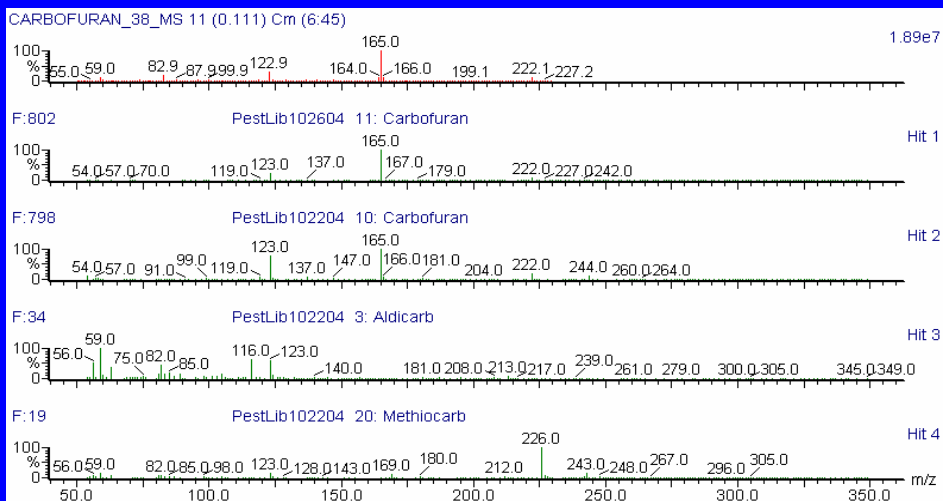
## Carbaryl Full Scan Spectrum



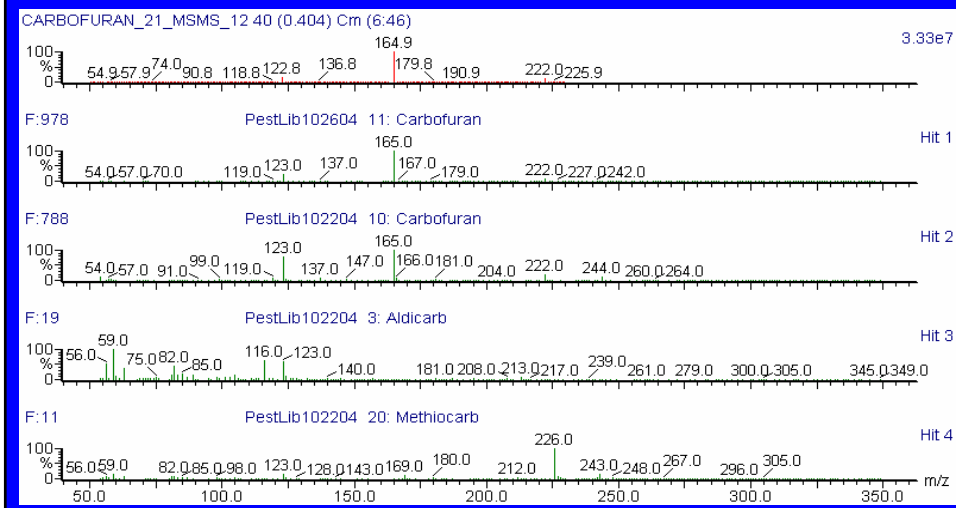
## Carbaryl Product Ion Spectrum of 202



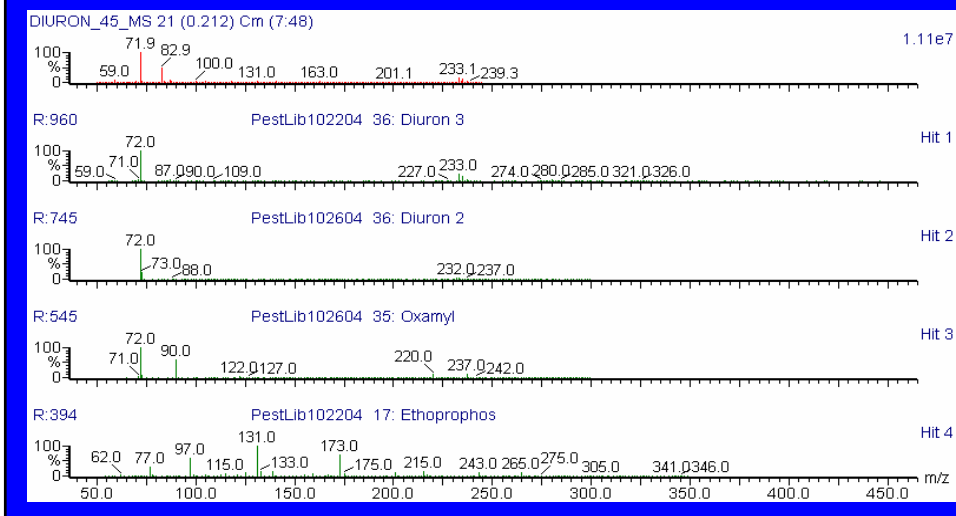
## Carbofuran Full Scan Spectrum



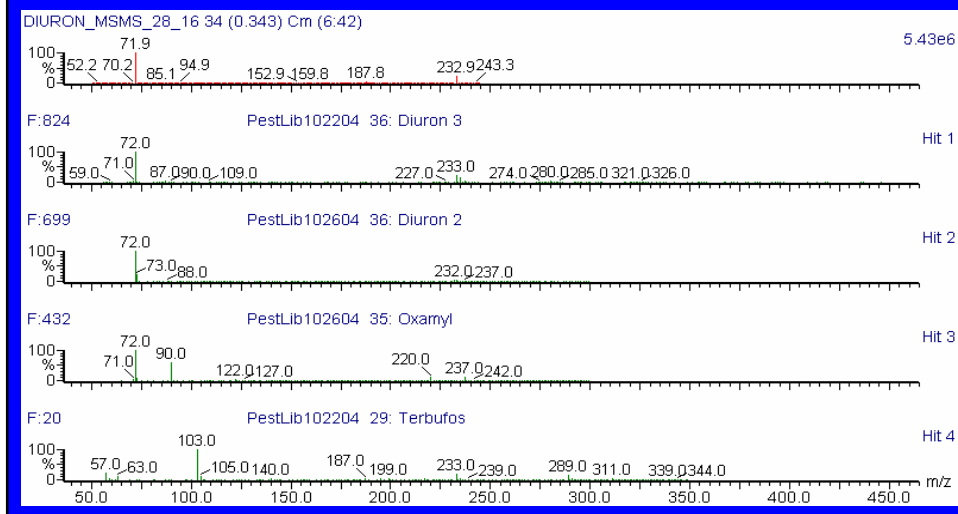
## Carbofuran Product Ion Spectrum of 222



## Diuron Full Scan Spectrum



## Diuron Product Ion Spectrum of 233

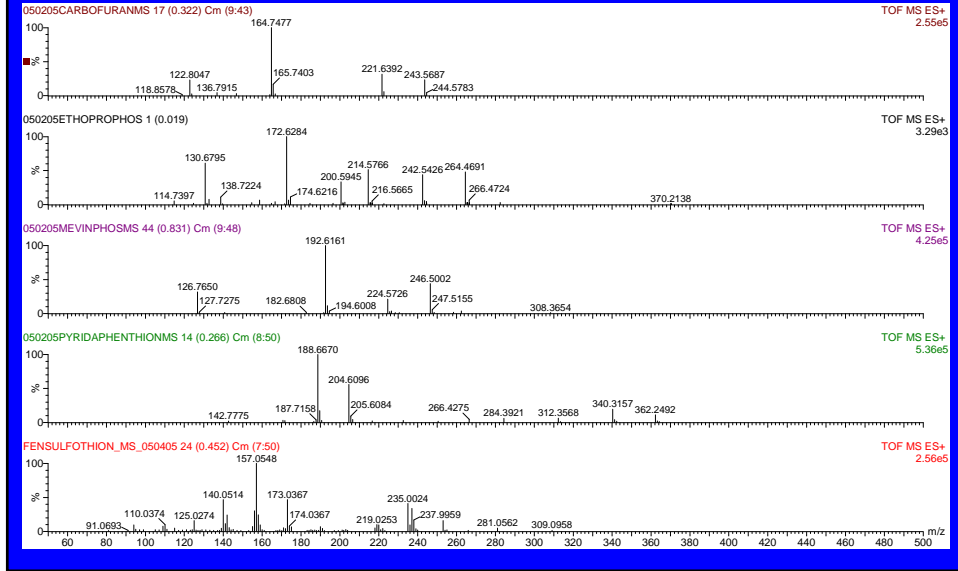


## Applicability for Time of Flight Data

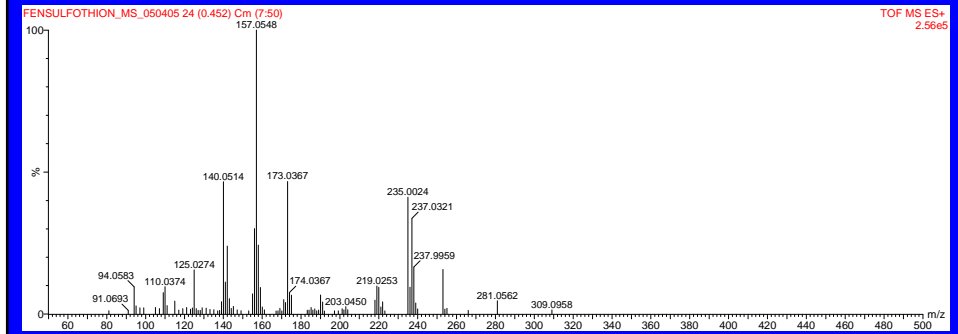
Marc Mills and Bryan Boulanger ORD-NRMRL Cincinnati, OH

- Quadrupole Time of Flight Mass Spectrometer (Q-ToF micro™)
- Exact mass MS measurement enables compounds to be more easily identified
- Great for “True Unknown” Analysis

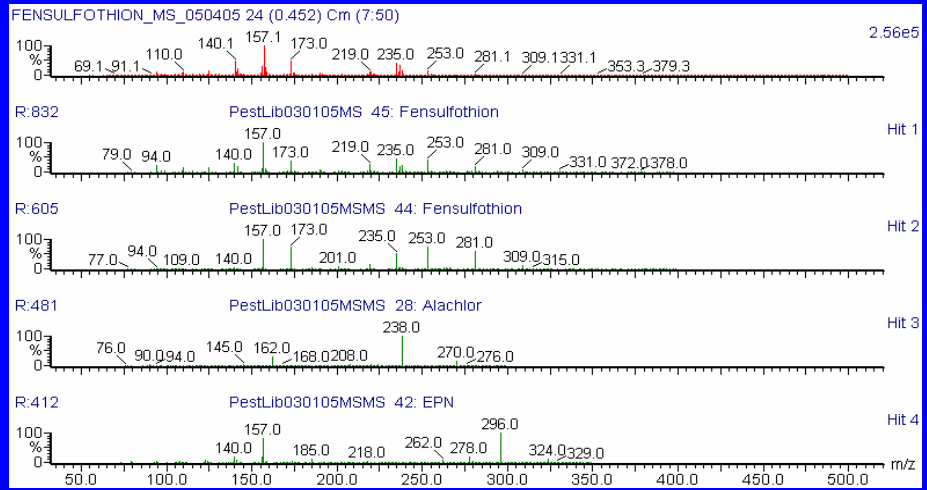
# TOF Spectra Data



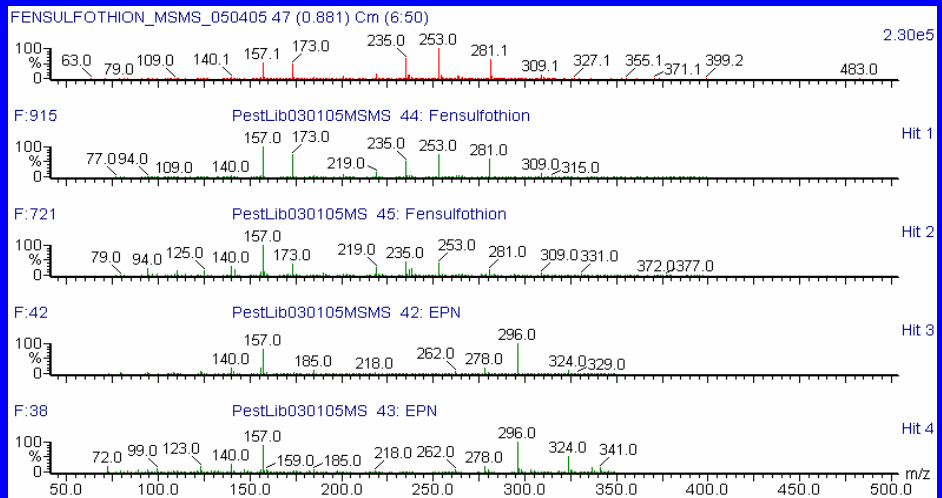
# Unknown MS TOF Spectrum



# MS TOF Match



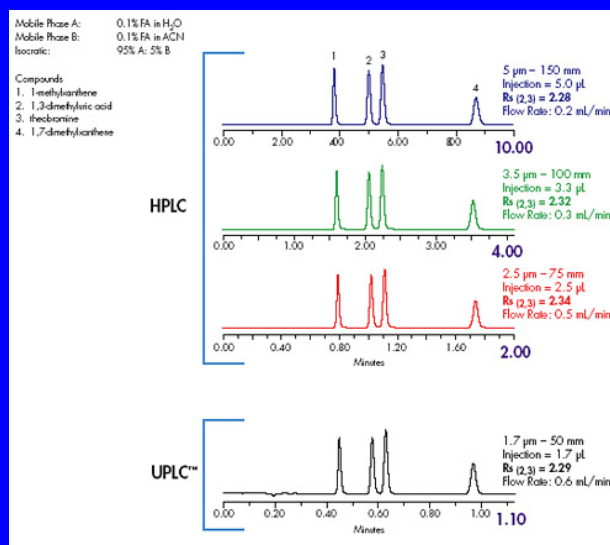
# Further Confirmation by MS/MS TOF



## Round Two

- Moving into BEH (Bridged Ethane-Silicon Hybrid Particles) Column Chemistry
- Directly Adaptable to UPLC/MS/MS
- Analyze Neutrals and Bases Together
- Currently New Library Contains 100 Neutral and Base Compounds
- Acidic Compounds will be analyzed separately

## XBridge™ HPLC Columns



The availability of multiple particle sizes and dimensions allows the optimization of the total cycle time without sacrificing resolution.

## New Collaborators

- US EPA ORD NERL- Las Vegas, NV
- State of Oregon Department of Environmental Quality
- Office of Indiana State Chemist
- Minnesota Department of Public Health
- Minnesota Department of Agriculture

## Minnesota Department Of Agriculture Comment

“We are interested as many of the compounds are pesticides. In our state, the Department of Agriculture regulates food and pesticides and the Department of Health regulates public drinking water. Except when the drinking water is used in food processing or is bottled then it is a food and is under Agriculture's jurisdiction. That's why both us and Health should look at participating. It's a food safety quilt, old discarded stuff, cut to shreds and sewn back together.”

*(Author- Viking Phil)*

## Conclusion

- Solution to the Problem! This will allow us to Screen Water Samples for Deleterious Organics in Environmental Waters
  - Drinking Water
  - Well Water
  - Clean Surface Waters
- Limit of Detection is Compound Dependent
- Improvements and additions to the libraries are continually being made as more applied science is conducted.
- Applicable and Transferable for LC/MS, LC/MS/MS, UPLC/MS/MS and TOF MS/MS

## If you have more interest...

- FREE Demonstration Today
- FREE Refreshments and Good Conversation with your Friends
- Waters New Technology for Laboratory Analysis- 1:30- 4:00 pm (Sawgrass Room- Jacaranda Hall)

## Contact Information

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