

Helping Contaminants Emerge: Non-targeted Analysis and Passive Sampling for Assessing Water Quality

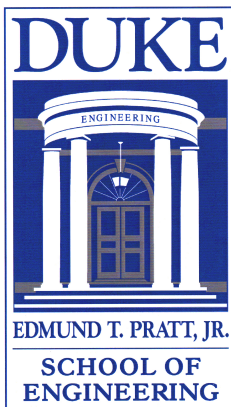
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Kirsten E. Overdahl¹, Jennifer Sun⁴, Rebecca Sutton⁴

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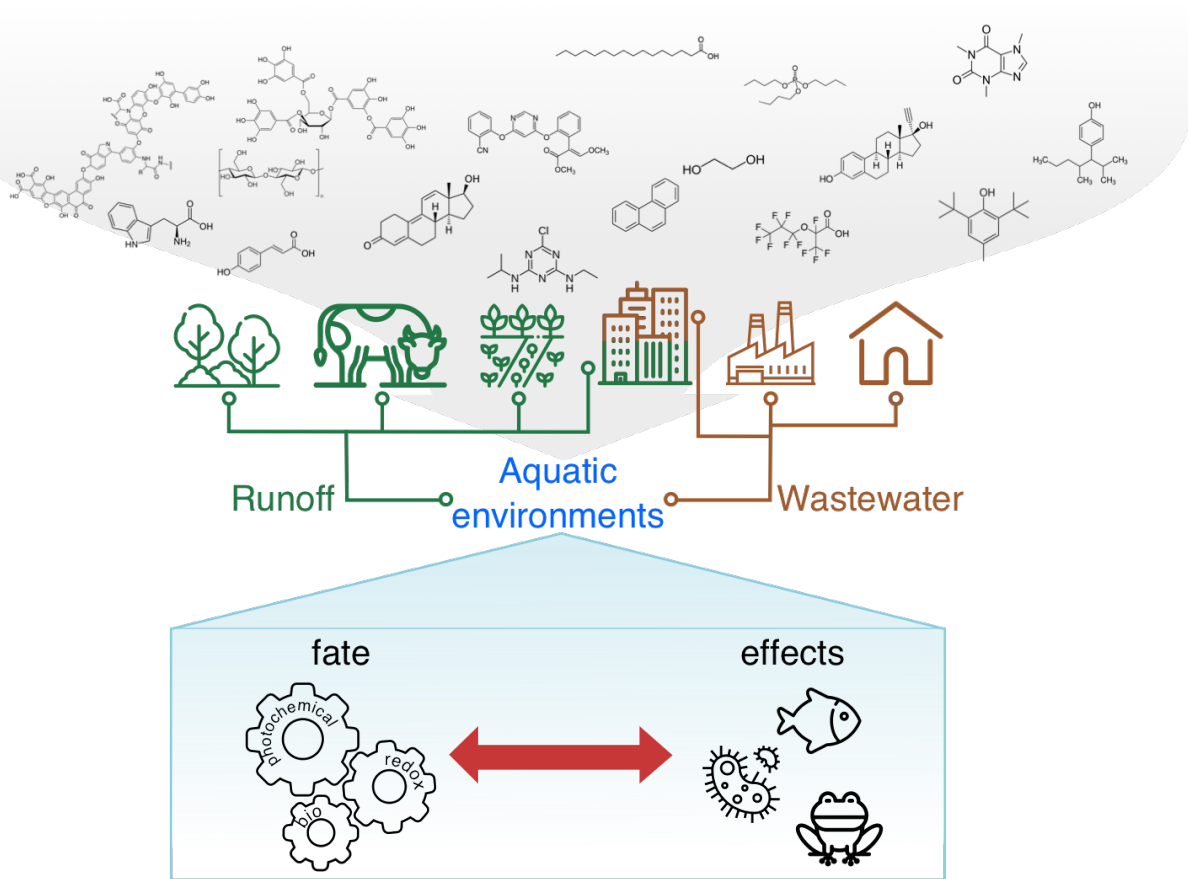
Estuary Institute



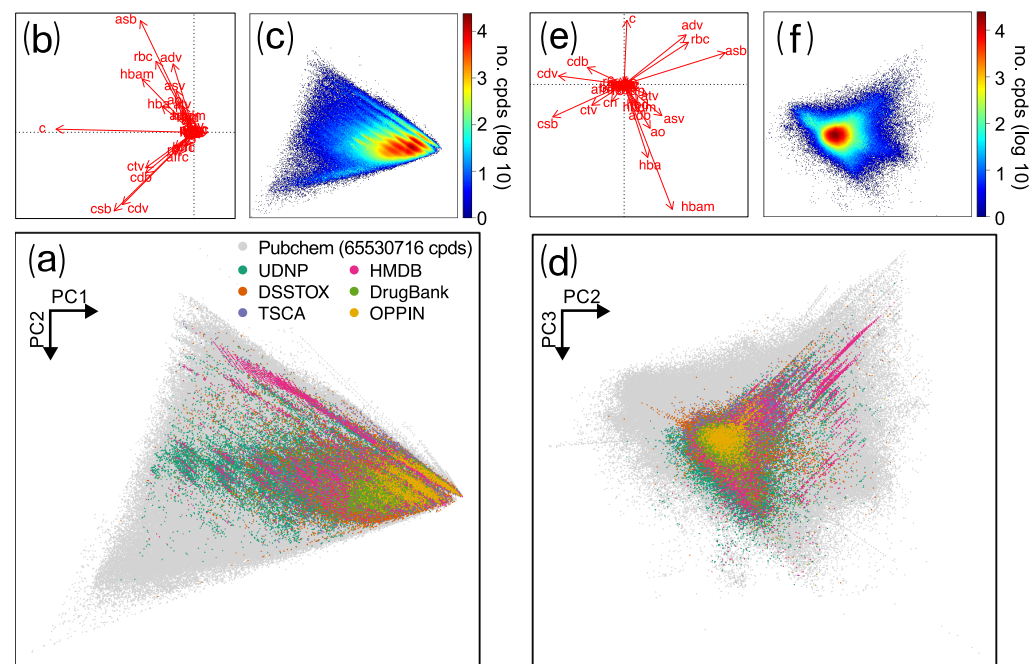
OIMA Brown Bag Webinar, April 26, 2018



Which organic pollutants occur in environmental waters?



- Chemical space is vast. How can we assess which chemicals in commerce are important as emerging pollutants in the aquatic environment?
- Chemical use, production, and regulation lists are incomplete.
- (Bio)transformation makes prioritization more difficult and compound identification challenging.



Targeted vs. Non-targeted analysis

Separation

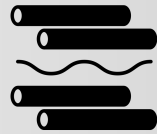
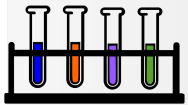
Detection

Signal

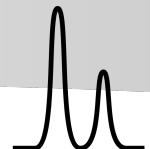
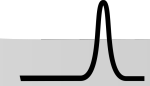
Outcome

Targeted Analysis

reference standards

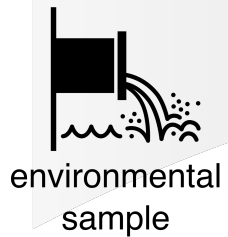


standard



sample

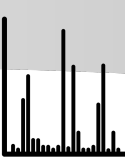
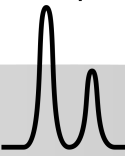
- identity
- concentration



environmental sample

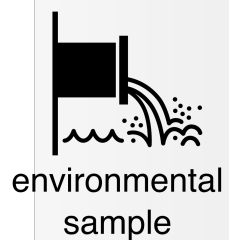
Non-targeted Analysis

sample

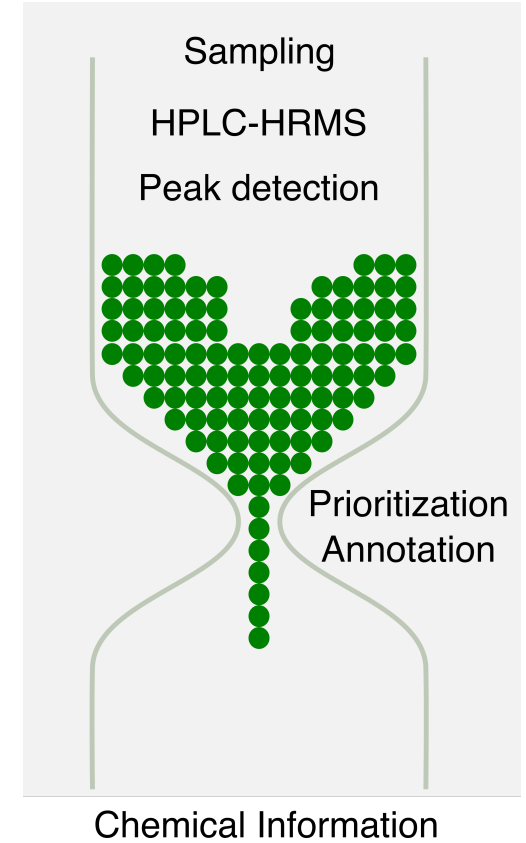


m/z

- molecular features (m/z @ ret. time)



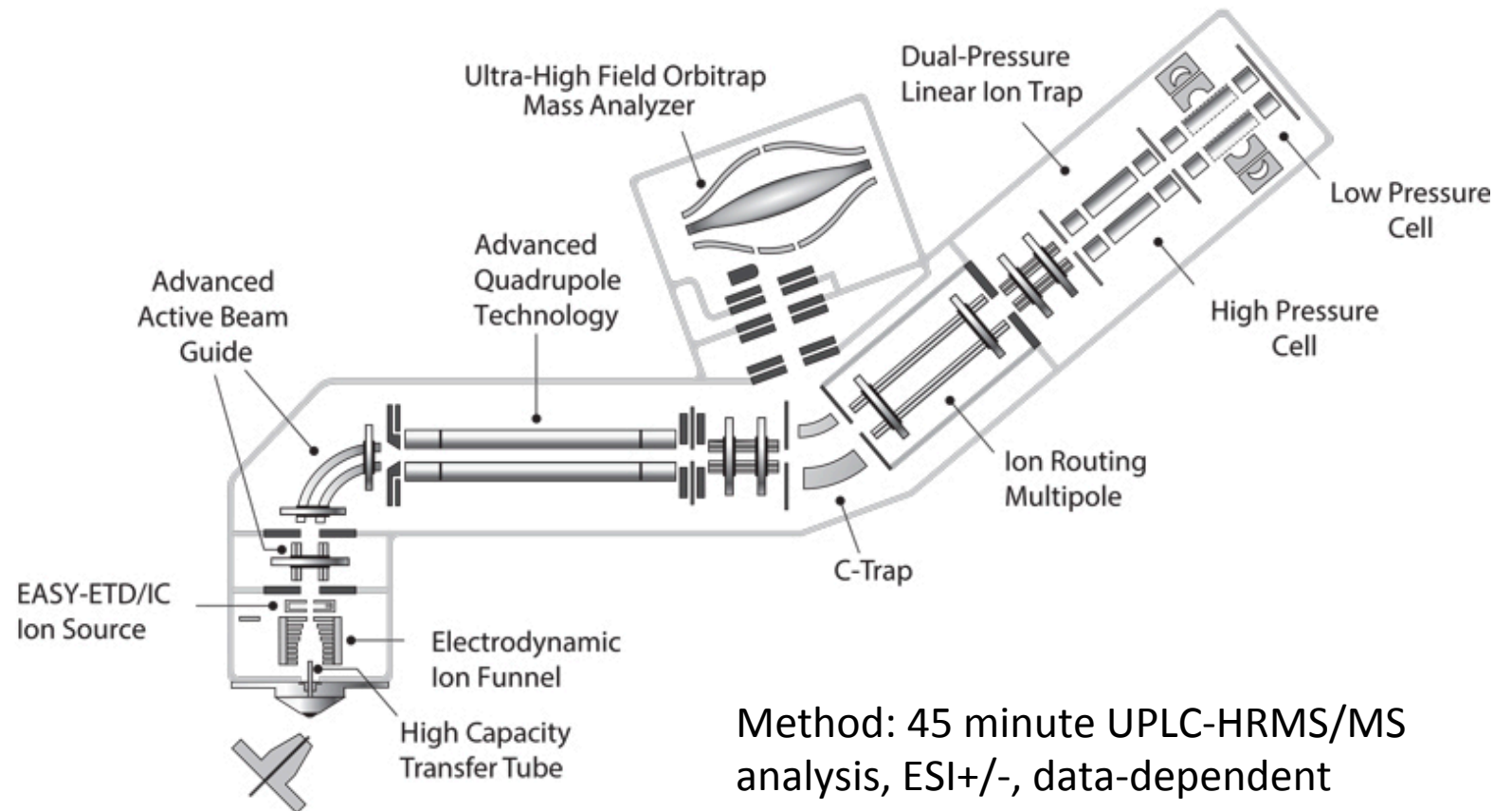
environmental sample



Ultra-high resolution mass spectrometry for non-targeted analysis



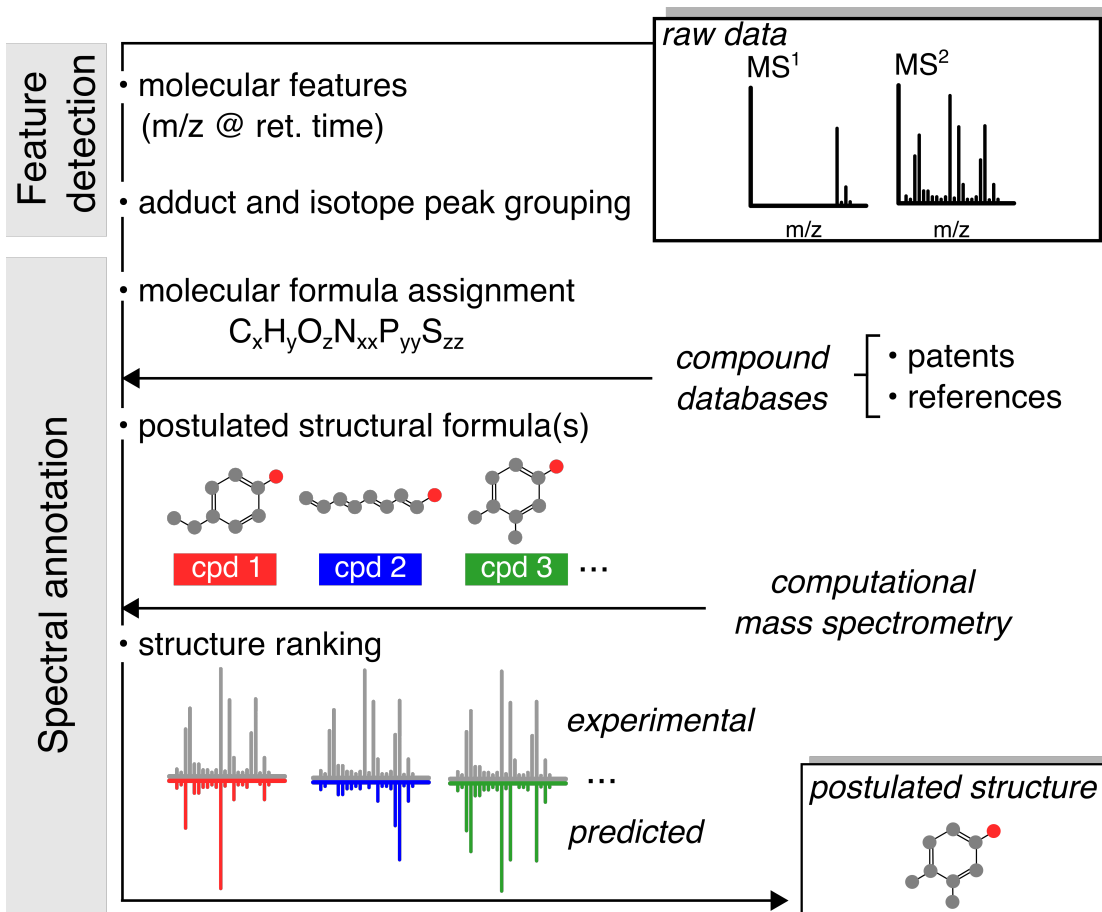
- Orbitrap Fusion Lumos mass spectrometer
- Critical resolution (500,000) and mass accuracy (< 1 ppm)
- Ultra-fast data-dependent MS/MS maximizes data acquisition rate
- MS³ capability for structural characterization
- Ion funnel gives maximum sensitivity



Method: 45 minute UPLC-HRMS/MS analysis, ESI+/-, data-dependent acquisition, internal mass calibration

Data analysis: The hard part!

Overall non-targeted analysis workflow:

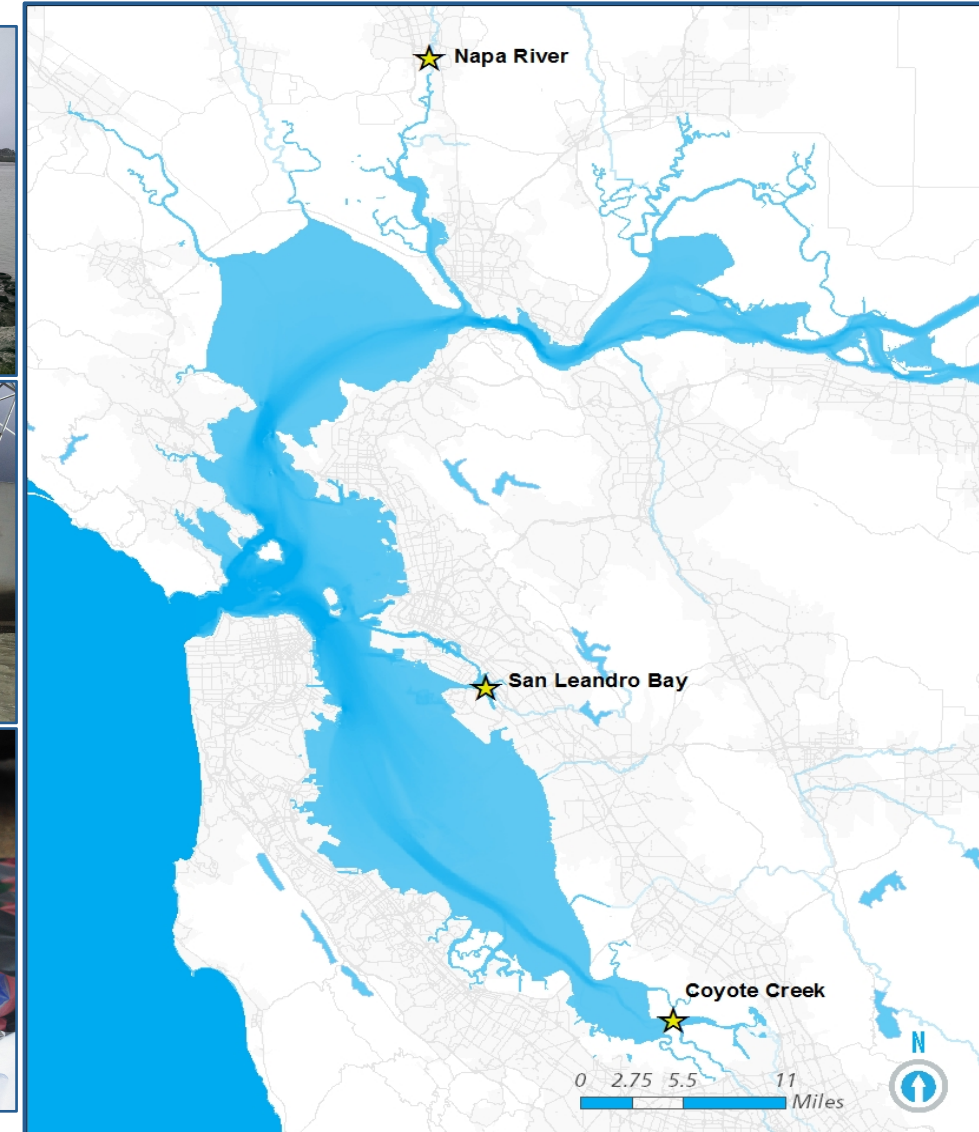


Objectives

- Utilize high-resolution mass spectrometry strategies to identify non-targeted polar organic pollutants in San Francisco Bay waters.
- Assess the performance of passive sampling vs. grab sampling strategies for non-targeted analysis of organic pollutants in Bay waters.
- Examine differences in organic pollutant occurrence and abundance among Bay waters with various pollutant sources.
- Perform reconnaissance of emerging contaminant occurrence in drinking water sources within three watersheds of North Carolina.

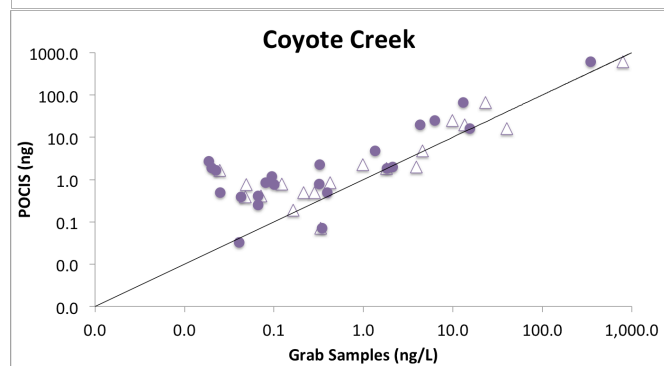
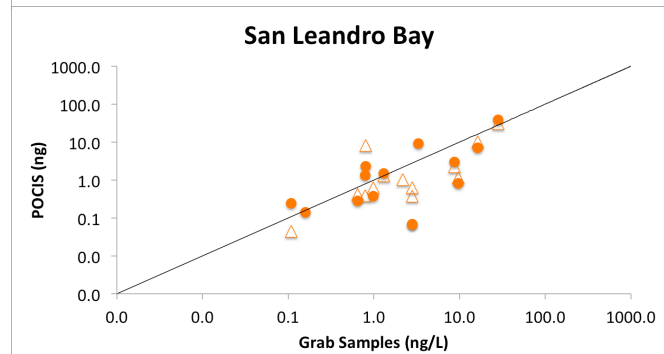
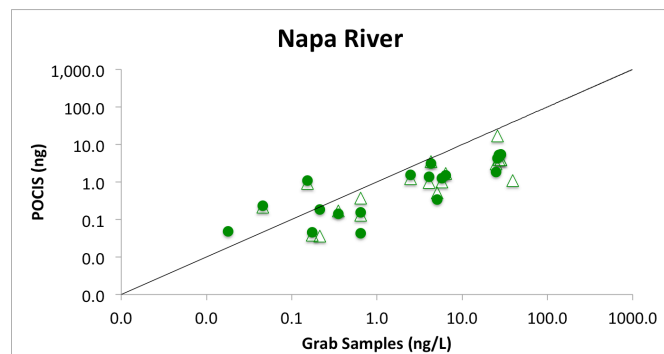
Emerging pollutants in SF Bay waters

- Ambient Bay sample collection:
 - Three sites were sampled: San Leandro Bay (March 2016), Napa River (April 2016), and Coyote Creek (Aug/ Sept. 2016)
 - POCIS were deployed for one month at each site.
 - Grab samples (4L volume, one collected in triplicate) were taken at sites on POCIS deployment & retrieval.
 - Field blanks collected for POCIS and grab samples.
- Four WWTP effluent grab samples were collected. Sampling sites were located near the San Leandro Bay and Coyote Creek sites



Target Analyte Quantitation

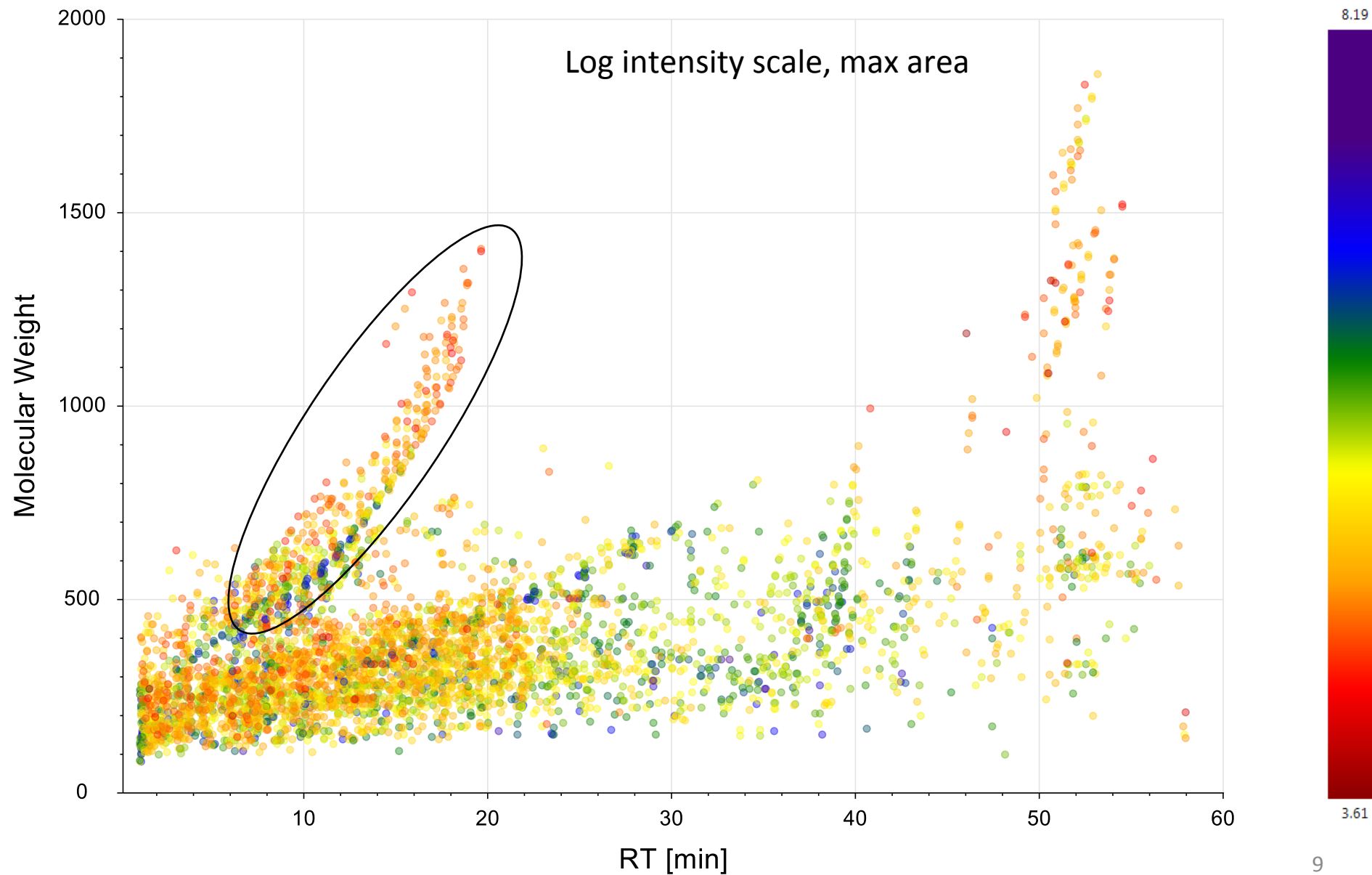
Concentrations in ng/L	Detection Limit	Napa River Deploy	Napa River Retrieval	San Leandro Bay Field Blank	San Leandro Bay Deploy	San Leandro Bay Retrieval	Coyote Creek Deploy	Coyote Creek Retrieval	WWTP Site A	WWTP Site B	WWTP Site C	WWTP Site D	WWTP Site D Field Blank
Agrochemicals:													
Allethrin	1.2				1.33 ± 0.13								
Atrazine	0.06		1.10 ± 0.072									0.296	
Azoxystrobin	0.6	3.09	3.51 ± 0.28								0.68 ± 0.29	3.30	
Benomyl	0.06								23.3				
Carbaryl	0.6	5.33	3.87 ± 0.27			0.628 ± 0.074	0.07						
Carbendazim	0.06	1.39	0.964	0.884 ± 0.38	2.97 ± 0.74	2.21 ± 0.28	6.29 ± 0.14	9.82 ± 0.15	86.6	39.2	40.5 ± 2.1	75.0	
Deet	0.6	1.82	2.98 ± 0.033	0.875 ± 0.37	7.13 ± 0.84	10.4 ± 1.8	4.31 ± 0.43	13.6 ± 0.84	264	61.3	65.0 ± 0.58	13.1	
Fluoxastrobin	0.06	1.10	0.913 ± 0.057	1.70 ± 0.28			0.40	0.29 ± 0.19		0.13	0.14		1.73
Fluridone	0.6	1.52	1.21 ± 0.019				1.83 ± 0.074	1.81 ± 0.20	1.56		3.30 ± 0.18	6.13	
Imazapyr	0.6								1.10				
Metalaxyl	0.06						0.07					0.17	
Prometon	0.06	0.34	0.512 ± 0.012	0.494 ± 0.019	1.47 ± 0.030	1.29 ± 0.074	0.319 ± 0.017	0.12 ± 0.12		0.61	0.42 ± 0.079	5.40	
Simazine	0.6	5.41	3.96 ± 0.20			0.63 ± 0.074							0.98
Sulfapyridine	0.6						1.36 ± 0.71	4.59 ± 1.1	50.4	49.5	144 ± 4.9	126	
Tertbutylazin-desethyl	0.6	5.41	3.95 ± 0.20										0.979
Propiconazole	0.6	0.14		3.03 ± 0.331	2.28 ± 0.12	8.14 ± 0.54							4.05 2.38
Pharmaceuticals:													
Carbamazepine	0.6	1.27	1.00 ± 0.11		0.822 ± 0.10	1.16 ± 0.067	13.1 ± 0.12	23.1 ± 1.1	107	125	101 ± 2.0	128	
Cetirizine	0.6	1.50	1.70 ± 0.24			1.03 ± 0.40	15.5 ± 0.41	39.9 ± 1.3	241	440	475 ± 24	454	
cis-Diltiazem	0.06	0.19		0.132 ± 0.007			0.33	0.98 ± 1.3		21.1	46.7 ± 4.1	45.0	
Citalopram	0.06	0.045		0.180 ± 0.014			0.095 ± 0.10			51.4	58.0 ± 0.92		
Dextromethorphan	0.06	0.02								18.3	23.2 ± 0.78		
Diphenhydramine	0.06	0.048		0.086 ± 0.009					0.12	18.8	34.8 ± 3.2	0.508	
Genistein	1.2				9.15 ± 0.11								
Irbesartan	0.6	0.04					2.11 ± 0.21	3.92 ± 0.98	26.3	24.1	70.2 ± 0.31	64.1	
Pramoxine	0.06	0.23	0.215 ± 0.016	0.543 ± 0.024				0.21 ± 0.32	3.68	6.58	0.40 ± 0.32	0.092	
Propranolol	0.06	0.29	0.179 ± 0.013	0.51 ± 0.029						16.4		0.093	
Rosuvastatin	0.6								49.1		1.65 ± 0.41	5.94	
Trimethoprim	0.06	0.16	0.131					0.43	41.0	3.81	5.67 ± 0.52	9.49	
Verapamil	0.6	0.10							7.05		7.72 ± 1.0	5.27	
Benzotriazole	0.06	4.28	16.9 ± 1.6	0.395 ± 0.021	38.1 ± 1.7	30.7 ± 3.7	346 ± 36	792 ± 9.4	1252	701	606 ± 12	1443	



- A variety of agrochemicals and pharmaceutical compounds were detected in WWTP effluents and Bay waters at typically ppt levels.
- Benzotriazole was the most abundant targeted micropollutant in Bay water (Coyote Creek), consistent with high levels in WWTP effluent.
- Target analyte levels in POCIS extracts generally correlated with water concentrations (grab samples) for all sites.

Non-targeted analysis: Data set characteristics

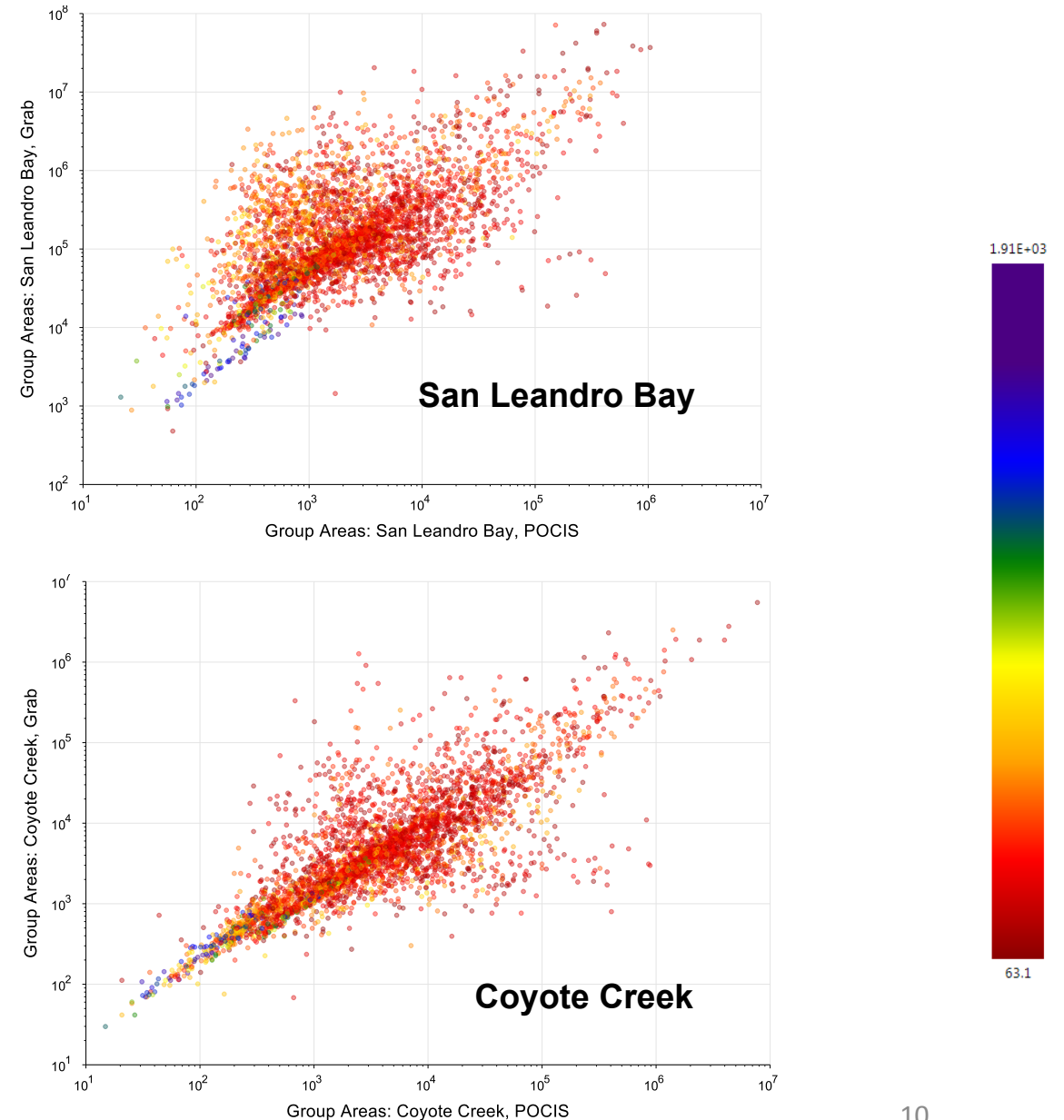
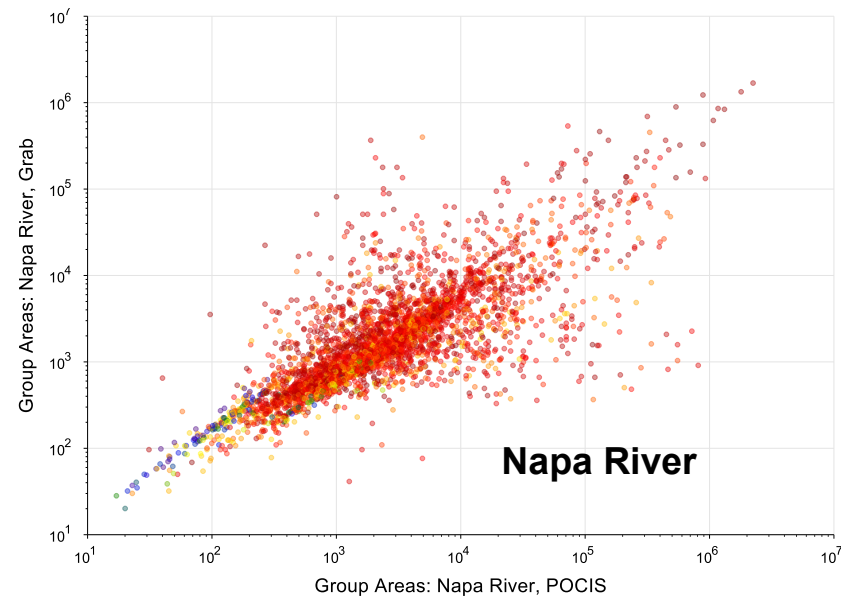
Total # compounds: **4965**
Not on target list: **4630**



Non-targeted analysis data overview: POCIS vs. Grab samples

How well do POCIS extract intensities correlate with intensities of compounds in grab samples?

- High MW, low abundance compounds correlate well at each location.
- Poor correlation is observed for higher abundance compounds in all locations.
- San Leandro Bay POCIS data underpredicts abundance in grab samples

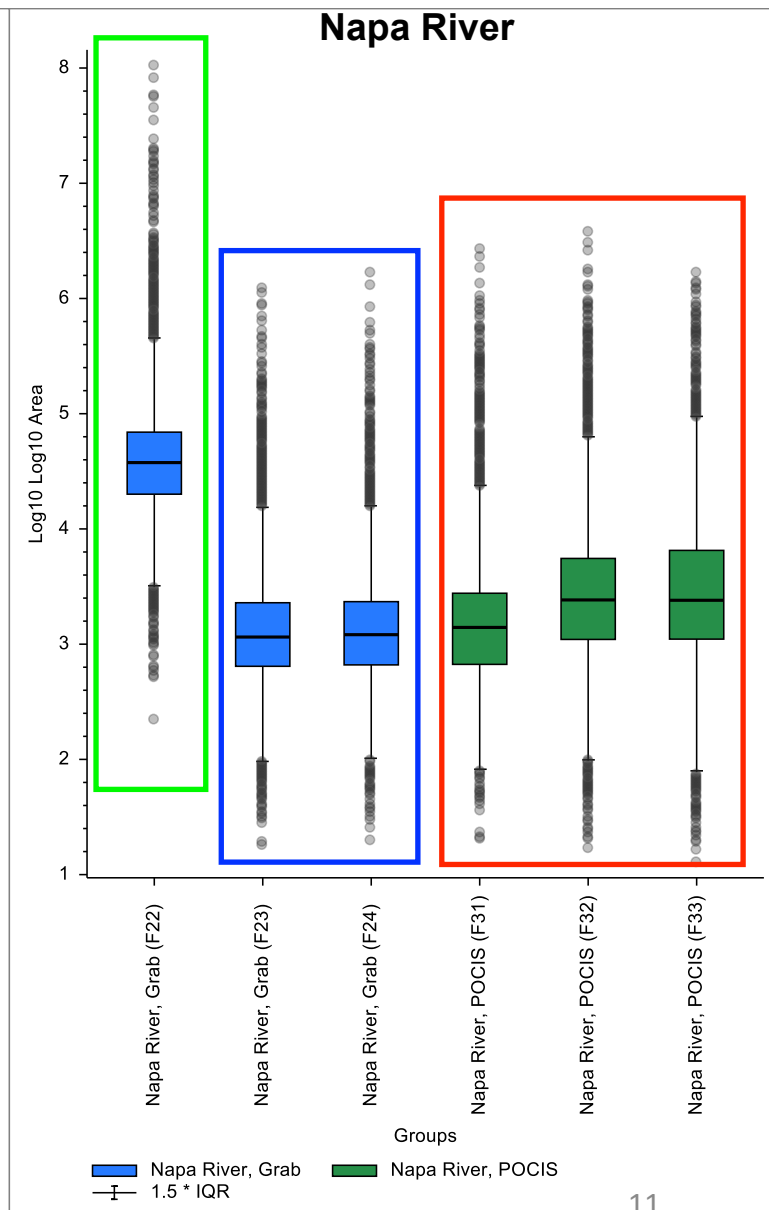
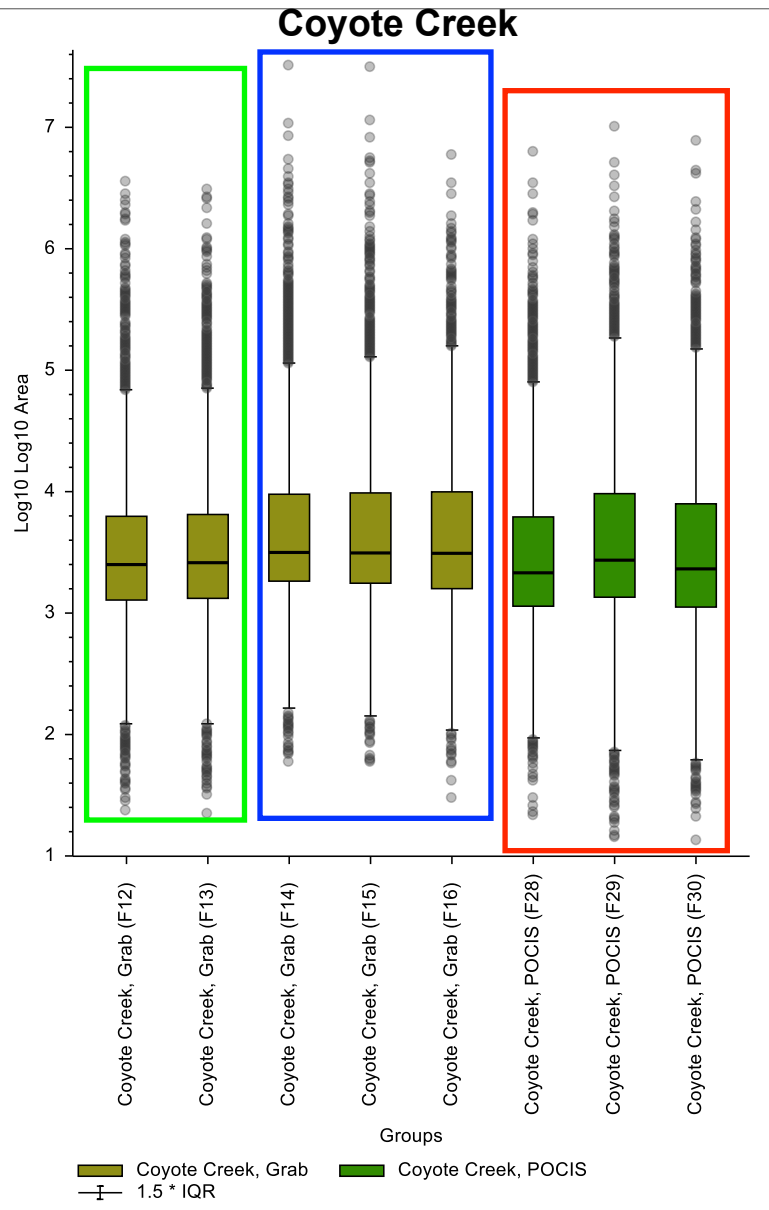
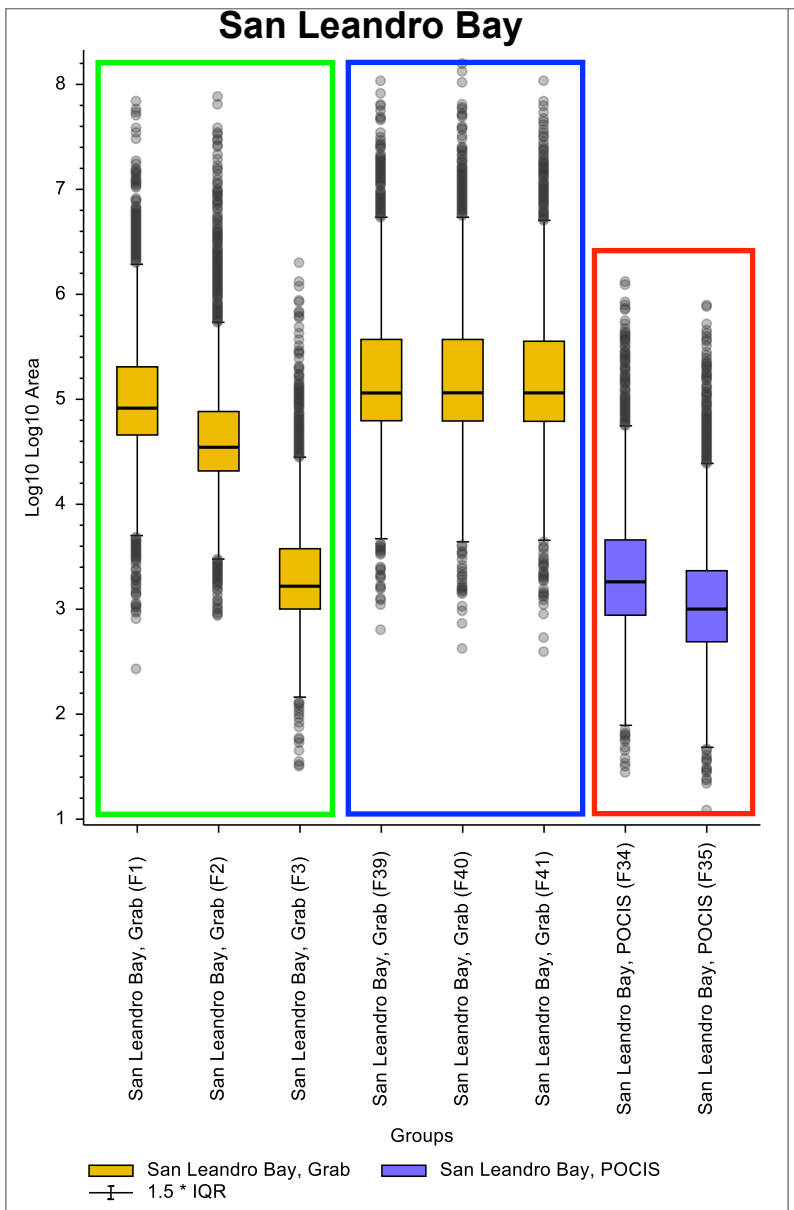


Non-targeted analysis data overview: POCIS and grab sample compound abundance by site

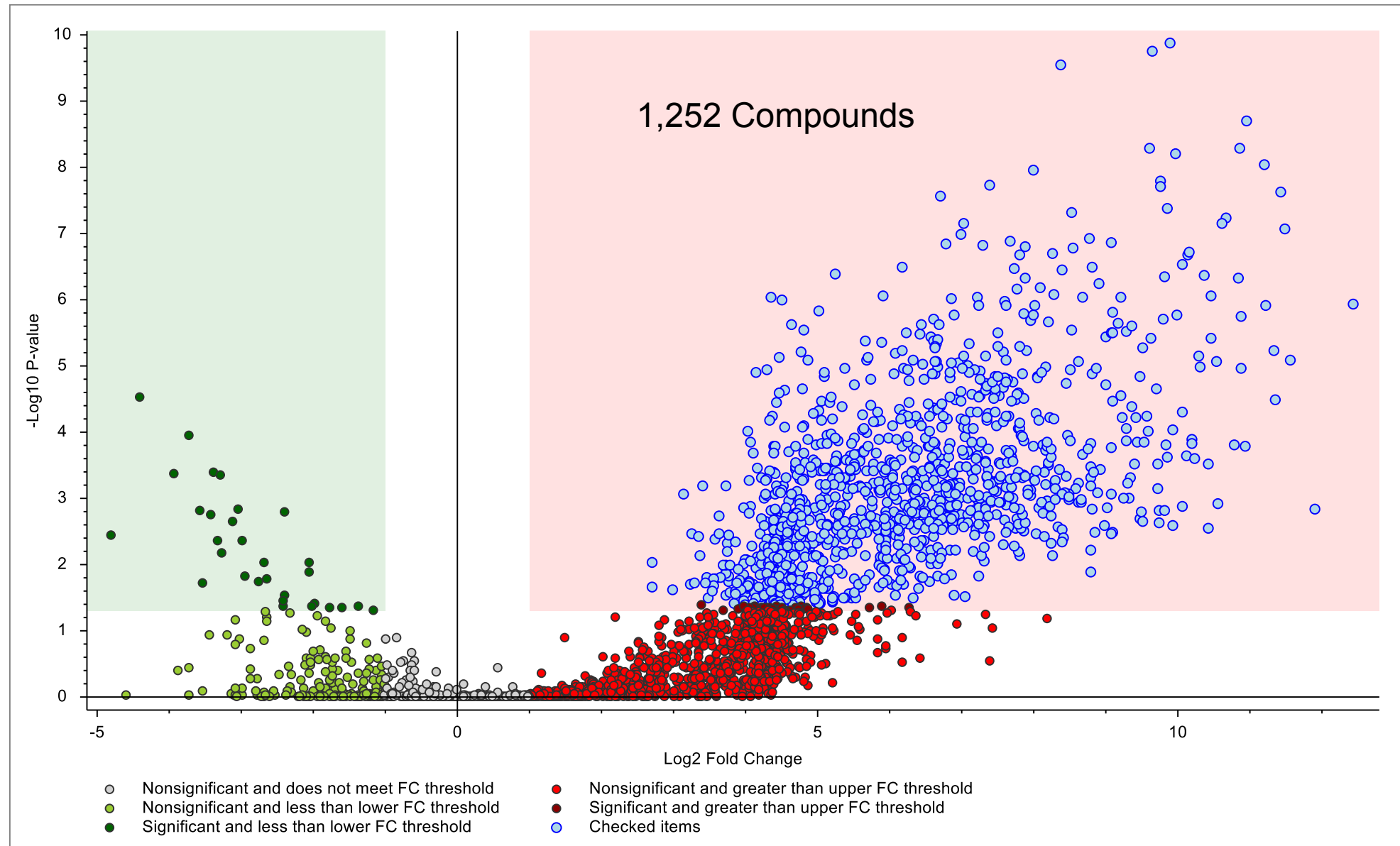
Deployment

Retrieval

POCIS



Differential analysis: Relative abundance of compounds in San Leandro Bay vs. WWTP Effluent (average)



Identifying compounds in San Leandro Bay water:

- Big picture: SLB is extremely complex: Higher pollutant burden than wastewater effluent!
- Large number (> 50) high confidence MS/MS library hits from mzCloud spectral library.
- Occurrence of many polyethoxylated compounds indicates untreated contaminant source.
- Highest abundance compounds identified are fungicide myclobutanil and several polymer/rubber additives.
- Pharmaceuticals and illicit drugs are also identified with high confidence.

Compound Discoverer 2.1.0.326

File Reporting Libraries View Window Help

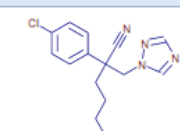
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Compounds Comounds per File Merged Features Features mzVault Results mzCloud Results ChemSpider Results Mass List Search Results Input Files

	Checked	Name	Formula	Annotation Sc	Molecular Weight	RT [min]	Area (Max.)	# MS2	# ChemSpider Results	# mzCloud Results	mzCloud Best Match
1	<input checked="" type="checkbox"/>	Myclobutanil	C15 H17 Cl N4	■■■■■	288.11446	28.935	57126323	6	14	1	81.4
2	<input checked="" type="checkbox"/>	N,N'-Diphenylguanidine	C13 H13 N3	■■■■■	211.11117	7.265	54003060	23	50	2	89.9
3	<input checked="" type="checkbox"/>	Caprolactam	C6 H11 N O	■■■□□	113.08419	3.350	52884350	28	145	1	77.6
4	<input checked="" type="checkbox"/>	PEG (12EO)		■■■□□	563.35120	10.912	19001426	1	0	1	87.9
5	<input checked="" type="checkbox"/>	PEG (12EO)		■■■□□	563.35140	10.833	19001426.067364939	1	0	1	87.9
6	<input checked="" type="checkbox"/>	PEG (13EO)		■■■□□	607.37757	11.770	16711576	1	1	1	85.1
7	<input checked="" type="checkbox"/>	PPG (10EO)		■■■□□	620.41070	27.612	6870340	1	1	1	75.8
8	<input checked="" type="checkbox"/>	Benzoylcegonine	C16 H19 N O4	■■■■■	289.13157	7.910	5410580	10	43	2	81.2
9	<input checked="" type="checkbox"/>	Cocaine	C17 H21 N O4	■■■■■	303.14726	11.853	3688306	5	63	1	84.0
10	<input checked="" type="checkbox"/>	N~5~-(Diaminomethylene)-L-ornith	C18 H36 N6 O4	■■■□□	400.27971	36.820	3445412	1	1	1	77.1
11	<input checked="" type="checkbox"/>	Paracetamol	C8 H9 N O2	■■■■■	151.06346	2.566	3181711	3	293	4	94.3
12	<input checked="" type="checkbox"/>	8-Hydroxyquinoline	C9 H7 N O	■■■□□	145.05285	4.207	2761085	9	91	1	77.3
13	<input checked="" type="checkbox"/>	Nobiletin	C21 H22 O8	■■■□□	402.13173	27.164	2615802	4	28	1	88.7
14	<input checked="" type="checkbox"/>	6-Methoxyquinoline	C10 H9 N O	■■■□□	159.06853	2.215	1883138	17	225	1	80.8
15	<input checked="" type="checkbox"/>	Benzothiazole	C7 H5 N S	■■■■■	135.01421	12.683	1765156	1	17	1	94.6
16	<input checked="" type="checkbox"/>	2-Hydroxysimazine	C7 H13 N5 O	■■■□□	183.11235	3.950	1763410	4	18	1	94.4
17	<input checked="" type="checkbox"/>	Cotinine	C10 H12 N2 O	■■■□□	176.09511	1.644	1317652	14	222	2	95.2
18	<input checked="" type="checkbox"/>	PEG (14EO)		■■■□□	651.40422	13.564	1006321	1	0	1	83.9
19	<input checked="" type="checkbox"/>	δ-Valerolactam	C5 H9 N O	■■■□□	99.06848	2.257	997237	1	86	3	83.5
20	<input checked="" type="checkbox"/>	N~5~-(Diaminomethylene)-L-ornith	C18 H36 N6 O4	■■■□□	400.28026	38.099	852226	1	1	1	76.5
21	<input checked="" type="checkbox"/>	2-[[Dimethylamino)methylidene]ind	C12 H13 N O	■■■□□	187.09985	3.390	782328	2	203	1	75.9
22	<input checked="" type="checkbox"/>	Caprolactam		■■■□□	113.08253	3.349	739621	1	0	1	77.6
23	<input checked="" type="checkbox"/>	Isoquinoline	C9 H7 N	■■■□□	129.05784	2.644	720716	3	23	1	83.4
24	<input checked="" type="checkbox"/>	PPG (9EO)		■■■□□	562.36932	35.702	426723	2	0	1	75.9
25	<input checked="" type="checkbox"/>	1-(1,3,3-Trimethyl-2,3-dihydro-1H-ir	C14 H17 N O	■■■□□	215.13080	6.820	279968	2	135	1	83.2

Hide Related Tables

Structure Proposals Comounds per File Predicted Compositions Merged Features Matched Patterns mzVault Results mzCloud Results ChemSpider Results Mass List Search Results

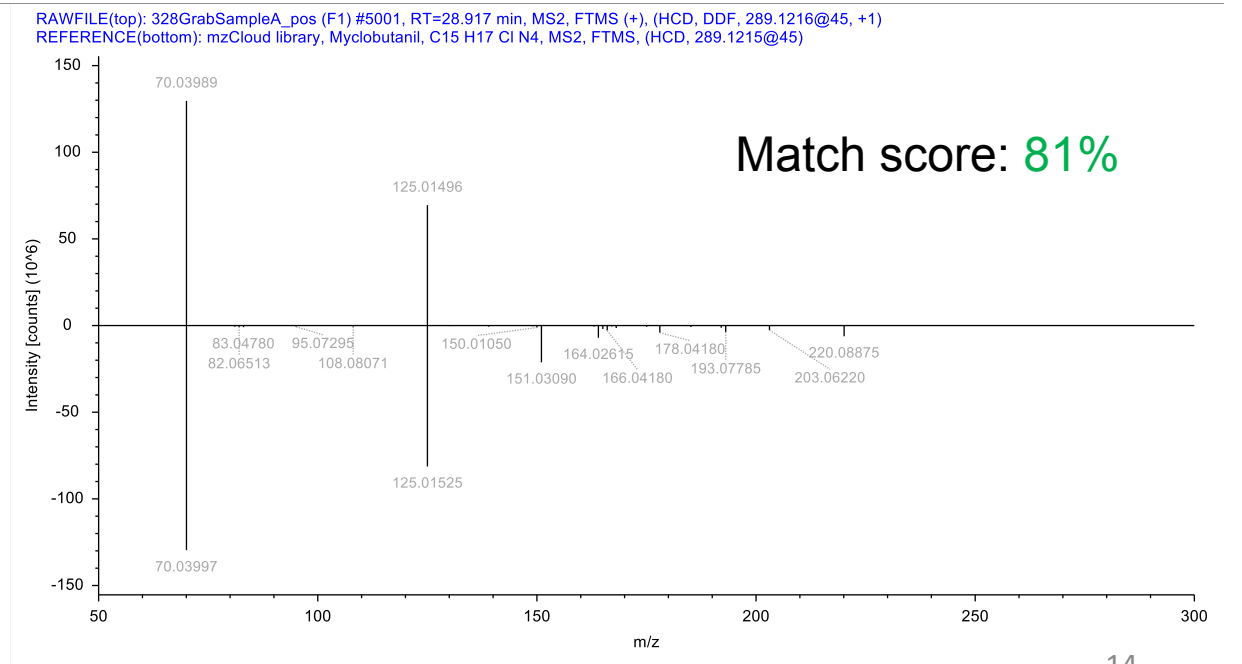
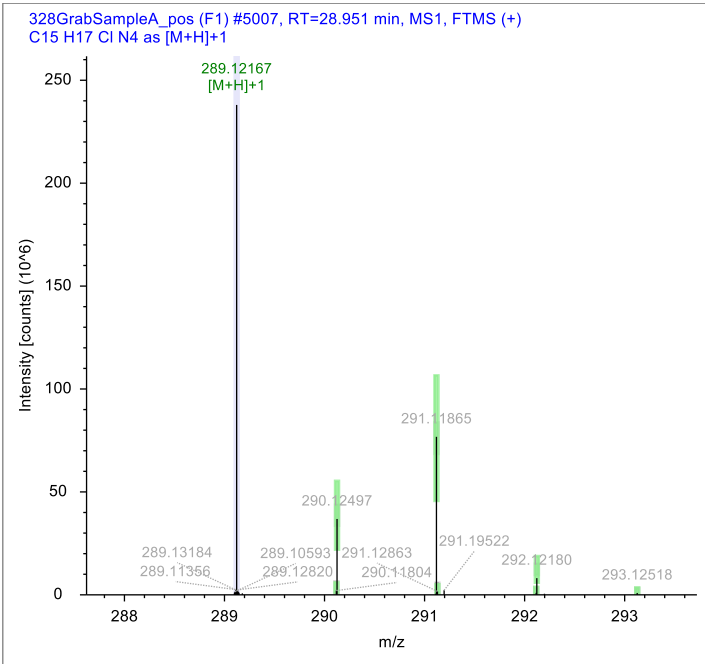
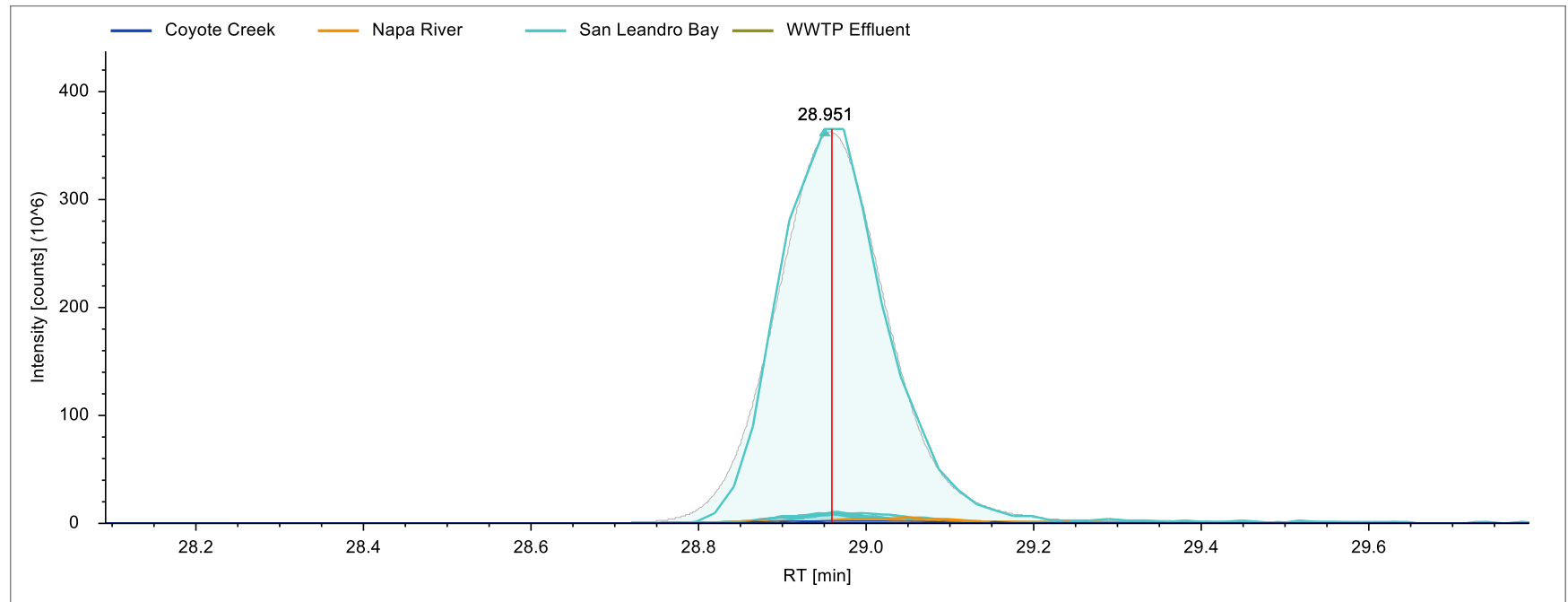
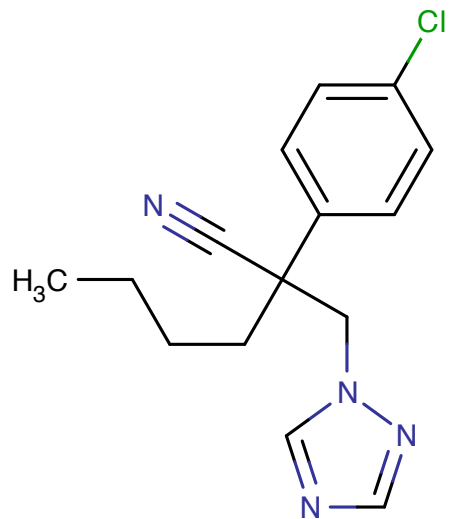
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1	<input checked="" type="checkbox"/>	■	-0.00028	-0.98	5001	1089	C15 H17 Cl N		288.11417	81.4	81.4	Myclobutanil

Show Related Tables

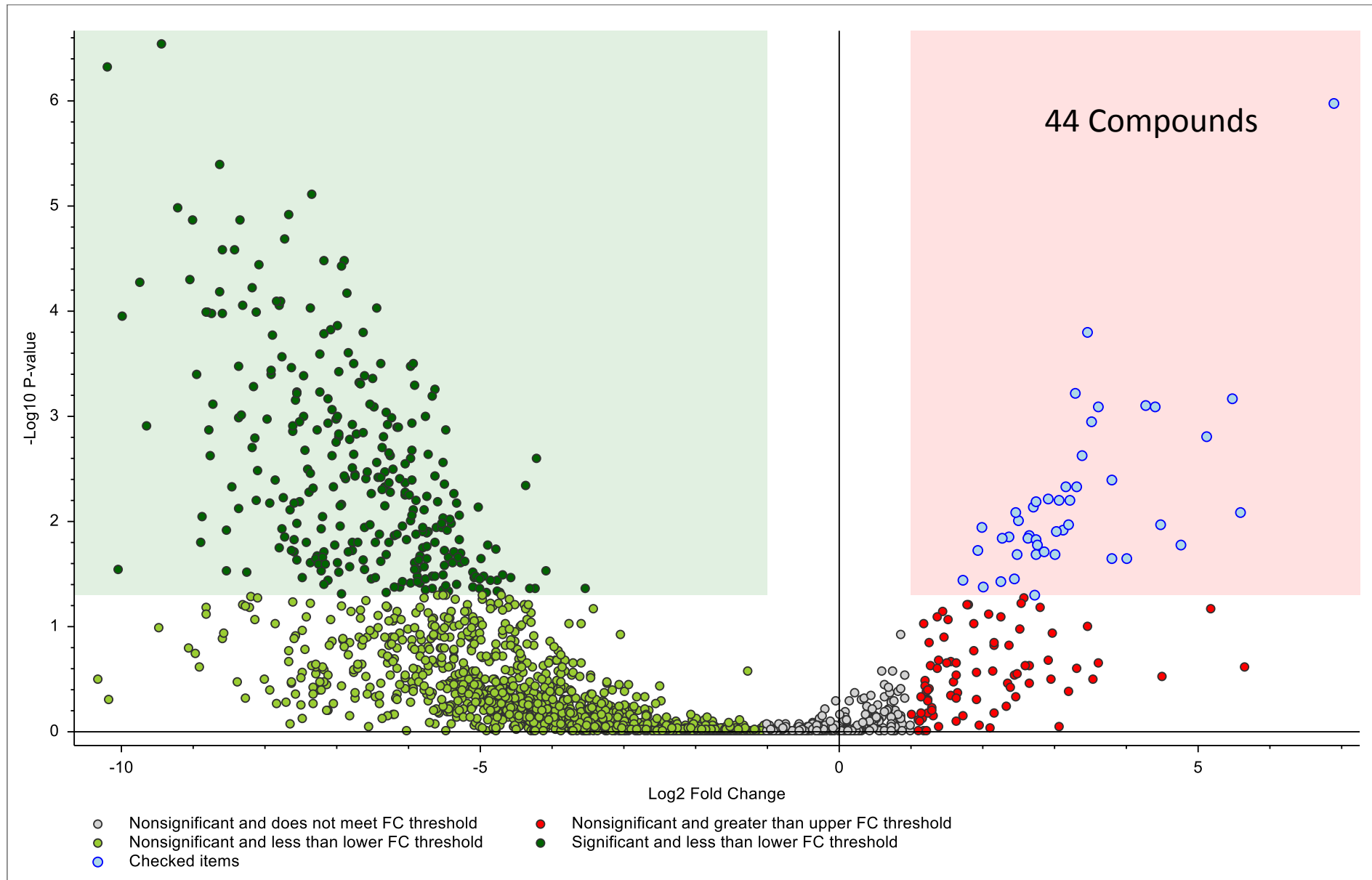
13

Myclobutanil: Conazole fungicide used to control fungal infections in table & wine grapes (primarily in California). Also used for cannabis cultivation.

<http://www.toxipedia.org/display/toxipedia/Myclobutanil>



Differential analysis: Relative abundance of compounds in Napa River vs. WWTP Effluent (average)



Identifying compounds in Napa River water:

- Far fewer compounds had elevated levels in Napa River water relative to WWTP effluent.
- Only one compound (2-hydroxysimazine) matched with high confidence to library spectra.
- Highly uncertain (mass-only) tentative identifications of other compounds indicates many natural products.
- No indication of significant wastewater pollutant sources in the Napa River.
- Several candidates had MS/MS spectra consistent with agrochemicals and commercial chemical transformation products.

Compound Discoverer 2.1.0.326

File Reporting Libraries View Window Help

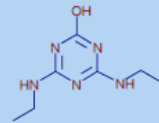
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Compounds Comounds per File Merged Features Features mzVault Results mzCloud Results ChemSpider Results Mass List Search Results Input Files

Checked	Name	Formula	Annotation Sc	Molecular Weight	RT [min]	Area (Max.)	# MS2	# ChemSpider Results	# mzCloud Results	mzCloud Best Match
<input checked="" type="checkbox"/>	1,9-Dihydroxy-3,6,11,14-tetraphenyl	C40 H26 O5	■ ■ ■ ■ ■	586.17842	28.700	19590570	7	3	0	
<input checked="" type="checkbox"/>	2-Amino-5,6,7,8-tetrahydro-4(1H)-q	C8 H11 N3 O	■ ■ ■ ■ ■	165.09052	2.054	13385868	5	93	0	
<input checked="" type="checkbox"/>	l-Phe-l-Phe	C18 H20 N2 O3	■ ■ ■ ■ ■	312.14813	29.180	11792074	2	66	0	
<input checked="" type="checkbox"/>	Butyl 4-[[4-nitrobenzoyl]amino]benz	C18 H18 N2 O5	■ ■ ■ ■ ■	342.12213	23.676	5515307	4	22	0	
<input checked="" type="checkbox"/>	1-carboxy-3-hydroxyadamantane	C11 H16 O3	■ ■ ■ ■ ■	196.11015	8.367	4966869	12	232	0	
<input checked="" type="checkbox"/>	Sulfolane	C4 H8 O2 S	■ ■ ■ ■ ■	120.02458	1.736	4862130	1	33	0	
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<input checked="" type="checkbox"/>	(+)-Abscisic acid	C15 H20 O4	■ ■ ■ ■ ■	264.13651	15.138	2704250	4	154	0	
<input checked="" type="checkbox"/>	Cinnamic acid	C9 H8 O2	■ ■ ■ ■ ■	148.05238	23.600	2454758	2	101	0	
<input checked="" type="checkbox"/>	3-[5-[[3-(2-carboxyethyl)-5-[[3-(2-ca	C35 H38 N4 O10	■ ■ ■ ■ ■	674.25945	16.979	2366026	3	3	0	
<input checked="" type="checkbox"/>	Lacosamide	C13 H18 N2 O3	■ ■ ■ ■ ■	250.13226	4.729	2331269	3	125	0	
<input checked="" type="checkbox"/>	Ancymidol	C15 H16 N2 O2	■ ■ ■ ■ ■	256.12144	17.140	2301205	4	136	1	57.0
<input checked="" type="checkbox"/>	1,9-Dihydroxy-3,6,11,14-tetraphenyl	C40 H26 O5	■ ■ ■ ■ ■	586.17674	28.778	2202900	1	3	0	
<input checked="" type="checkbox"/>	Methoxyfenozide	C22 H28 N2 O3	■ ■ ■ ■ ■	368.21060	29.181	1961423	2	34	0	
<input checked="" type="checkbox"/>	4-Butoxy-3-ethoxybenzoic acid	C13 H18 O4	■ ■ ■ ■ ■	238.12067	12.107	1883955	5	163	0	
<input checked="" type="checkbox"/>	2-Hydroxysimazine	C7 H13 N5 O	■ ■ ■ ■ ■	183.11235	3.950	1763410	4	18	1	94.4
<input checked="" type="checkbox"/>	6,7-Dimethoxy-2,2-dimethylchrome	C13 H16 O3	■ ■ ■ ■ ■	220.11008	14.018	1711739	5	281	0	
<input checked="" type="checkbox"/>	Tolmetin	C15 H15 N O3	■ ■ ■ ■ ■	257.10520	17.134	1649184	6	199	0	
<input checked="" type="checkbox"/>	2,2,2-Trifluoro-N-[3-methyl-1-(2-ph	C22 H25 F3 N2 O	■ ■ ■ ■ ■	390.19147	29.137	1378679	3	1	0	
<input checked="" type="checkbox"/>	Oxadixyl	C14 H18 N2 O4	■ ■ ■ ■ ■	278.12685	5.874	1240518	13	72	0	
<input checked="" type="checkbox"/>	Methyl 3,5-di-tert-butyl-4-hydroxyb	C16 H24 O3	■ ■ ■ ■ ■	264.17314	34.758	1237294	1	80	1	53.0
<input checked="" type="checkbox"/>	(2E)-2-(1,3-Benzothiazol-2-yl)-3-[9-r	C26 H18 N4 O2 S	■ ■ ■ ■ ■	450.11500	3.948	1230553	6	7	0	
<input checked="" type="checkbox"/>	Ethyl 6-nitro-2-oxo-4-phenyl-1,2-dil	C18 H14 N2 O5	■ ■ ■ ■ ■	338.09080	20.873	1150352	4	40	0	
<input checked="" type="checkbox"/>	3-Ethyl 1-(2-methyl-2-propanyl) 3-b	C20 H29 N O4	■ ■ ■ ■ ■	347.21024	35.125	1111333	4	13	0	

Hide Related Tables

Structure Proposals Comounds per File Predicted Compositions Merged Features Matched Patterns mzVault Results mzCloud Results ChemSpider Results Mass List Search Results

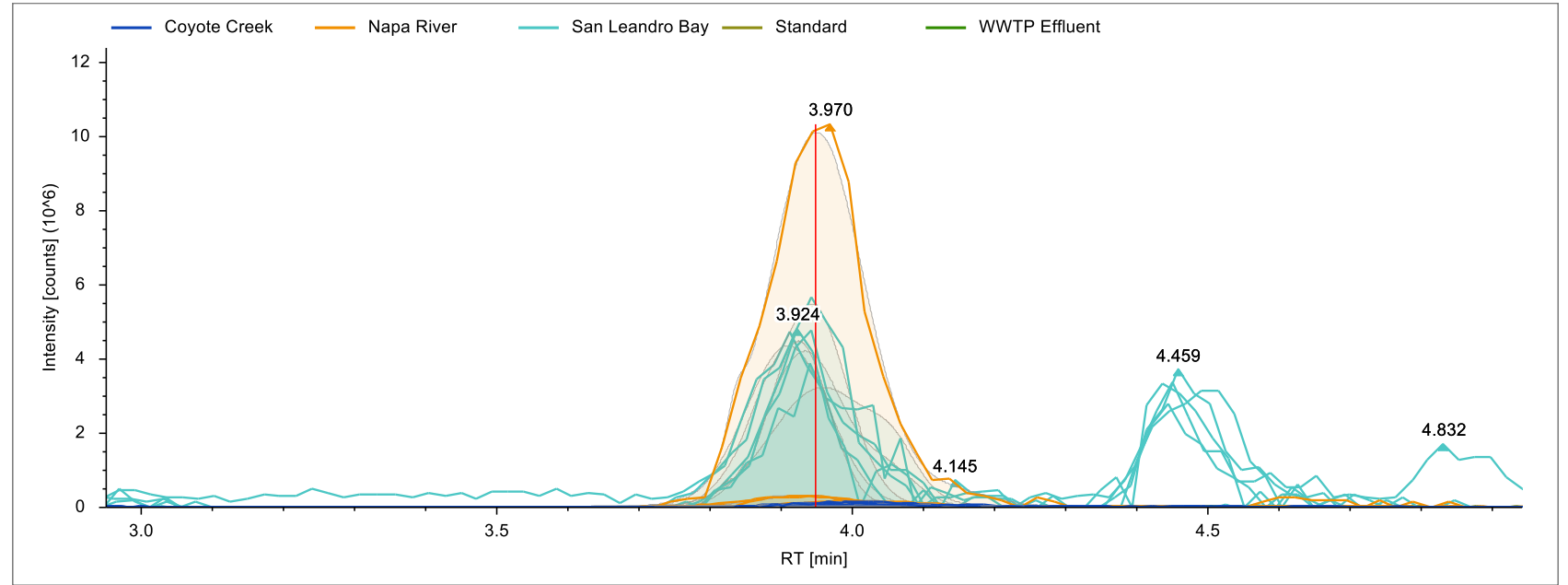
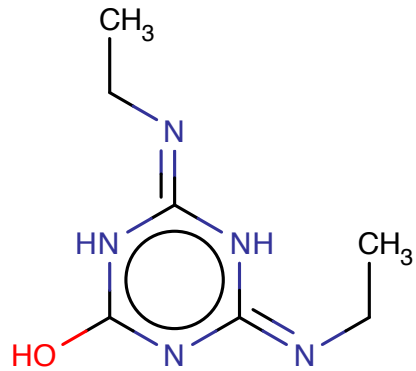
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<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	-0.00034	-1.84	490	1135	C7 H13 N5 O		183.11201	94.4	94.4	2-Hydroxysimazine

Show Related Tables

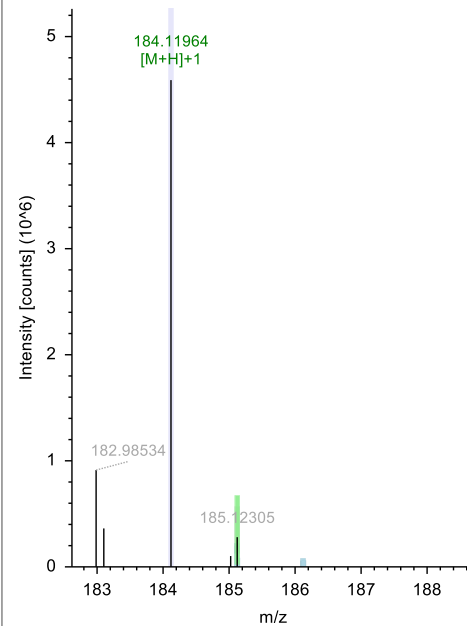
16

2-Hydroxysimazine:

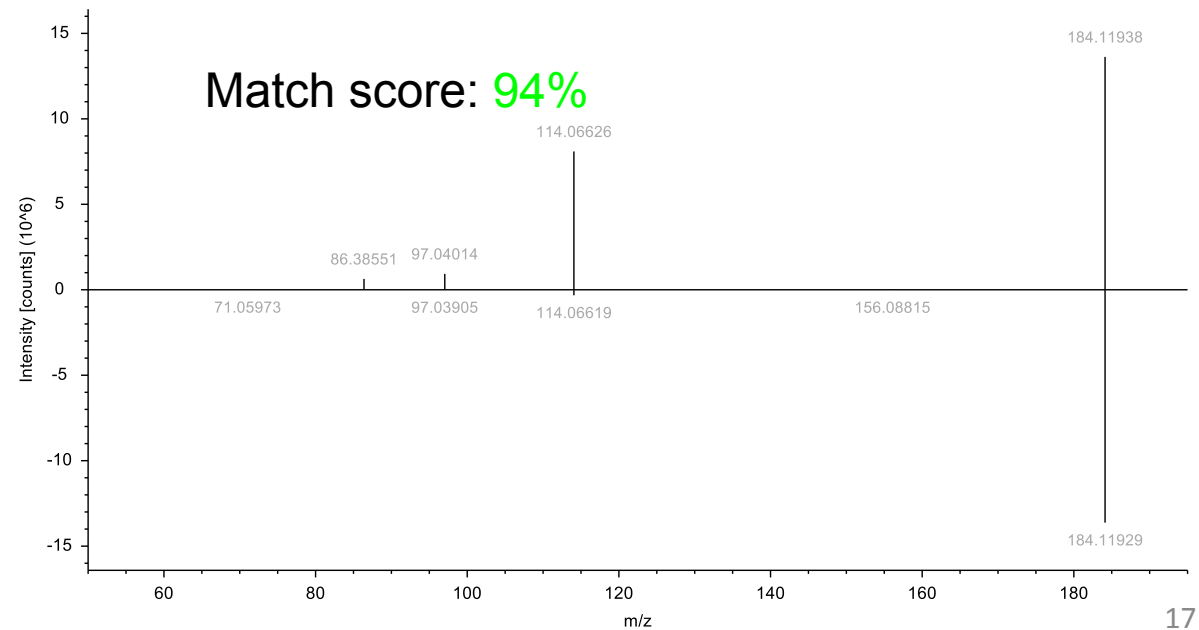
Transformation product of the widely-used triazine herbicide simazine (broadleaf weed and grass killer).



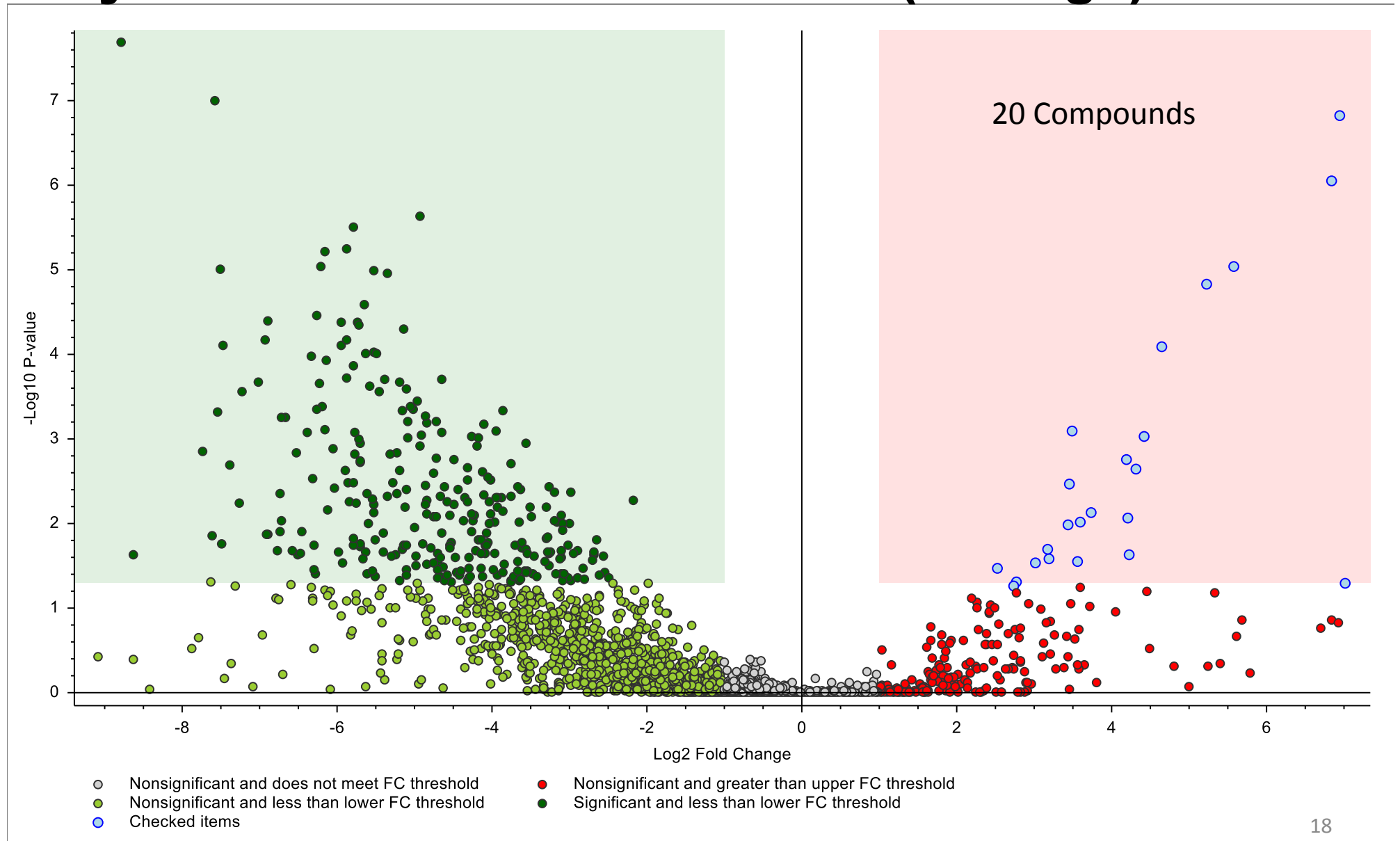
NapaRiverDeployGrab_pos (F22) #489, RT=3.870 min, MS1, C7 H13 N5 O as [M+H]⁺1



RAWFILE(top): NapaRiverDeployGrab_pos (F22) #490, RT=3.877 min, MS2, FTMS (+), (HCD, DDF, 184.1196@45, +1)
REFERENCE(bottom): mzCloud library, 2-Hydroxysimazine, C7 H13 N5 O, MS2, FTMS, (HCD, 184.1193@30)



Differential analysis: Relative abundance of compounds in Coyote Creek vs. WWTP Effluent (average)



Identifying compounds in Coyote Creek water:

- Few unique compounds in Coyote Creek.
- Most detected compounds are also found in high abundance in WWTP effluent (Coyote Creek resembles diluted effluent).
- No high confidence identifications from Spectral library.
- Several low-confidence spectral library matches can be examined.

Compound Discoverer 2.1.0.323

File Reporting Libraries View Window Help

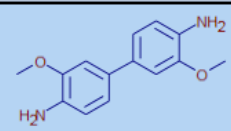
Start Page x SFEL_2017_0324 x

Compounds Comounds per File Merged Features Features mzVault Results mzCloud Results ChemSpider Results Mass List Search Results Input Files

	Checked	Name	Formula	Annotation Sc	Molecular Weight	RT [min]	# MS2	Area (Max.)	mzCloud Best Match	# mzCloud Results	# ChemSpider Results
1	<input checked="" type="checkbox"/>	2-Methyl-2-propanyl 4-(4-hydroxyb	C14 H27 N O3	■ ■ ■ ■ ■	257.19905	33.965	4	4121099		0	31
2	<input checked="" type="checkbox"/>	5-(3-Bromo-4-methoxyphenyl)-1H-1	C8 H7 Br N4 O	■ ■ ■ ■ ■	253.97973	1.134	1	2071379		0	6
3	<input checked="" type="checkbox"/>	Methylprednisolone	C22 H30 O5	■ ■ ■ ■ ■	374.20927	33.689	7	1192510		0	20
4	<input checked="" type="checkbox"/>			■ ■ ■ ■ ■	139.99927	1.140	5	872810		0	0
5	<input checked="" type="checkbox"/>	(6beta,7alpha)-7,11,12-Trihydroxy-6-	C20 H26 O5	■ ■ ■ ■ ■	346.17801	30.548	2	839555		0	23
6	<input checked="" type="checkbox"/>	4-Butoxy-3-ethoxybenzoic acid	C13 H18 O4	■ ■ ■ ■ ■	238.12055	16.266	3	734564		0	163
7	<input checked="" type="checkbox"/>	1,1'-hexane-1,6-diylbis(1H-pyrrole-2	C14 H16 N2 O4	■ ■ ■ ■ ■	276.11110	7.263	16	711474		0	68
8	<input checked="" type="checkbox"/>	3,3'-Dimethoxybenzidine	C14 H16 N2 O2	■ ■ ■ ■ ■	244.12116	10.838	2	457741	52.0	1	199
9	<input checked="" type="checkbox"/>	N-(tert-Butoxycarbonyl)-3-methyl-L-	C11 H21 N O4	■ ■ ■ ■ ■	231.14719	6.110	8	414957		0	67
10	<input checked="" type="checkbox"/>	N-(2-Methyl-2-propanyl)-N-[(Z)-1-c	C10 H14 N2 O2	■ ■ ■ ■ ■	194.10581	1.889	7	255782		0	278
11	<input checked="" type="checkbox"/>	Methylenedioxybenzylpiperazine	C12 H16 N2 O2	■ ■ ■ ■ ■	220.12129	3.402	9	236827		0	237
12	<input checked="" type="checkbox"/>	N-Boc-L-proline	C10 H17 N O4	■ ■ ■ ■ ■	215.11582	3.588	8	225642		0	77
13	<input checked="" type="checkbox"/>	Benalaxyl	C20 H23 N O3	■ ■ ■ ■ ■	325.16774	14.913	8	179097		0	67
14	<input checked="" type="checkbox"/>			■ ■ ■ ■ ■	473.28339	4.606	2	172332		0	0
15	<input checked="" type="checkbox"/>	1-Phenylhexahydropyridazine-3,6-di	C10 H10 N2 O2	■ ■ ■ ■ ■	190.07421	7.636	1	99523	55.5	1	370
16	<input checked="" type="checkbox"/>	Crocin	C20 H24 O4	■ ■ ■ ■ ■	328.16732	26.368	2	94433		0	41
17	<input checked="" type="checkbox"/>	Atropine	C17 H23 N O3	■ ■ ■ ■ ■	289.16790	12.468	4	84052	62.0	3	67
18	<input checked="" type="checkbox"/>	3-(n-methyl-p-((p-nitrophenyl)azo)a	C16 H15 N5 O2	■ ■ ■ ■ ■	309.12264	11.684	7	82655		0	12
19	<input checked="" type="checkbox"/>	3-morpholino-2-(morpholinomethyl	C12 H21 N3 O2	■ ■ ■ ■ ■	239.16358	6.206	1	54734		0	28
20	<input checked="" type="checkbox"/>	6-Hydroxy-N-[[[2-methyl-2-propany	C11 H21 N O5	■ ■ ■ ■ ■	247.14200	4.392	7	48172		0	10
21	<input checked="" type="checkbox"/>	2-Methyl-2-propanyl 3-(hydroxymet	C11 H18 F3 N O3	■ ■ ■ ■ ■	269.12399	3.592	8	47493		0	2

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Structure Proposals Comounds per File Predicted Compositions Merged Features Matched Patterns mzVault Results mzCloud Results ChemSpider Results Mass List Search Results

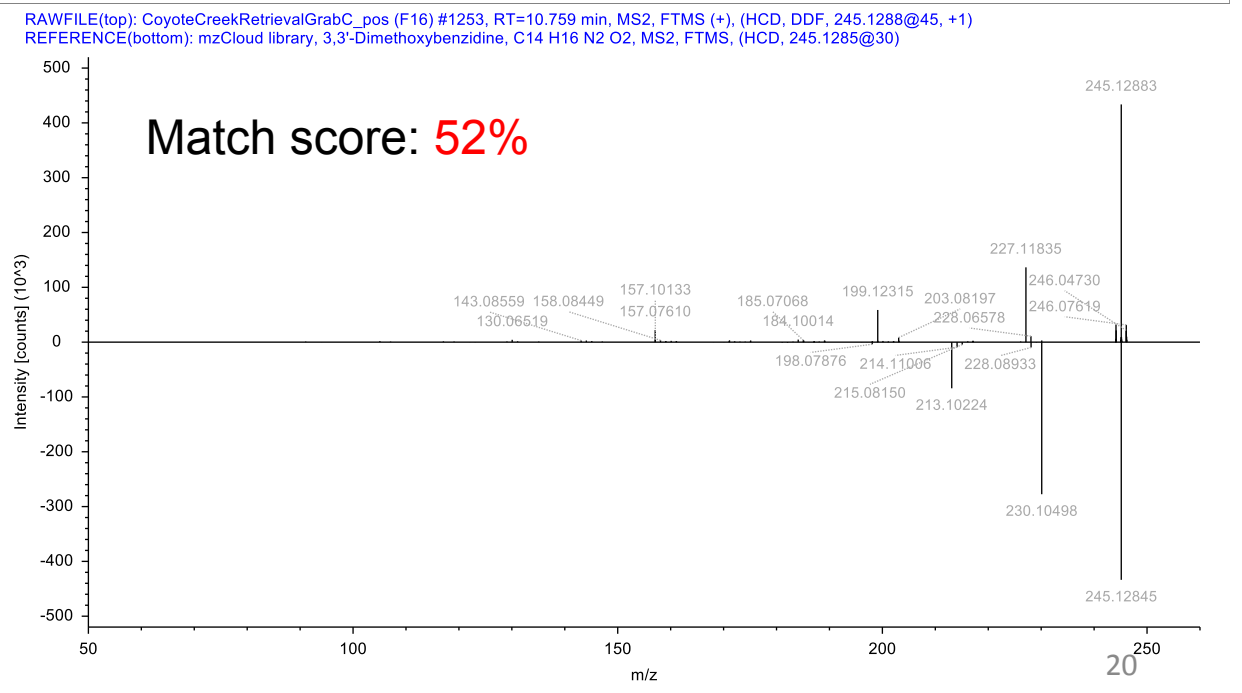
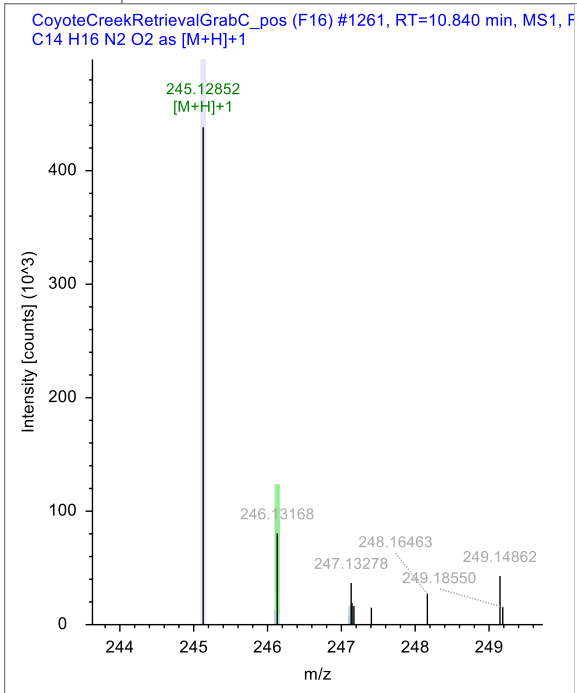
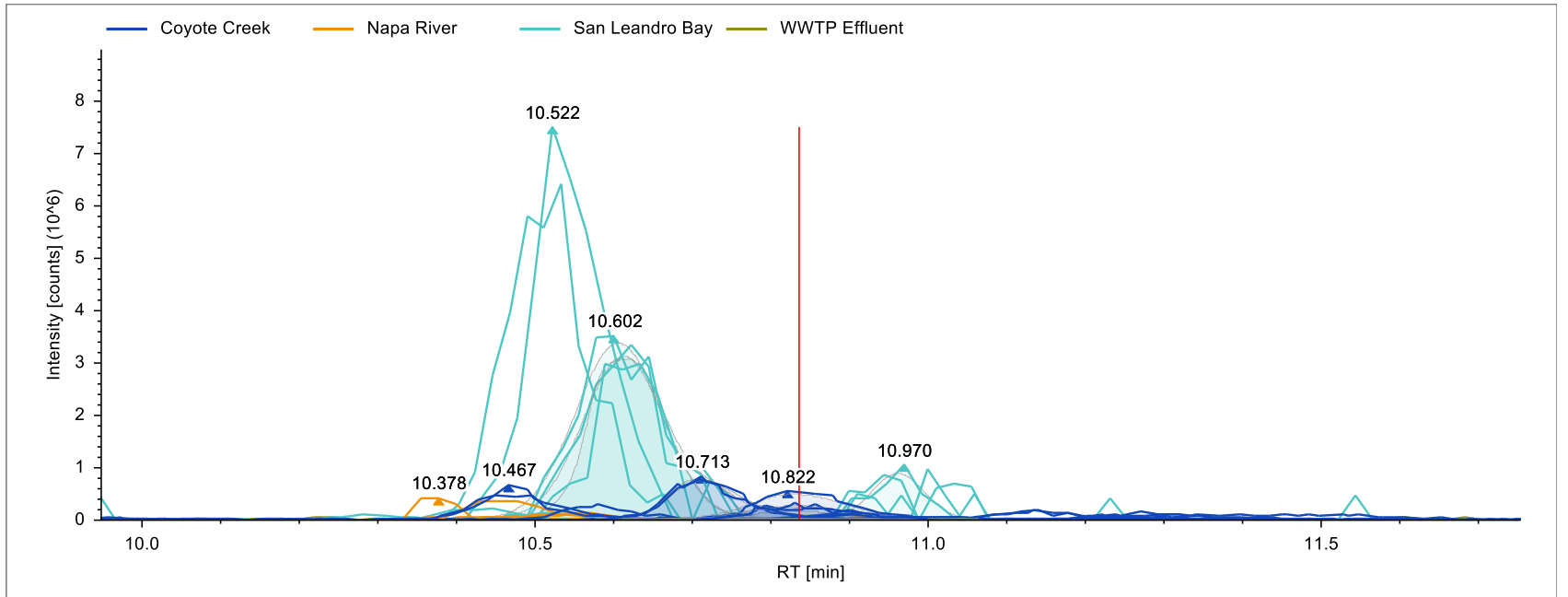
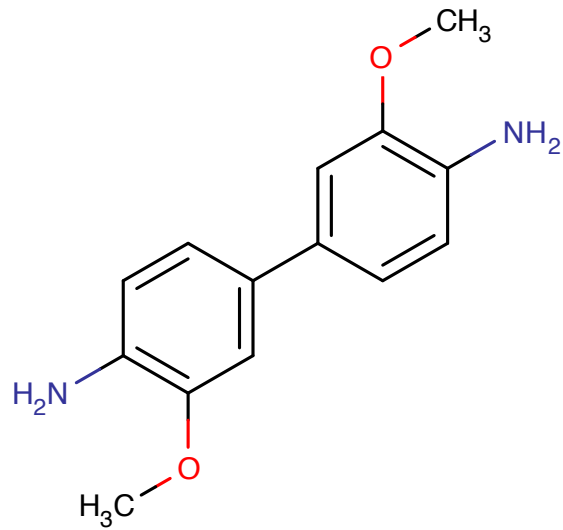
	Checked	Compound	ΔMass [Da]	ΔMass [ppm]	Scan Number	mzCloud ID	Formula	Structure	Molecular Weight	Match	Best Match	Name
1	<input type="checkbox"/>	■	0.00002	0.09	1253	1842	C14 H16 N2 O2		244.12118	52.0	59.6	3,3'-Dimethoxybenzidine

Show Related Tables

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3,3'-Dimethoxybenzidine?

This could be a reductive transformation product of diazobenzene dyes, but the spectral match is relatively poor.



Can library match be verified by *in silico* fragmentation?

Computational fragment tree analysis by Sirius 3.4 supports the assignment of $C_{14}H_{16}N_2O_2$ as candidate molecular formula...

The screenshot displays the Sirius 3.4.1 (build 6) software interface. The top menu bar includes options like Import, Batch Import, Load Workspace, Save Workspace, Compute All, Export Results, CSI:FingerId, Jobs, Settings, Bug Report, and About. The main window is divided into several sections:

- Left Panel (Filter):** Lists search results with details for each entry, including ionization type, parent mass, and the presence of 1 ms1 and 1 ms2 spectra. The top entry is 586.1784@28.68 with ionization [M + H]⁺ and parent mass 587.19 Da. The bottom entry is 244.1212@10.84 with ionization [M + H]⁺ and parent mass 245.13 Da.
- Top Panel (Results Table):** A table with columns: Rank, Molecular Formula, Score, Isotope Score, and Tree Score. The top result is Rank 1, Molecular Formula C₁₄H₁₆N₂O₂ + H, Score 47.31, Isotope Score 3.42, and Tree Score 43.88.
- Bottom Left Panel (Spectrum):** A mass spectrum plot showing relative intensity (0.0 to 1.0) versus mass/charge in Da (100 to 225). The base peak is at m/z 245.1285 Da.
- Bottom Right Panel (Nodes):** A fragmentation tree diagram starting from the parent ion C₁₄H₁₆N₂O₂ (245.1285 Da). The tree branches into various fragment ions based on losses of H₂O, CH₂N₂, C₂H₄N₂, C₃H₆, and CO. The root node is highlighted in green.

Can library match be verified by *in silico* fragmentation?

... however, structural fragment matching does not support identity of compound as 3,3'-dimethoxybenzidine.

An alternative identity may be Amidate (Etomidate), an anesthetic/hypnotic compound used in medical procedures. This must be considered a tentative identification.

The screenshot displays the Sirius 3.4.1 (build 6) software interface. The top menu bar includes options like Import, Batch Import, Load Workspace, Save Workspace, Compute All, Export Results, CSI:FingerId, Jobs, Settings, Bug Report, and About. The main window is divided into several sections:

- Filter:** A search filter box.
- Table of Results:** A table with 6 columns showing mass spectrometry data for different compounds. The first row is highlighted in green, indicating a match: **1 C₁₄H₁₆N₂O₂ + H⁺** with a score of 47.306630.
- XLogP filter:** A slider set to -2.00 to 8.00.
- Database Selection:** A row of checkboxes for various databases: Biocyc, biological, CHEBI, custom, GNPS, HMDB, HSDB, KEGG, KNApSAcK, Maconda, MeSH, Natural Products, PubChem, PubMed, and training set.
- Chemical Structure 5282:** A chemical structure of Etomidate (Amidate) is shown with a score of e^{-4813} . The structure is CCOC(=O)C1=CN=C(C2=CC=CC=C2)N1.
- Chemical Structure 6079:** A chemical structure of N-beta-Naphthylaminomethyl-L-alanine is shown with a score of e^{-4819} . The structure is CC(C)C(=O)NCCNc1ccc2ccccc12.
- Substructures and Databases:** For each structure, there are substructure grids and database buttons (e.g., training set, HMDB, CHEBI, ZINC bio, KEGG, MeSH, biological, GNPS for 5282; MeSH for 6079).

Alternative approaches for prioritizing compounds for identification:

- Chlorine isotope matching on molecular ion clusters.
- This approach uses allows “identity-blind” focus on chlorine and/or bromine-containing compounds in samples.
- Spectral library matches were available for only 18 “Cl”-pattern matched compounds in the SF Bay sample set.
- Most of these compounds were pharmaceuticals and/or agrochemicals.

Compound Discoverer 2.1.0.323

File Reporting Libraries View Window Help

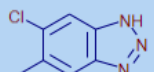
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Compounds Compounds per File Merged Features Features mzVault Results mzCloud Results ChemSpider Results Mass List Search Results Input Files

	Checked	Name	Formula	Annotation Sc <input type="checkbox"/>	Molecular Weight	RT [min]	# MS2	Area (Max.)	Pattern Matches <input type="checkbox"/>	mzCloud Best Match	# mzCloud Results	# ChemSpider Results
1	<input type="checkbox"/>	Myclobutanil	C15 H17 Cl N4	<input type="checkbox"/>	288.11446	28.960	5	56539079	<input type="checkbox"/>	81.4	1	14
2	<input type="checkbox"/>	Phenol, 2,2'-thiobis[4-tert-butyl-	C20 H26 O2 S	<input type="checkbox"/>	330.16526	13.652	5	3753773	<input type="checkbox"/>	57.2	2	7
3	<input type="checkbox"/>	Losartan	C22 H23 Cl N6 O	<input type="checkbox"/>	422.16199	21.611	8	3195331	<input type="checkbox"/>	87.6	1	5
4	<input type="checkbox"/>	Losartan	C22 H23 Cl N6 O	<input type="checkbox"/>	422.16291	22.043	3	1258201	<input type="checkbox"/>	68.2	1	1
5	<input type="checkbox"/>	Losartan	C22 H23 Cl N6 O	<input type="checkbox"/>	422.16229	21.192	6	839837	<input type="checkbox"/>	86.1	1	3
6	<input type="checkbox"/>	Losartan	C22 H23 Cl N6 O	<input type="checkbox"/>	422.16201	22.062	2	700336	<input type="checkbox"/>	84.9	1	7
7	<input type="checkbox"/>	Clindamycin	C18 H33 Cl N2 O5 S	<input type="checkbox"/>	424.17984	15.238	6	393633	<input type="checkbox"/>	99.8	1	13
8	<input type="checkbox"/>	6-Chloro-5-methyl-1H-1,2,3-benzotriazole	C7 H6 Cl N3	<input type="checkbox"/>	167.02501	15.466	3	376255	<input type="checkbox"/>	79.6	3	100
9	<input type="checkbox"/>	Temazepam	C16 H13 Cl N2 O2	<input type="checkbox"/>	300.06639	22.242	12	352804	<input type="checkbox"/>	91.8	1	35
10	<input type="checkbox"/>	Clopidogrel carboxylic acid	C15 H14 Cl N O2 S	<input type="checkbox"/>	307.04332	10.068	5	301450	<input type="checkbox"/>	95.3	1	24
11	<input type="checkbox"/>	4-Chloro-N-[5-(2-methyl-2-propoxy)]	C14 H15 Cl N2 O2	<input type="checkbox"/>	278.08228	10.826	4	267665	<input type="checkbox"/>	50.4	1	25
12	<input type="checkbox"/>	Fenofibric acid	C17 H15 Cl O4	<input type="checkbox"/>	318.06572	30.088	1	255075	<input type="checkbox"/>	68.0	1	25
13	<input type="checkbox"/>	Phenol, 2,2'-thiobis[4-tert-butyl-	C20 H26 O2 S	<input type="checkbox"/>	330.16594	7.349	1	135556	<input type="checkbox"/>	59.4	2	15
14	<input type="checkbox"/>	Clindamycin	C18 H33 Cl N2 O5 S	<input type="checkbox"/>	424.17967	14.977	1	131760	<input type="checkbox"/>	97.3	1	2
15	<input type="checkbox"/>	Brilliant blue FCF	C37 H36 N2 O9 S3	<input type="checkbox"/>	748.15797	18.072	1	122568	<input type="checkbox"/>	85.8	1	1
16	<input type="checkbox"/>	Acamprosate	C5 H11 N O4 S	<input type="checkbox"/>	181.04070	19.579	2	119509	<input type="checkbox"/>	69.7	1	24
17	<input type="checkbox"/>	PIQUIZIL	C19 H26 N4 O4	<input type="checkbox"/>	374.19548	18.505	2	94898	<input type="checkbox"/>	50.9	1	7
18	<input type="checkbox"/>	Metoclopramide	C14 H22 Cl N3 O2	<input type="checkbox"/>	299.14025	9.989	3	70759	<input type="checkbox"/>	85.1	1	3

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Structure Proposals Compounds per File Predicted Compositions Merged Features Matched Patterns mzVault Results mzCloud Results ChemSpider Results Mass List Search Results

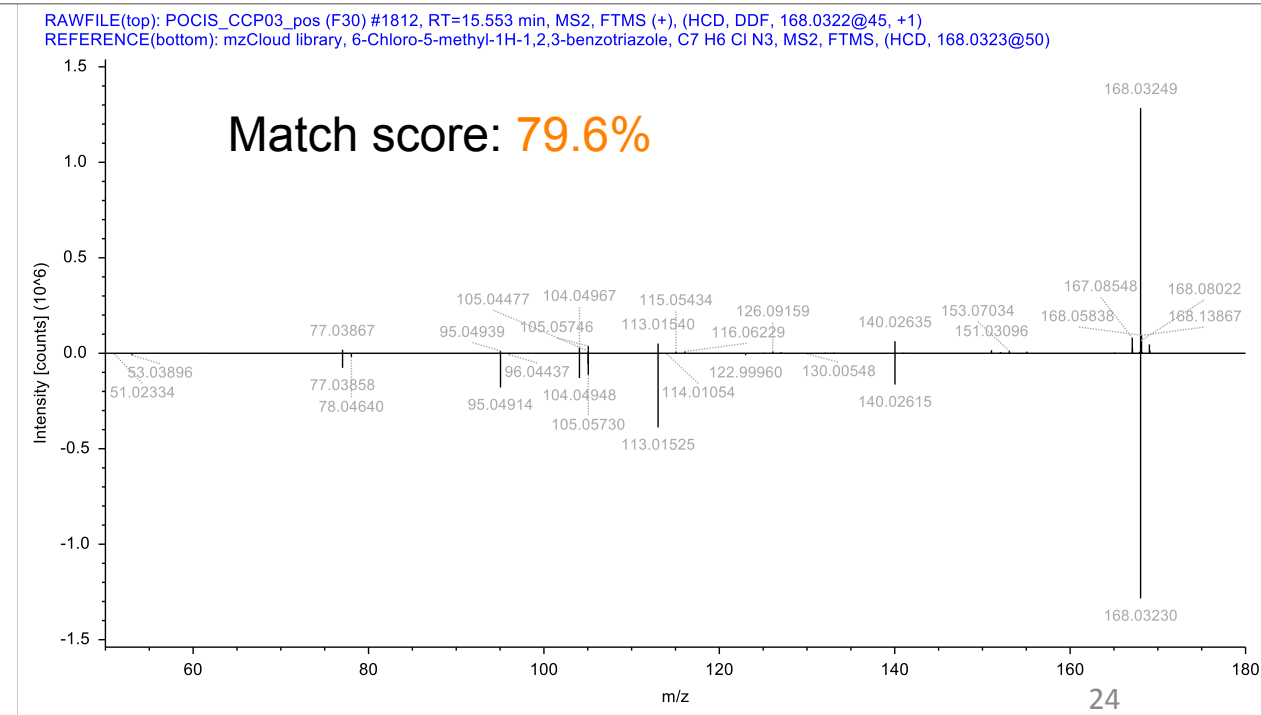
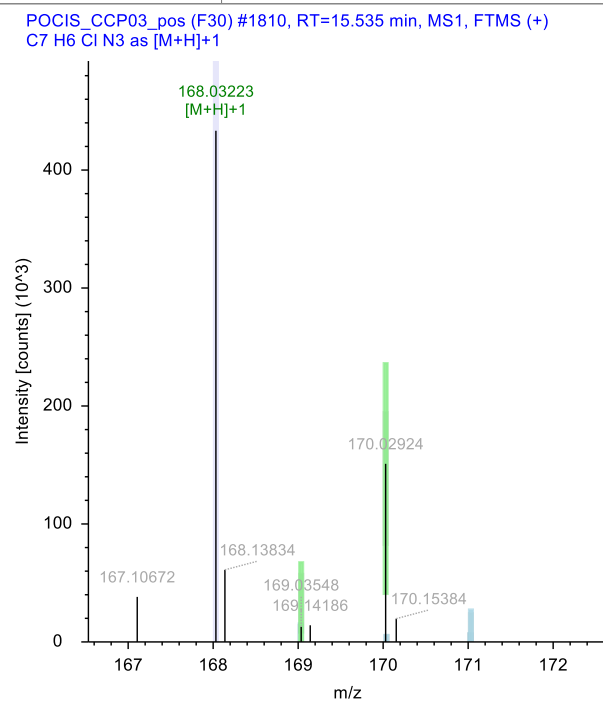
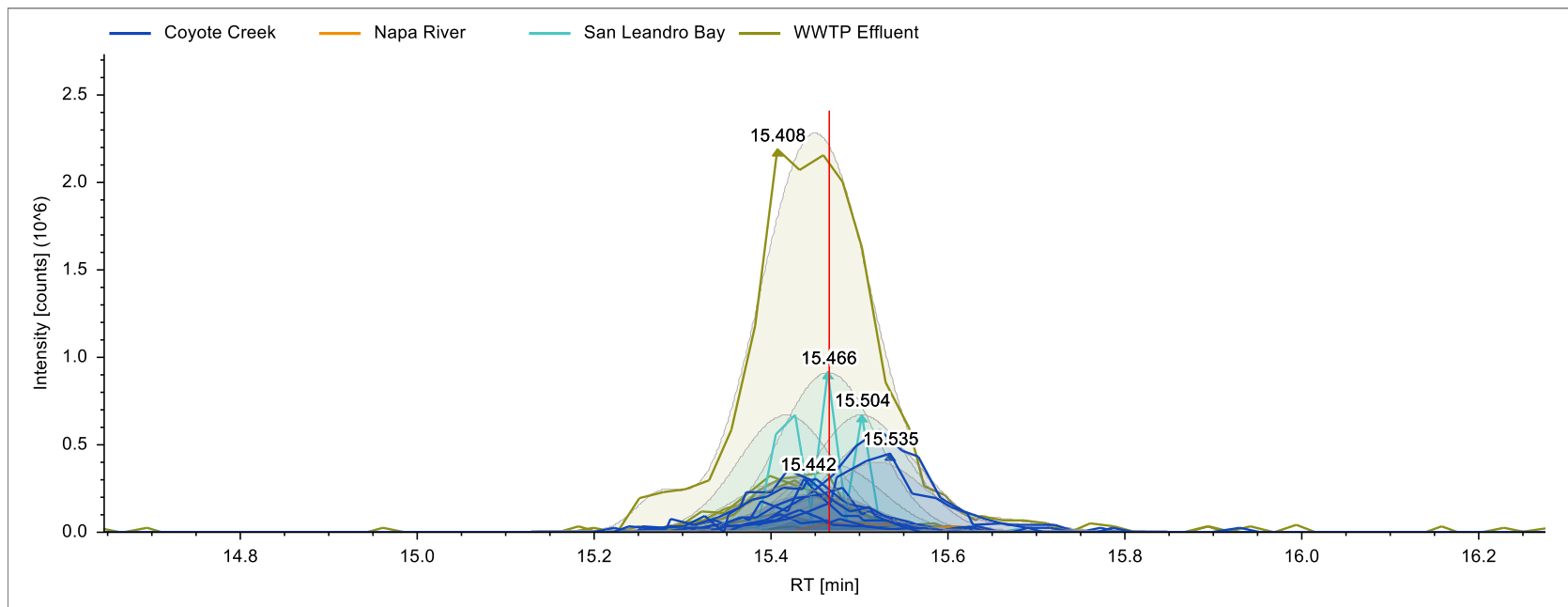
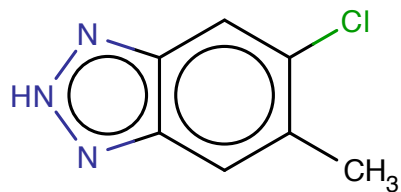
Checked	Compound	ΔMass [Da]	ΔMass [ppm]	Scan Number	mzCloud ID	Formula	Structure	Molecular Weight	Match	Best Match	Name
<input type="checkbox"/>	<input type="checkbox"/>	0.00002	0.10	1812	3729	C7 H6 Cl N3		167.02502	79.6	79.6	6-Chloro-5-methyl-1H-1,2,3-benzotriazole

Show Related Tables

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6-Chloro-5-methyl-1H-1,2,3-benzotriazole:

Chlorinated benzotriazoles are used in polymer additive UV inhibitors. This may be a precursor or transformation product of such polymer additives.



Conclusions: SF Bay

- Bay waters are impacted by a variety of organic pollutant sources.
- Stormwater seems to be an important contributor of organic pollutant burdens in some areas of the Bay, as illustrated by findings of abundant polyethoxylated compounds and additive chemicals in San Leandro Bay.
- Agrochemicals and natural products appear to be the organic compounds most specifically detected in the Napa River.
- Passive sampling and discrete grab-sampling are complementary approaches for non-targeted analysis of ambient waters.

Future directions

- Statistically-sound, comprehensive identification of organic pollutants in water using a harmonized workflow: *in silico* MS/MS and evidence-based prioritization (moving beyond library matching).
- Application of computational transformation product prediction from identified “leads” for drilling-down into possible pollutant TP identifications.
- Enhanced prioritization of compound identification using pairwise differential analysis among spatial and temporal samples.
- Annotation of compound identifications by molecular ontology, functional use, and toxicity data/predictions using open-source tools including EPA CompTox Dashboard.

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ThermoFisher
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