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National Environmental Monitoring
Virtual Conference
August 3rd, 2020

Non-Target and Suspect-Screening of Reuse Water by Large-Volume Injection Liquid Chromatography and High Resolution Mass Spectrometry.
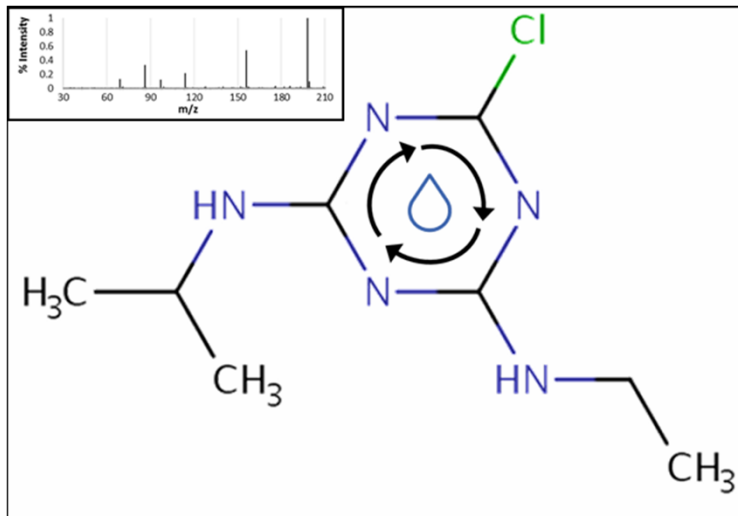
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PROTECTING, MAINTAINING AND IMPROVING THE HEALTH OF ALL MINNESOTANS

CLEAN WATER LAND & LEGACY AMENDMENT

CLEAN WATER LAND & LEGACY AMENDMENT

- Water scarcity is increasing

- Alternative water sources need to be investigated

- Wastewater, stormwater, industrial water, roof-runoff, grey water, etc.

- Irrigation, cleaning, cooling, toilet-flushing

- Understand chemical makeup

Targeted versus Non-target Analysis

- Pre-selection of analytes

- Robust and sensitive

- Quantitative

- Limited in scope

- Reliance on reference standards

- Continued development and validation

# Targeted versus Non-target Analysis
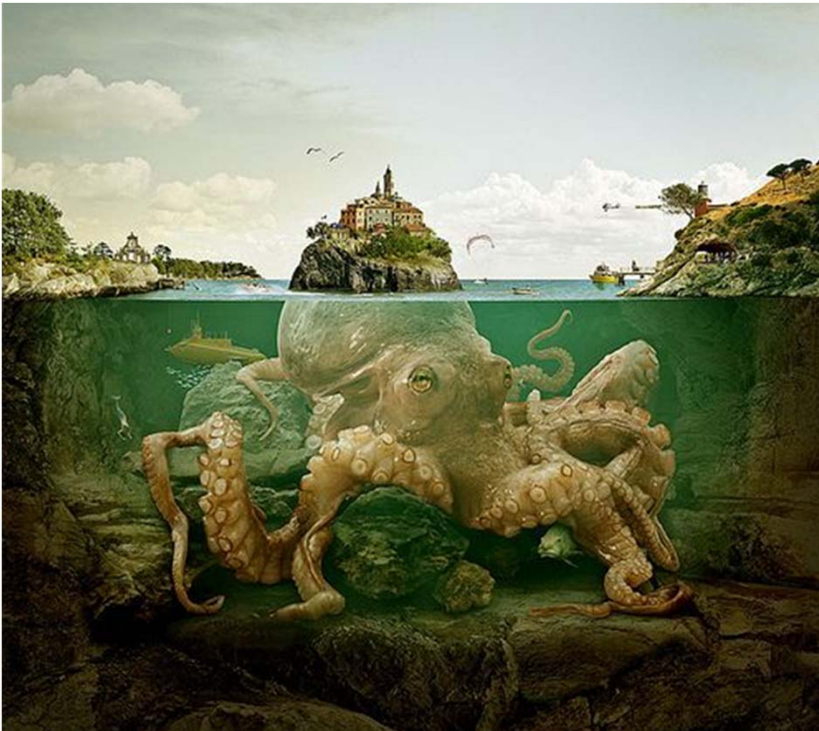
- Suspect selection

- Reference standards not required

- Expansive in scope

- Retroactive analysis

- Sample/site comparisons

- Initial development and validation

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- Development choices bias results

  - Sample Preparation, Chromatography, Mass Spectrometer, Prioritization, and Data Processing

- Qualitative

- Less sensitive than targeted methods

- Reference standards required for unequivocal IDs

- Data intensive

- Laborious data analysis

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Non-target and Suspect Screen Overview
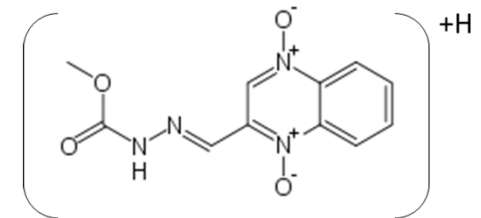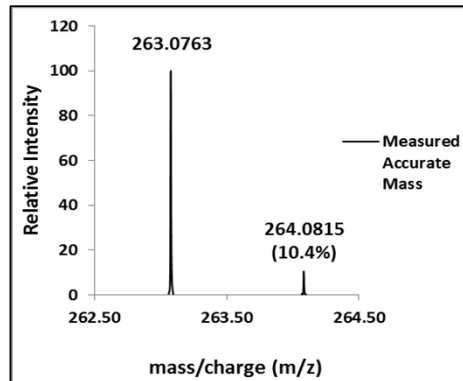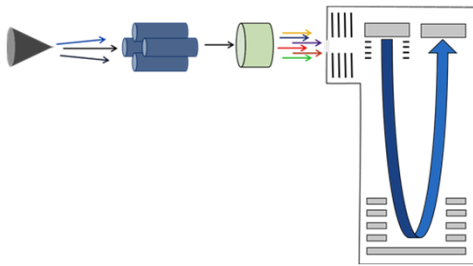
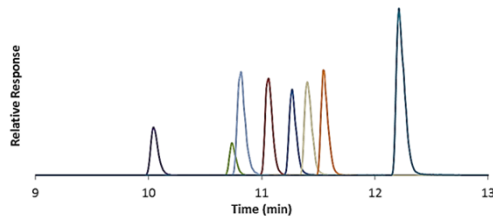- Quadrupole time-of-flight mass spectrometer

- Molecular formula information

- Molecular formula information

- Structural information

- Carbadox

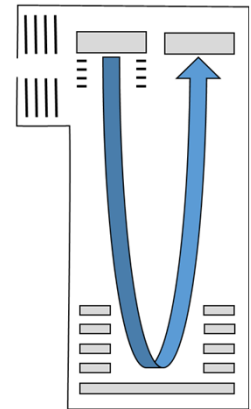- $[C_{11}H_{10}N_4O_4+H]^+$

- 263.0775 Da

Instrumental Parameters

HPLC

- Inject 650 µL (LVI)

- Biphenyl Column (+)

- HPH Phenyl-Hexyl Column (-)

- 25 minute gradient

- Rt stability was within 0.2 min

Mass Spectrometry

- (MS1) 110-875 m/z

- (MS2) 30-850 m/z

- 25 Da SWATH Windows

- 34K Resolution @ ~350 m/z

- Mass error < 3.5 ppm

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7

Retention Time Models

RM1 (Retention Model 1)
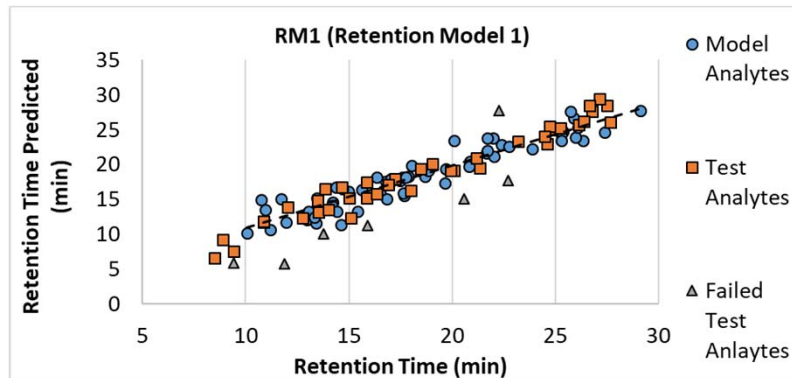
- $11+1.9(\log K_{ow})+0.26(\alpha)-5.4(i°)-0.56(iHBD)$

- $R^2 = 0.89$

- $R^2_{Pred} = 0.87$

- Standard Error = 1.67 min

RM2 (Retention Model 2)
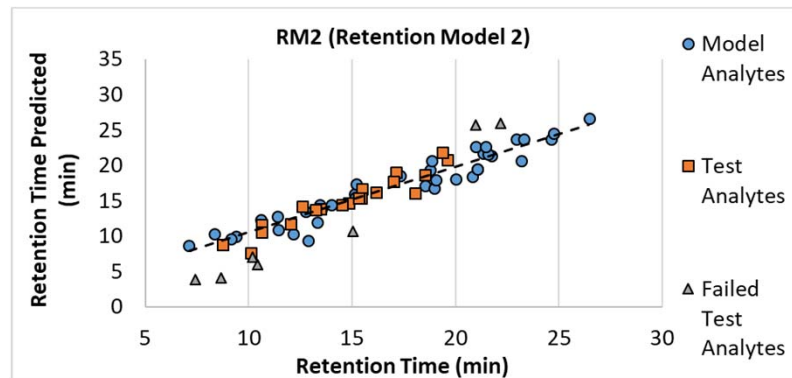
- $8.9 +2.1(\log K_{ow})+0.27(\alpha)-3.1(i°)-1.1(iHBD)-2.6(Carb)$

- $R^2 = 0.93$

- $R^2_{Pred} = 0.87$

- Standard Error = 1.50 min

Samples

Name	Site Description	Water Source	Water Use
A	University Campus	Drinking Water	Irrigation
B	Gov. Campus/Park/DWTP	Stormwater & Filter Backwash	Irrigation
C	Baseball Field	Roof Runoff	Irrigation/Toilets
D	Retail Center	Stormwater	Irrigation
E	Casino	Wastewater & Stormwater	Irrigation
F	Wastewater Treatment Plant	Wastewater	Cooling/Irrigation/Street Sweeping
G	Townhome Development	Stormwater	Irrigation
H	Motor Pool Garage	Roof Runoff	Irrigation /Vehicle Washing

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Feature Reduction and Metrics

Data Reduction	Before	Remaining	Total % Reduction
Integration Quality < 0.5	87462	47729	45
Peak Height < 10000	47729	19205	78
Non-Dups	19205	17030	81
Peak Area RPD < 35	17030	16524	81
Adducts (Na, K)	16524	15036	83
Isotopes	15036	12698	85
Peak Height < 80x Reference	12698	11183	87
Manual Removal	11183	9070	90

Sample	Total Features	Total Area (Millions)	Average Area/Feature	% Unique Features
A	972	716	740000	83
B	304	160	530000	36
C	551	206	370000	52
D	774	159	210000	59
E	763	195	260000	53
F	1513	441	290000	85
G	395	94	240000	55
H	347	128	370000	68

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A = drinking water; B = stormwater (govt. campus and park) and DWT backwash; C = rainwater (roof runoff); D = stormwater (commercial area); E = wastewater and stormwater (casino); F = wastewater (municipal); G = stormwater (residential); H = rainwater (roof runoff).

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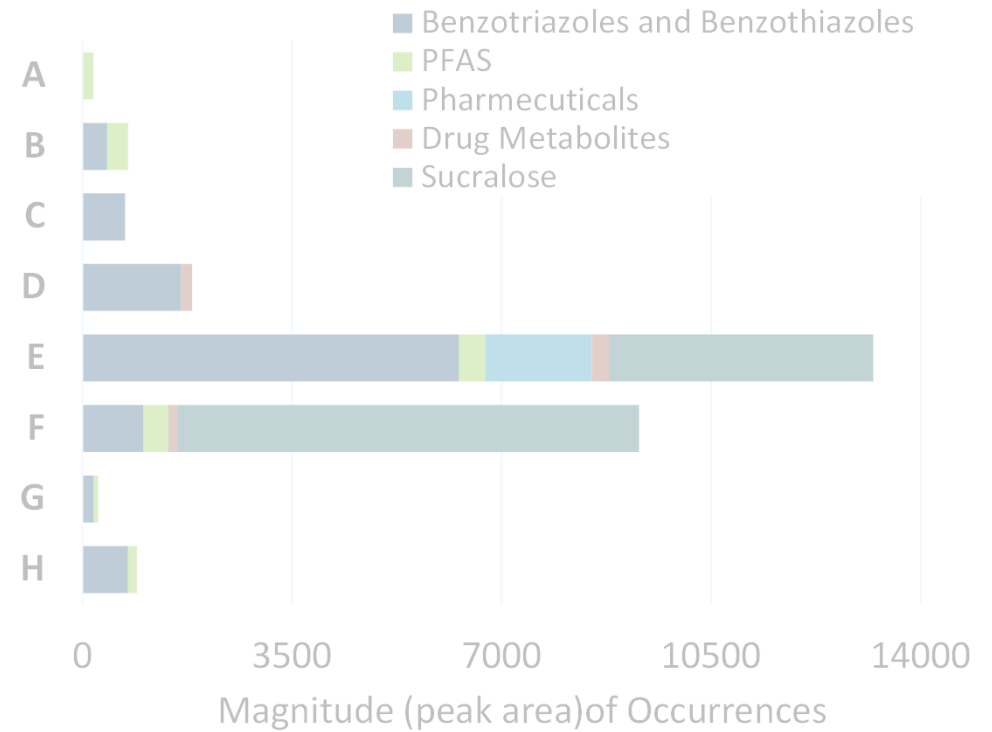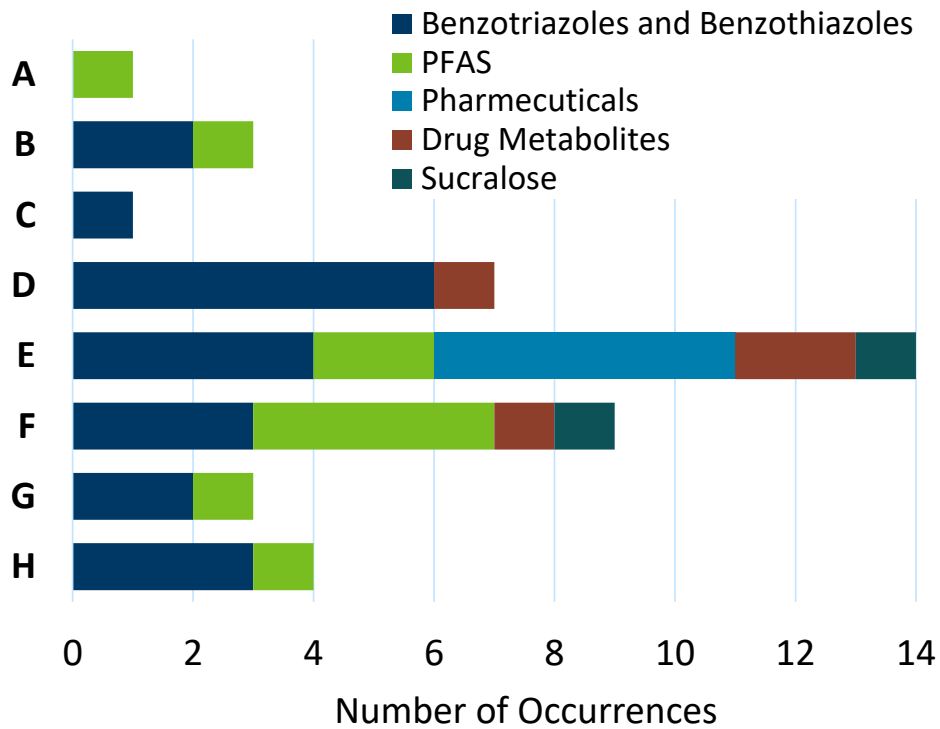# Principal Component Analysis
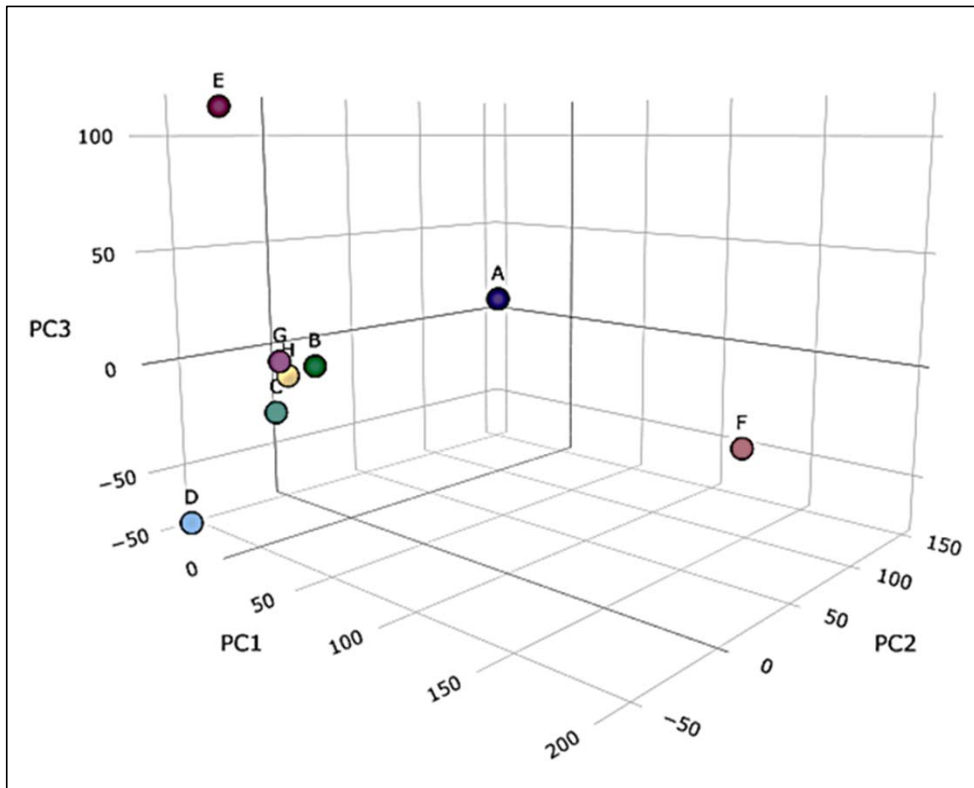
| Sample Name - Description	PC1 (31%)	PC2 (22%)	PC3 (13%)
A - City Drinking Water	-50	152	6.2
B - Stormwater (Gov. Campus and Park) and DWTB	-25	0.7	-6.7
C - Rainwater (Roof Runoff)	-26	-21	-25
D - Stormwater (Commercial/Retail Area)	-35	-62	-70
E - Wastewater and Stormwater (Casino)	-15	-51	112
F - Wastewater (Municipal)	197	17	-7.8
G - Stormwater (Residential Area)	-24	-20	-1.2
H - Rainwater (Roof Runoff)	-22	-17	-8.0

Suspect Screening

- Suspect compounds were from an in-house chemical stock

    - Rx, corrosion inhibitors, PFAS, drugs, oxy-PAHs, artificial sweeteners, hormones, and industrial compounds

- Results are biased based on selection criteria

- 20 out of 97 of the compounds were detected

- All detected compounds are unequivocally confirmed

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Suspect Screening (cont.)

Left chart — X-axis: Number of Occurrences (0–14). Y-axis categories A–H.
Legend: Benzotriazoles and Benzothiazoles, PFAS, Pharmecuticals, Drug Metabolites, Sucralose

Right chart — X-axis: Magnitude (peak area) of Occurrences (0–14000). Y-axis categories A–H.
Legend: Benzotriazoles and Benzothiazoles, PFAS, Pharmecuticals, Drug Metabolites, Sucralose

A = drinking water; B = stormwater (govt. campus and park) and DWT backwash; C = rainwater (roof runoff); D = stormwater (commercial area); E = wastewater and stormwater (casino); F = wastewater (municipal); G = stormwater (residential); H = rainwater (roof runoff).

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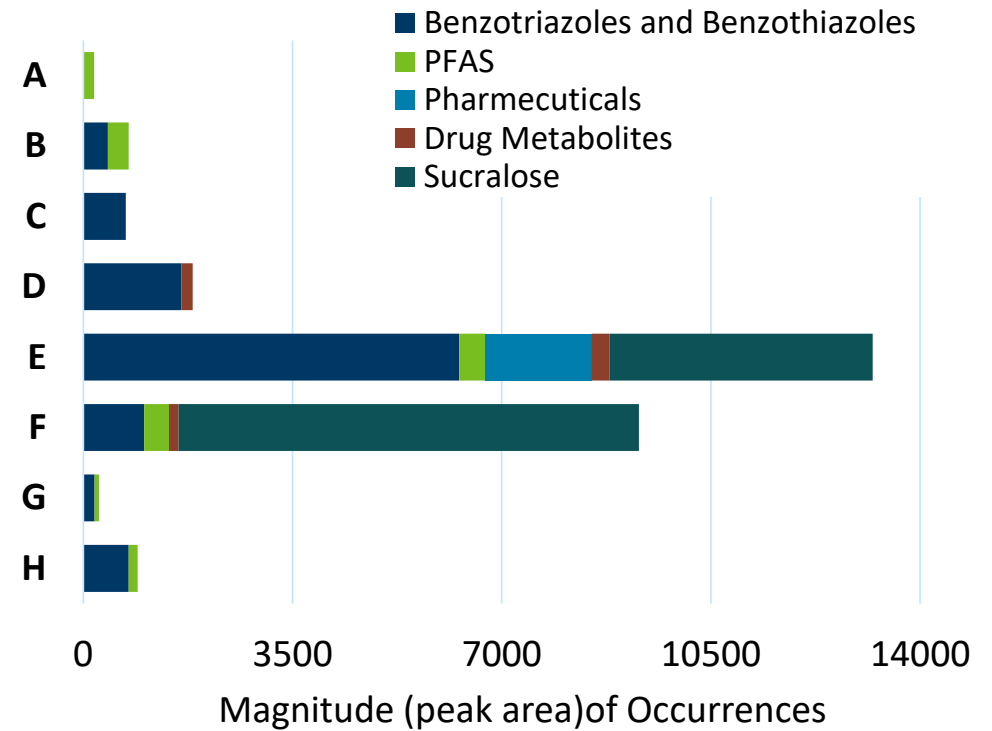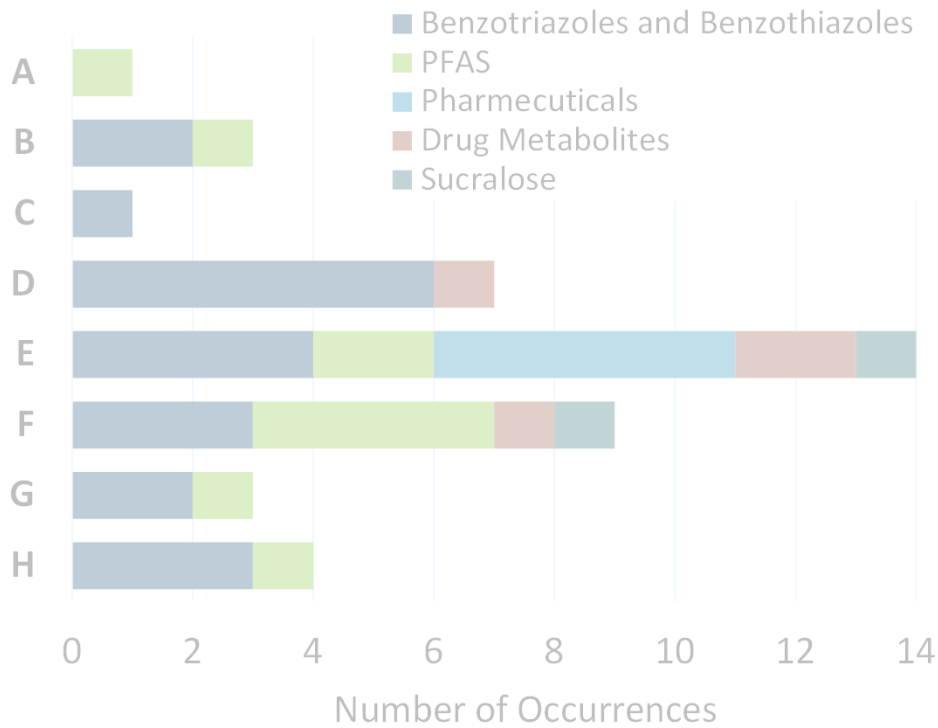
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- Identification confidence is based on the Schymanski scale

  - (1) Confirmed, (2) Probable, (3) Tentative, (4) Formula, (5) Feature

- Identifications were prioritized by library matches, peak area, and frequency of detection

  - ~ 4000 compound HRMS library from SCIEX

  - 15,000 + compound library from NIST

  - mzCloud; MetFrag; U.S. EPA Chemistry Dashboard; PubChem

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Non-Target (cont.)

- 58 features triggered library matches considered probable-identifications

  - 44 confirmed; seven refuted; seven not assessed (six matched RM predictions)

  - 17 pharmaceuticals; 19 pesticides; six industrial compounds; six biological compounds; three personal use compounds

- Features prioritized by area and frequency are focused on unique confirmed (1) or probable identifications (2).

  - 12 features were confirmed and two were probable (both matched RM predictions)

  - Nine industrial compounds; three pesticides; one pharmaceutical; one personal use compound

Non-Target (cont.)
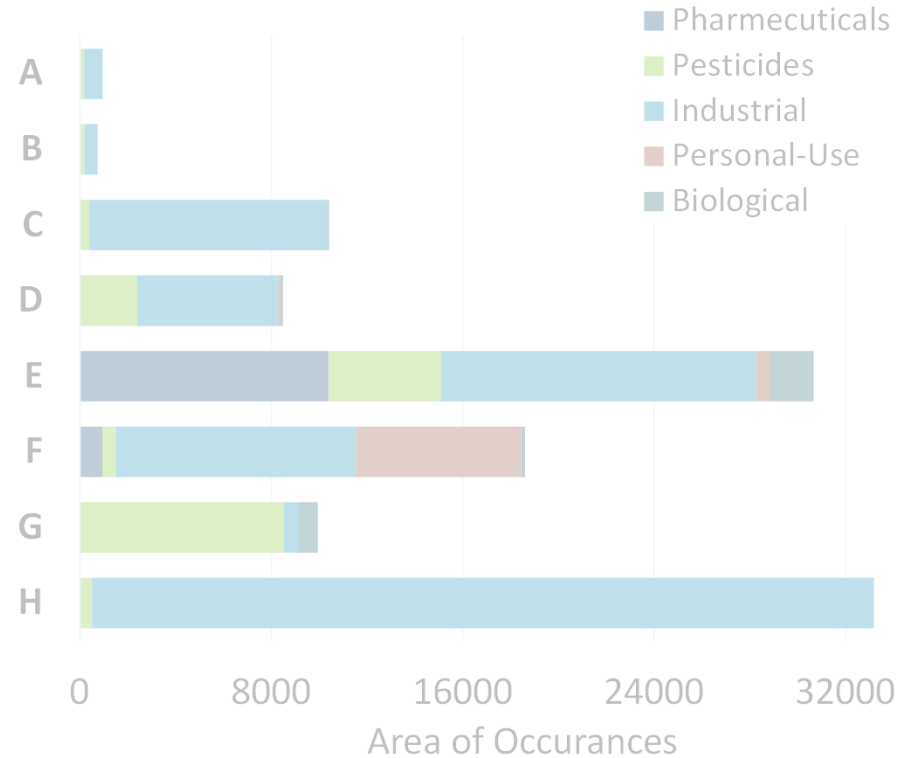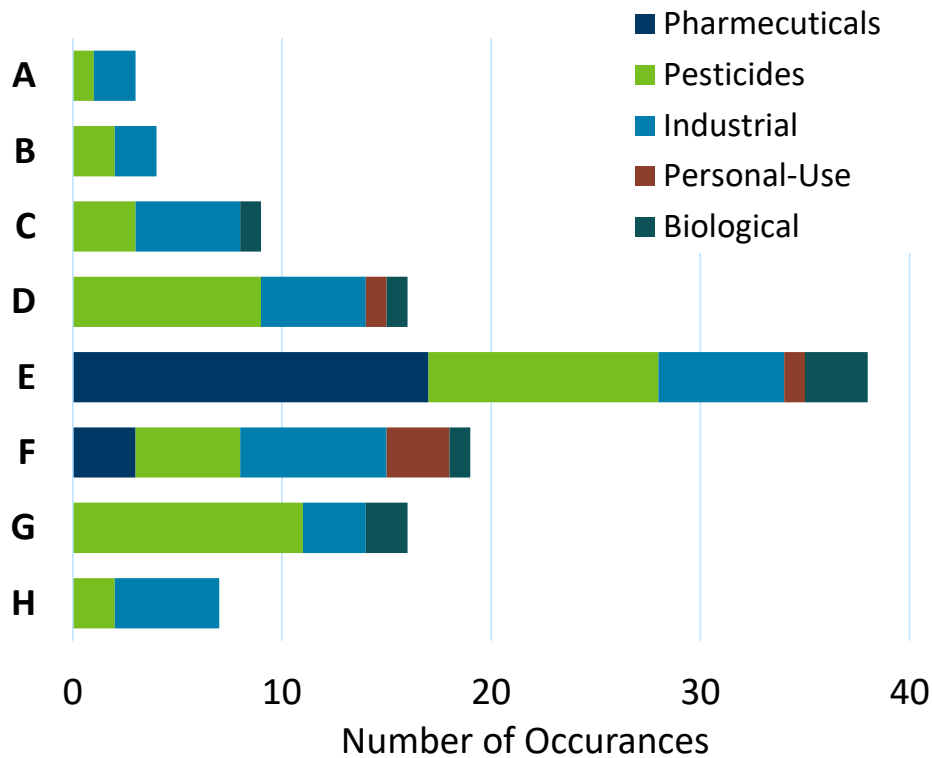
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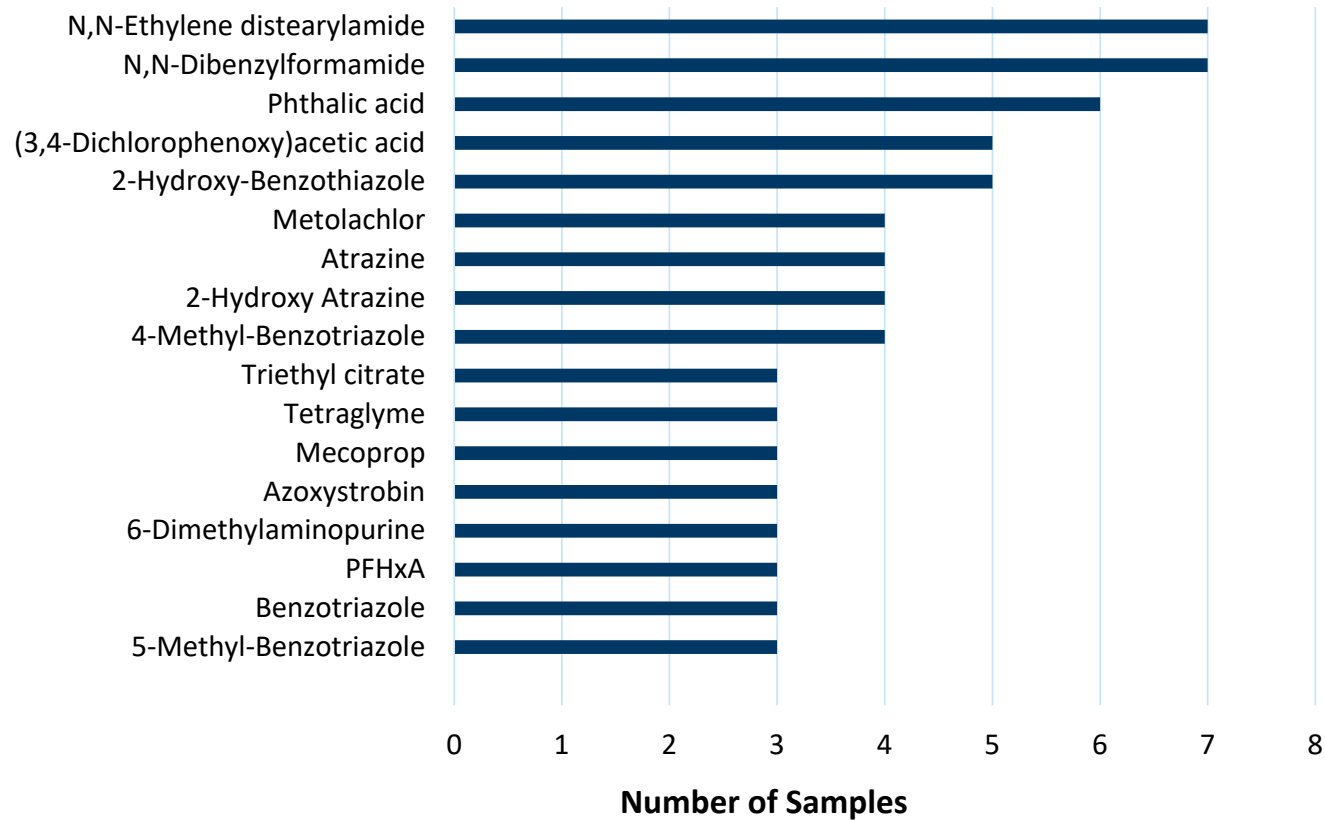
Non-Target (cont.)
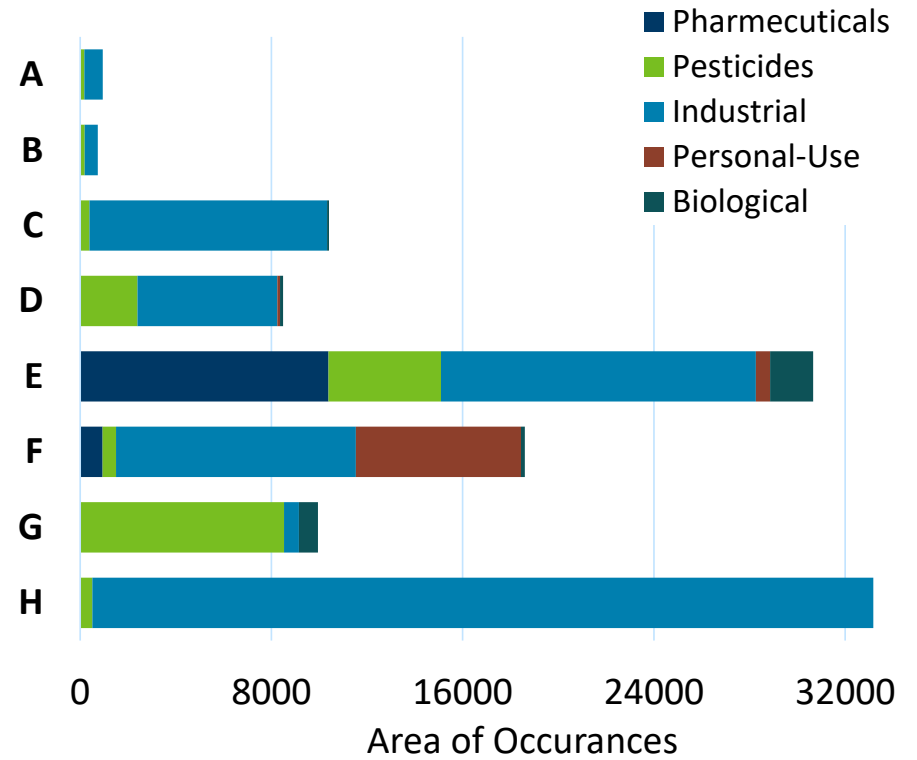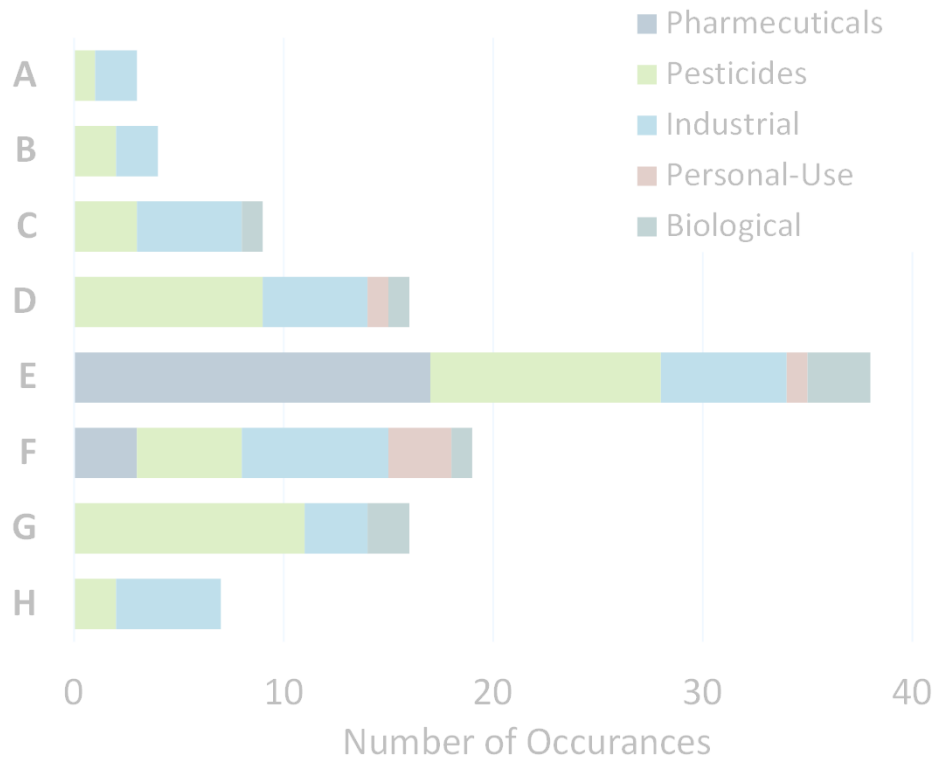
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Non-Target (cont.)

Frequently Confirmed Compounds

Number of Samples

Implications

- LVI facilitates direct sample analysis and reduces method bias

- Retention models add confidence

- Feature metrics reveal sample differences

- 82 compounds were identified or probable

- Library hits resulted in the most identifications

  - Need widely curated and open-access library data bases.

- Water reuse for irrigation or other practices increases the exposure to CECs.

  - One site uses roof runoff for community garden irrigation

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Acknowledgments

- Clean Water Land and Legacy Amendment

- Nancy Rice

- Anita Anderson

- Satoshi Ishii

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- Paul Moyer

- Stefan Saravia

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Thank you.

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