Profiling Environmental Contaminants in Water using GC-Q/TOF

National Environmental Monitoring Conference 2019

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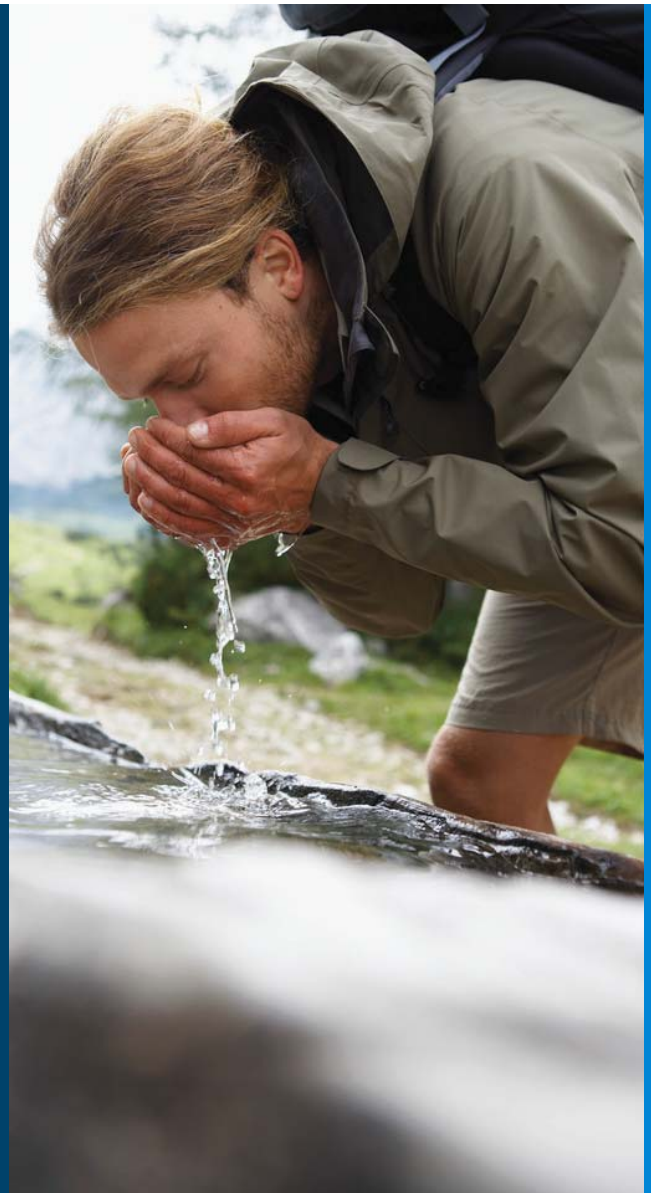


Agilent
Trusted Answers

The Agilent 7250 GC/Q-TOF

High Resolution and Mass Accuracy

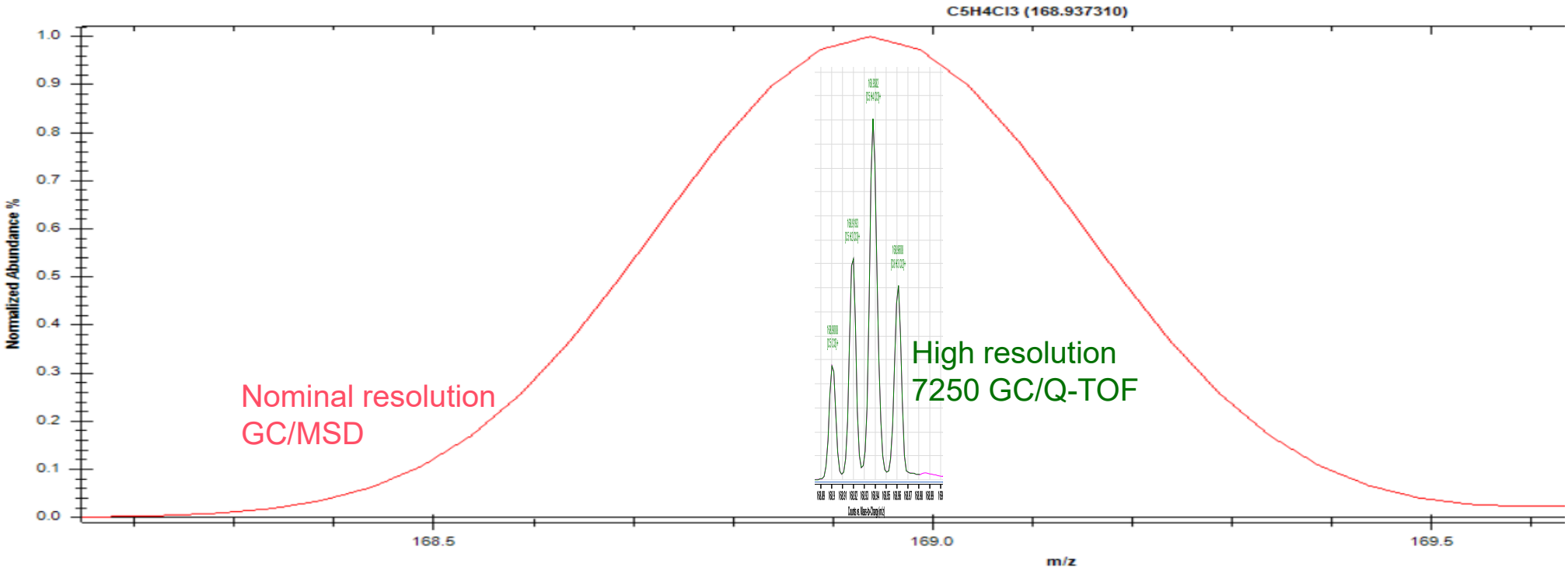
Reproducible Spectral Performance

Simultaneous High Resolution and Wide Dynamic Range

Quick Vent (10 min to vent, 10 min to pump down)

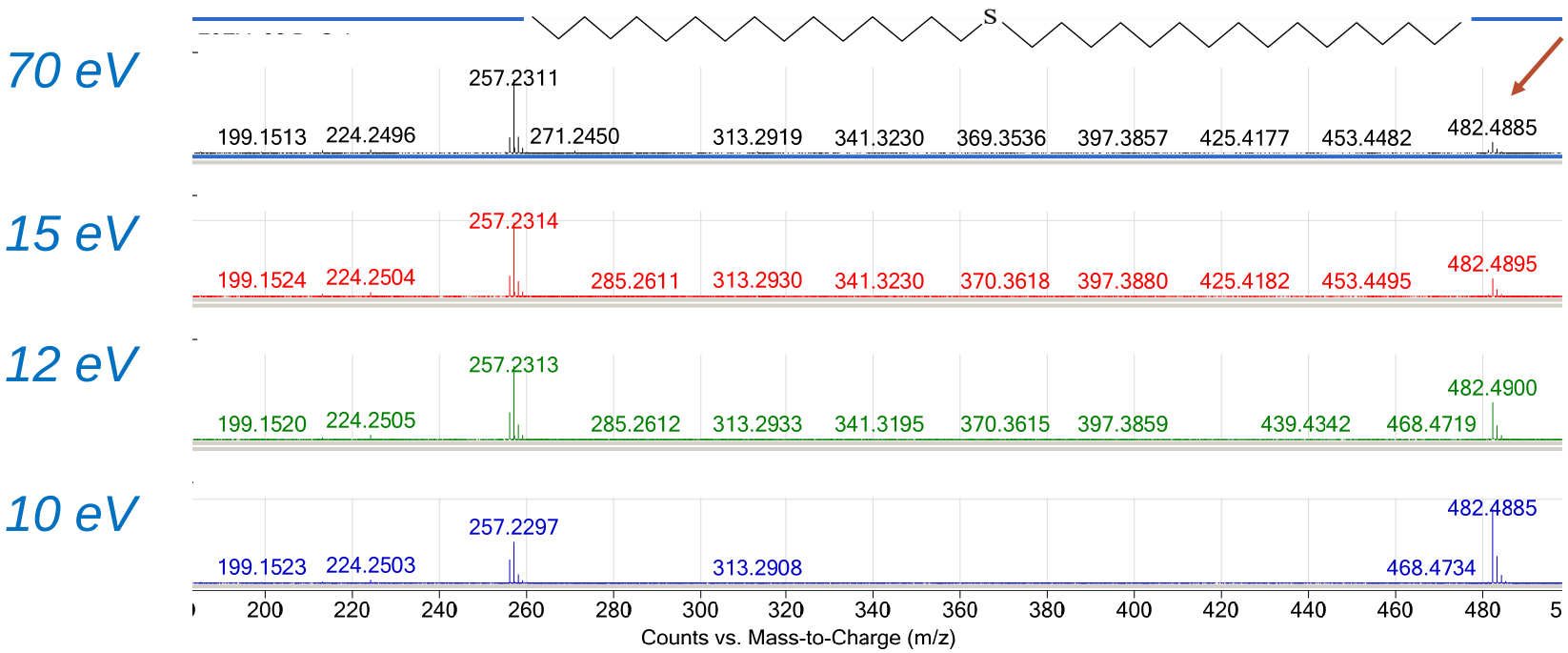
Low Energy EI capability, Sensitive Detection MS/MS experiments with Q-TOF

Resolving Power: Nominal *vs* High Resolution



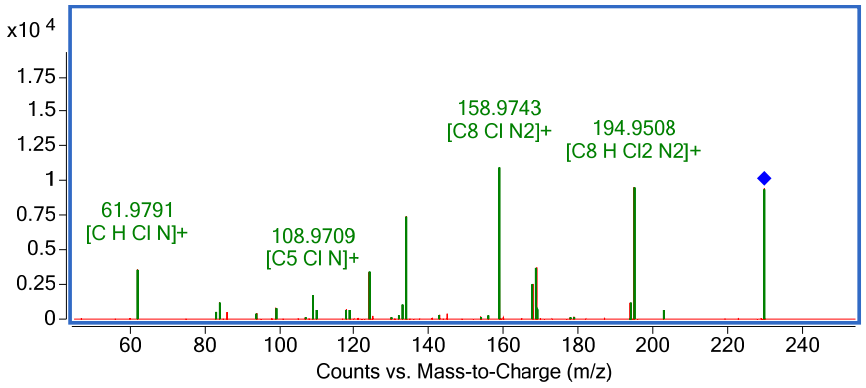
Low Electron Energy for Confirmation of Molecular Ion in Unknowns Identification

Hexadecyl sulfide: C32H66S

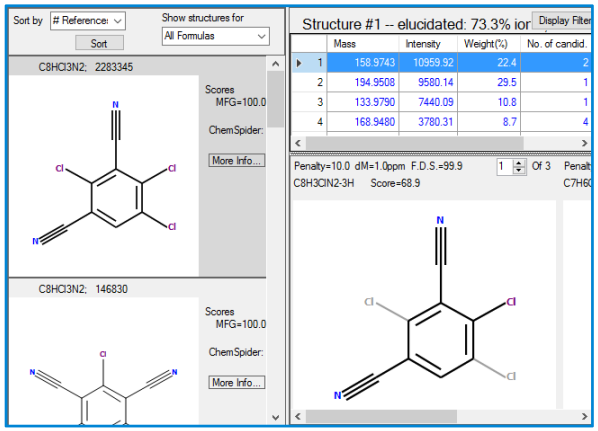


Using MS/MS Capability for Unknowns Identification
Don’t skip the ‘Q’ in Q/TOF

MS/MS CID @ 35 eV



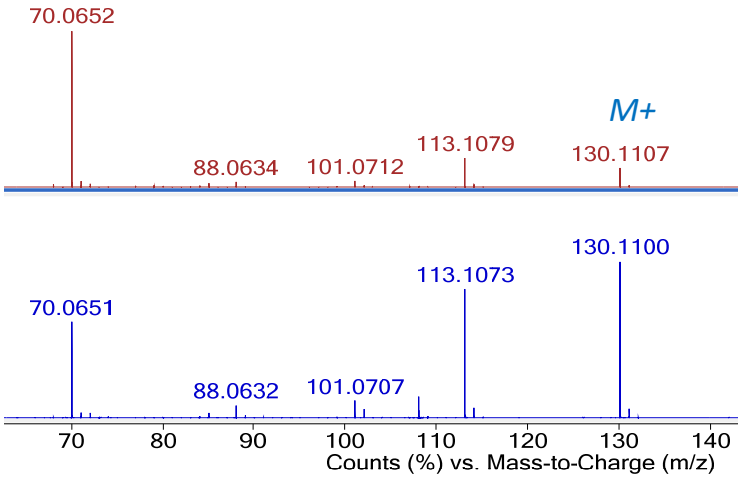
Molecular Structure Correlator (MSC)



Most likely: ***2,4,5-Trichloroisophthalonitrile***.
A degradation product of Chlorothalonil

Comparison between CI and Low Energy EI Spectra

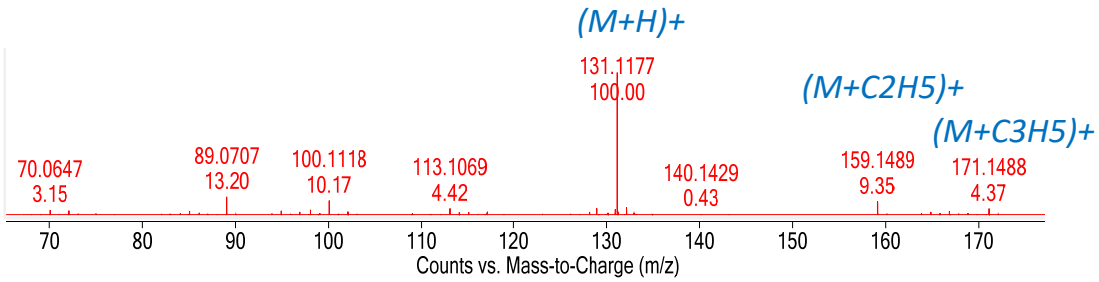
N-nitroso-N-propyl-1-Propanamine



70 eV

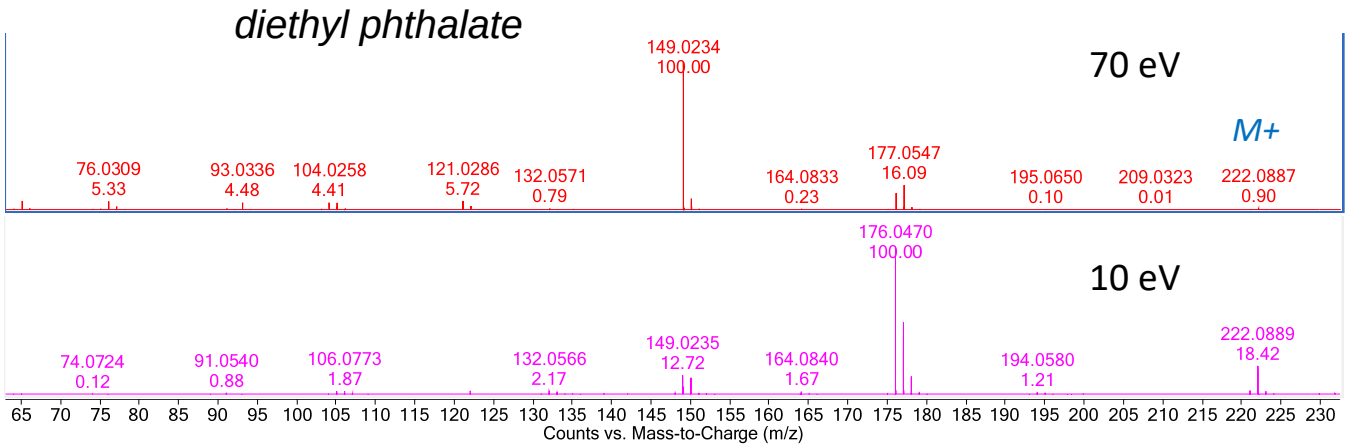
12 eV

EI

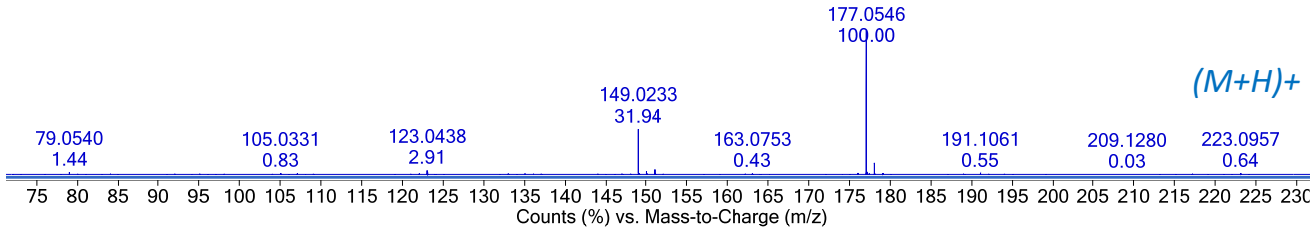


PCI

Comparison between CI and Low Energy EI Spectra



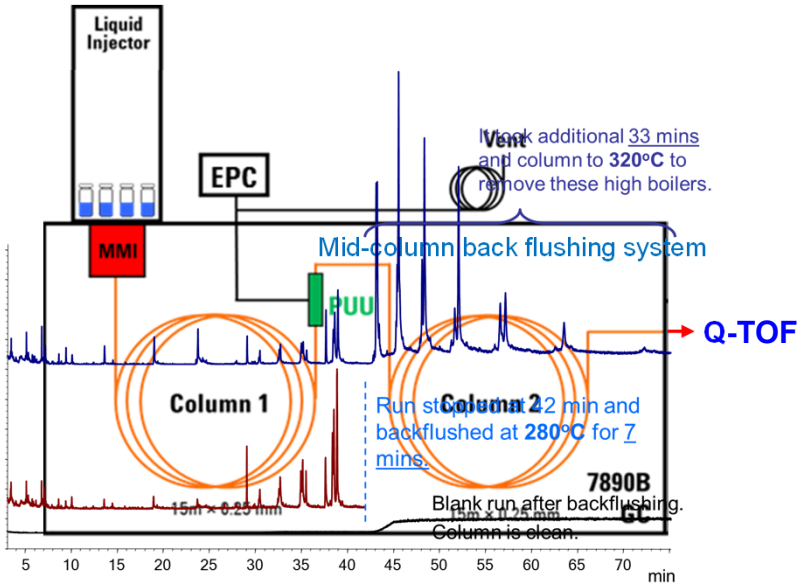
EI

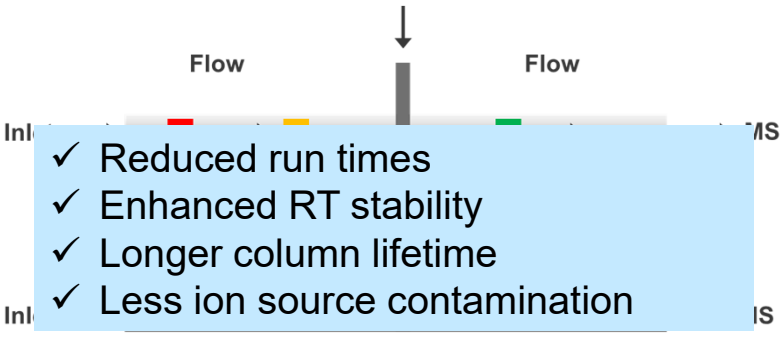


PCI

GC/Q-TOF Method Conditions
Mid-column Backflush

GC and MS Conditions:	EI	Negative CI	Positive CI
GC	8890		
Column	2 x HP-5MS UI, 15 m, 0.25 mm, 0.25 µm		
Inlet	MMI, 4-mm UI liner single taper w wool		
Injection volume	1 µL		
Injection mode	Cold splitless		
Inlet temperature	60°C for 0.2 min; 600°C/min to 320°C		
Oven temperature program	60°C for 1 min; 40°C/min to 170°C, 10°C/min to 310°C, 3 min hold		
Carrier gas	Helium		
Column 1 flow	~1.2 mL/min		
Column 2 flow	~ 1.4 mL/min		
Backflushing conditions	5 min (Post-run), 310 °C (Oven), 50 psi (Aux EPC pressure), 2 psi (Inlet pressure)		
Transfer line temperature	280°C		
Mass range	50 to 650 m/z		
Spectral acquisition rate	5 Hz		
Quadrupole temperature	150°C		
Source temperature	280°C	150°C	280°C
Electron energy	70 eV	250 eV	100 eV
Emission current	5 µA	10 µA	15 µA





- ✓ Reduced run times
- ✓ Enhanced RT stability
- ✓ Longer column lifetime
- ✓ Less ion source contamination

Pressure drops at inlet

GC/Q-TOF Method Conditions & Sampling

GC and MS Conditions:	EI	Negative CI	Positive CI
GC	8890		
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Inlet	MMI, 4-mm UI liner single taper w wool		
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Backflushing conditions	5 min (Post-run), 310 °C (Oven), 50 psi (Aux EPC pressure), 2 psi (Inlet pressure)		
Transfer line temperature	280°C		
Mass range	50 to 650 m/z		
Spectral acquisition rate	5 Hz		
Quadrupole temperature	150°C		
Source temperature	280°C	150°C	280°C
Electron energy	70 eV	250 eV	100 eV
Emission current	5 µA	10 µA	15 µA

Sample Collection

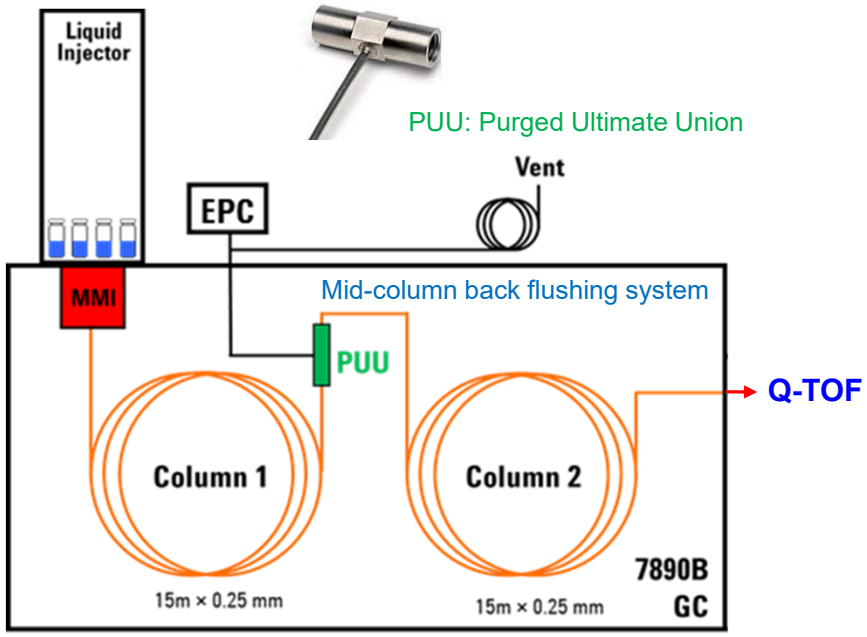
Replicate samples of Wastewater effluent collected on four different days.

First two days of WW samples displayed acute toxicity with WET testing; while other two showed no toxicity

Sample Preparation

- 1 L samples filtered with 0.45 um GF/F
- Solid phase extraction with ethyl acetate and methanol elution
- Filters were sonicated and extracted with hexane/acetone
- Both extracts combined and spiked with DBOFB as ISTD

Configuration Optimized with Backflushing



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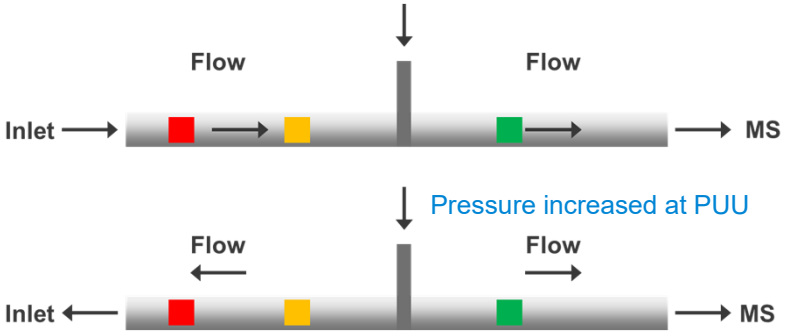
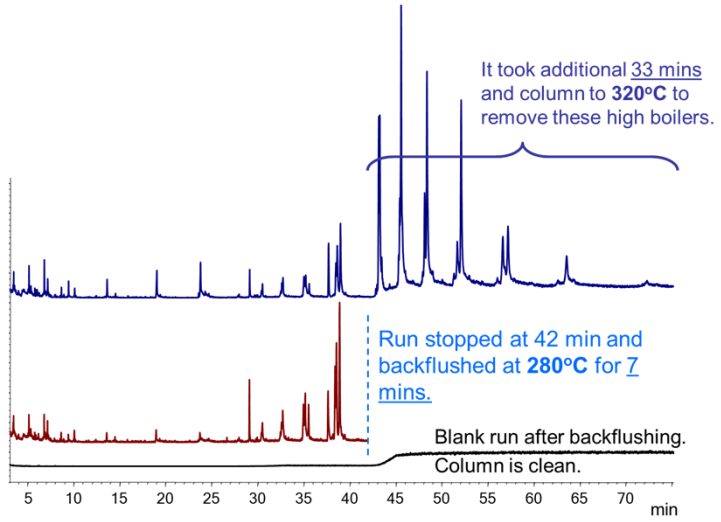


Pressure drops at inlet



Screening Workflow with GC/Q-TOF

Acquire full-spectrum data

Targeted Method

Yes

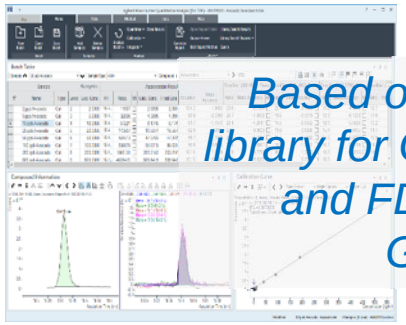
Calibrate?

No

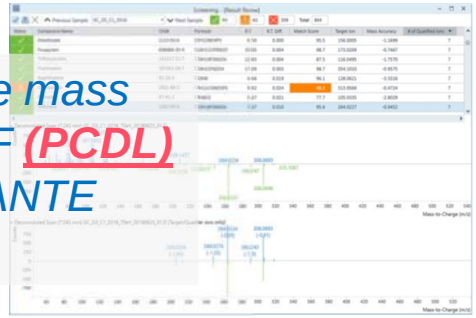
Targeted Quantitation

Suspect Screening

Non-Targeted Screening



*Based on accurate mass library for GC/Q-TOF **(PCDL)** and FDA and SANTE Guidelines*

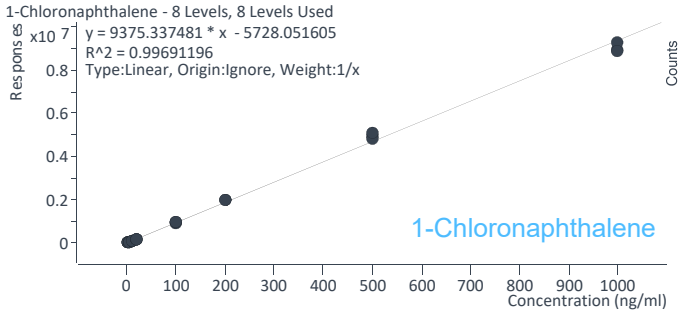


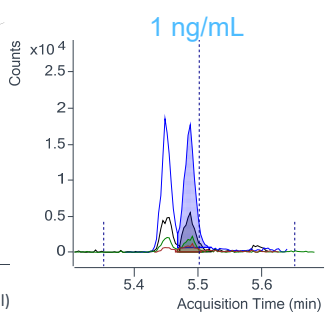
NIST

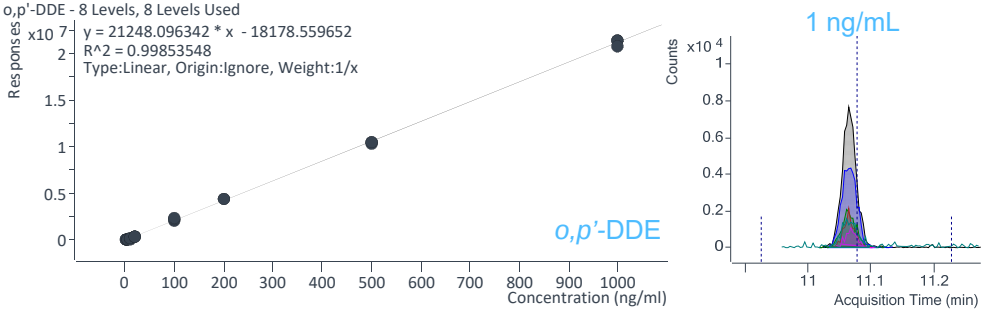
Deconvolution followed by matching public libraries

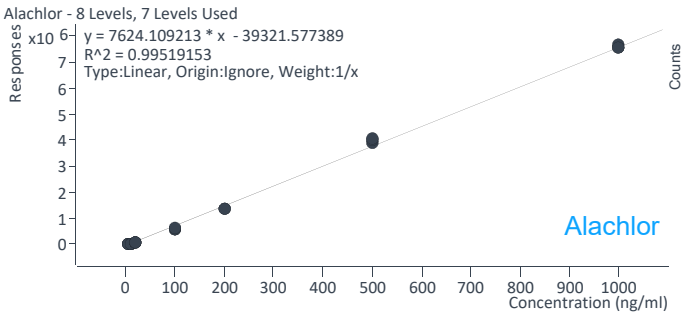
One Software

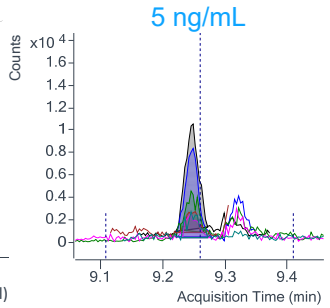
Calibration Range (1-1000 ng/mL)

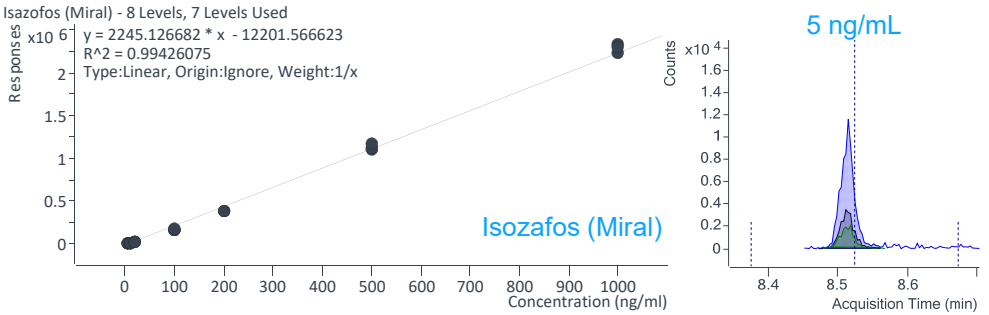


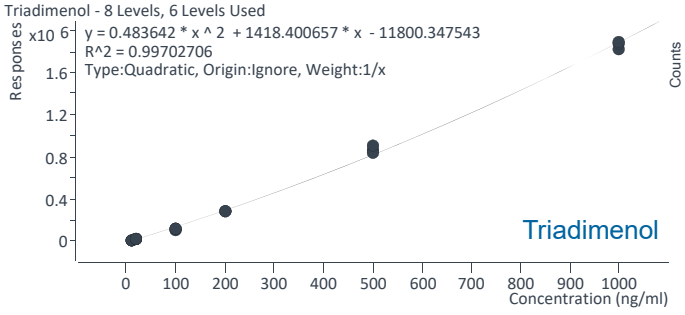


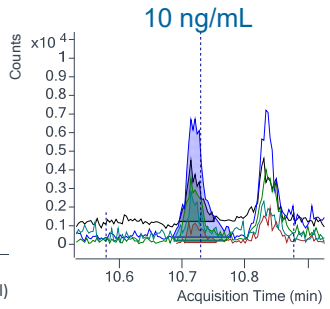


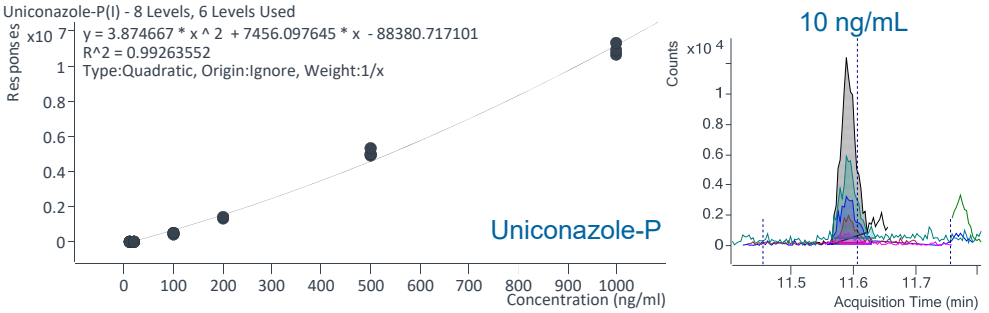






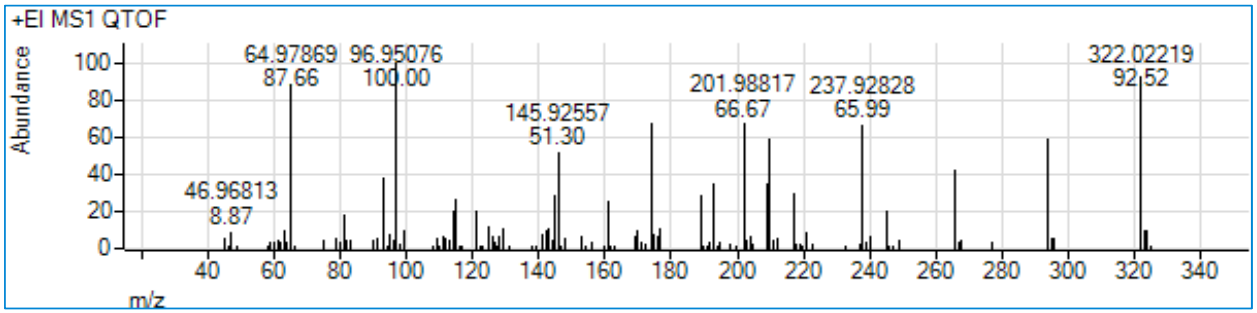


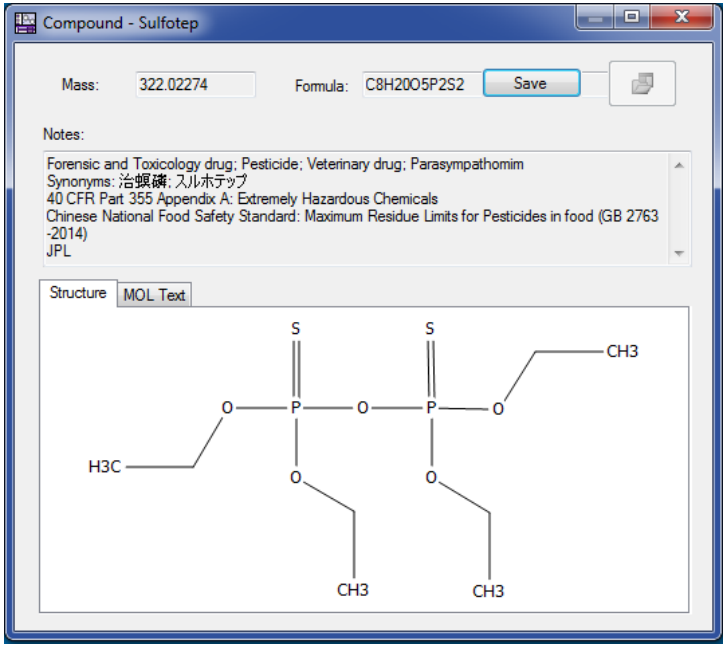


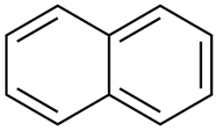


GC/Q-TOF Accurate Mass Library *(PCDL)*

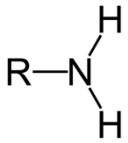
- Over **1,000** compounds
- High Resolution Spectra
- Expert curation
- Includes a wide range of environmental contaminants and pesticides



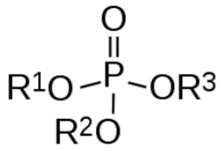




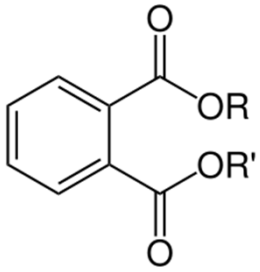
PAHs



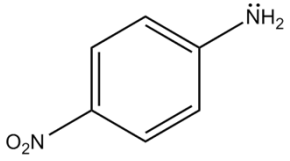
Amines



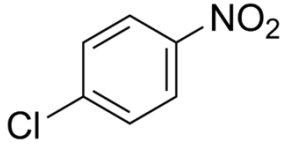
Organophosphates



Phthalates



Nitroanilines

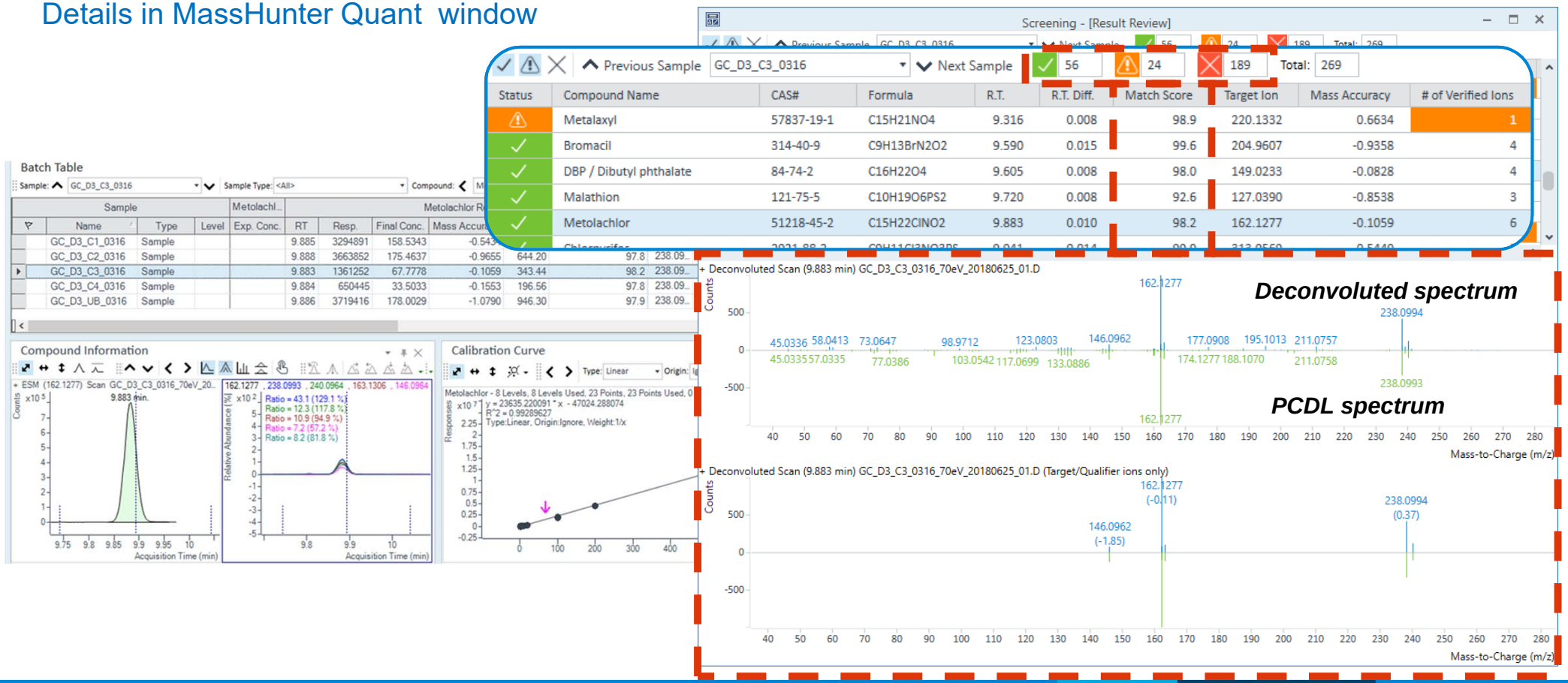


Chloronitrobenzenes

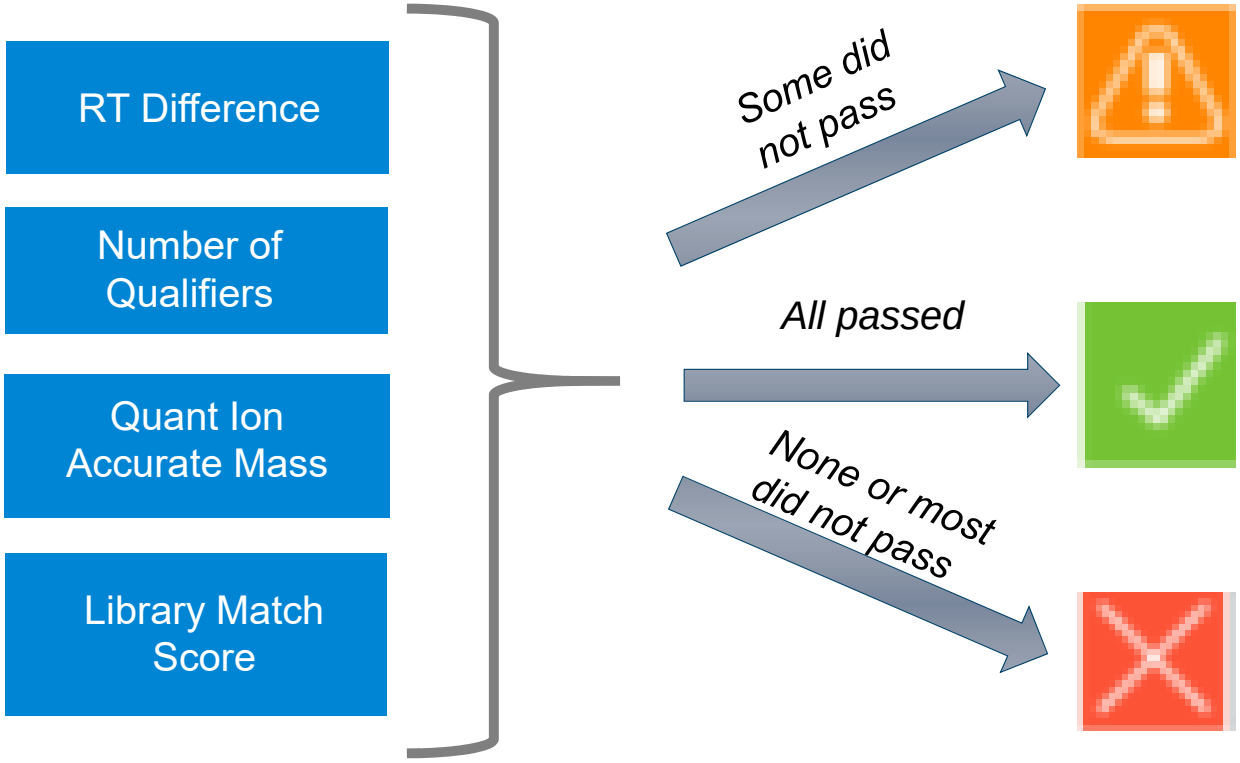
Screening: Results Review

Details in MassHunter Quant window

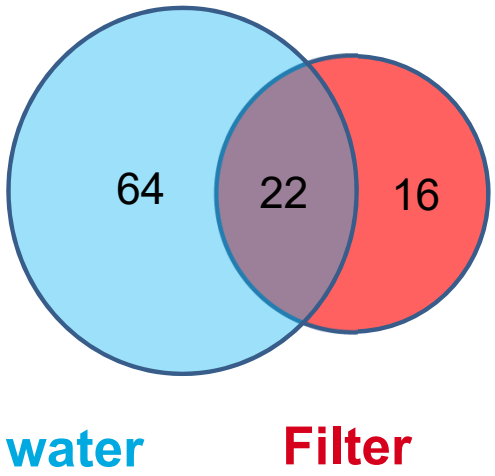
Summary in Screening window



Criteria for Compound Verification



The Impact of Sample Filtration

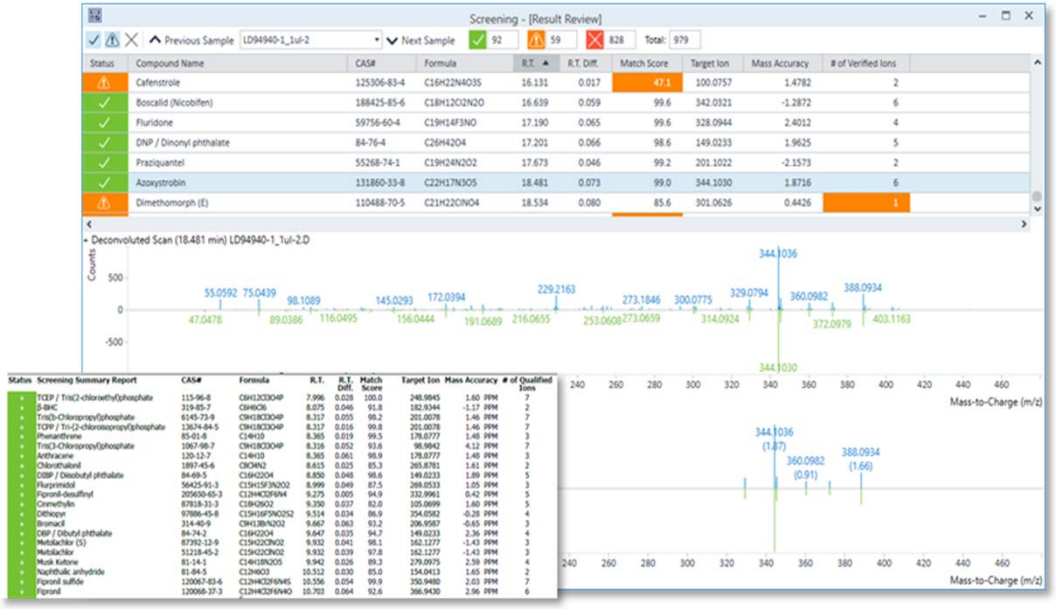


Compounds uniquely identified in the filter extract:

Diphenylamine (DFA)
Hexachlorobenzene
Pentachloroaniline
Fluoranthene
Pyrene
Nonachlor-trans
p,p'-DDD
Dihexylphthalate
Bifenthrin
Chrysene
cis-Permethrin
trans-Permethrin
Benzo[b]fluoranthene
Benzo[a]pyrene
Dinonylphthalate
Indeno[1,2,3-cd]pyrene

Suspect Screening with EI

- 90 compounds identified in each wastewater effluent using the PCDL in EI mode
- Criteria:
  - Mass accuracy: <4 ppm
  - Match Score: >75
  - RT difference:
  - No. of Ions:



Sample	80 % mortality						20 % mortality						0 % mortality					
	LD94940-1			LD94940-2			LD94941-1			LD94941-2			LD94943-1			LD94943-2		
Compound Name	Response	Mass Error	Library Match score	Response	Mass Error	Library Match score	Response	Mass Error	Library Match score	Response	Mass Error	Library Match score	Response	Mass Error	Library Match score	Response	Mass Error	Library Match score
TBEP / Tris(2-butoxyethyl) phosphate	2013504	2.8	99.9	1502528	3.9	99.9	1289372	2.5	99.9	1559301	3.8	99.9	787113	3.1	99.9	784473	3.8	99.9
tert-Butylphenyldiphenylphosphate	16799	2.1	92.9	4948	3.2	74.6	2828	1.1	82.5	10468	0.8	91.9	2950	1.3	70.6	2766	0.8	91.9
Chlorantraniliprole	6298	0.2	76.8	5330	2.0	79.4	3572	1.7	63.2	3494	1.8	66.4	3458	1.1	52.4	2710	1.8	66.4
Flurprimidol	16518	1.3	80.4	15240	0.5	76.4	10698	2.6	73.7	12065	2.1	80.2	6038	2.0	74.2	4976	2.1	80.2
Paclobutrazol	16985	0.9	96.8	15763	1.6	98.7	10725	0.9	92.4	12090	2.1	94.9	9106	1.8	79.1	8448	2.1	94.9
TBZ / Thiabendazole	1570235	1.4	99.7	1536170	2.4	99.7	1282402	0.6	99.7	1368732	2.2	99.8	774093	0.6	99.7	675439	2.2	99.8
Azoxystrobin	134463	1.8	99.1	139960	3.0	98.9	109579	1.4	98.9	119004	1.7	98.8	104804	1.7	89.9	94511	1.7	98.8

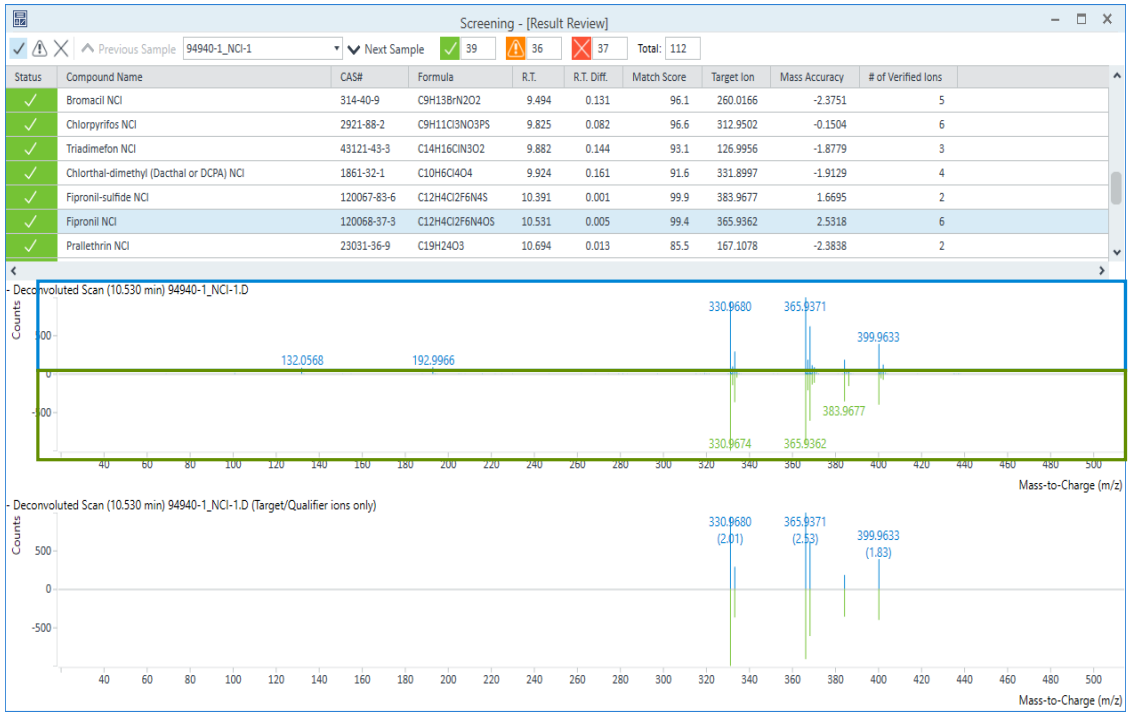
EI PCDL-based screening in MassHunter. Automatically verified compounds are labeled in green. The compounds that need additional review are in orange

Compounds in EI that were identified with large difference in response values between toxic and non-toxic samples

Suspect Screening with NCI

~40 additional compounds identified in NCI with suspect screening

NCI PCDL-based screening in MassHunter.



Actual spectra obtained from sample

Standard spectra in Agilent PCDL

Suspect Screening with NCI

80% mortality **20% mortality** **0% mortality**

Sample	94940-1		94940-2		94941-1		94941-2		94943-1		94943-2	
Compound Name	Response	Library Match Score	Response	Library Match Score	Response	Library Match Score	Response	Library Match Score	Response	Library Match Score	Response	Library Match Score
Deltamethrin	16837	71.4	14202	42.7	6474	83.7	4138	36.3	3253	56.5	4294	36.3
Endosulfan sulfate	3372	98.8	3013	91.9	12182	98.9	11865	99.1	18103	98.8	15859	99.1
Fipronil sulfone	1184481	99.4	989538	99.3	1058932	99.4	898204	99.3	1218463	99.4	1089462	99.3
Chlorfenvinphos	22450	94.7	13196	86.3	16668	94.4	14469	94.7	14757	95.7	12834	94.7
Fipronil	1312800	98.7	1269915	97.7	1255881	97.1	1307988	96.8	1519654	95.8	1350814	96.8
Fipronil-sulfide	201344	99.9	192041	100.0	224062	100.0	218654	100.0	241255	100.0	274001	100.0
Chlorthal-dimethyl	1730		1409	94.1	1468	94.3	1489	96.2	2204	80.0	1807	96.2
Triadimefon	22376	94.1	16547	94.2	19705	96.0	17006	96.4	18710	97.2	16675	96.4
Malathion	474	86.6	249	86.6	0	-	0	-	0	-	0	-
Fipronil-desulfinyl	128886	97.8	111722	97.8	122423	97.8	119001	97.9	164450	97.8	135773	97.9
Chlorothalonil	23789	99.4	12226	99.2	14367	99.0	15765	99.1	14714	99.2	12680	99.1
BHC-beta	36573	88.4	19696	91.7	25594	81.4	19439	84.7	23983	69.4	13527	84.7
Dicloran	30089	92.3	33303	93.2	34005	92.6	39632	93.8	44118	95.1	35911	93.8
Hexachlorobenzene	13573	99.3	10353	99.6	11863	99.3	9934	99.1	14371	98.7	12048	99.1
Trifluralin	10334	86.6	11119	94.1	12089	94.2	11454	92.8	13550	94.5	9293	92.8
2,4-Dinitrotoluene	81406	90.1	91627	89.0	75770	84.4	67256	83.3	43423	91.1	41979	83.3
2,4,6-Trichlorophenol	2551498	92.2	2250861	91.6	2525758	91.5	2544336	91.4	2707308	91.2	2736603	91.4

Compounds in NCI that were identified with large difference in response values between toxic and non-toxic samples

Workflow Strategy

Acquire full-spectrum data

Targeted Method

Yes

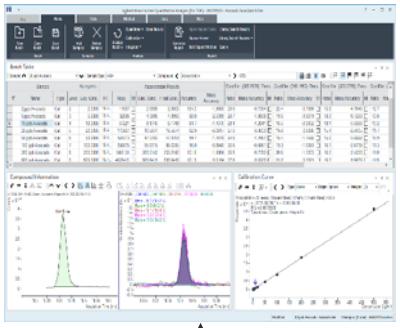
Calibrate?

No

Targeted Quantitation

Suspect Screening

Non-targeted Analysis

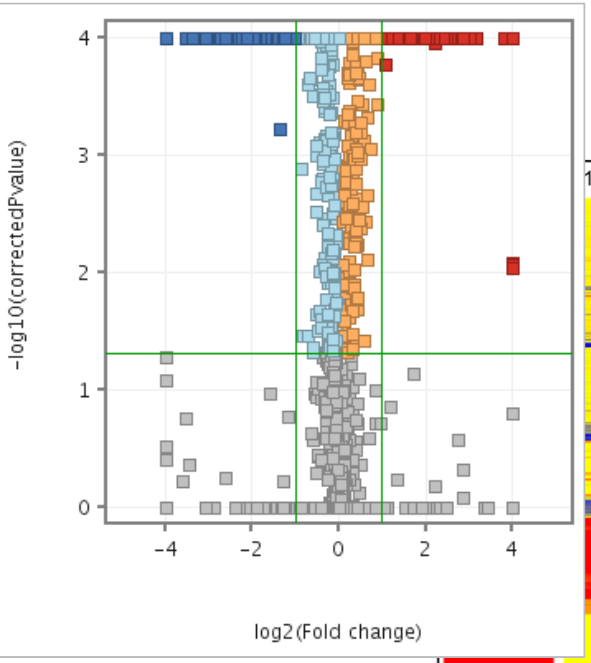


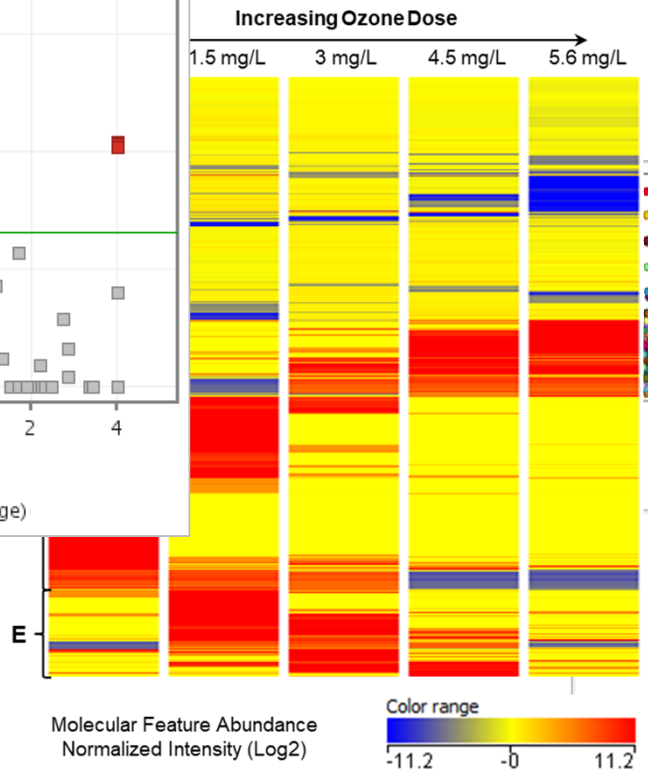
NIST

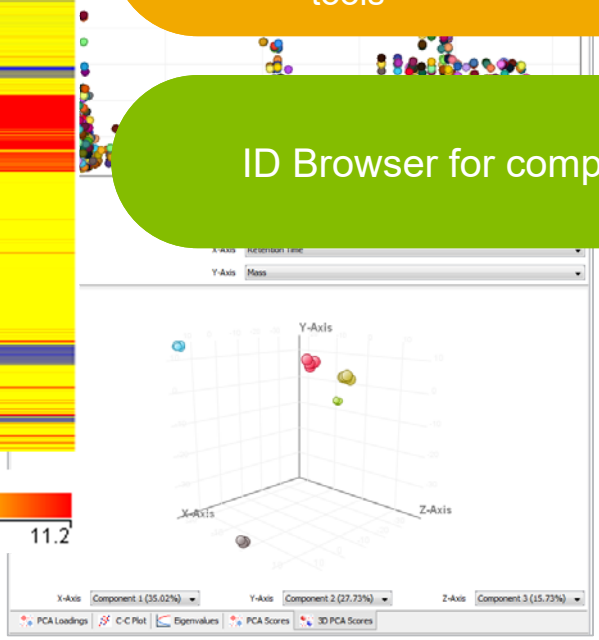
Deconvolution followed by matching public libraries

Suspect added to target

Mass Profiler Professional





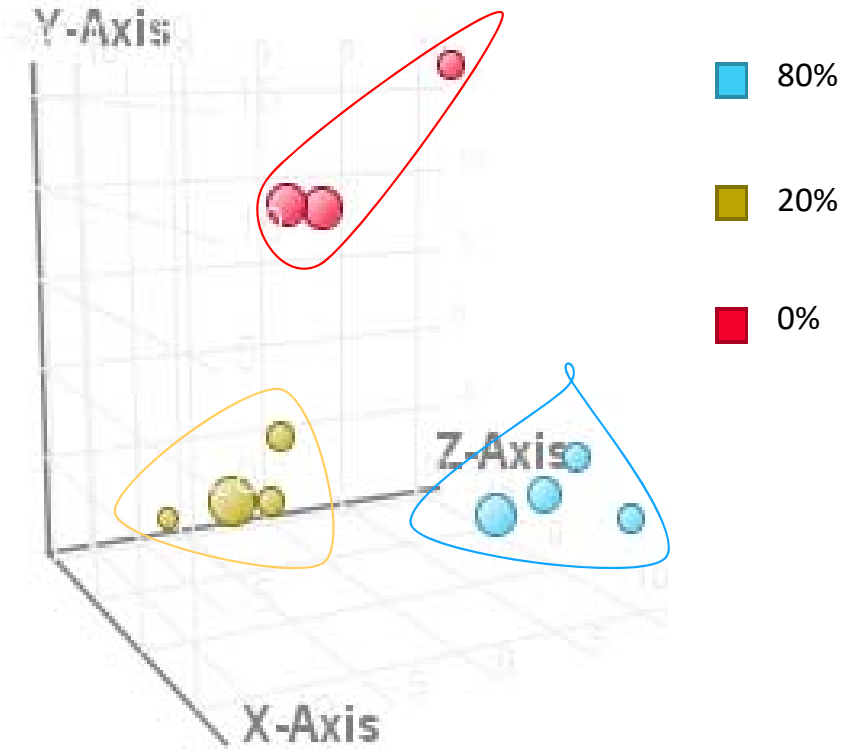


Import, store, and visualize
- Agilent LC/MS TOF, Q-TOF, and QQQ
- Agilent GC/MS Quad, QQQ, and Q-TOF
- Agilent ICP-MS, Generic file import (NMR data)

Performs many types of statistical analysis
ANOVA, clustering, PCA, class prediction tools

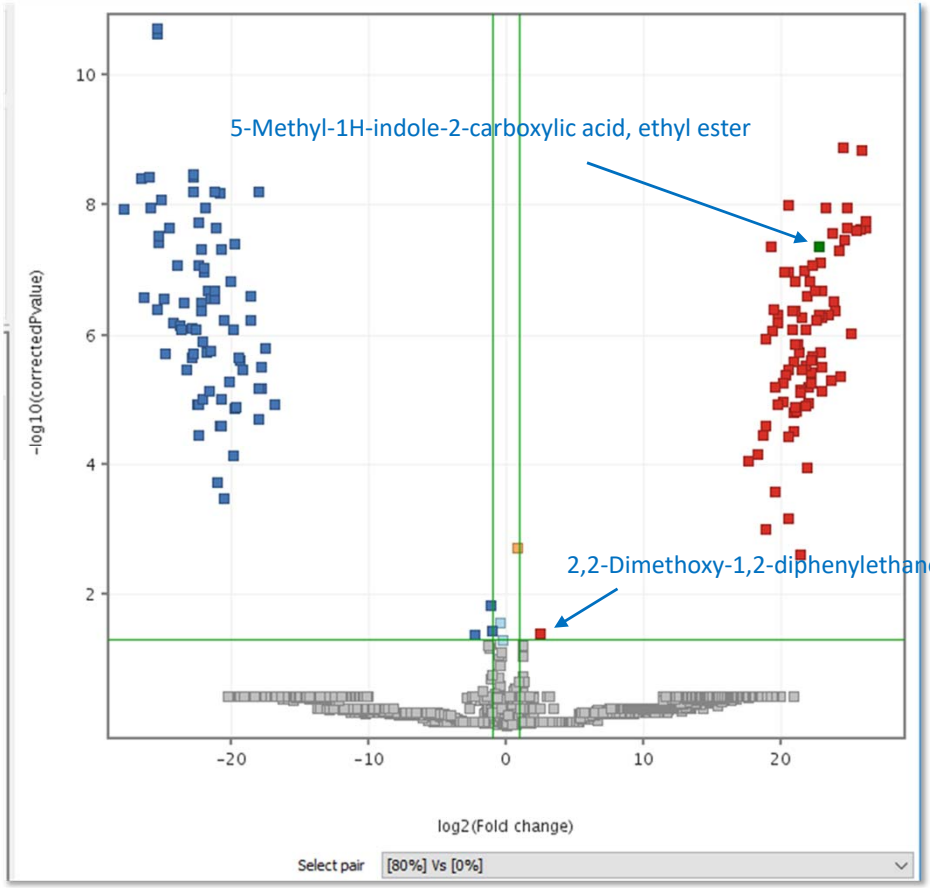
ID Browser for compound identification

Unknown Analysis using MPP
Principal Component Analysis



Unknown Analysis using MPP Volcano Plot

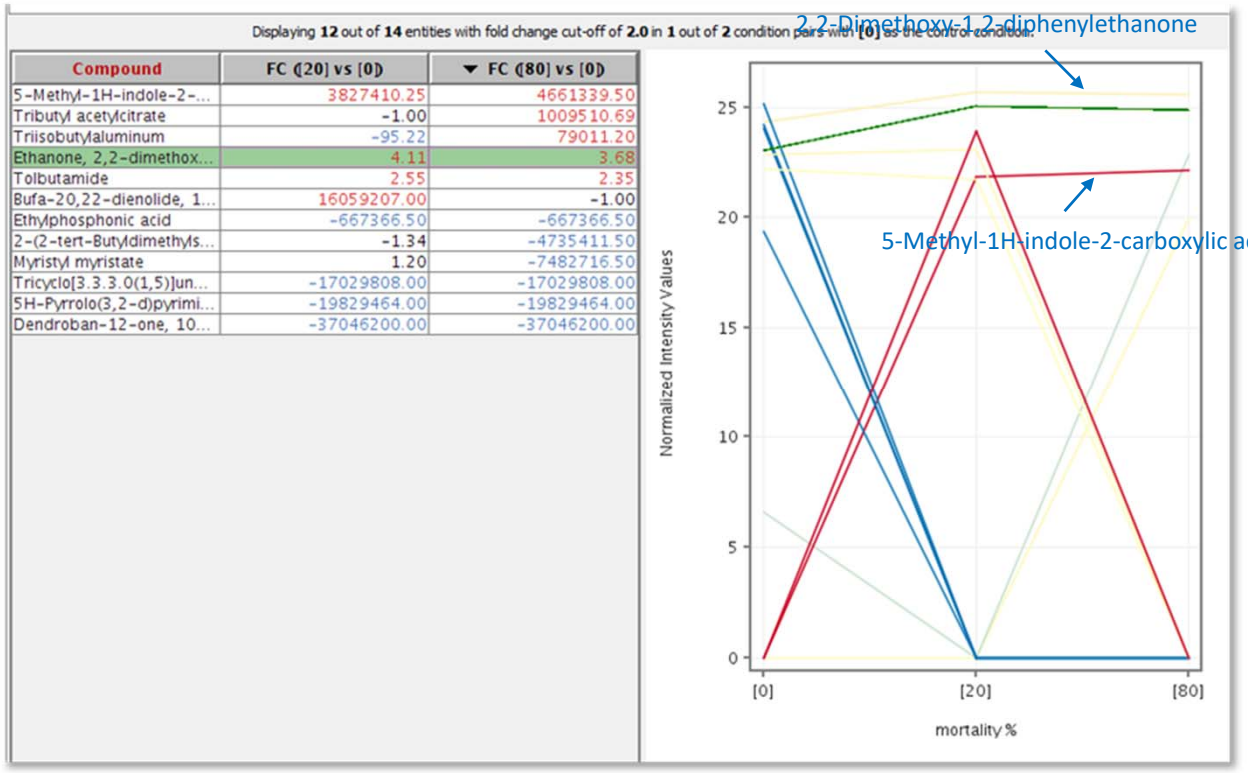
Compounds uniquely present at higher levels statistically in 0% mortality samples



Compounds uniquely present at higher levels statistically in 80% mortality samples

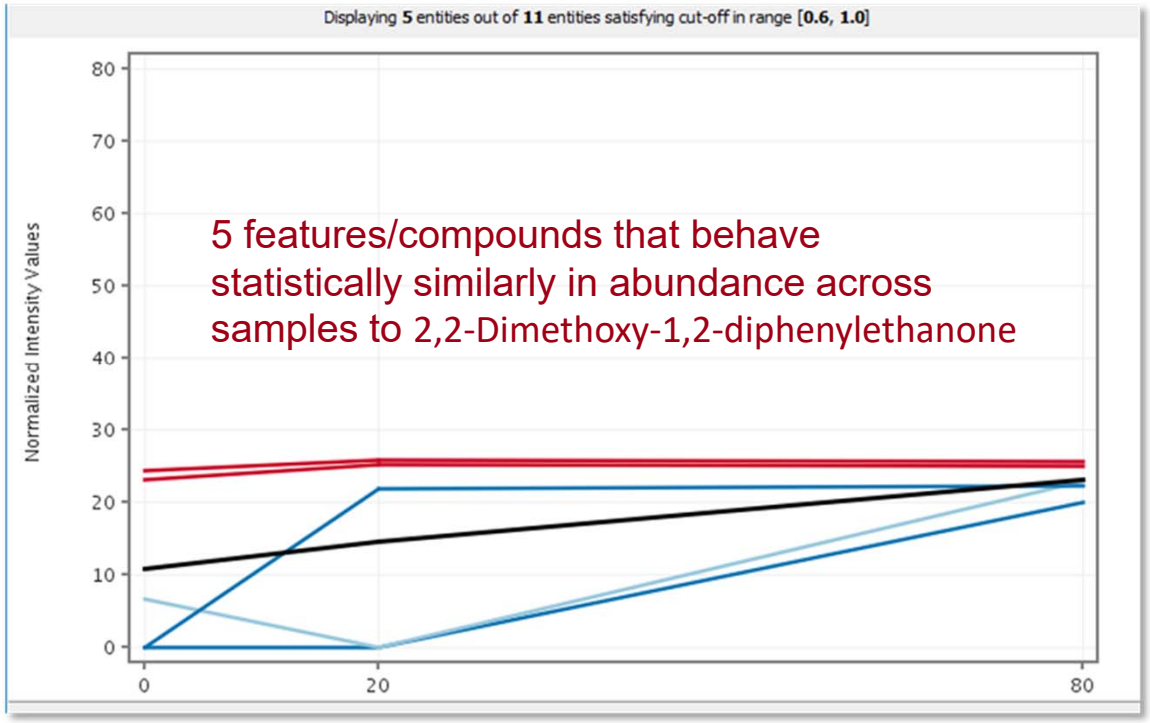
Compounds present at similar levels in both sets of samples

Unknown Analysis using MPP
Fold Change Analysis



Compound	FC ([20] vs [0])	FC ([80] vs [0])
5-Methyl-1H-indole-2-...	3827410.25	4661339.50
Tributyl acetylcitrate	-1.00	1009510.69
Triisobutylaluminum	-95.22	79011.20
Ethanone, 2,2-dimethox...	4.11	3.68
Tolbutamide	2.55	2.35
Bufa-20,22-dienolide, 1...	16059207.00	-1.00
Ethylphosphonic acid	-667366.50	-667366.50
2-(2-tert-Butyldimethyls...	-1.34	-4735411.50
Myristyl myristate	1.20	-7482716.50
Tricyclo[3.3.3.0(1,5)]un...	-17029808.00	-17029808.00
5H-Pyrrolo(3,2-d)pyrimi...	-19829464.00	-19829464.00
Dendroban-12-one, 10...	-37046200.00	-37046200.00

Unknown Analysis using MPP Correlation Analysis

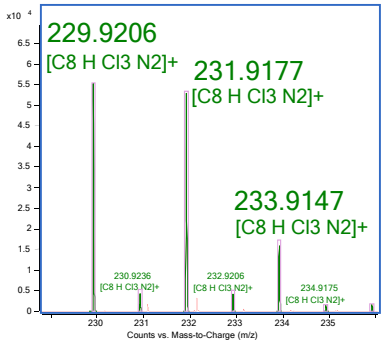


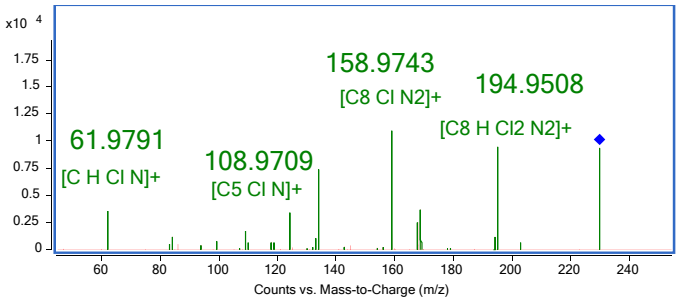
Entities | Attributes

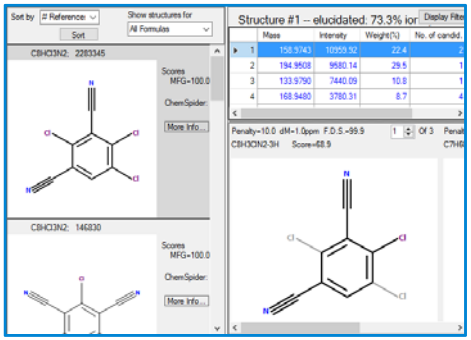
Compound	Similarity	Alignment Value	Annotations	CAS Number
Tributyl acetylcit...	0.97073	Tributyl acetylcit...	Tributyl acetylcit...	77-90-7
Ethanone, 2,2-d...	0.64071	Ethanone, 2,2-d...	Ethanone, 2,2-d...	24650-42-8
Triisobutylalumi...	0.86491	Triisobutylalumi...	Triisobutylalumi...	100-99-2
Tolbutamide	0.63448	Tolbutamide	Tolbutamide [C...	64-77-7
5-Methyl-1H-in...	0.70139	5-Methyl-1H-in...	5-Methyl-1H-in...	1000318-48-3

Unknowns Structure Elucidation

Step 1: Confirm M^+ | Step 2: Confirm fragment ions | Step 3: Structure elucidation on candidate







low electron energy @ 12 eV

MS/MS CID @ 35 eV

Most likely: ***2,4,5-Trichloroisophthalonitrile***.
A degradation product of Chlorothalonil

The compound was identified using Molecular Structure Correlator tool with accurate mass product ion spectrum as an input

Conclusions

- A comprehensive workflow that includes targeted quantitation, suspect screening as well as a non-targeted approach was applied to screen for environmental pollutants in water samples
- An accurate mass GC/Q-TOF library was used to successfully set up the target extraction method to perform both target quantitation and suspect screening for pesticides and environmental contaminants
- Low energy EI and accurate mass MS/MS facilitate untargeted screening and structure elucidation of unknowns

Acknowledgement

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Thanks for your attention!