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

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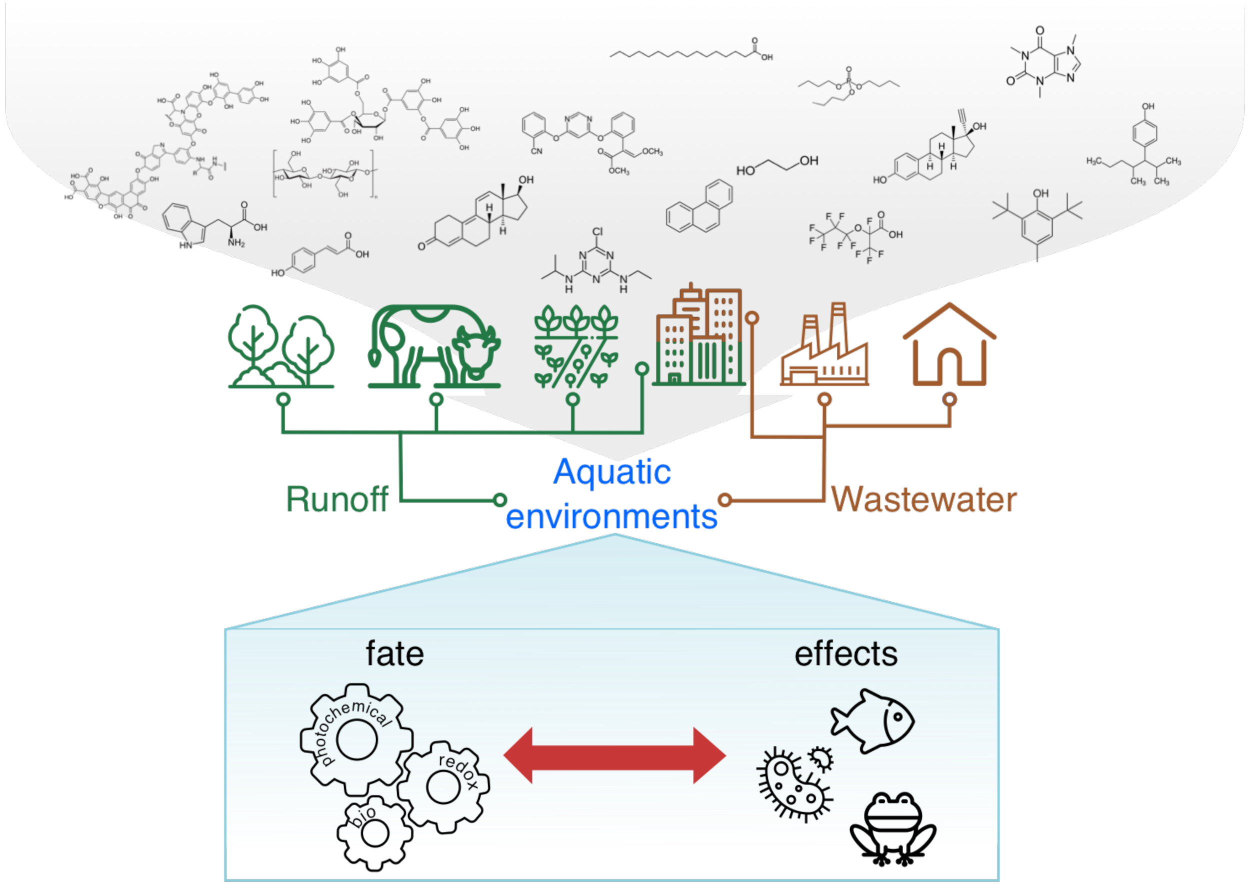
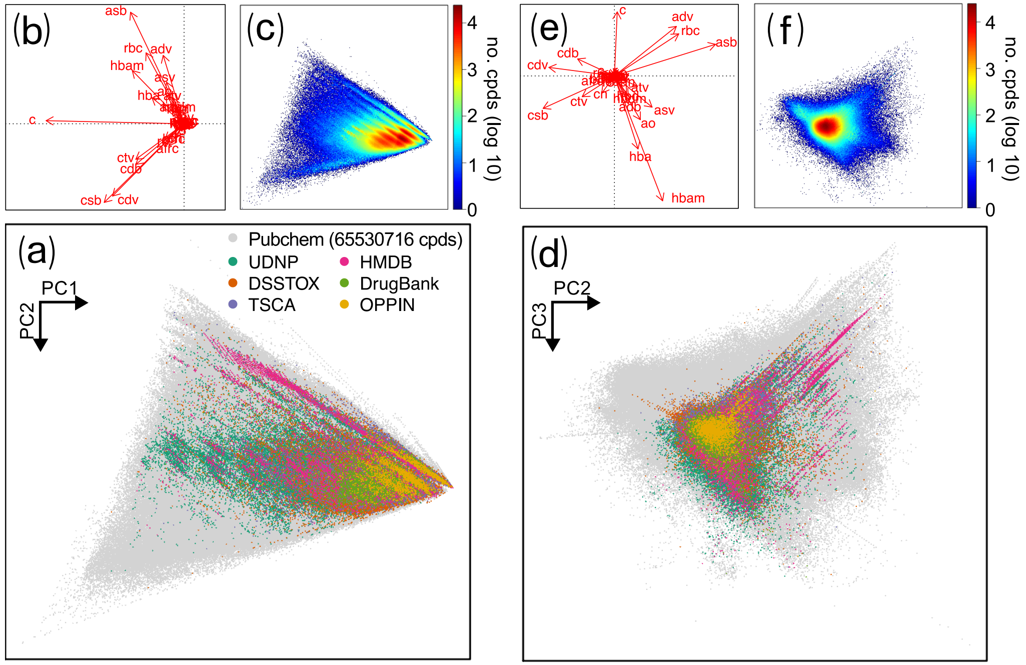
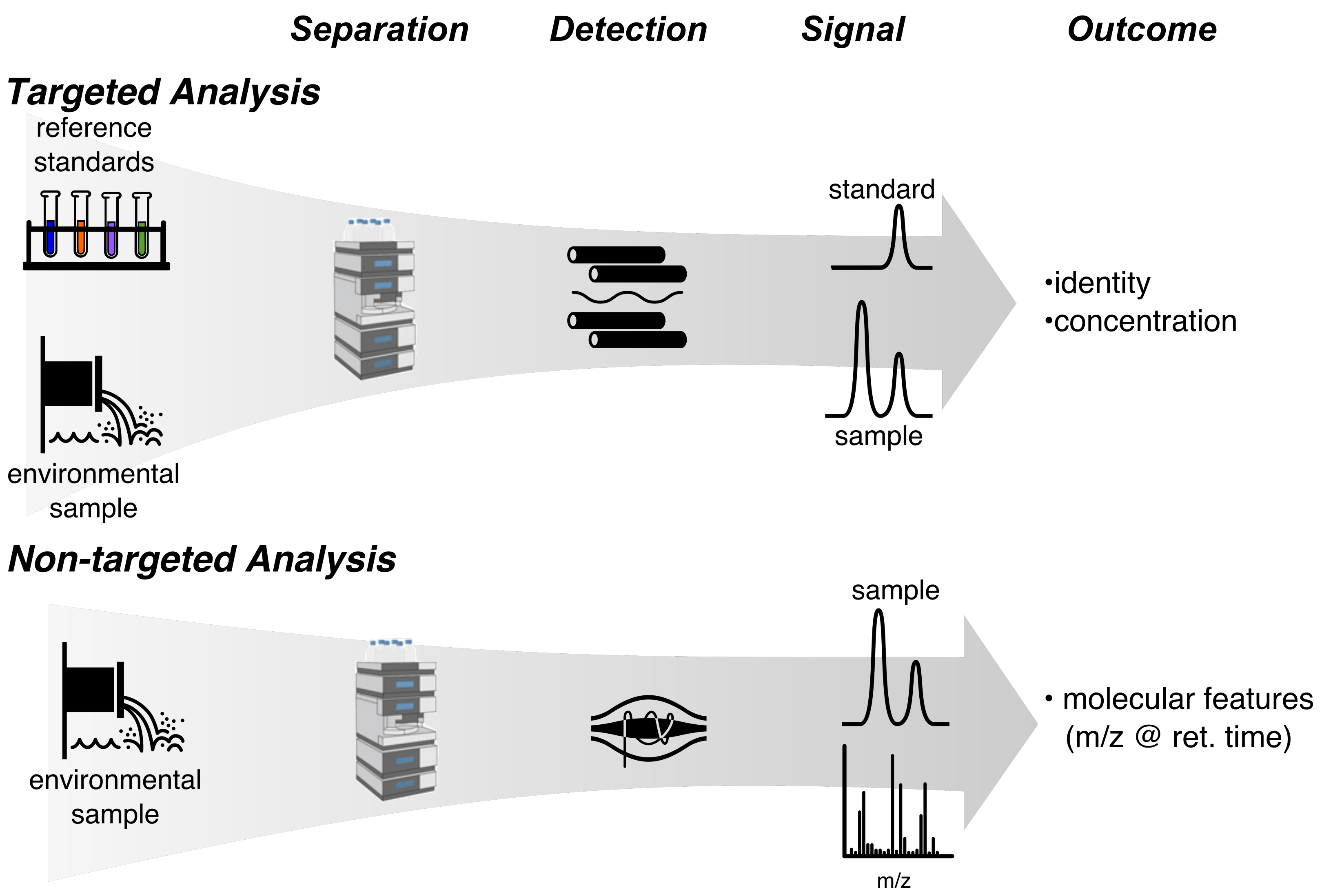
Which organic pollutants occur in environmental waters?

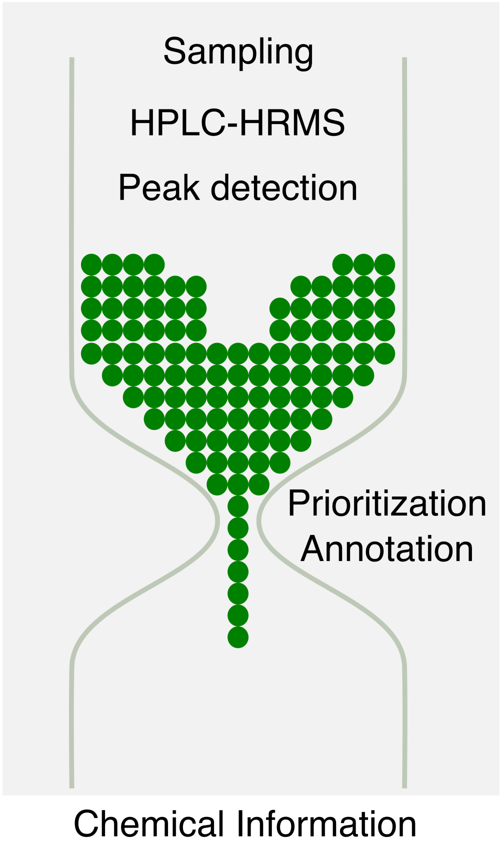


- Chemical space is vast. How can we assess which chemicals in commerce are important as emerging pollutants in the aquatic environment?
- Chemical use, production, and regulation lists are incomplete.
- (Bio)transformation makes prioritization more difficult and compound identification challenging.



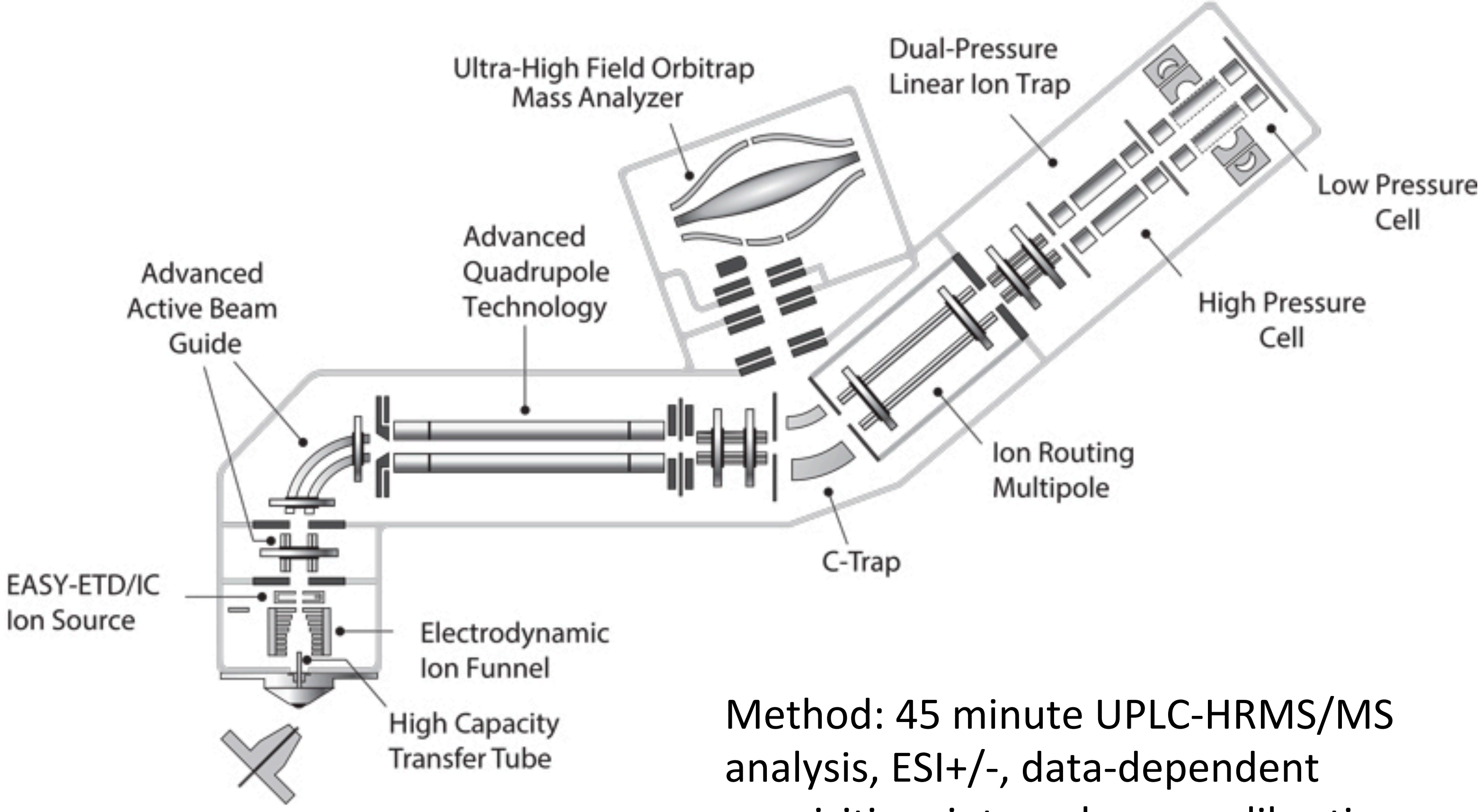
Targeted vs. Non-targeted analysis





Ultra-high resolution mass spectrometry for non-targeted analysis

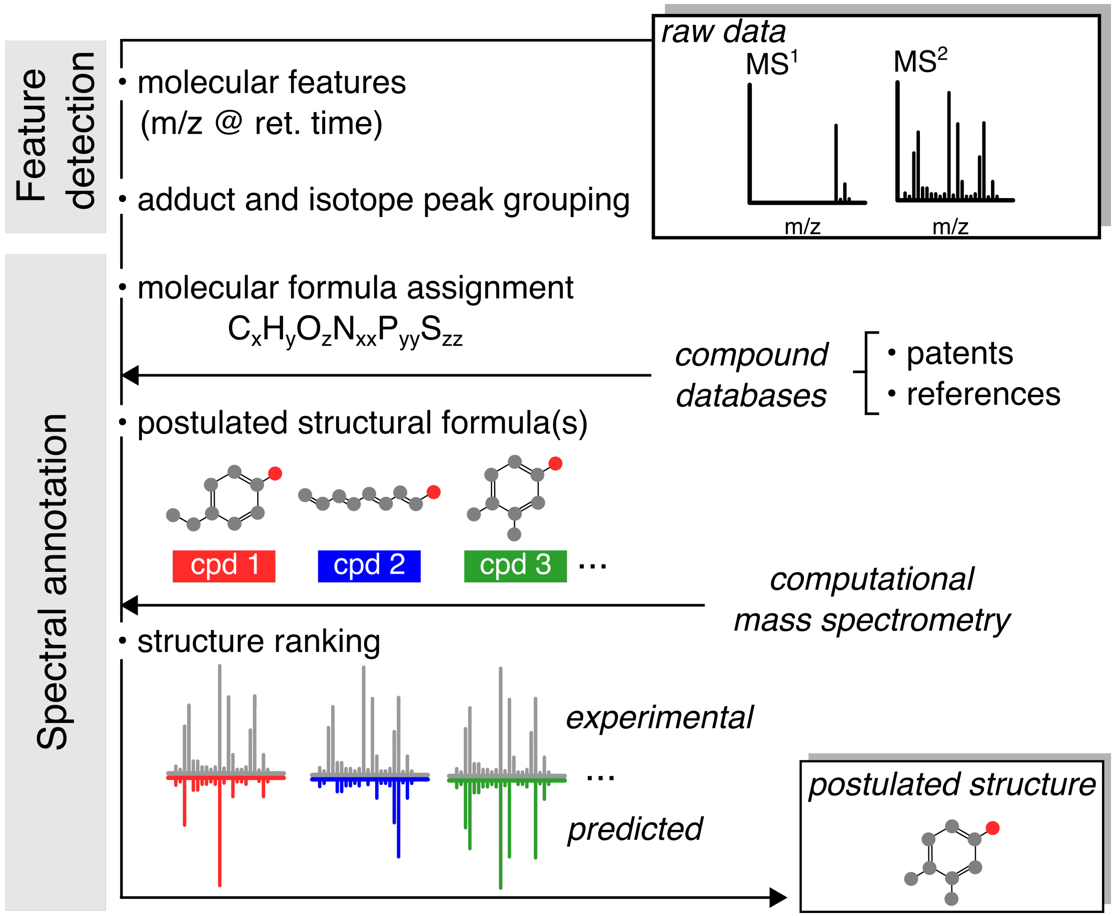
- Orbitrap Fusion Lumos mass spectrometer
- Critical resolution (500,000) and <u>mass accuracy</u> (< 1 ppm)
- Ultra-fast data-dependent MS/MS maximizes data acquisition rate
- MS^3 capability for structural characterization
- Ion funnel gives maximum sensitivity



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

Data analysis: The hard part!

<u>Overall non-targeted analysis workflow:</u>

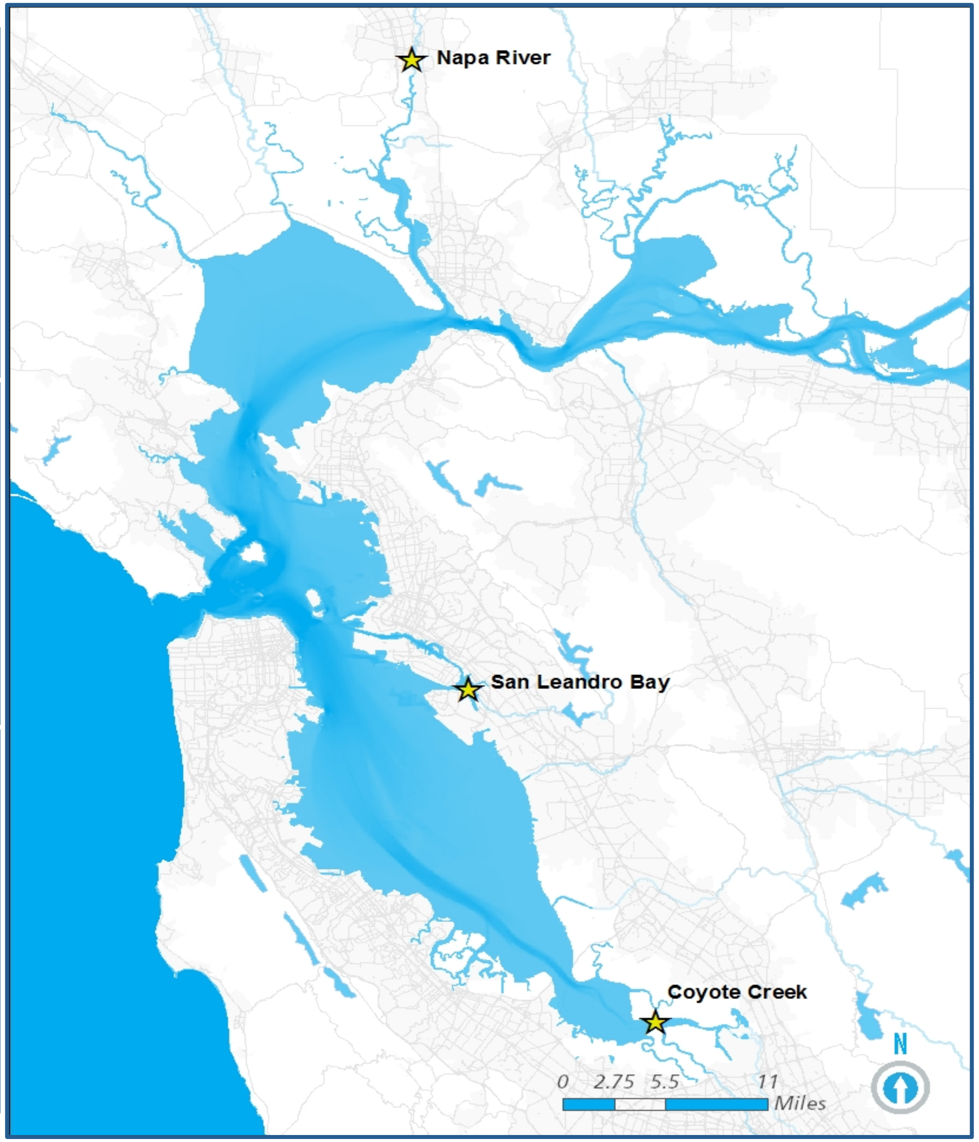


Objectives

- 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.

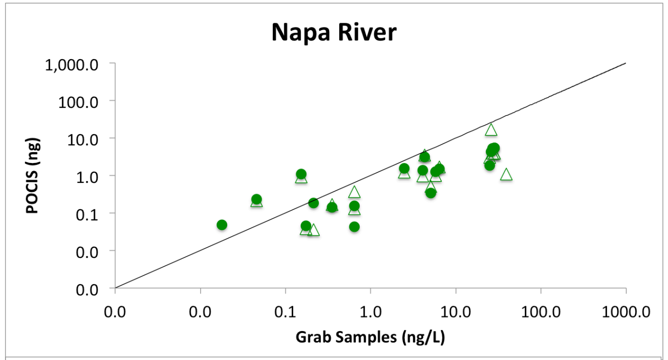
Emerging pollutants in SF Bay waters

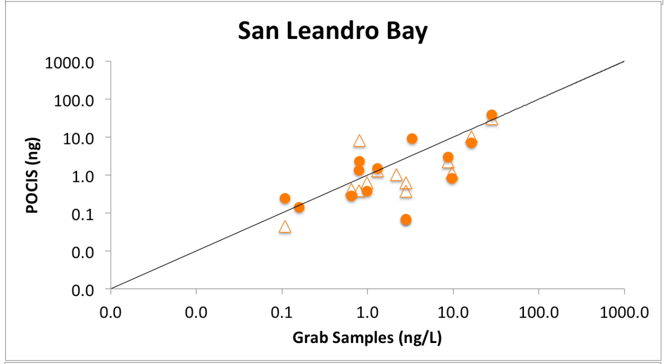
- Ambient Bay sample collection:
  - Three sites were sampled: San Leandro Bay (March 2016), Napa River (April 2016), and Coyote Creek (Aug/ Sept. 2016)
  - POCIS were deployed for one month at each site.
  - Grab samples (4L volume, one collected in triplicate) were taken at sites on POCIS deployent & 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

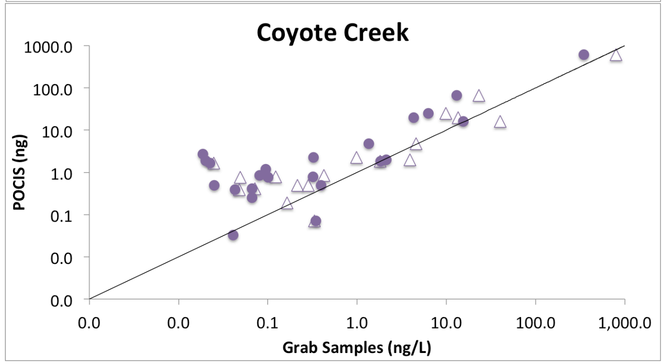


Target Analyte Quantitation

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	
Propanolol	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	



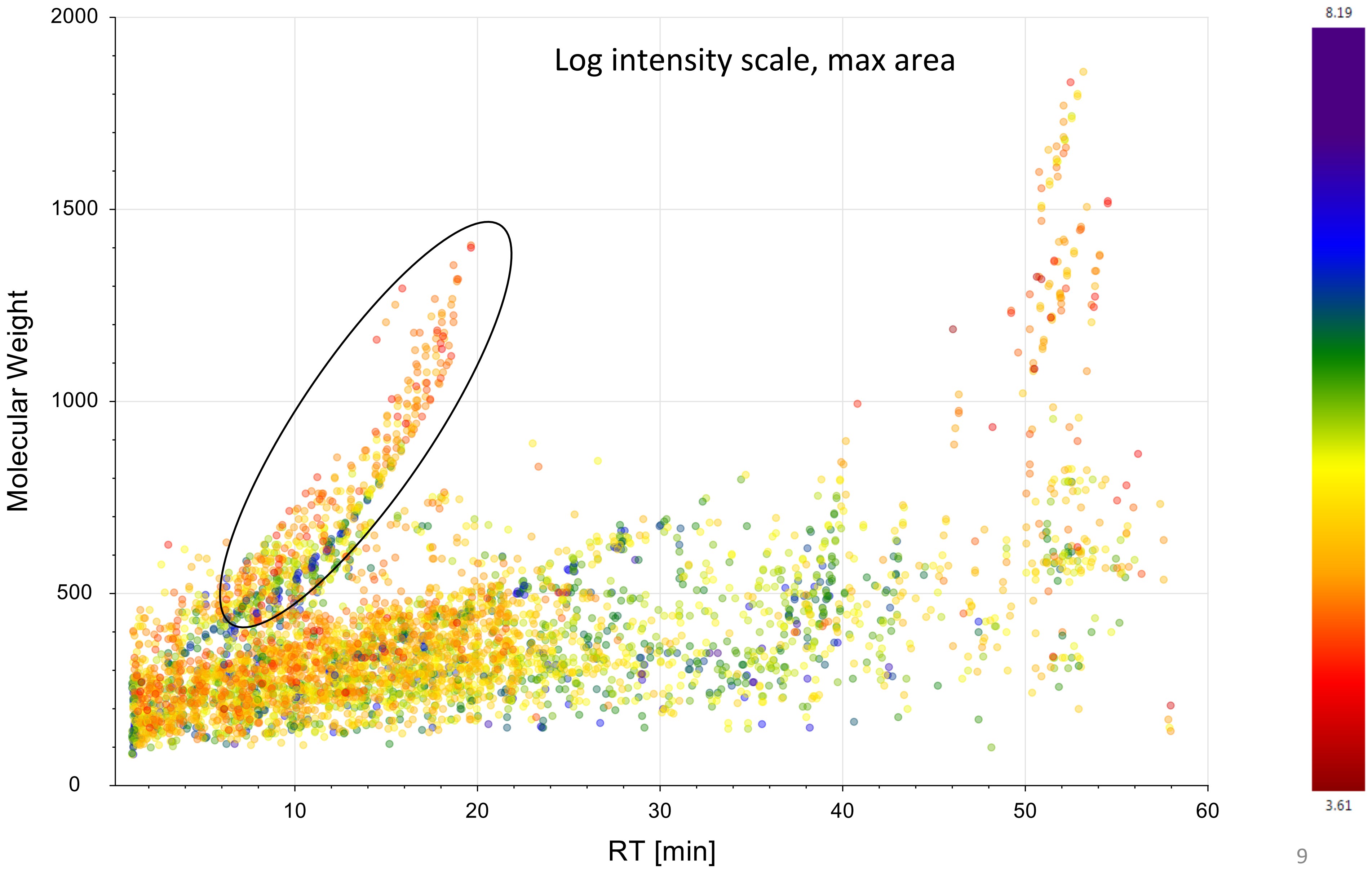




- A variety of agrochemicals and pharmaceutical compounds were detected in WWTP effluents and Bay waters at typically ppt levels.
- Benzotriazole was the most abundant targeted micropollutant in Bay water (Coyote Creek), consistent with high levels in WWTP effluent.
- Target analyte levels in POCIS extracts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

Non-targeted analysis: Data set characteristics

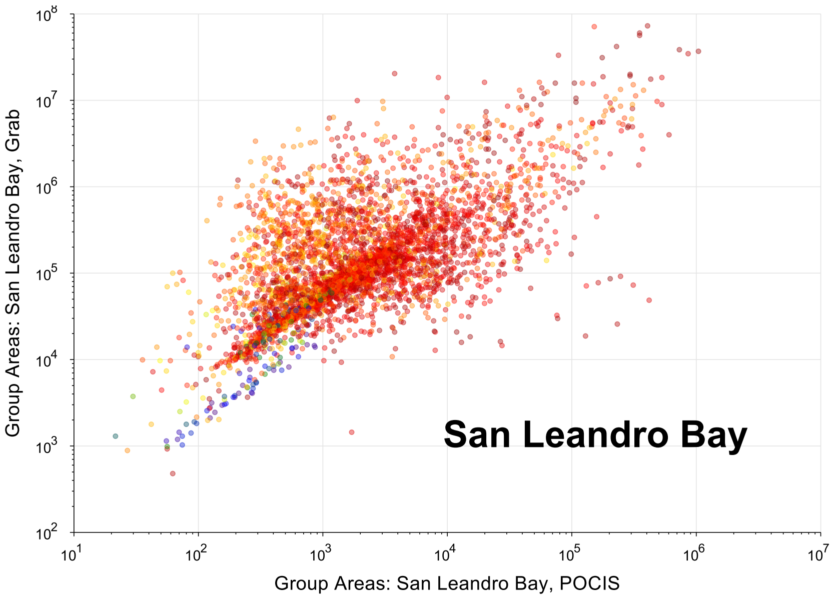
Total # compounds: **4965**
Not on target list: **4630**

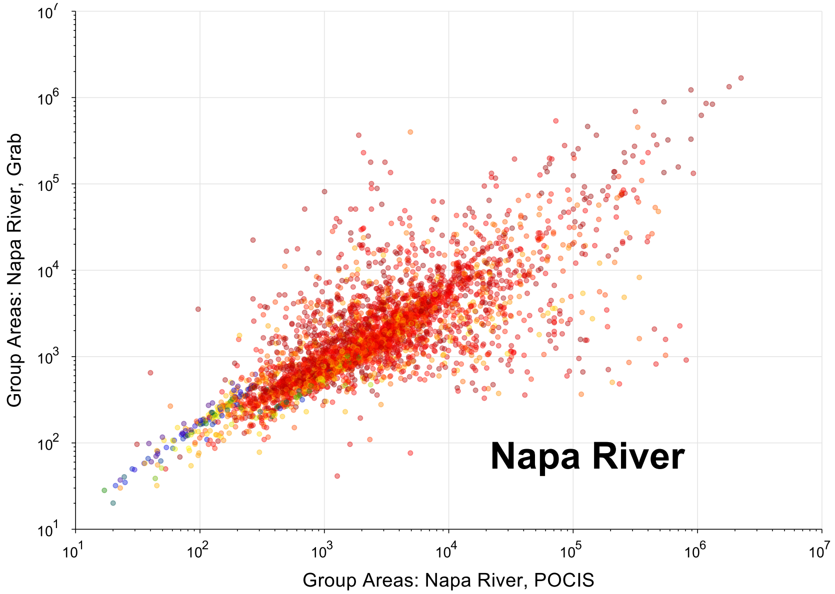


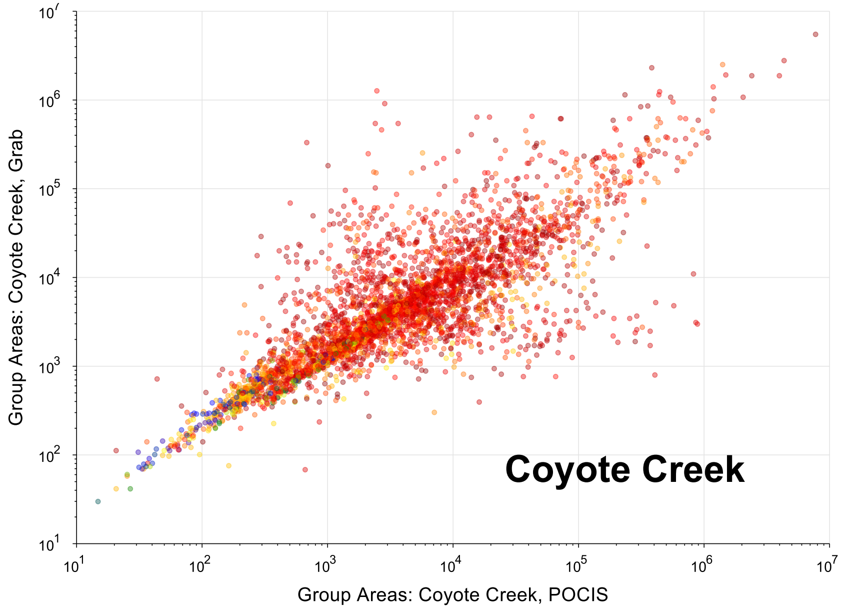
Non-targeted analysis data overview: POCIS vs. Grab samples

<u>How well do POCIS extract intensities correlate with intensities of compounds in grab samples?</u>

- 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





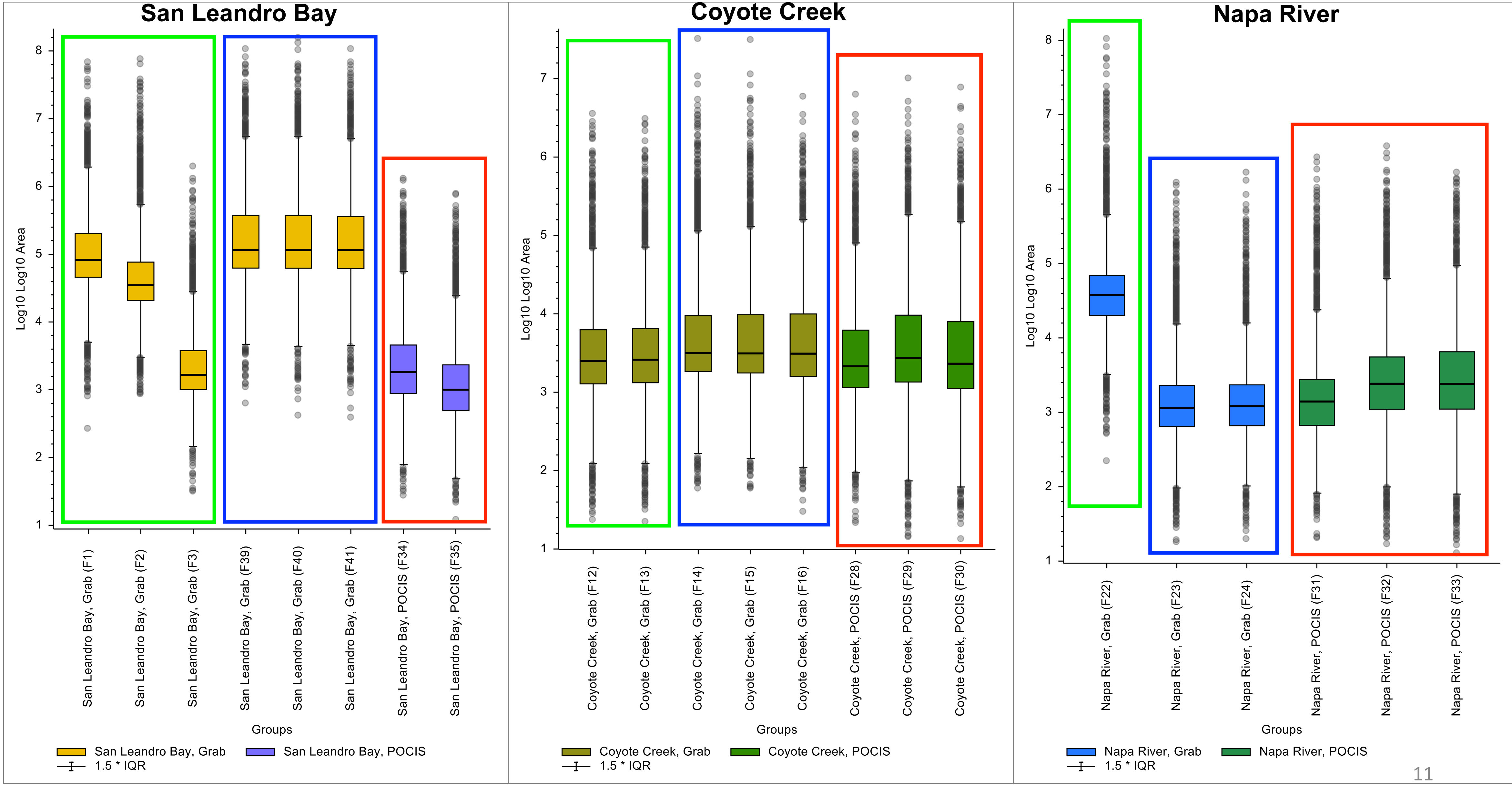


Non-targeted analysis data overview: POCIS and grab sample compound abundance by site

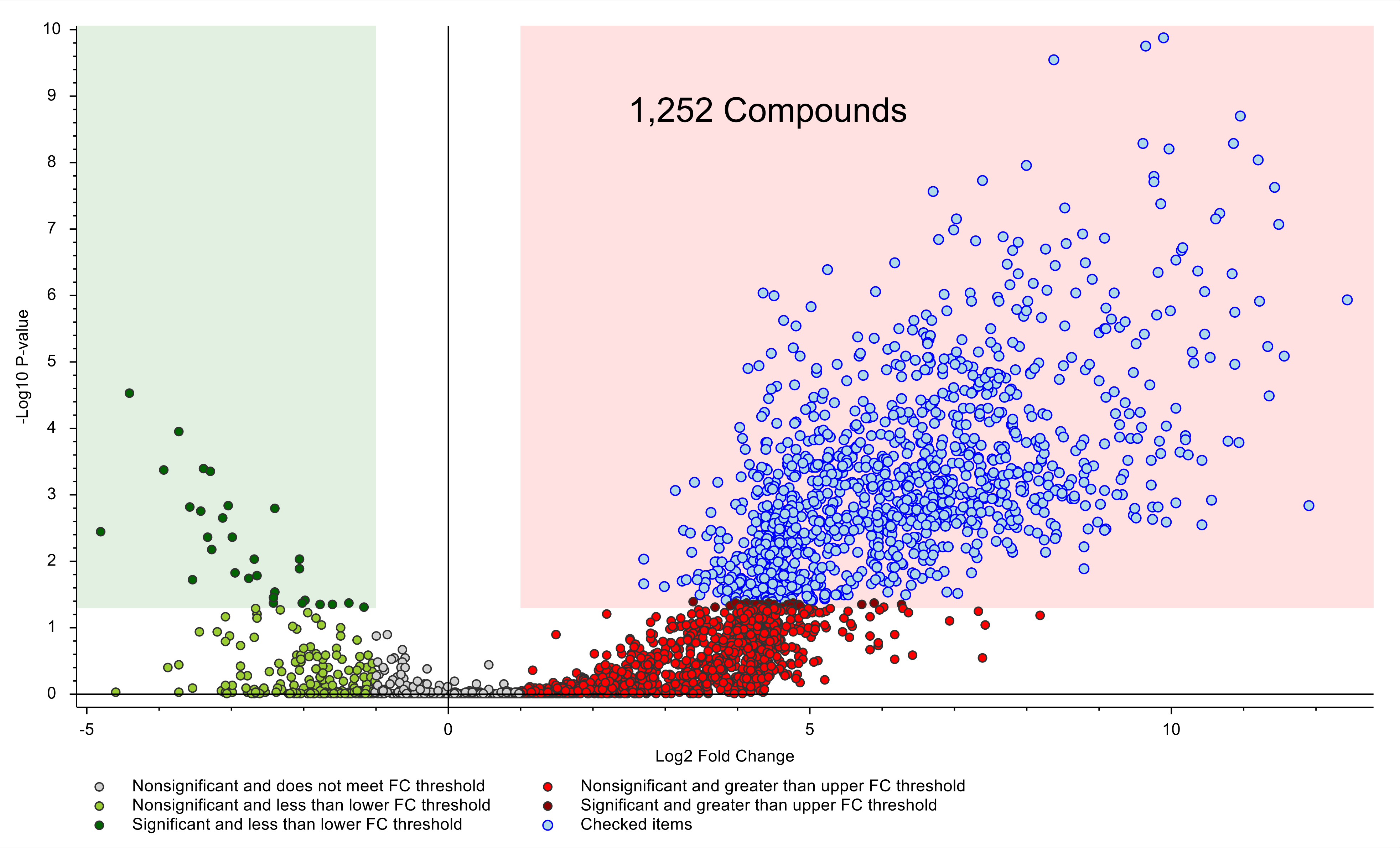
Deployment

Retrieval

POCIS

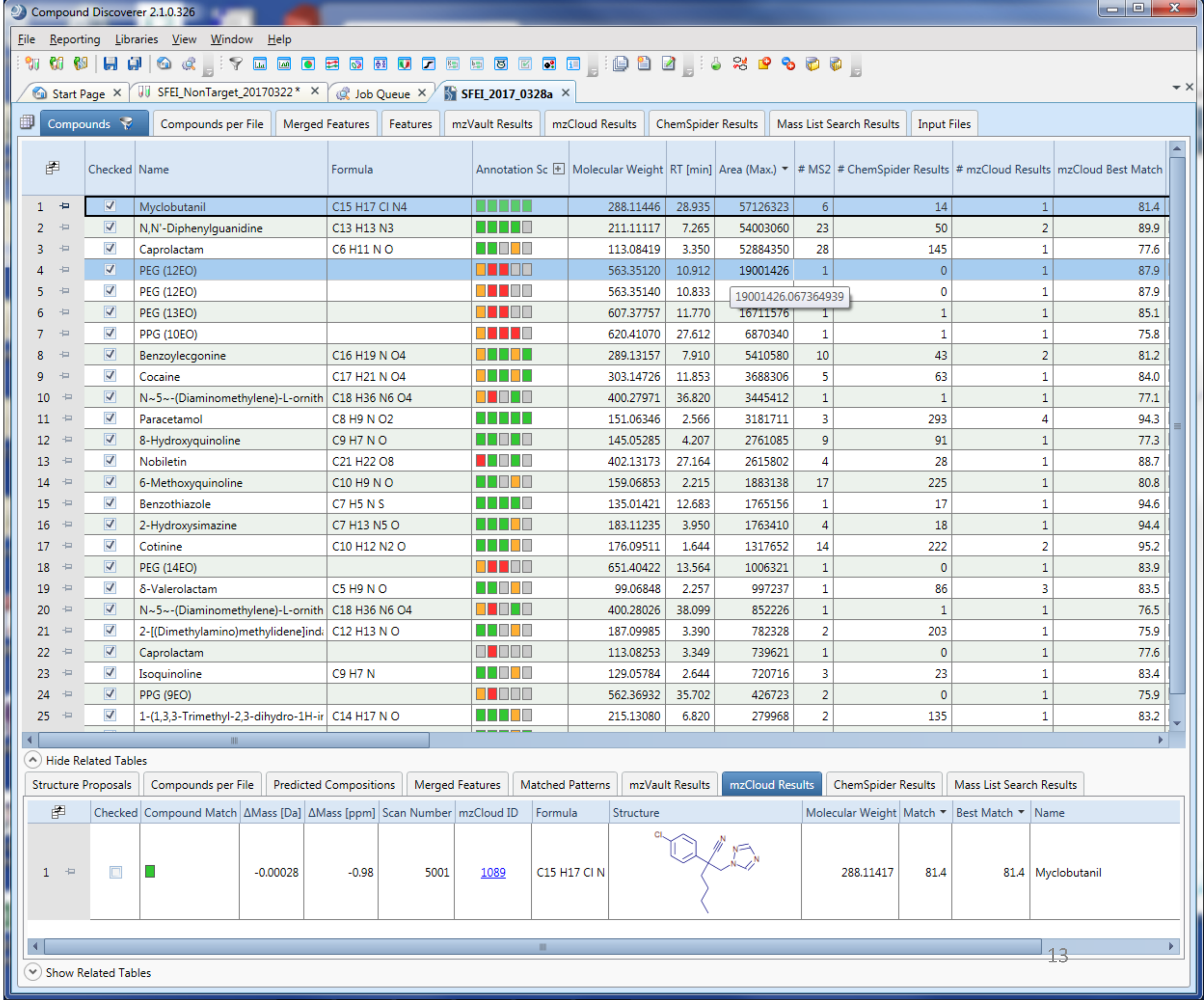


Differential analysis: Relative abundance of compounds in San Leandro Bay vs. WWTP Effluent (average)



<u>Identifying compounds in San Leandro Bay water:</u>

- 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.

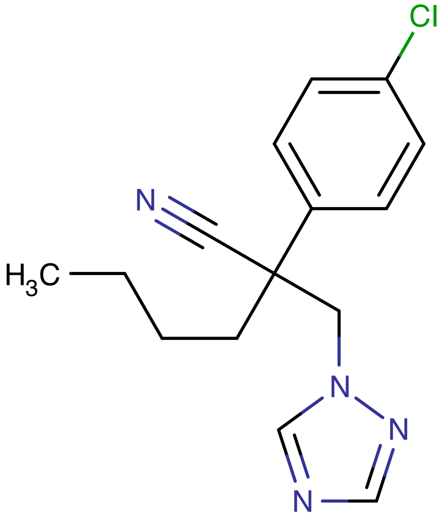


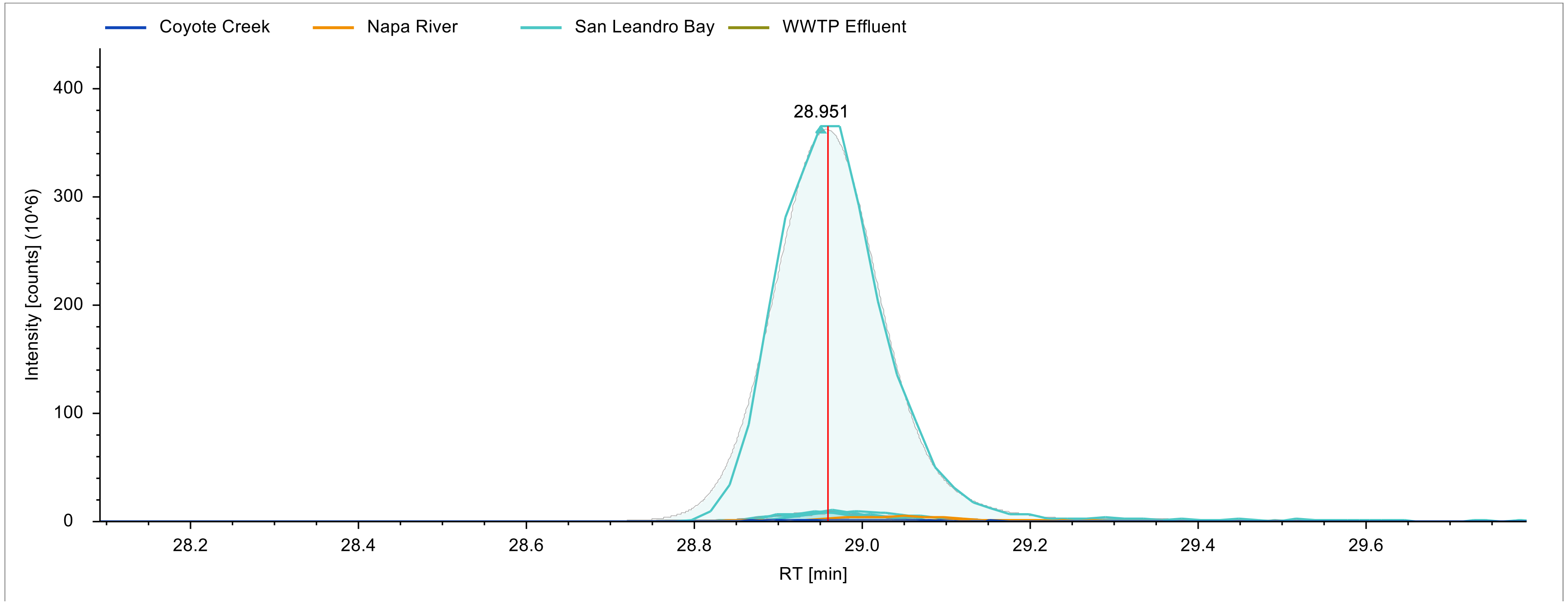
	Checked	Name	Formula	Annotation Sc	Molecular Weight	RT [min]	Area (Max.)	# MS2	# ChemSpider Results	# mzCloud Results	mzCloud Best Match
1	✓	Myclobutanil	C15 H17 Cl N4		288.11446	28.935	57126323	6	14	1	81.4
2	✓	N,N'-Diphenylguanidine	C13 H13 N3		211.11117	7.265	54003060	23	50	2	89.9
3	✓	Caprolactam	C6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

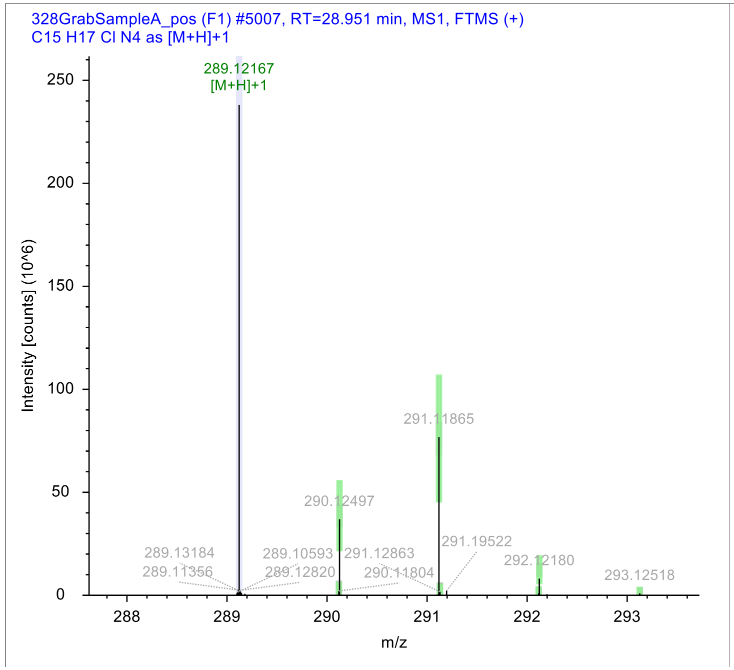
	Checked	Compound Match	ΔMass [Da]	ΔMass [ppm]	Scan Number	mzCloud ID	Formula	Structure	Molecular Weight	Match	Best Match	Name
1	☐		-0.00028	-0.98	5001	1089	C15 H17 Cl N		288.11417	81.4	81.4	Myclobutanil

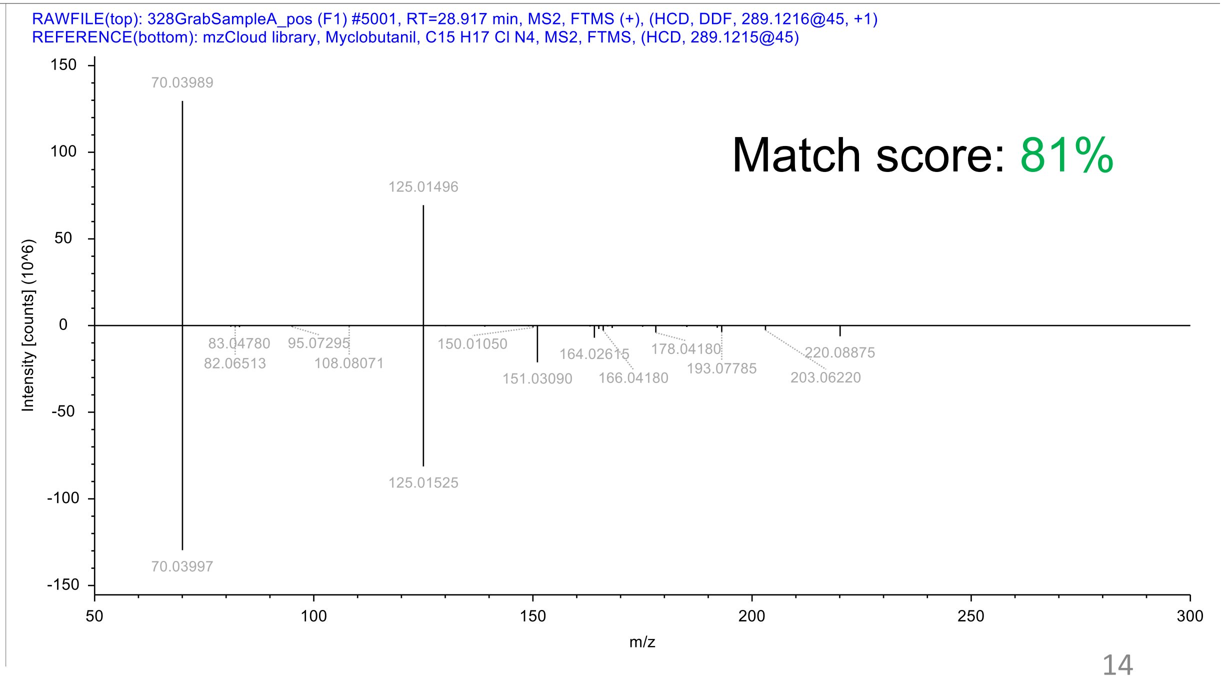
Myclobutanil: Conazole fungicide used to control fungal infections in table & wine grapes (primarily in California). Also used for cannabis cultivation.

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

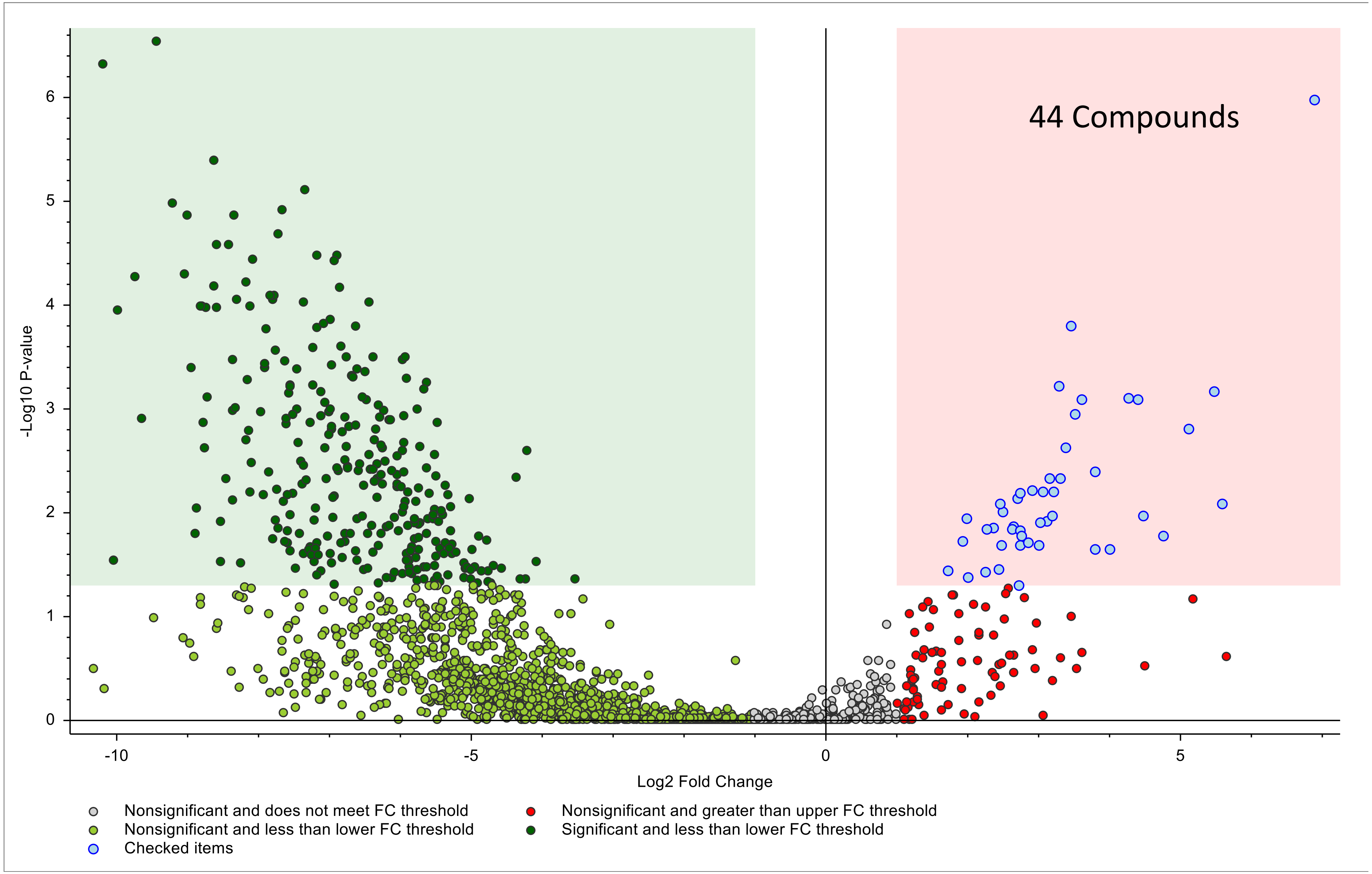






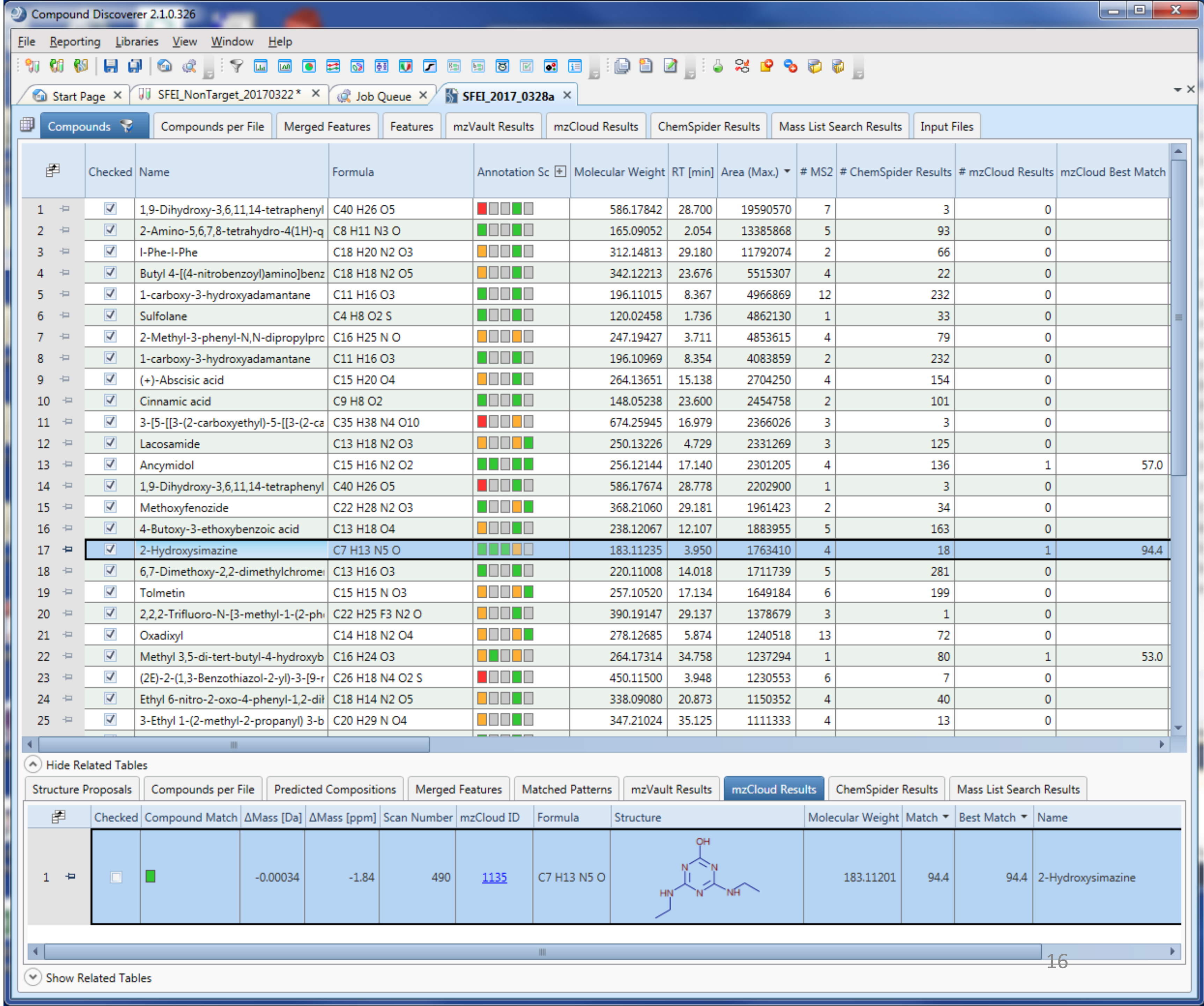


Differential analysis: Relative abundance of compounds in Napa River vs. WWTP Effluent (average)

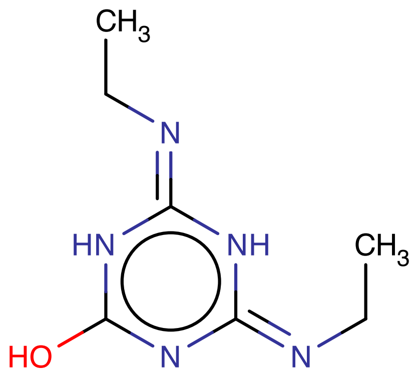


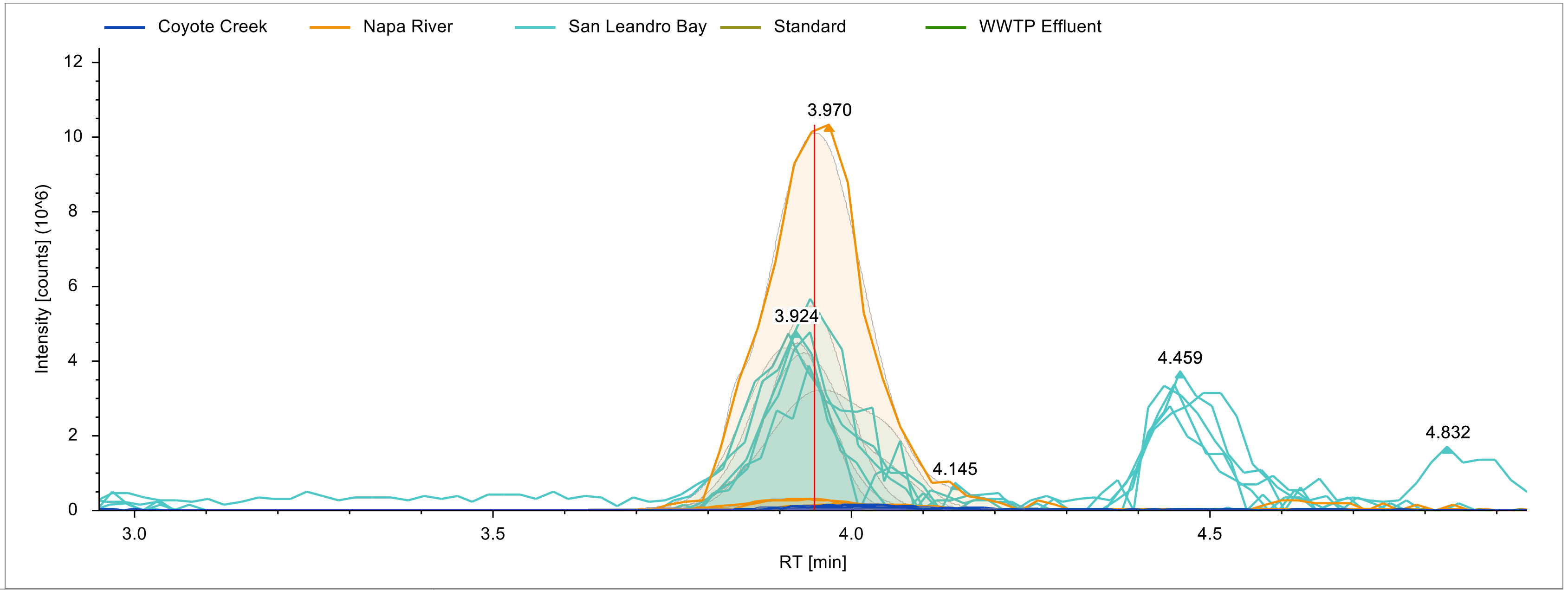
Identifying compounds in Napa River water:

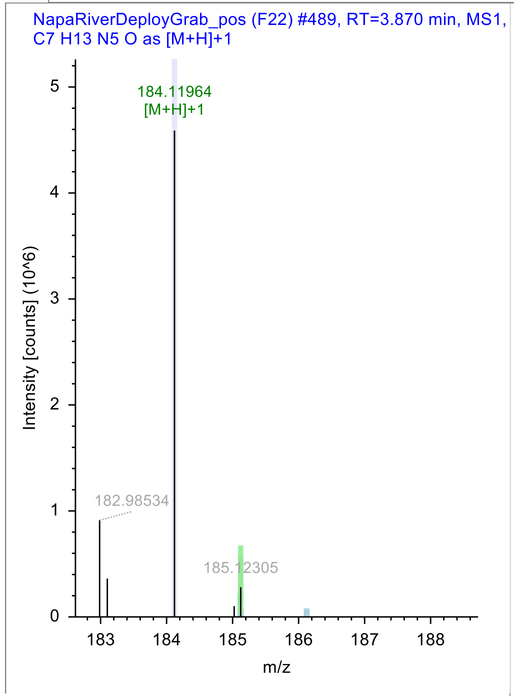
- Far fewer compounds had elevated levels in Napa River water relative to WWTP effluent.
- Only one compound (2-hydroxysimazine) matched with high confidence to library spectra.
- Highly uncertain (mass-only) tentative identifications of other compounds indicates many natural products.
- No indication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

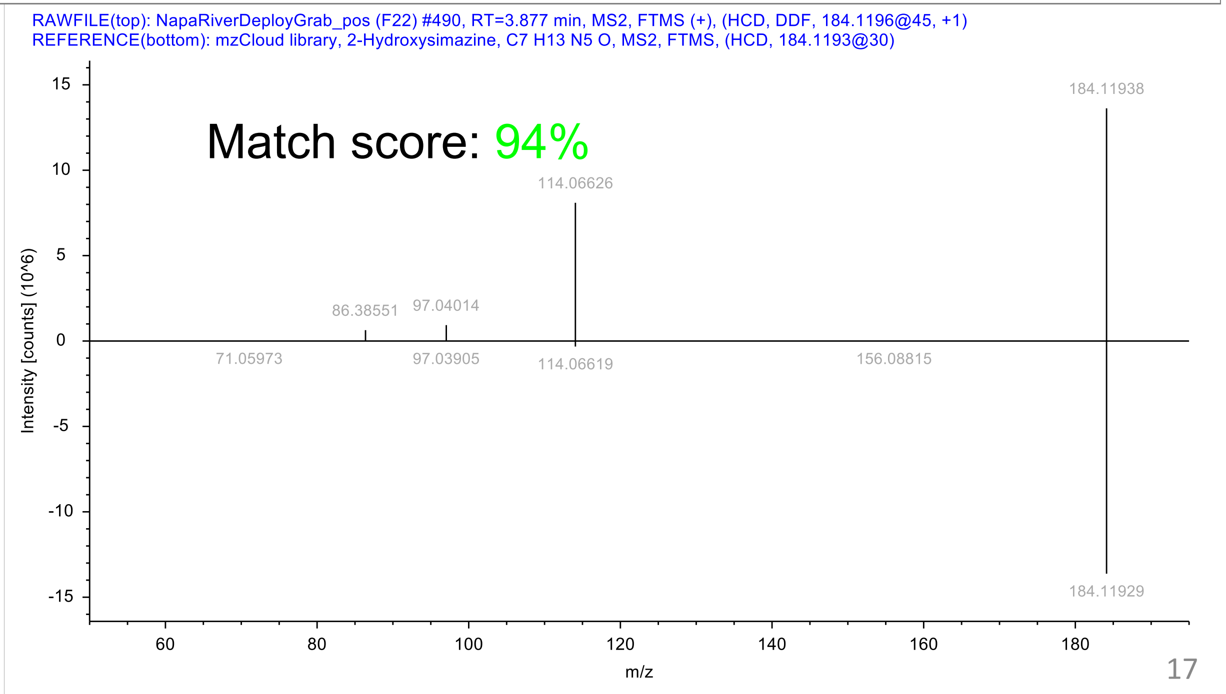


2-Hydroxysimazine: Transformation product of the widely-used triazine herbicide simazine (broadleaf weed and grass killer).

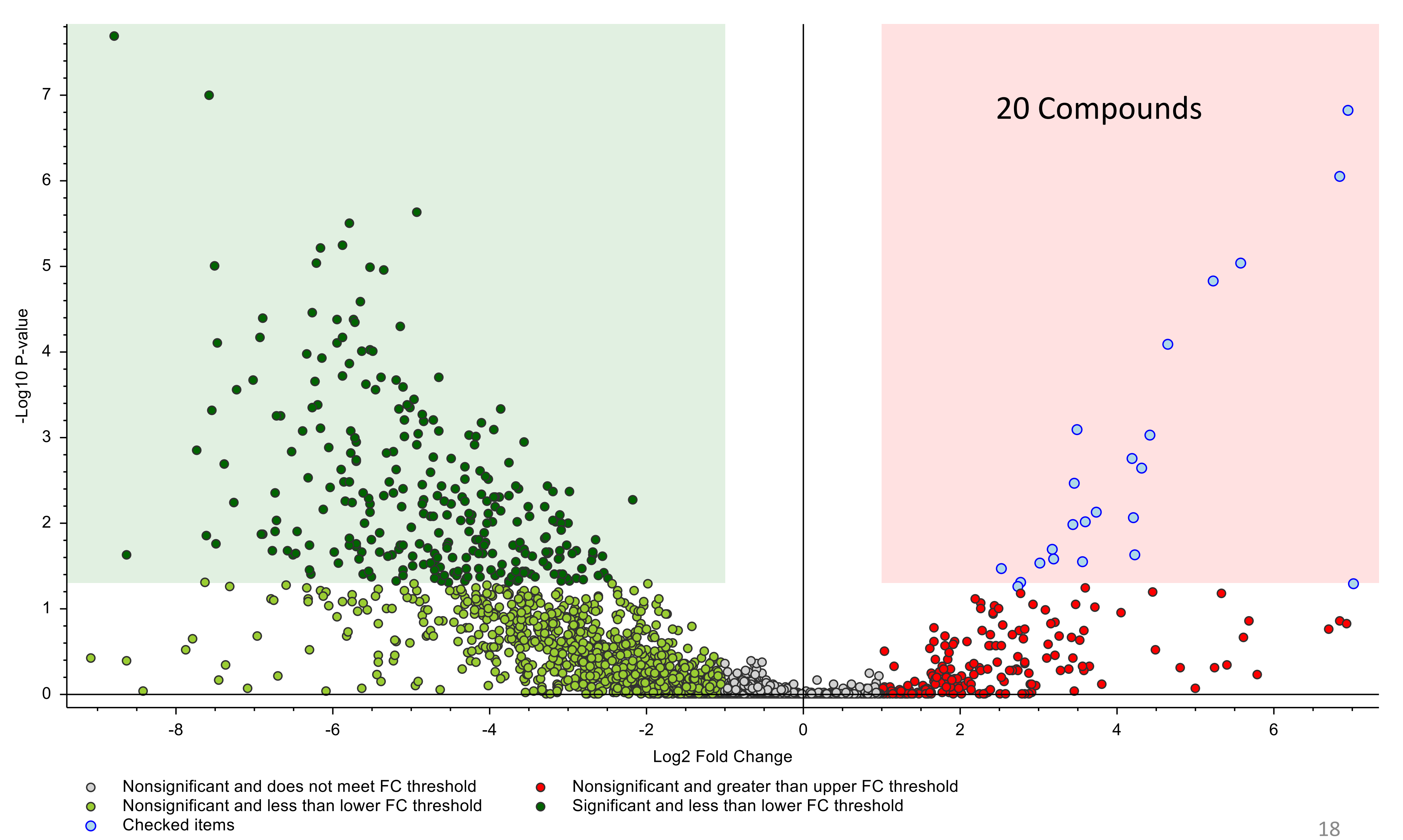






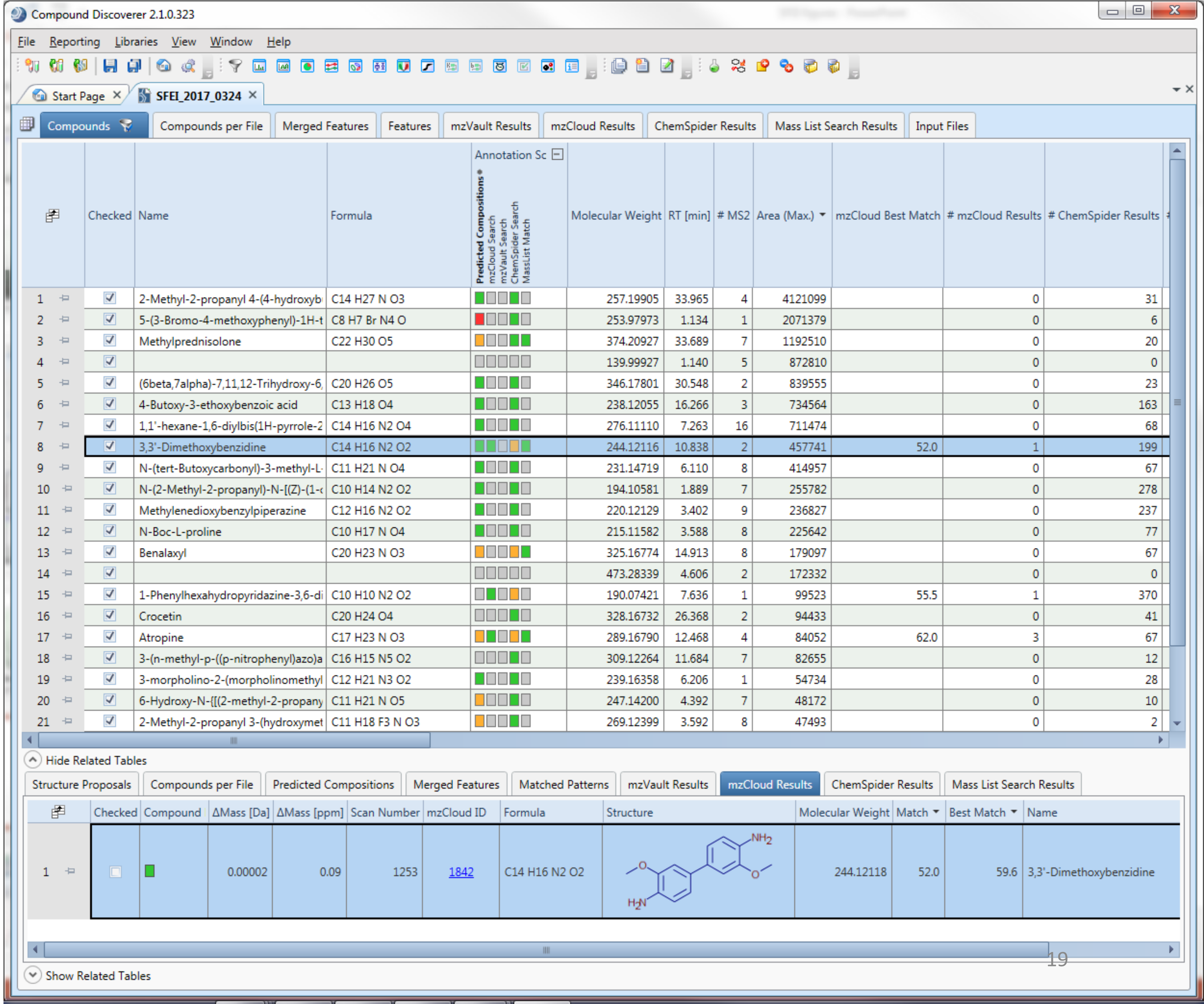


Differential analysis: Relative abundance of compounds in Coyote Creek vs. WWTP Effluent (average)

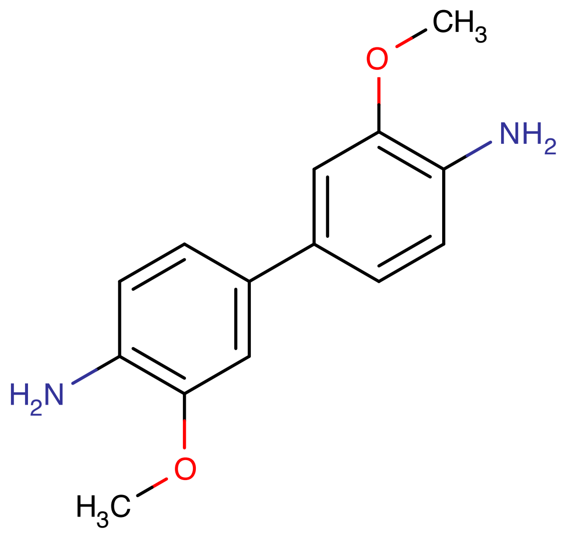


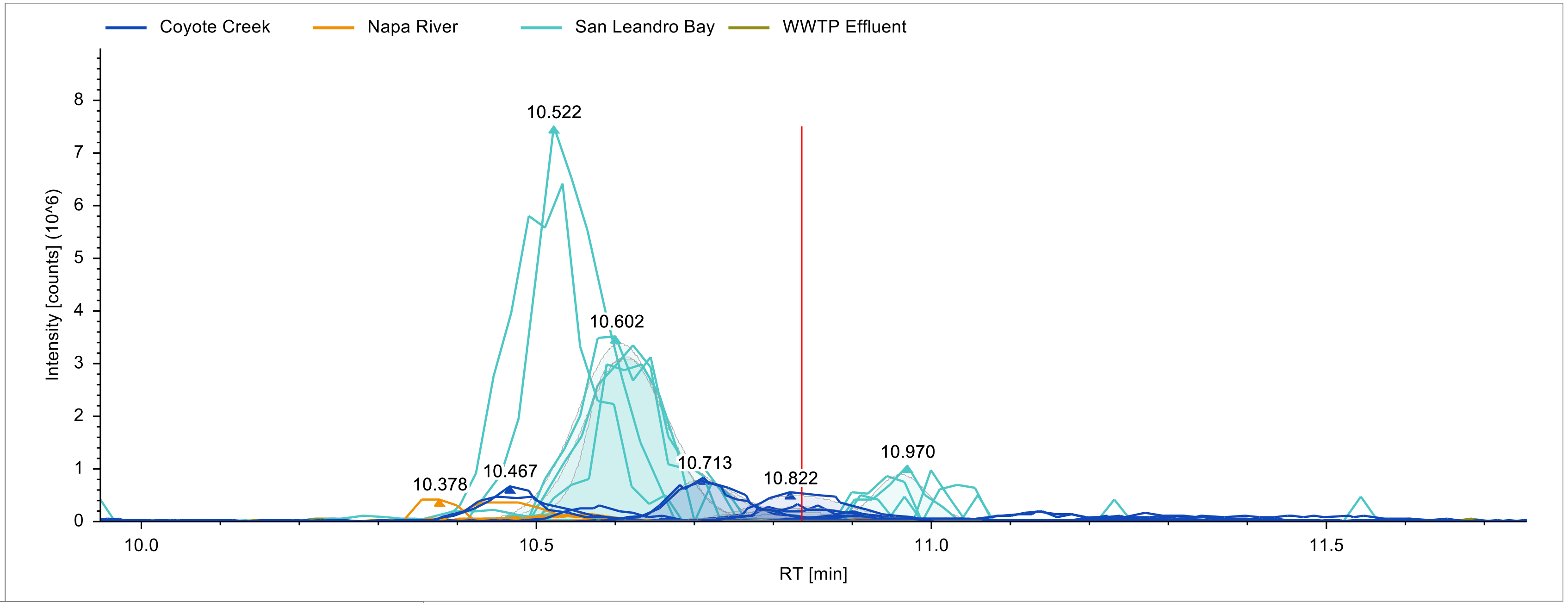
Identifying compounds in Coyote Creek water:

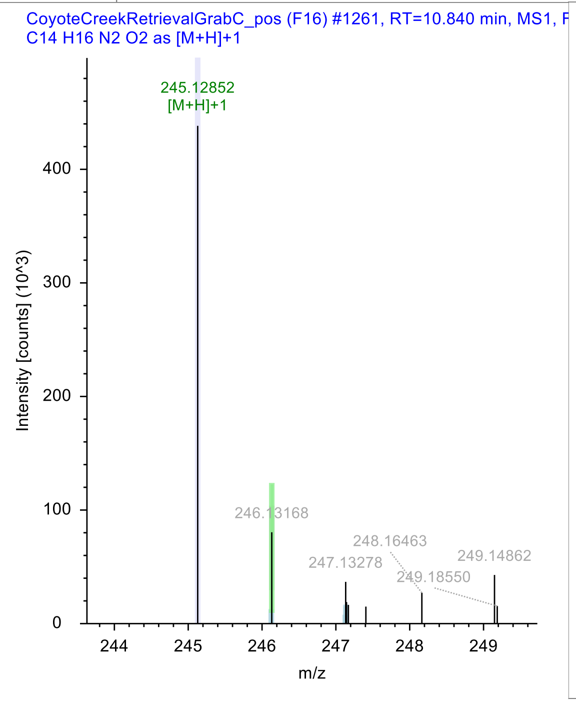
- Few unique compounds in Coyote Creek.
- Most detected compounds are also found in high abundance in WWTP effluent (Coyote Creek resembles diluted effluent).
- No high confidence identifications from Spectral library.
- Several low-confidence spectral library matches can be examined.

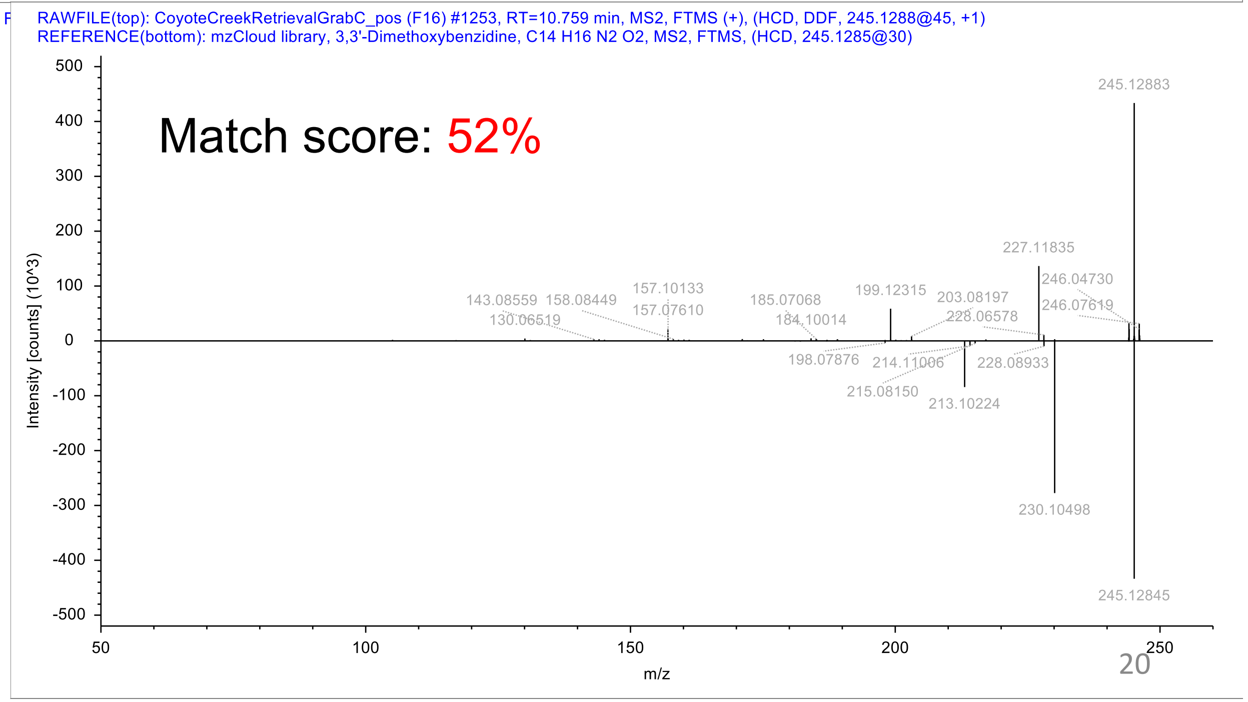


3,3’-Dimethoxybenzidine?: This could be a reductive transformation product of diazobenzene dyes, but the spectral match is relatively poor.









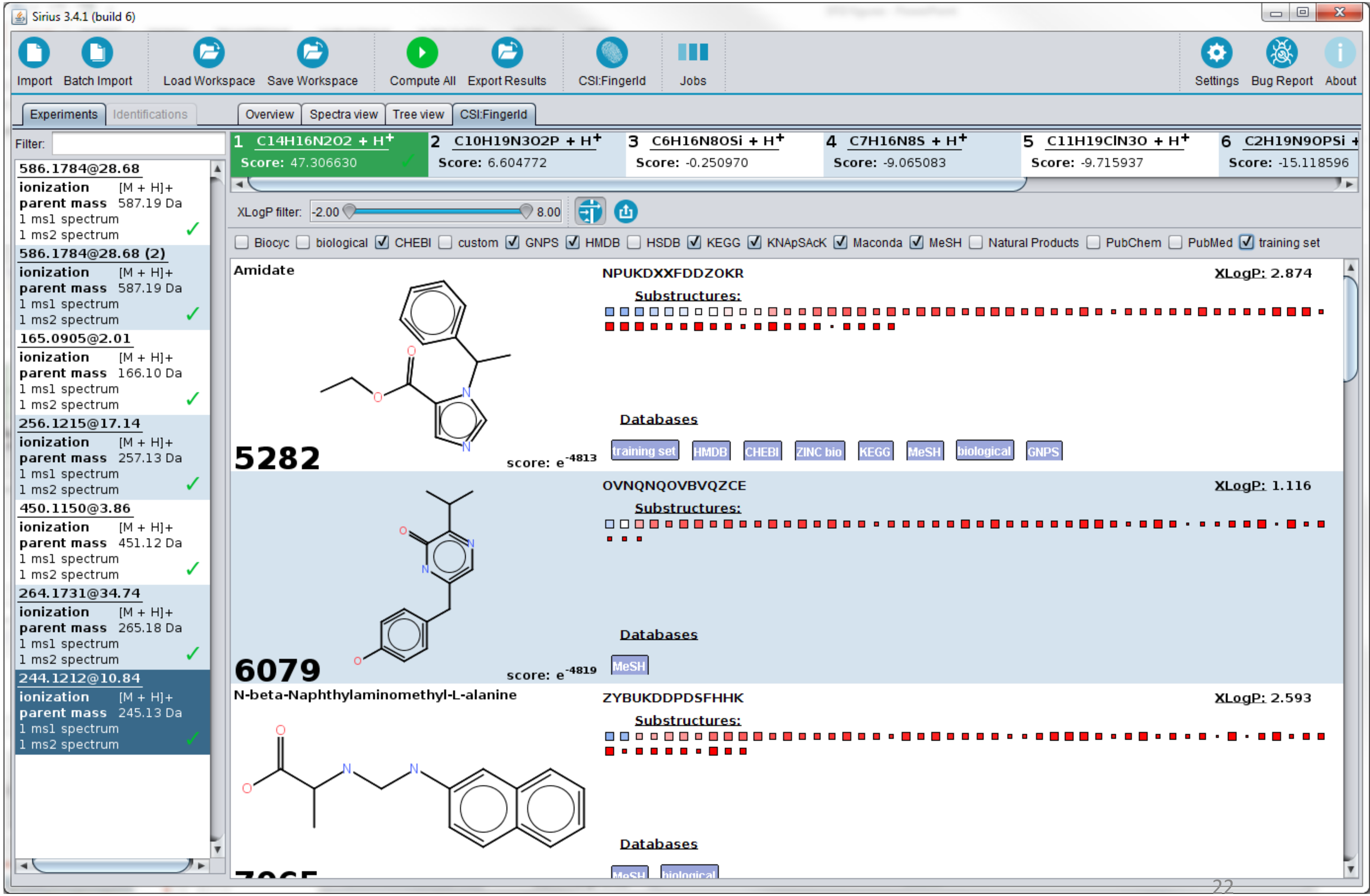
Can library match be verified by *in silico* fragmentation?

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

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

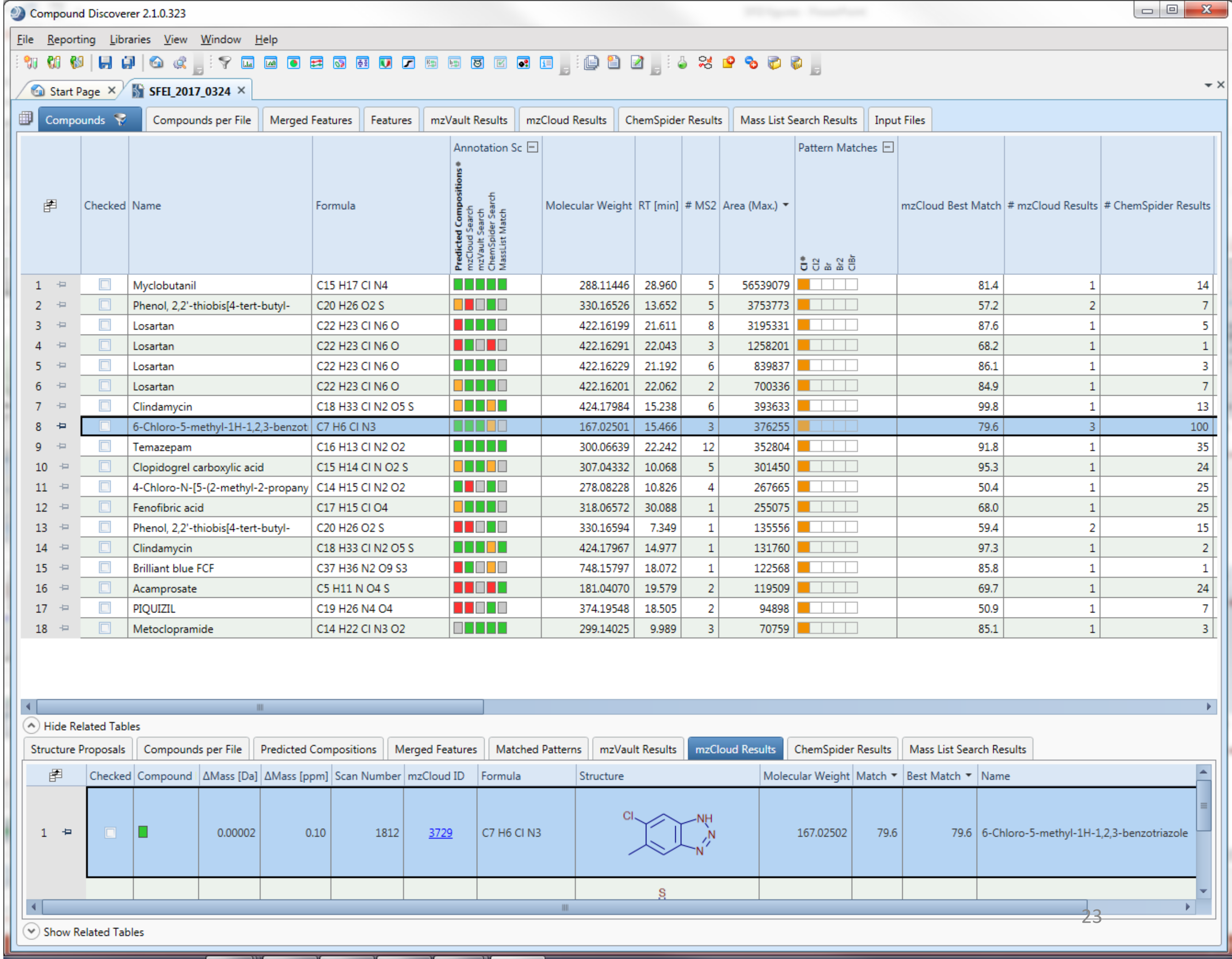
… however, structural fragment matching does not support identity of compound as 3,3'-dimethoxybenzidine.

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



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.

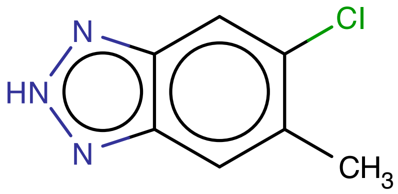


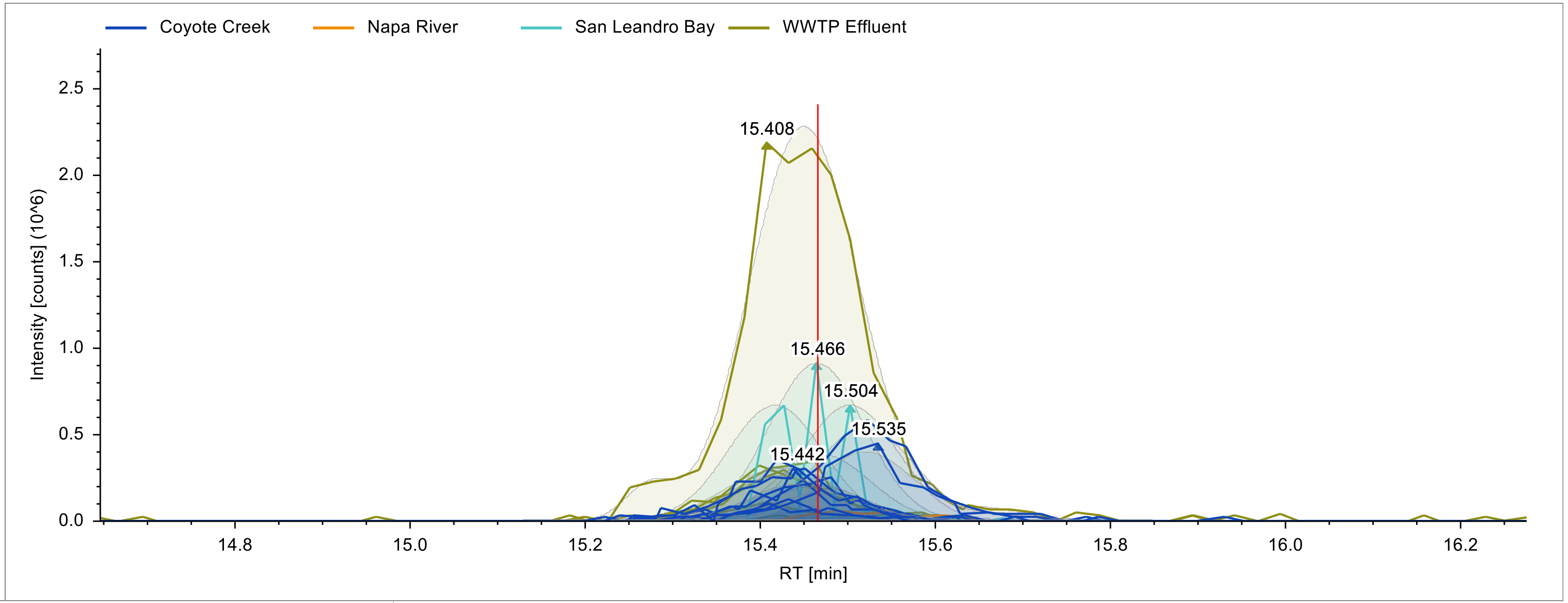
	Checked	Name	Formula	Molecular Weight	RT [min]	# MS2	Area (Max.)	mzCloud Best Match	# mzCloud Results	# ChemSpider Results
1		Myclobutanil	C15 H17 Cl N4	288.11446	28.960	5	56539079	81.4	1	14
2		Phenol, 2,2'-thiobis[4-tert-butyl-	C20 H26 O2 S	330.16526	13.652	5	3753773	57.2	2	7
3		Losartan	C22 H23 Cl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

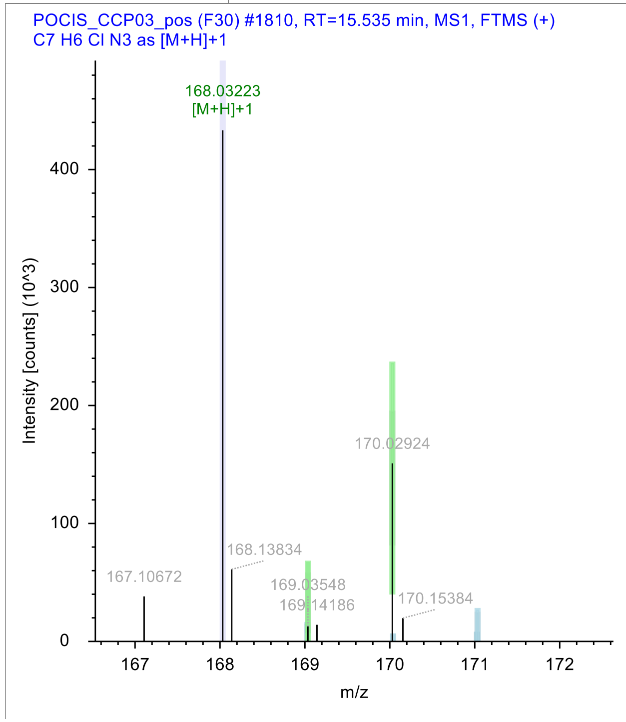
	Checked	Compound	ΔMass [Da]	ΔMass [ppm]	Scan Number	mzCloud ID	Formula	Structure	Molecular Weight	Match	Best Match	Name
1			0.00002	0.10	1812	3729	C7 H6 Cl N3		167.02502	79.6	79.6	6-Chloro-5-methyl-1H-1,2,3-benzotriazole

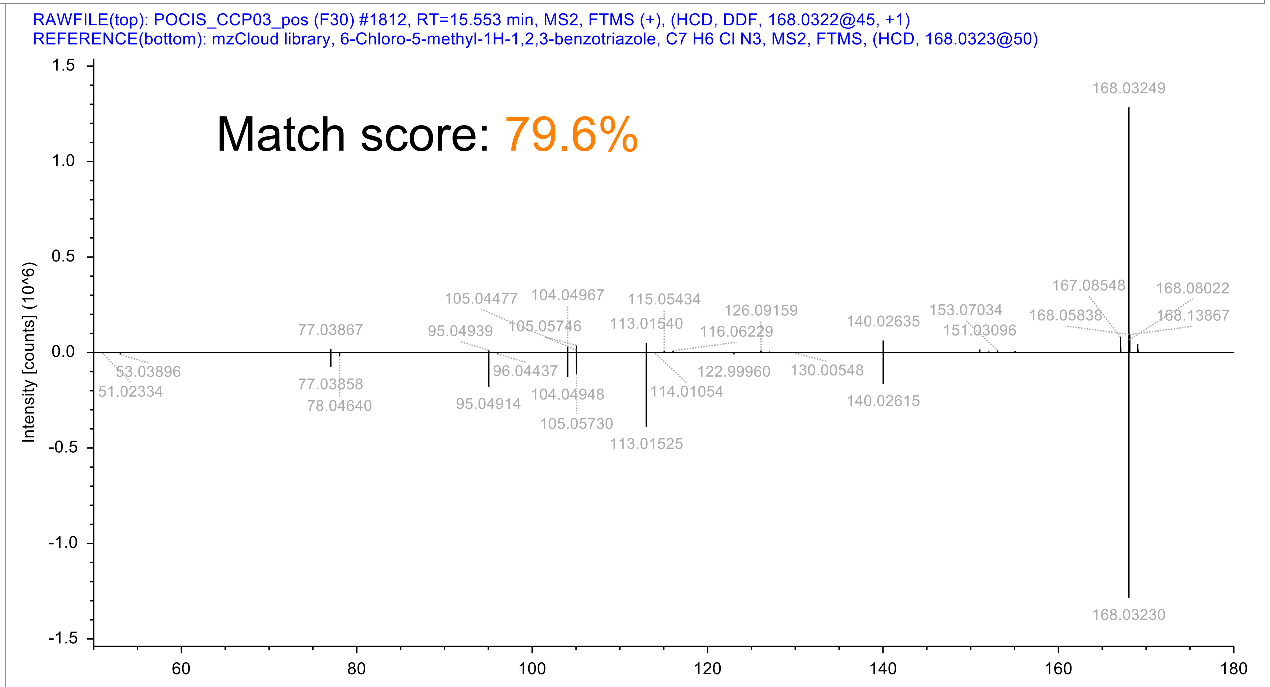
6-Chloro-5-methyl-1H-1,2,3-benzotriazole:

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 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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