High Resolution LC-QTOF MS for the Screening of Unknown Contaminants in Biological and Environmental Samples

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Department of Toxic Substance Control

9/28/16

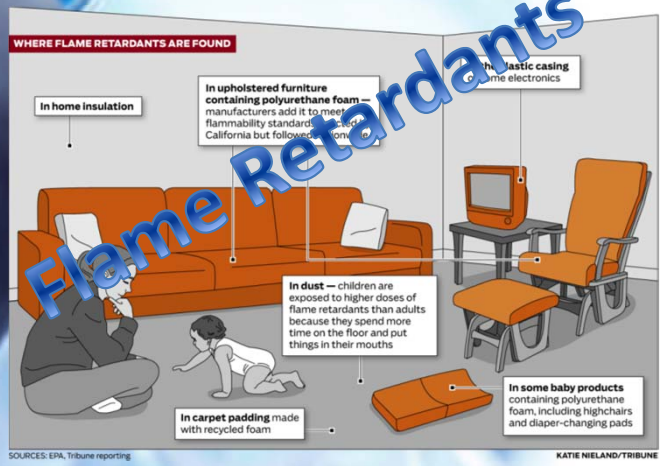
Why QTOF Screening?

Beyond the targeted analytes list

Poly and perfluoroalkyl substances

Consumer Products

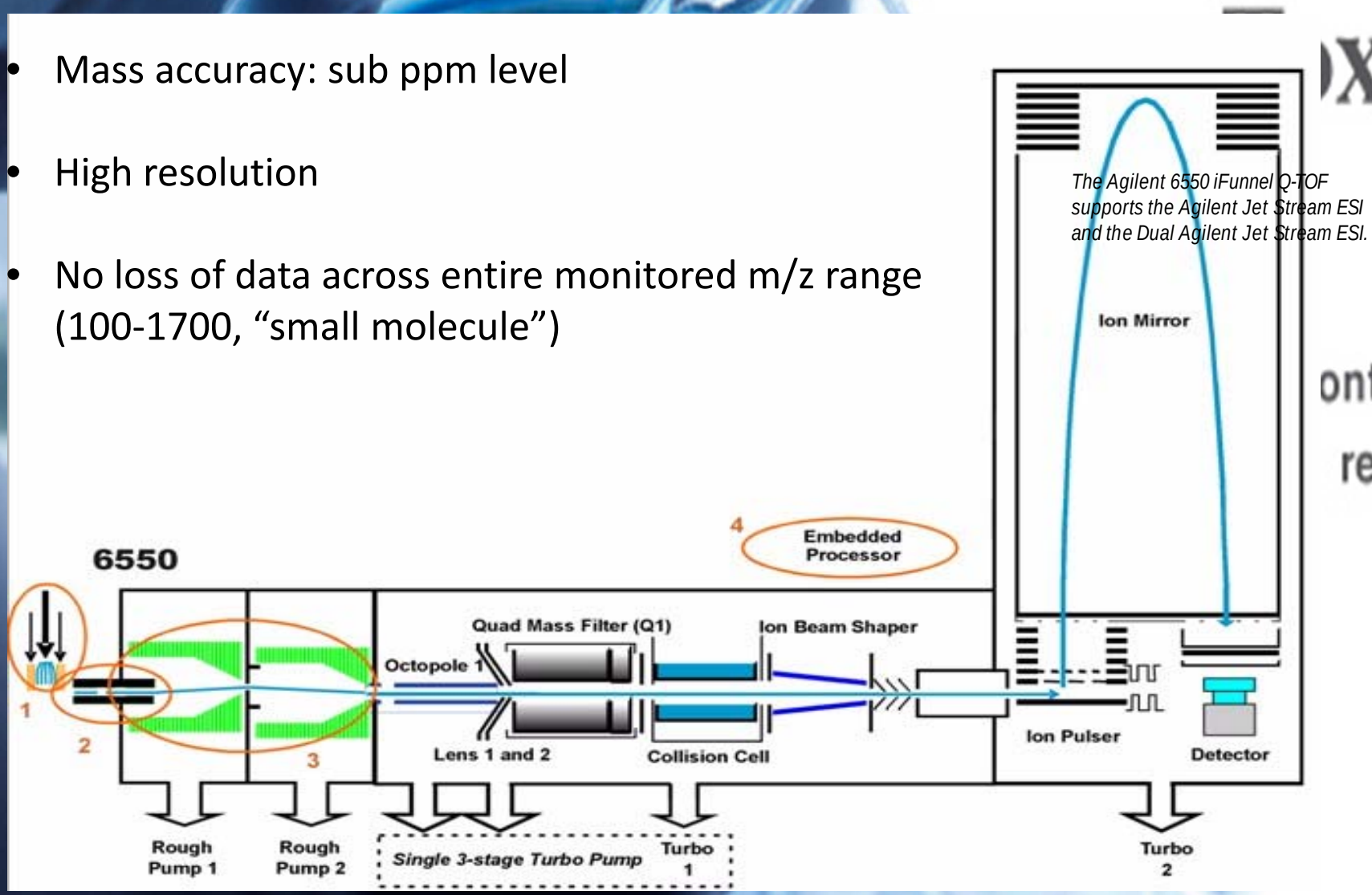
Pesticides



Flame Retardants

Agilent 6550 LC QTOF

- Mass accuracy: sub ppm level
- High resolution
- No loss of data across entire monitored m/z range (100-1700, “small molecule”)



Presentation Outline

- System and Projects Overview
- Target Suspect Screening ->Non-target Suspect Screening -> Semi-Unknown approach
- Projects Summary (biological and environmental samples)

    1. SFO waste water screening
    2. Cat serum screening
    3. Foam Project

1. Target Suspect Screening : Samples with interested Targeted Chemical Family

FBF (find by formula) algorithm

2. Non Target Suspect Screening: not limited to one particularly family but expand to broader non target contaminants

MFE (molecular feature extraction) algorithm

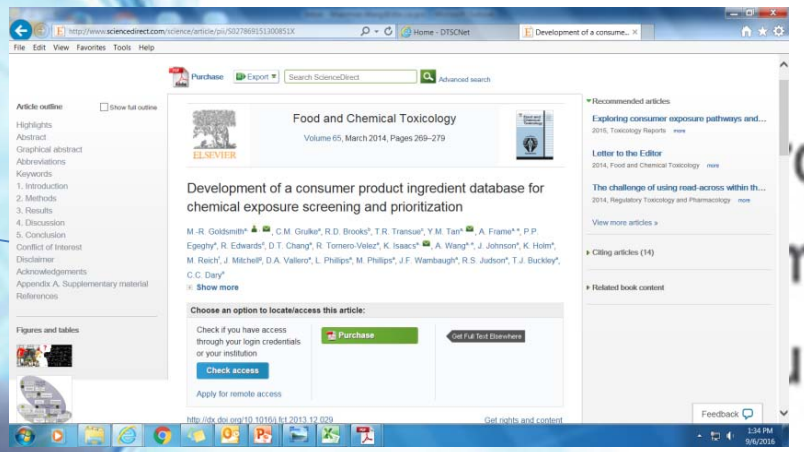
3. Semi-Unknown Approach : beyond current databases

Projects Overview

- SFO waste water : historical usage and current annual testing of AFFF (aqueous fire fighting foam) at SFO result in high concentration of PFASs in waste water. What are those precursors/intermediates in the WW in the treatment plants before discharge to bay? Target Suspect Screening
- Cat serum exposome screening: a pilot project to set up biomonitoring/serum sample method on the QTOF. Convenient samples of 5 HT cats and 5 Normal cats serum. compounds profiles comparison between HT and Normal cats. Non Target Suspect + Semi Unknown
- Participate in the safer consumer product projects (SB-1019 labeling): help confirm if there is no presence of OPFRs, as well as expand identification of other FRs present in fabric/foams: in the process of summarize into a standard method. Target Suspect Screening

Library

1. organo-phosphate flame retardants (OPFR) library: 92
2. Expanded PFAS library: 244
3. In House LCMS library (using consumer product chemicals index, combine with our in-house chemicals and up to date literatures): ~2500
4. EPA Tox21 library (DSSTox _ToxCastRelease_20151019) : ~ 9000
5. EOA library (Environmental organic acids, Collaboration Project with UCSF): 744



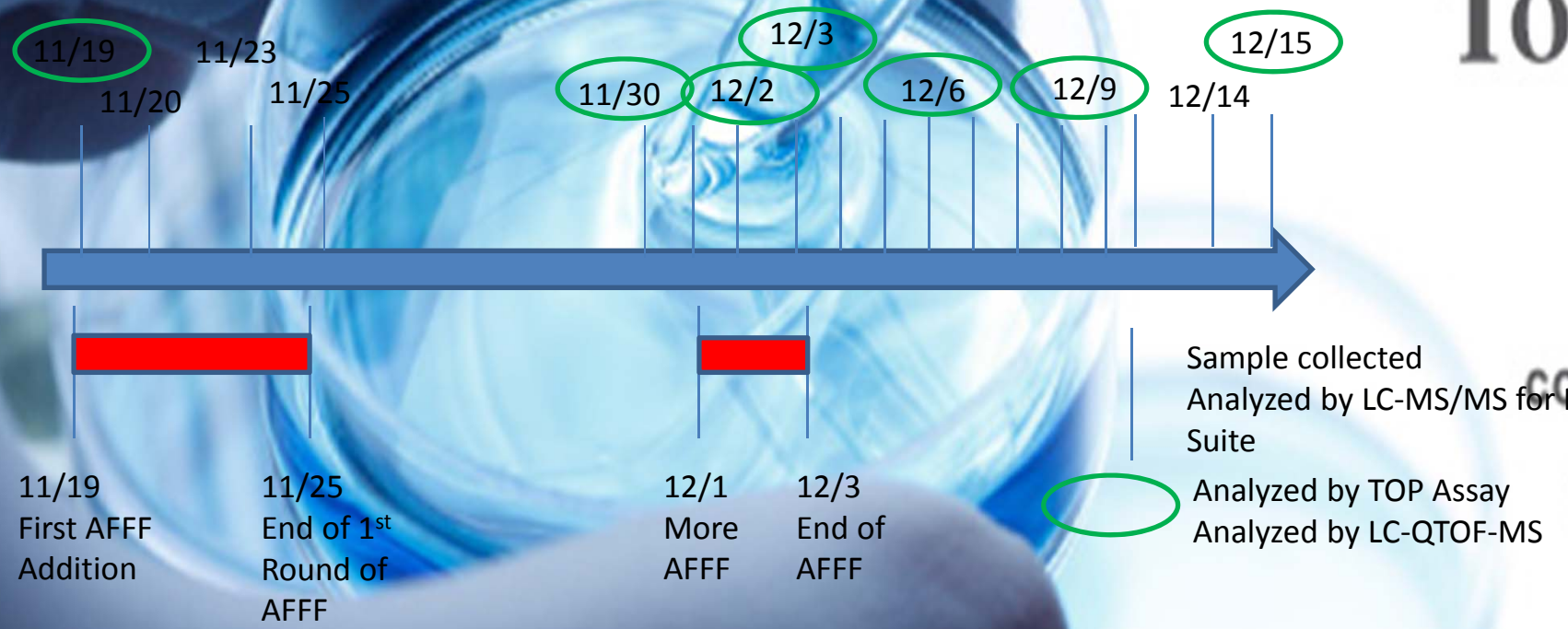
3. GoldSmith et. al. Food Chem. Toxicol., 2014, 269-279
4. Richard et.al., Chem. Res. Toxicol. , 2016, 1225-1251

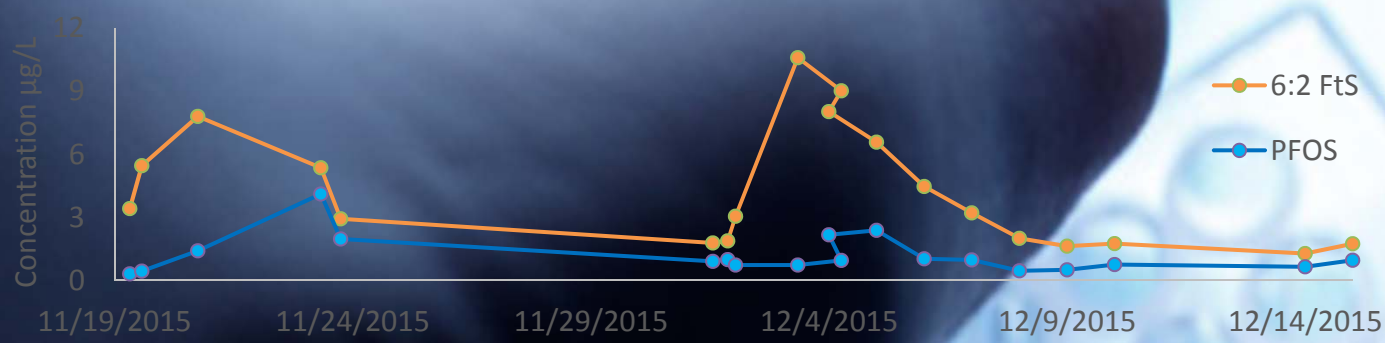
SFO Waste Water Screening: Target Suspect Screening

- A spin-off project from our LC-MS/MS methods of PFAS analysis

Erika Houtz et al, 2016 ACS presentation, San Diego

Timeline of Sampling (2015)





Total PFAS level (TOP assay) much higher than total concentration of targeted PFAS measured in the waste water

Targeted PFAS

????

QTOF: Expanded PFAS library

Total← TOP assay

workflow

Full Scan TIC, triplicate data set

Inspect Data Quality, RT shift, peak intensity

FBF (Find By Formula using appropriate parameters), search against PFAS library

MPP for compounds Grouping and Alignment, and blank substraction

Recursive Qual Analysis

1. Generate Compound list
2. Inspect Chromatography
3. Inspect Mass spectrum
4. review overall score

Further Identification and confirmation

1. Literature search
2. Standard comparison
3. MS/MS spectra

Identification of Novel Fluorinated Surfactants in Aqueous Film Forming Foams and Commercial Surfactant Concentrates

Lisa Anne D'Agostino, and Scott Andrew Mabury

Environ. Sci. Technol., **Just Accepted Manuscript** • Publication Date (Web): 20 Nov 2013

Downloaded from http://pubs.acs.org on November 27, 2013

105 compounds identified in AFFF samples: C3-C15

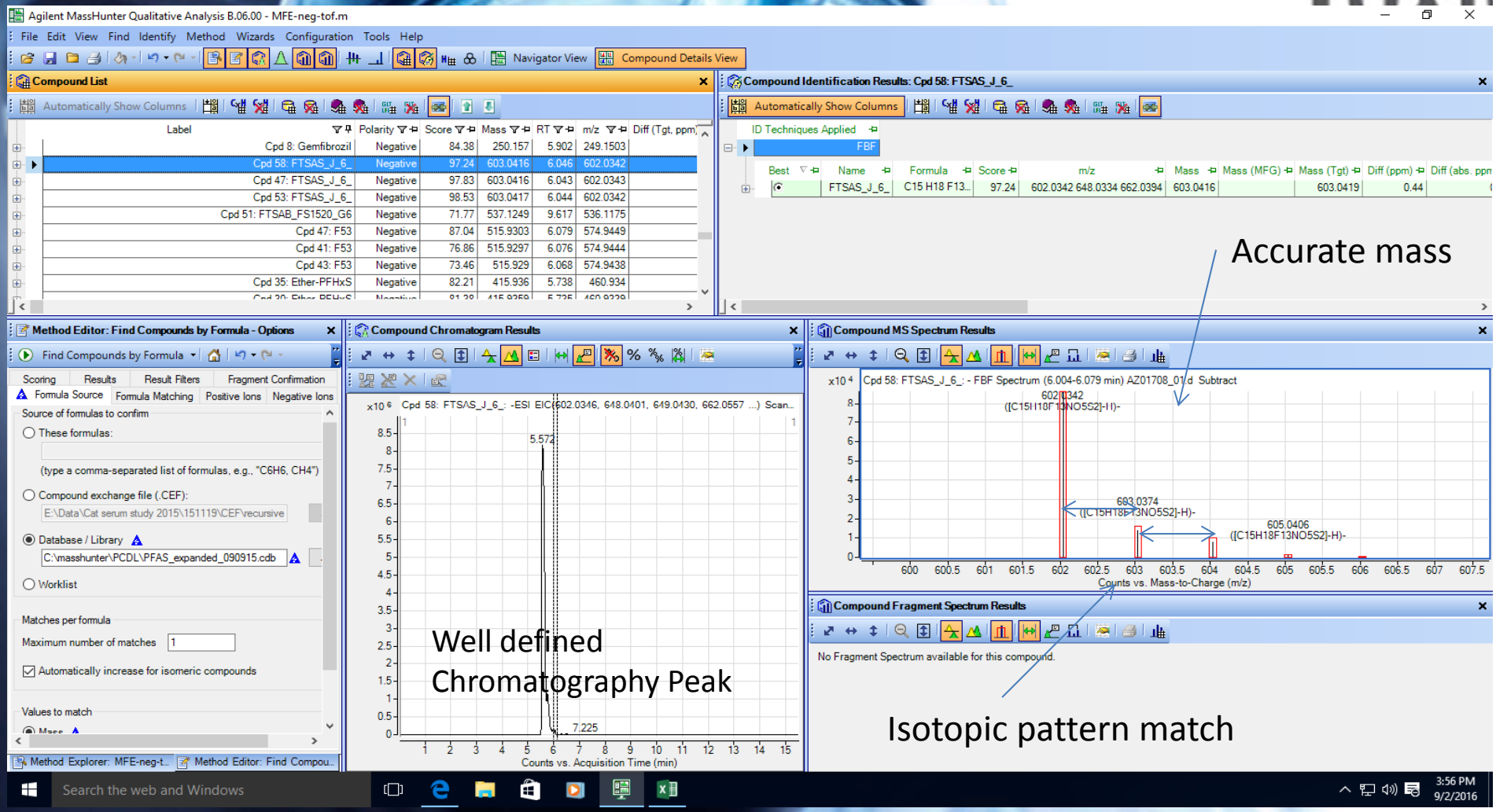
Table 1: Accurate masses used in assigning molecular formulae to congeners with the highest apparent abundance for each structure

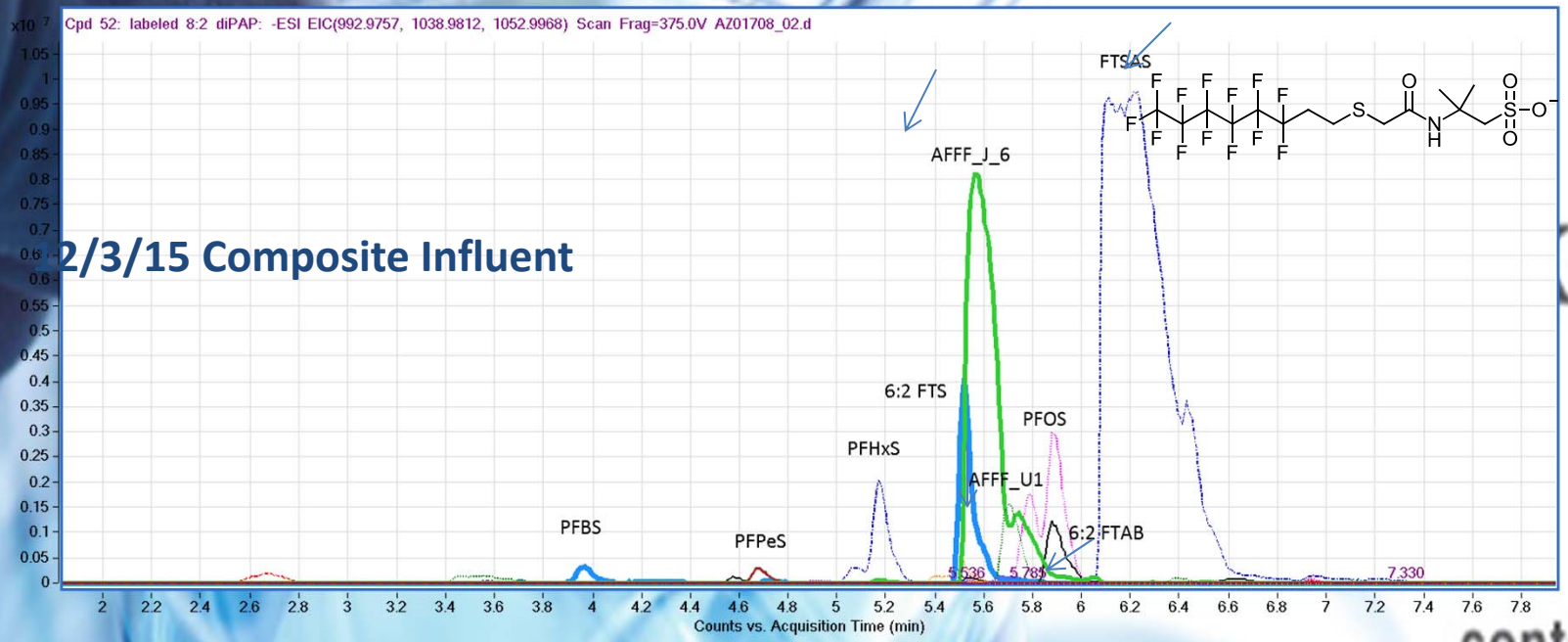
Structure	*n* =	Observed *m/z*	Formula	Theoretical *m/z*	Error (mDa)
A	8	549.0829	$C_{14}H_{14}ON_2F_{17}^{+1}$	549.0829	0.0
B	8	607.0887	$C_{16}H_{16}O_3N_2F_{17}^{+1}$	607.0884	0.3
H	6	553.1185	$C_{16}H_{22}O_2N_2SF_{13}^{+1}$	553.1189	-0.4
I	6	586.0392	$C_{15}H_{17}O_4NS_2F_{13}^{-1}$	586.0397	-0.5
J	6	602.0343	$C_{15}H_{17}O_5NS_2F_{13}^{-1}$	602.0346	-0.3
K	6	451.0041*	$C_{11}H_8O_2SF_{13}^{-1}$	451.0043	-0.2
L	6	571.0926	$C_{15}H_{20}O_4N_2SF_{13}^{+1}$	571.0931	-0.5
M	6	513.0875	$C_{13}H_{18}O_2N_2SF_{13}^{+1}$	513.0876	-0.1
N	5	414.0921	$C_{12}H_{15}O_2NF_{11}^{+1}$	414.0922	-0.1
O	5	432.0825	$C_{12}H_{14}O_2NF_{12}^{+1}$	432.0827	-0.2
P	6	496.0974	$C_{14}H_{19}ONSF_{13}^{+1}$	496.0974	0.0
Q	6	512.0923*	$C_{14}H_{19}O_2NSF_{13}^{+1}$	512.0923	0.0
R/S	6	557.0780	$C_{14}H_{18}O_4N_2SF_{13}^{+1}$	557.0774	0.6
T	6	485.0566	$C_{11}H_{14}O_2N_2SF_{13}^{+1}$	485.0563	0.3
U	6	629.0996	$C_{17}H_{22}O_6N_2SF_{13}^{+1}$	629.0986	1.0
V	8	601.0445	$C_{13}H_{14}O_3N_2SF_{17}^{+1}$	601.0448	-0.3

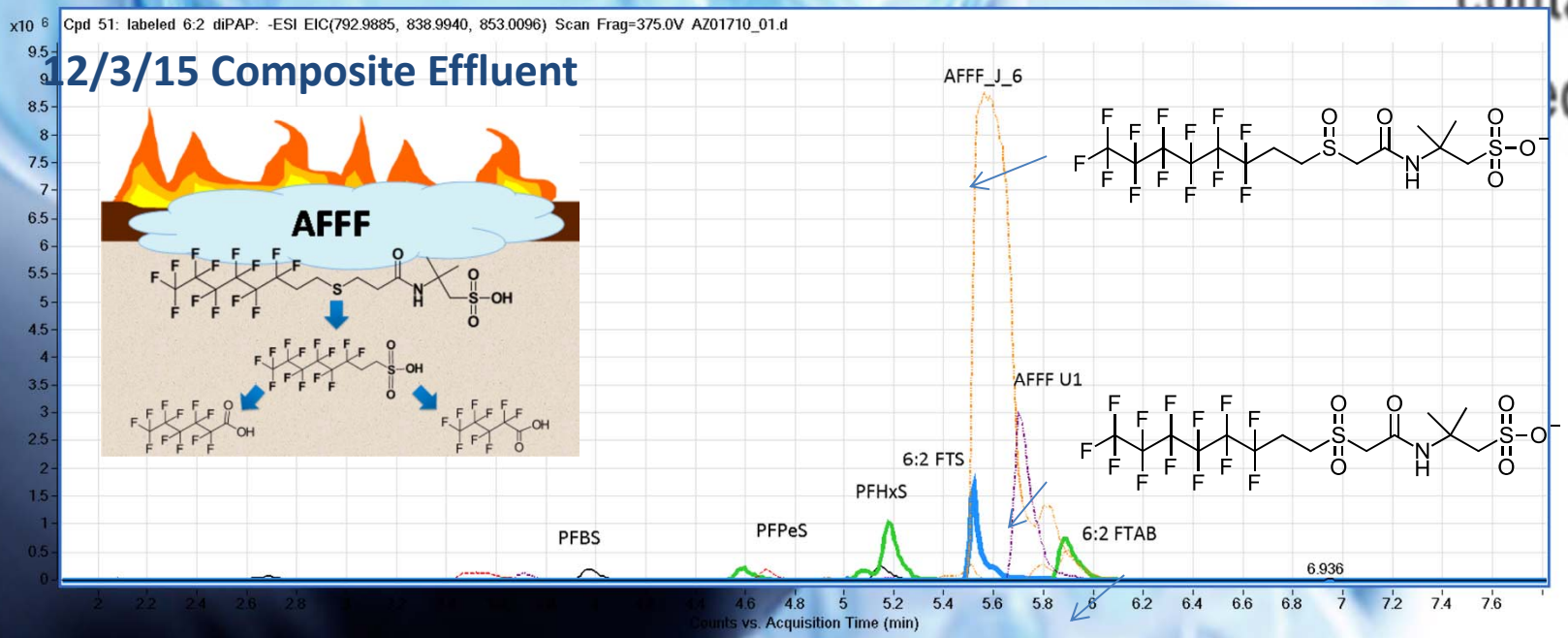
Expanded PFAS library

A database was compiled, which contained the masses and elemental formulas for all potential AFFF fluorochemicals identified in patents.

Qual


Agilent MassHunter Qualitative Analysis B.06.00 - MFE-neg-tof.m
Compound List
Compound Identification Results: Cpd 58: FTSAS_J_6_
Method Editor: Find Compounds by Formula - Options
Compound Chromatogram Results
Compound MS Spectrum Results
Compound Fragment Spectrum Results
Cpd 58: FTSAS_J_6_: - FBF Spectrum (6.004-6.079 min) AZ01708_01.d Subtract
Counts vs. Mass-to-Charge (m/z)
Counts vs. Acquisition Time (min)
No Fragment Spectrum available for this compound.
Accurate mass
Well defined
Chromatography Peak
Isotopic pattern match





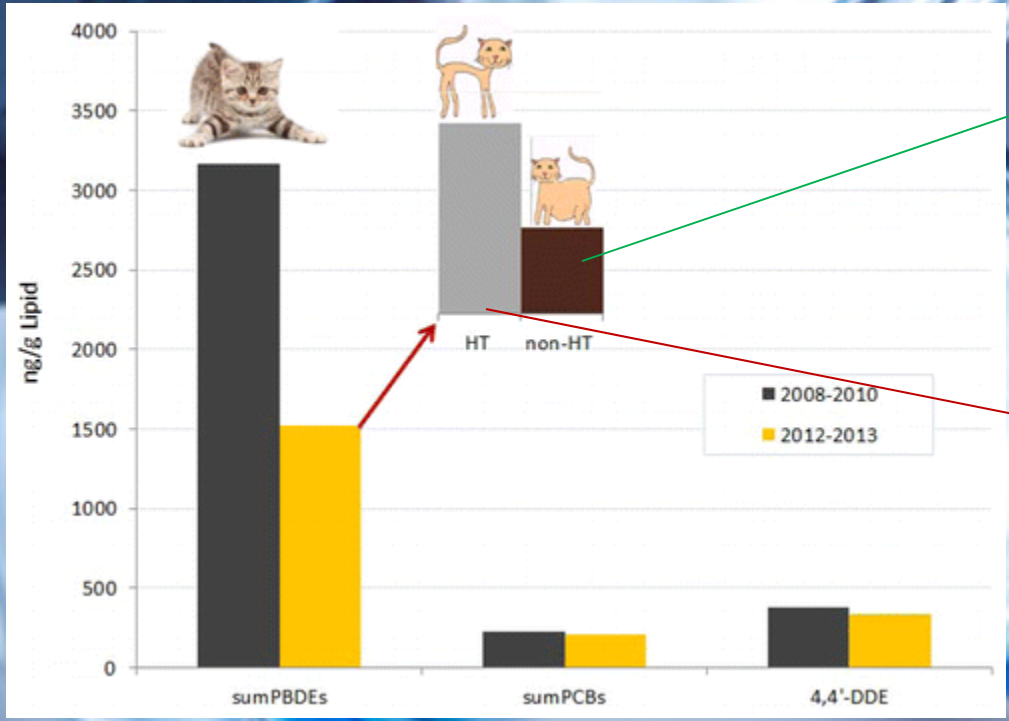
Katie C. Harding-Marjanovic et al., Environ. Sci. Technol. 2015, 49, 7666−7674

SFO WW Screening

- Expand the analytes list beyond the original LC-MS/MS method: found precursors, intermediates, and new replacement PFASs: <u>ADONA, Ether-PFHxS, Ketone-PFOS</u>
- Help confirmation of the sources and transformation pathways
- Working on Compare WW profiles from different sources: MPP analysis

Cat serum exposome screening

- Previously, we have used LC-MS/MS serum method to analyzed 12 PFAS compounds in cat serum samples.
- Pilot project for biological sample QTOF screening



Normal Cats Control (5)

HT Cats Case (5)

Toxi

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contam

redu

Article

Previous Ar

Temporal Changes of PBDE Levels in California House Cats and a Link to Cat Hyperthyroidism

Weihong Guo*†, Stephen Gardner‡, Simon Yen§, Myrto Petreas†, and June-Soo Park†

† California Department of Toxic Substances Control, California Environmental Protection Agency, 700 Heinz Avenue, Berkeley, California 94710, United States,

‡ VCA Albany Animal Hospital, 1550 Solano Avenue, Albany, California 94707, United States

§ Campus Veterinary Clinic, 1807 M.L.K. Jr Way, Berkeley, California 94709, United States

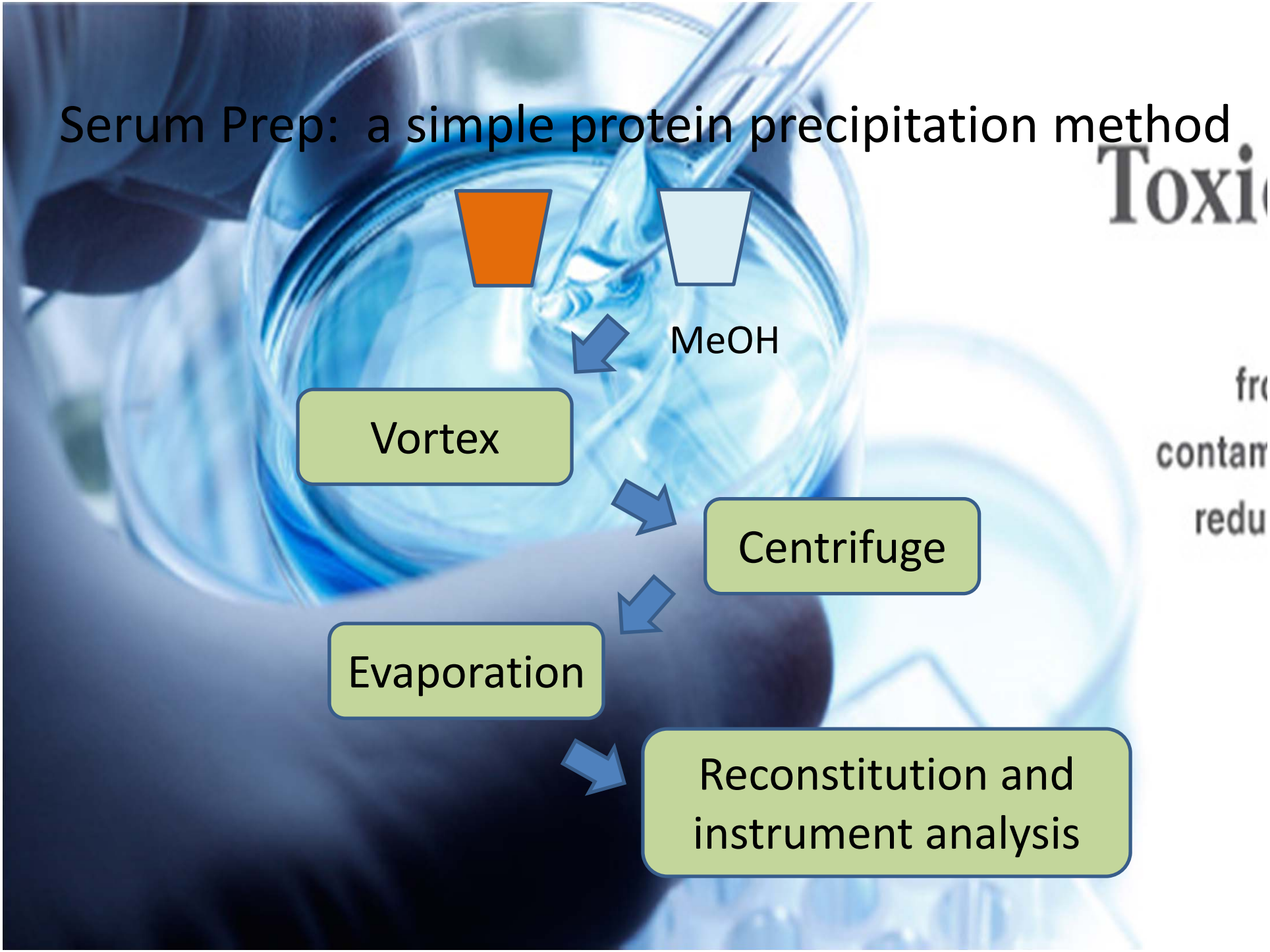
Questions Asked For This Study

1. Exposome of Cat Serum: Non Target Suspect Screening: Beyond the 12 common PFCAs ;

High production volume (HPV) chemicals, pharmaceuticals, pesticides, poly and perfluoroalkyl substances(PFAS) and flame retardants

2. Are there detectable profile differences between the control and case groups?

Serum Prep: a simple protein precipitation method



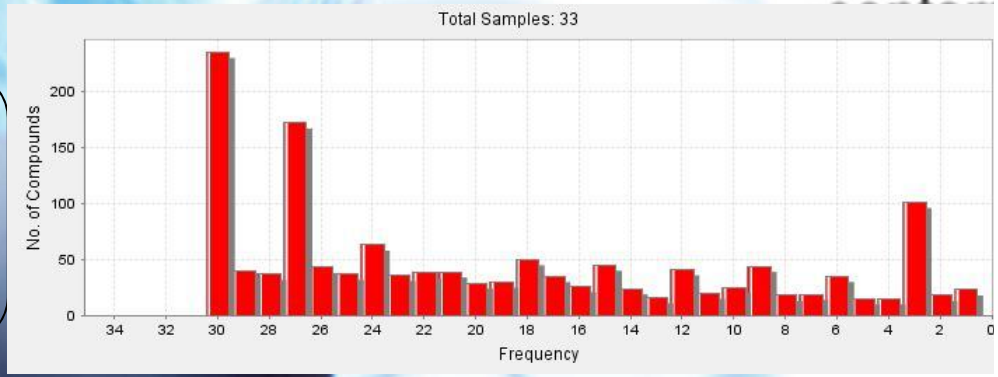
the MPP (Mass Profiler Professional) non targeted workflow

Full Scan TIC, triplicate injections

MFE (Molecular Feature Extraction) with appropriate parameters

Peak filter
Mass tolerance
RT tolerance
Ion species

Export to MPP, Grouping and Alignment: Features aligned across all data sets



Apply Quality Control Filters, Blank subtraction, Recursive analysis

Exposome

Export for Identification:
exact mass, isotope pattern, retention time

1. Search against databases
2. Unknowns: elemental formula generation

Profile

Profiles Comparison MPP

1. Group as control and case
2. Comparison using volcanic plot, $p < 0.05$, fold > 2

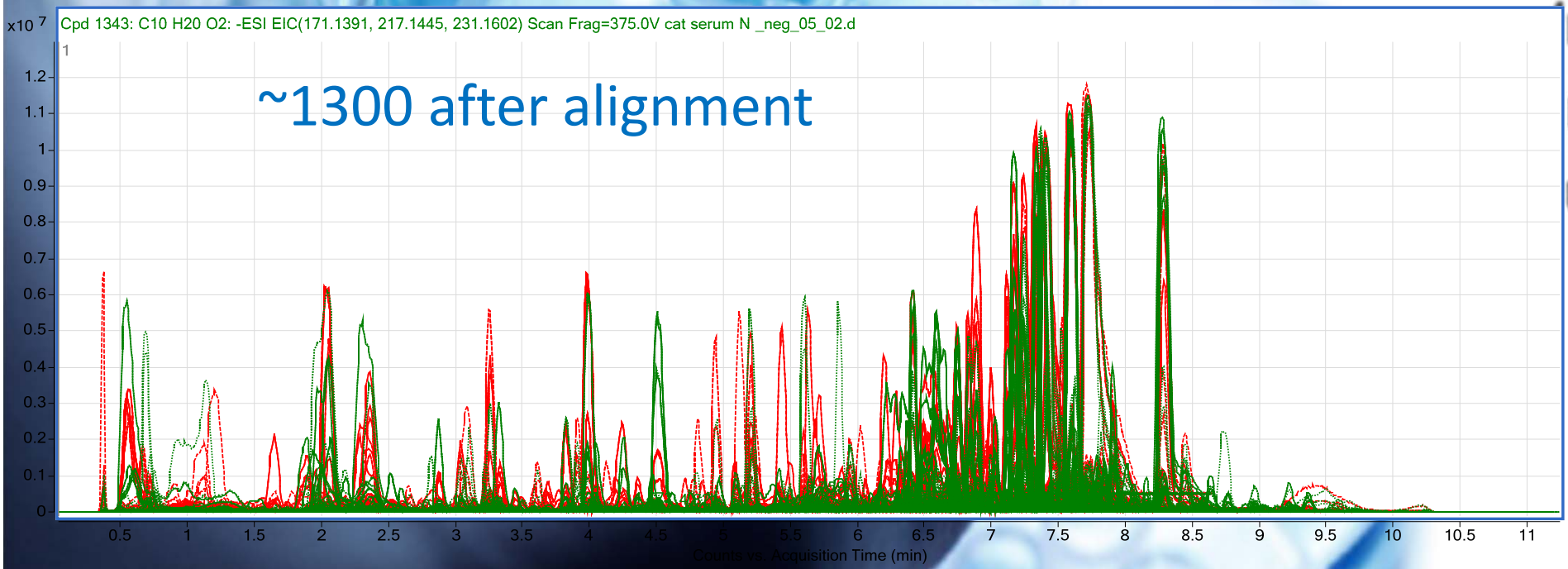
Identify features that are up or down regulated between case and control groups

Further Identification and confirmation

1. Search Chemspider/Pubmed and/or Merck Index (search by formula, by accurate mass)
2. MS/MS spectra

Cat Exposome: All 10 samples

Feature: Mass@RT



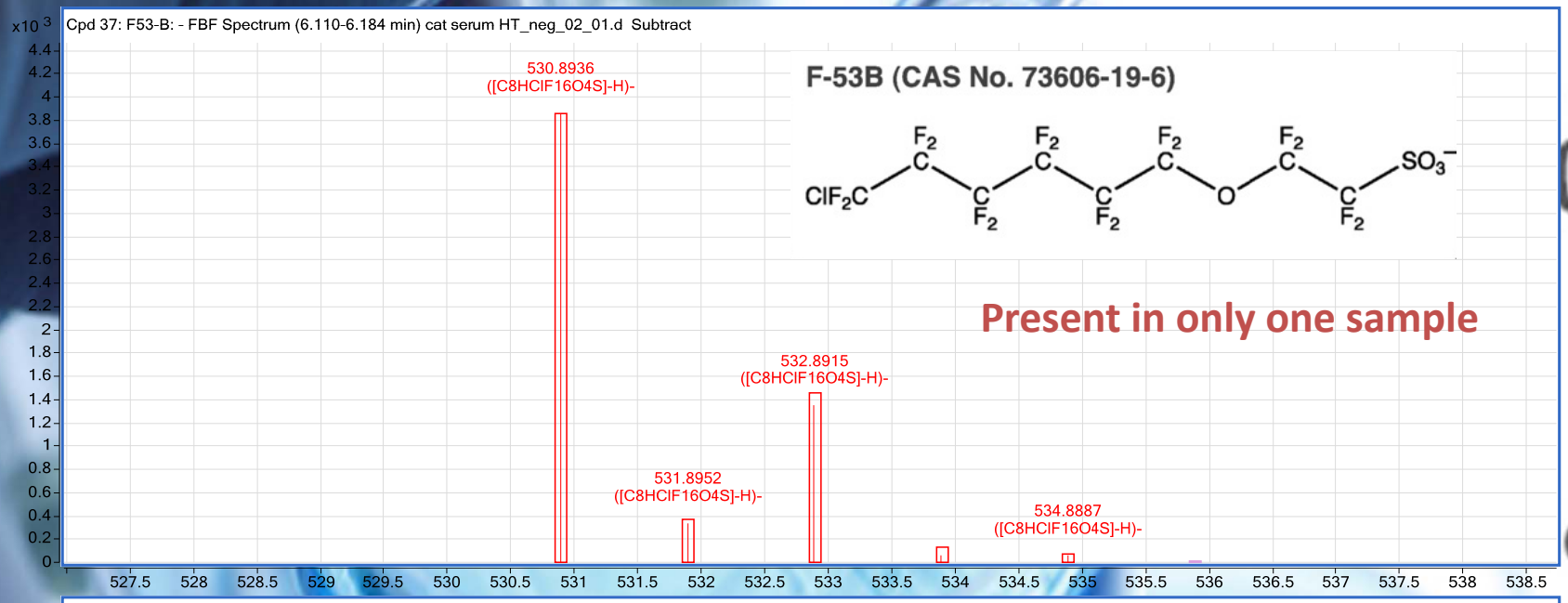
Food preservative and additives

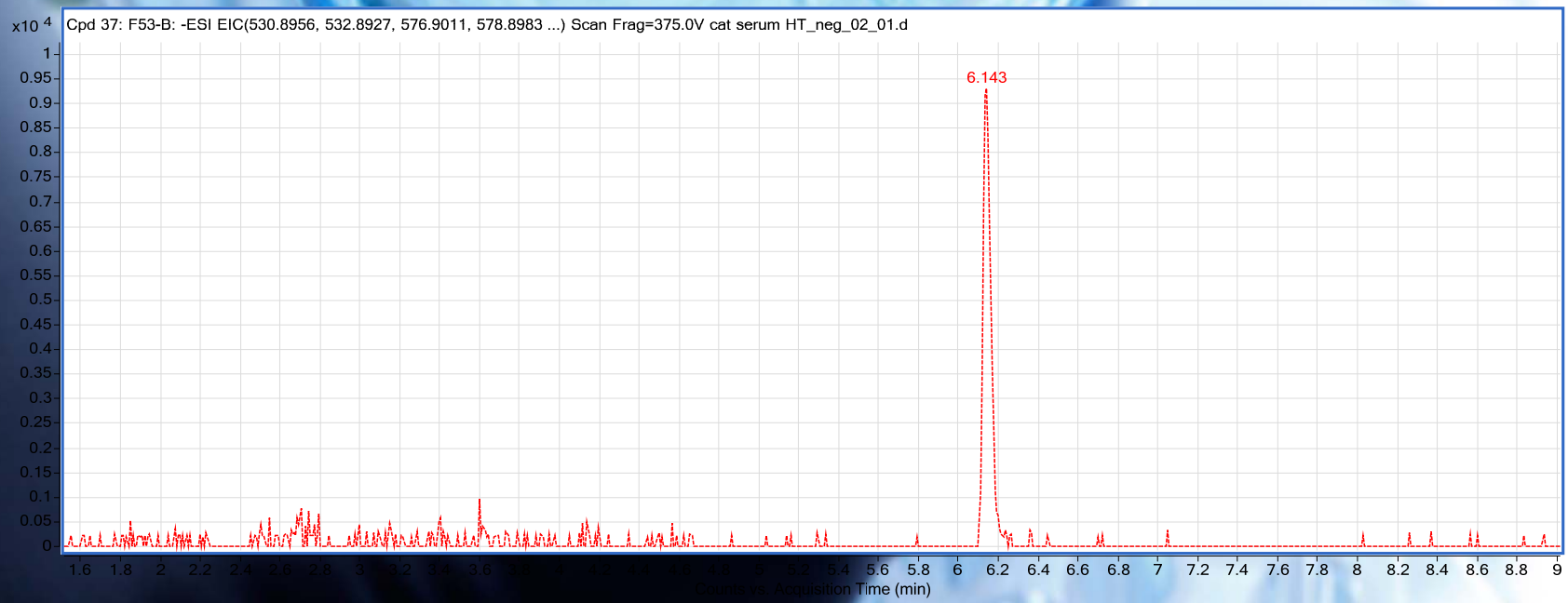
PFBS

PFHxS

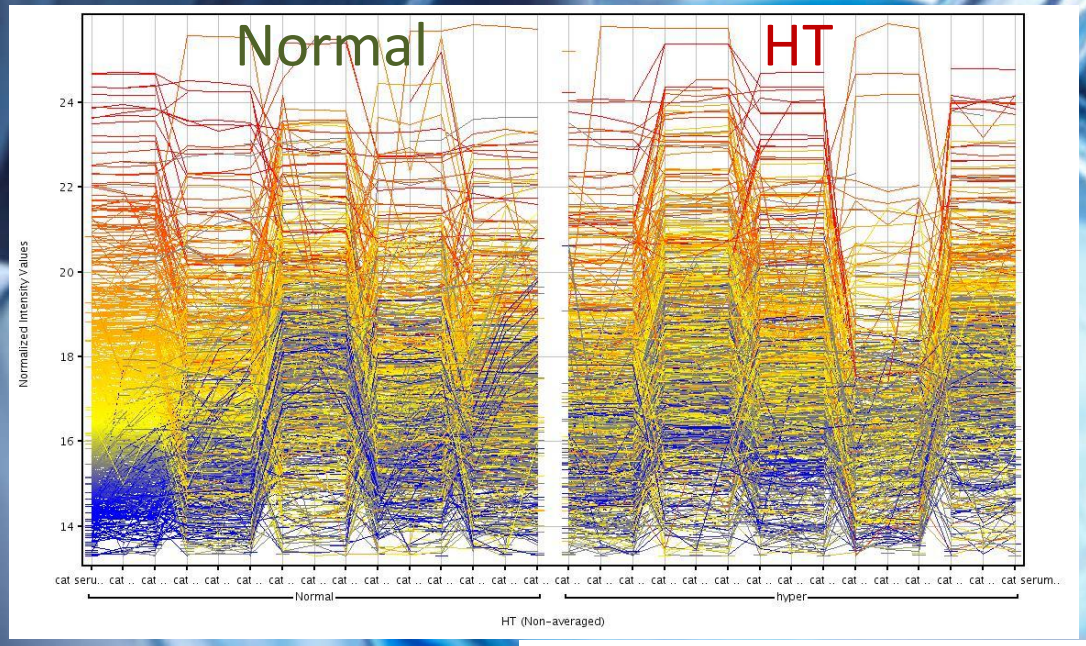
6:2 FTS

Cpd	Label	Name	RT	Formula	Score	Mass
488	Cpd 4	benzoic acid	2.105	C7 H6 O2	99.14	122.0367
94	Cpd 9	allyl caproate	3.449	C9 H16 O2	99.03	202.1213
47	Cpd 4	1-octen-3-ol	2.593	C8 H16 O	99.02	174.1258
57	Cpd 5	1-octen-3-ol	2.88	C8 H16 O	98.94	174.1261
366	Cpd 3	2,2,6,6-tetramethyl-4-methylideneheptane	6.813	C12 H24	98.49	214.1927
66	Cpd 6	tert-butylhydroquinone	3.046	C10 H14 O2	98.48	166.0993
763	Cpd 7	1,2-benzisothiazol-3(2H)-one	7.567	C7 H5 N O S	98.3	151.0092
35	Cpd 3	2-{[2,2-dimethyl-3-(oxiran-2-ylmethoxy)propoxy]me	2.353	C11 H20 O4	98.27	216.1364
302	Cpd 3	decene	6.194	C10 H20	98.27	186.1623
490	Cpd 4	perfluoro-1-butanesulfonate	4.028	C4 H F9 O3 S	98.24	299.9508
116	Cpd 1	ID 0273 - from: Env. Science Technol., 2013, 47 (10),	3.822	C11 H18 O	98.21	166.1359
713	Cpd 7	1,2-benzisothiazol-3(2H)-one	7.201	C7 H5 N O S	98.18	151.0094
7	Cpd 7	allyl caproate	0.724	C9 H16 O2	98.16	202.1205
583	Cpd 5	dibutyl adipate	4.585	C14 H26 O4	98.13	258.1835
166	Cpd 1	dibutyl adipate	4.511	C14 H26 O4	98.06	258.1832
493	Cpd 4	1,1,2,2,3,3,4,4,5,5,6,6,6-Tridecafluoro-1-hexane-sulfc	5.249	C6 H F13 O3 S	98.06	399.9433
1266	Cpd 1	Lufenuron	6.954	C17 H8 Cl2 F8 N2 O3	97.98	509.9781
130	Cpd 1	ethenyl decanoate	3.95	C12 H22 O2	97.97	244.1679
139	Cpd 1	ethenyl decanoate	4.054	C12 H22 O2	97.96	244.168
293	Cpd 2	2,6,8-Trimethyl-4-nonanone ID# 204	6.104	C12 H24 O	97.96	244.204
87	Cpd 8	diisopropyl adipate	3.332	C12 H22 O4	97.89	230.1518
771	Cpd 7	ID 0270 - from: Env. Science Technol., 2013, 47 (10),	8.28	C35 H70 O2	97.86	568.5426
128	Cpd 1	1,2-benzisothiazol-3(2H)-one	3.906	C7 H5 N O S	97.85	151.0093
4	Cpd 4	butyl 2-methylprop-2-enoate	0.579	C8 H14 O2	97.73	188.1052
191	Cpd 1	dibutyl adipate	4.968	C14 H26 O4	97.69	258.1834
423	Cpd 4	DL-12-hydroxy stearic acid	7.205	C18 H36 O3	97.64	300.2669
470	Cpd 4	palmitic acid	7.794	C16 H32 O2	97.44	256.2404
228	Cpd 2	2,2,4-trimethyl-1-[(2-methylpropanoyl)oxy]pentan-3	5.43	C16 H30 O4	97.41	286.2148
67	Cpd 6	isoeugenol	3.045	C10 H12 O2	97.4	210.0892
288	Cpd 2	decene	6.079	C10 H20	97.15	186.1616
213	Cpd 2	17alpha-Estradiol	5.216	C18 H24 O2	97.05	318.183
734	Cpd 7	1H, 1H, 2H, 2H-perfluorooctane sulfonate (6:2 FTS)	5.618	C8 H5 F13 O3 S	97.04	427.9748

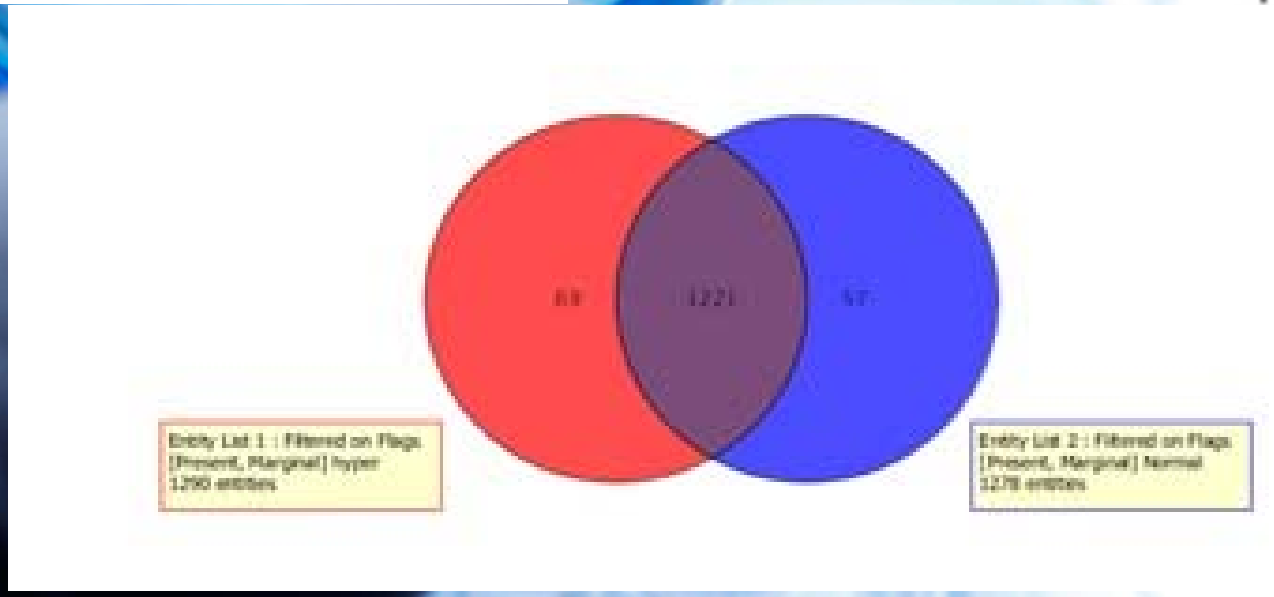

Cpd 37: F53-B: - FBF Spectrum (6.110-6.184 min) cat serum HT_neg_02_01.d Subtract
x10 3
530.8936
([C8HClF16O4S]-H)-
531.8952
([C8HClF16O4S]-H)-
532.8915
([C8HClF16O4S]-H)-
534.8887
([C8HClF16O4S]-H)-
F-53B (CAS No. 73606-19-6)
ClF2C
SO3−
Present in only one sample


Cpd 37: F53-B: -ESI EIC(530.8956, 532.8927, 576.9011, 578.8983 ...) Scan Frag=375.0V cat serum HT_neg_02_01.d
x10 4
6.143
Counts vs. Acquisition Time (min)

HT vs. Normal: Profiles comparison

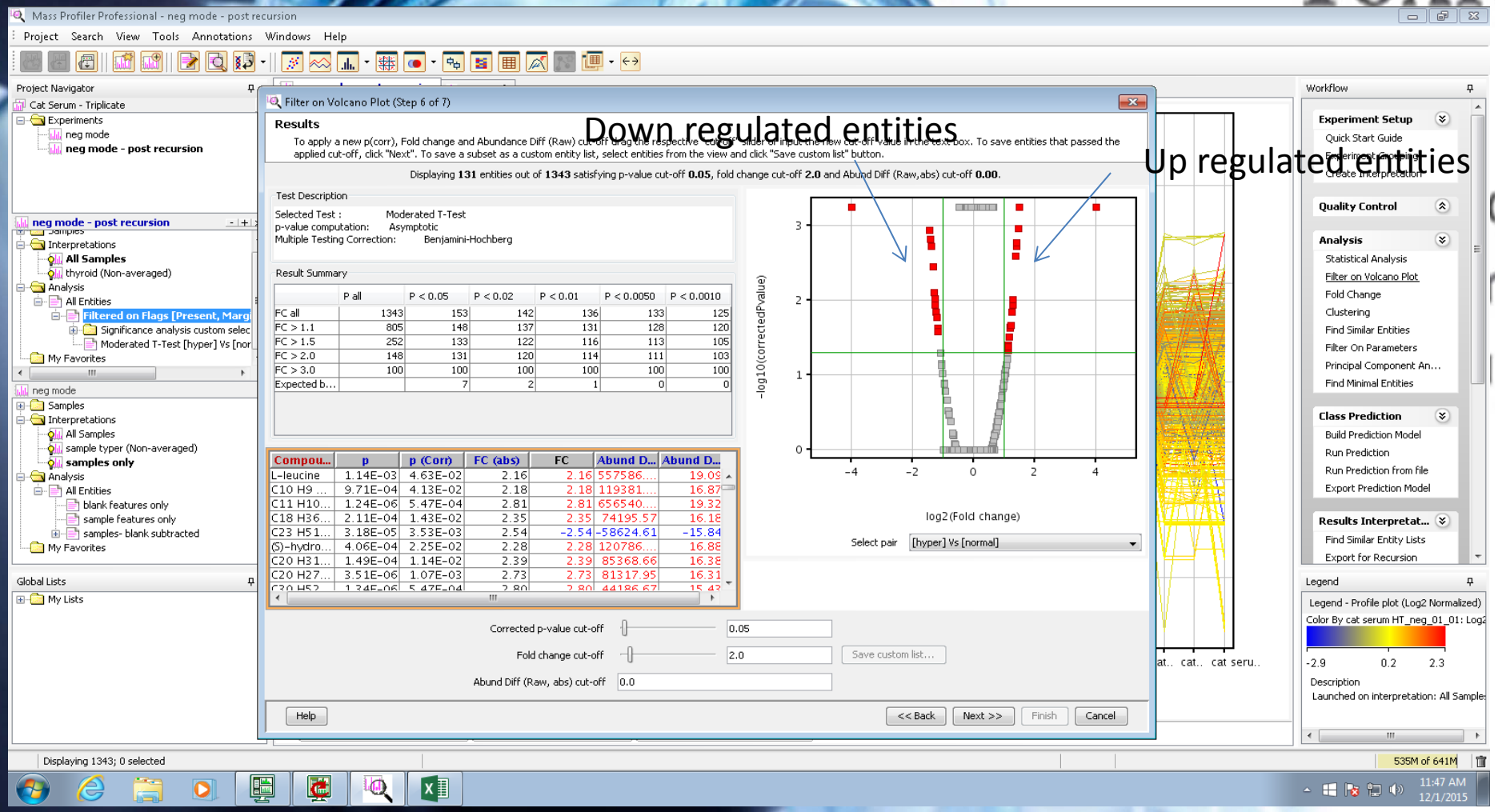


Venn diagram, HT vs. Normal



Volcano plot: Up vs Down Regulation

5 vs 5: very limited sample size



Profile comparison: HT vs. Normal

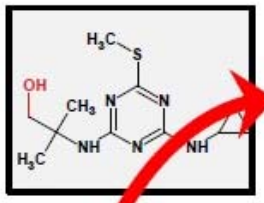
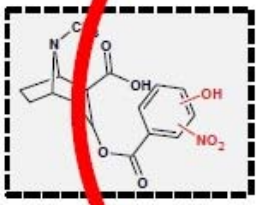
ID Browser Up regulated, Score > 80

Name	Formula	Mass	RT	Score	comment
Duloxetine	C18 H19 N O S	343.1241	5.617	96.76	depression drug
PFOSA	C8 H2 F17 N O2 S	498.953	6.568	96.73	PFAS
PFOSAA	C10 H4 F17 N O4 S	556.9582	6.236	95.59	PFAS
Catechol	C6 H6 O2	110.0368	0.905	87.47	phenol/pesticide
L-leucine	C6 H13 N O2	131.0947	0.619	86.9	dietary supplement
acetaminophen	C8 H9 N O2	151.0634	1.458	86.75	pharmaceutical
texanol	C12 H24 O3	216.1722	5.738	86.44	coalescent for latex paints
myristic acid	C14 H28 O2	228.2093	7.208	85.83	common fatty acid
acetophenone	C8 H8 O	120.0569	5.286	85.56	common fragrant ketone used in fragance
(S)-hydroprene	C17 H30 O2	266.2239	7.274	85.37	insecticide
dodecyl methacrylate	C16 H30 O2	254.2236	7.644	82.19	an ester that might be used in PCP

eawag aquatic research

Identification with (LC-HR)MS/MS – Confidence?

Proposed levels for MS and MS/MS data

Example	Identification confidence	Minimum data requirements
	***Level 1: Confirmed structure*** by reference standard	MS, MS^2, RT, Reference Std.
	Level 2: Probable structure a) by library spectrum match b) by diagnostic evidence	MS, MS^2, Library MS^2 MS, MS^2, Exp. data
	***Level 3: Tentative candidate(s)*** structure, substituent, class	MS, MS^2, Exp. data
$C_6H_{[illegible]}N_3O_4$	***Level 4: Unequivocal molecular formula***	MS isotope/adduct
192.0757	***Level 5: Exact mass*** of interest	MS

Schymanski, Jeon, Gulde, Fenner, Ruff, Singer & Hollender (2014) *ES&T*, 48 (4), 2097-2098. DOI: 10.1021/es5002105

Profile comparison: HT vs. Normal

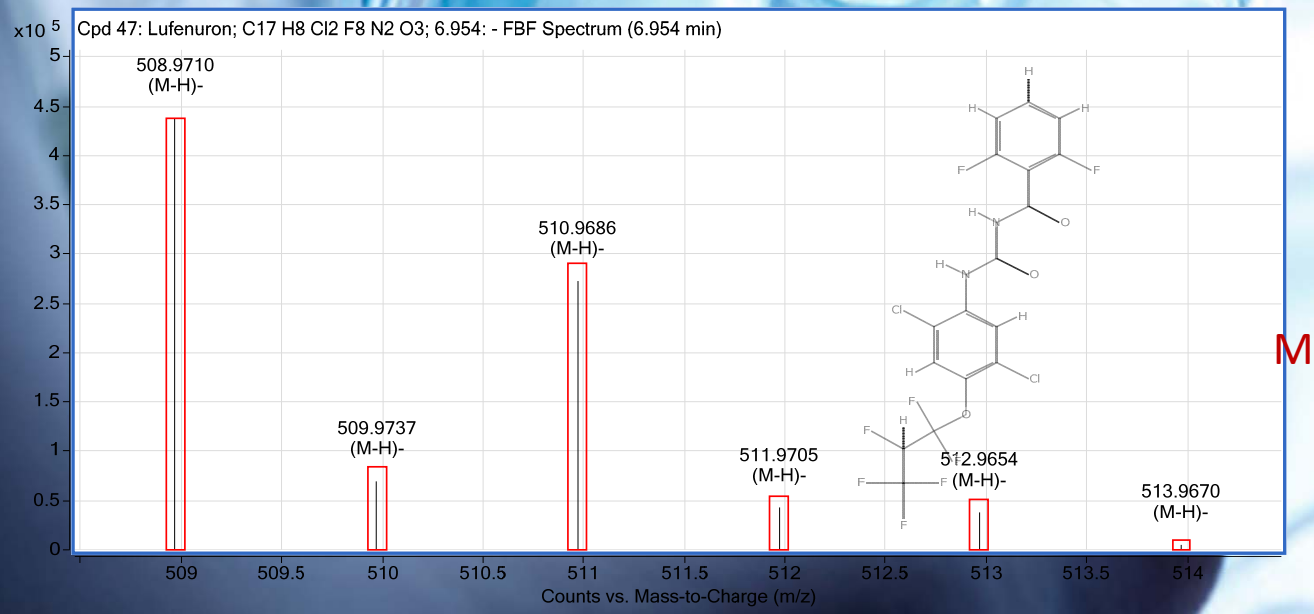
ID Browser Up regulated, Score > 80

Name	Formula	Mass	RT	Score	comment
3 Duloxetine	C18 H19 N O S	343.1241	5.617	96.76	depression drug
PFOSA 1	C8 H2 F17 N O2 S	498.953	6.568	96.73	PFAS
PFOSAA 1	C10 H4 F17 N O4 S	556.9582	6.236	95.59	PFAS
Catechol	C6 H6 O2	110.0368	0.905	87.47	phenol/pesticide
L-leucine	C6 H13 N O2	131.0947	0.619	86.9	dietary supplement
acetaminophen	C8 H9 N O2	151.0634	1.458	86.75	pharmaceutical
texanol	C12 H24 O3	216.1722	5.738	86.44	coalescent for latex paints
myristic acid	C14 H28 O2	228.2093	7.208	85.83	common fatty acid
acetophenone	C8 H8 O	[illegible].0569	5.286	85.56	common fragrant ketone used in fragance
(S)-hydroprene	C17 H30 O2	266.2239	7.274	85.37	insecticide
dodecyl methacrylate	C16 H30 O2	254.2236	7.644	82.19	an ester that might be used in PCP

3-4

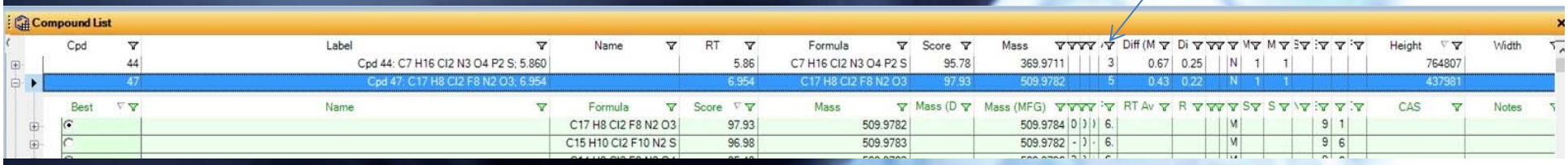
Tentatively identified compounds, Down regulated

Name	Formula	Mass	RT	Score	comment
Lufenuron	C17 H8 Cl2 F8 N2 O3	509.9782	6.954	97.98	flea control agent
(+)-isostearic acid	C18 H36 O2	284.271	7.826	93.26	fatty acid, PCP



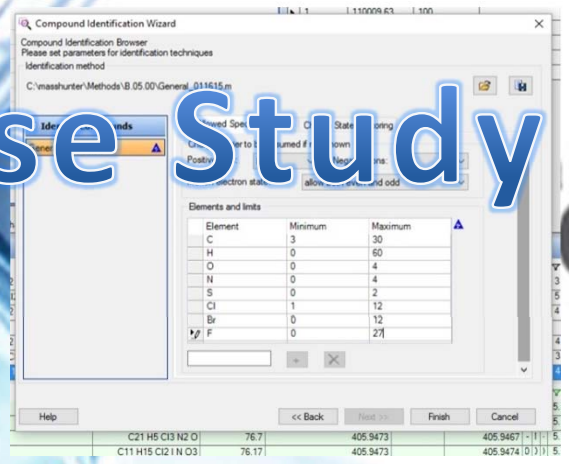
Database match

Molecular Formula Generation



Semi-Unknowns: Those that have No Database match

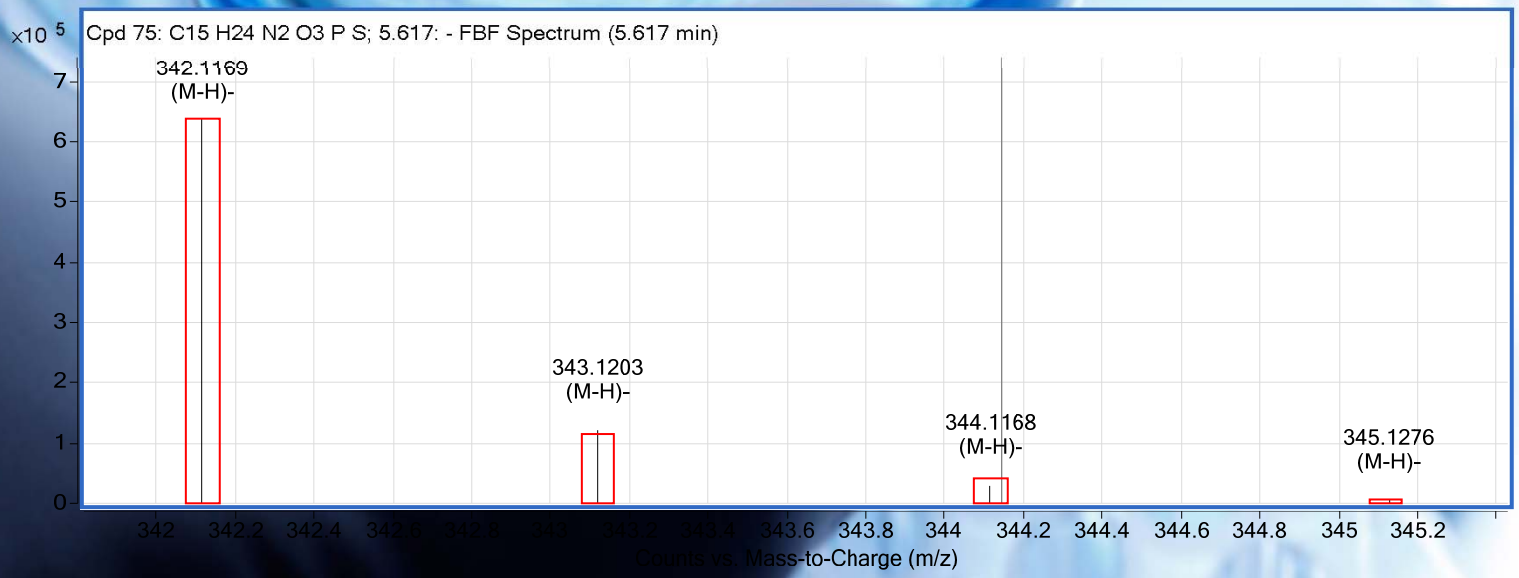
Case Study #1



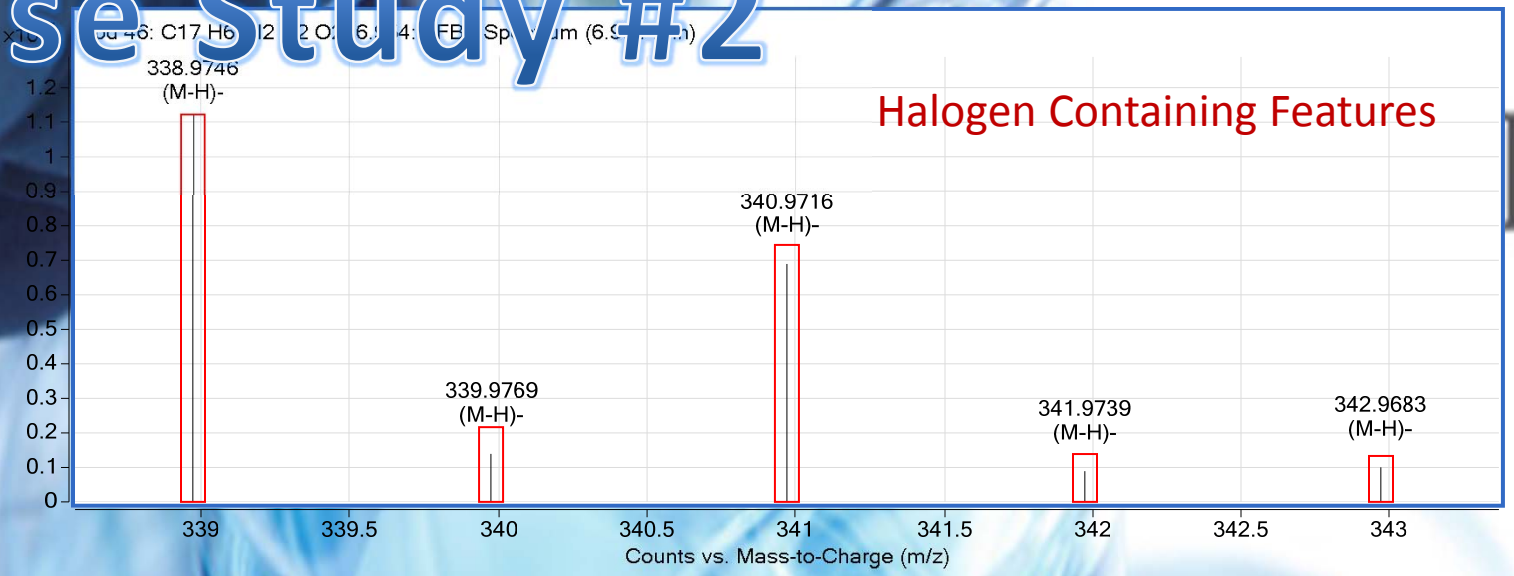
20 Formula Generated

TRUE		C15 H24 N2 O3 P S	97.8	343.1243		343.1245	0.59	0.59	0.2	5.617
FALSE		C19 H21 N O3 S	97.01	343.1243		343.1242	-0.14	0.14	-0.05	5.617

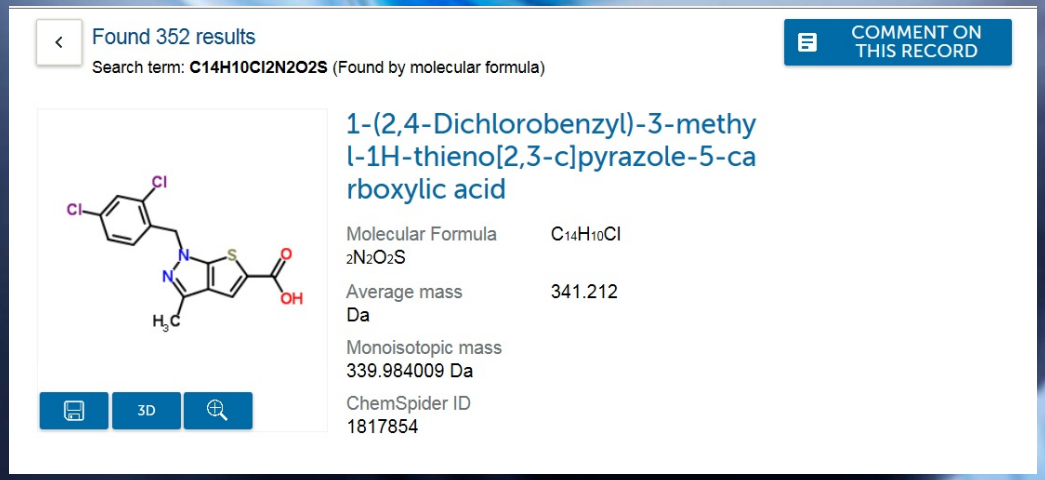
2570 Formula match in ChemSpider



Case Study #2

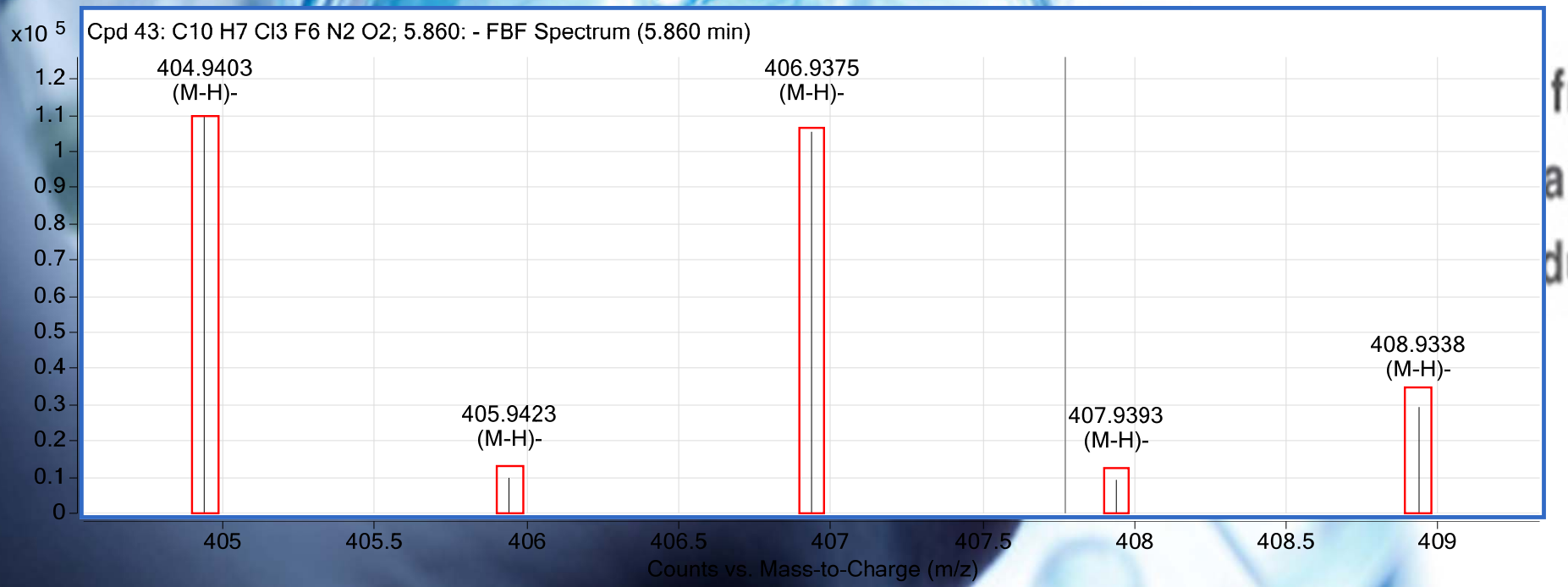


18 formulas → $C_{14}H_{10}Cl_2N_2O_2S$



352 hits, ChemSpider

Case Study #3


Cpd 43: C10 H7 Cl3 F6 N2 O2; 5.860: - FBF Spectrum (5.860 min)
x10 5
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
404.9403
(M-H)-
405.9423
(M-H)-
406.9375
(M-H)-
407.9393
(M-H)-
408.9338
(M-H)-
405
405.5
406
406.5
407
407.5
408
408.5
409
Counts vs. Mass-to-Charge (m/z)

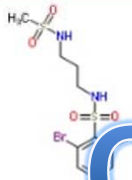
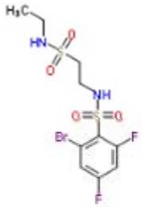
C10H13BrF2N2O4S2

FILTER

Search Hits Limit: 100

Found 3 results

Search term: **C10H13BrF2N2O4S2** (Found by molecular formula)

ID	Structure	Molecular Formula	Molecular Weight	# of Data Sources	# of References	# of PubMed	# of RSC
49665410		$C_{10}H_{13}BrF_2N_2O_4S_2$	407.252	1	1	0	0
54898253		$C_{10}H_{13}BrF_2N_2O_4S_2$	407.252	1	1	0	0

Only R/D usage

USING MASS SEARCH

Merck Index: search by formula mass, low resolution

EPA CompTox Dashboard: Search by monoisotopic Mass, high resolution

EPA comptox dashboard, mass search

Case Study #3

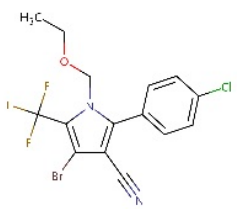
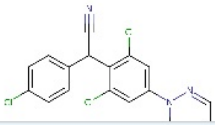
46 hit

EPA United States Environmental Protection Agency | Home | Advanced Search | Search CompTox Dashboard

View Selected | Hide Isotopes

Searched by Mass and single component chemicals: Found 46 results for '405.9483 ± 0.035 amu'.

Download as: CSV | Excel | SDF

Structure	Preferred Name	CAS-RN	QC Level	Number of Sources	Monoisotopic Mass	Mass Difference	
	Chlorfenapyr	122453-73-0	DSSTox Low	24	405.969538	0.0212	☐
	Diclazuril	101831-37-2	DSSTox Low	10	405.979109	0.0308	☐

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EPA comptox dashboard, mass search

405.9483 ± 0.0035

Case Study #3

1 hit

CompTox Dashboard | T | Phenol, 4,4'-(2,2,2-trichloro | TCMDC-124158 | C14H9Cl3 | Wang, Miaomiao@DTSC - C

comptox.epa.gov/dashboard/dsstoxdb/results?utf8=%E2%9C%93&mass=2&mass1=405.9483&mass2=0.0035&single_component=1

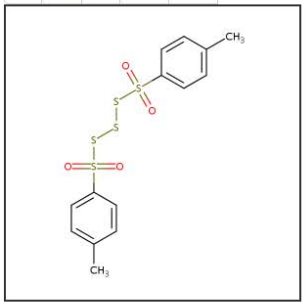
EPA United States Environmental Protection Agency | Home | Advanced Search | Search CompTox Dashboard

Submit Comment | Share | Copy

Trisulfide, bis[(4-methylphenyl)sulfonyl]

5692-44-4 | DTXSID10399230

Searched by Mass and single component chemicals: Found 1 result for '405.9483 ± 0.0035 amu'.



Intrinsic Properties

Molecular Formula: C14H14O4S5 | Find All Chemicals

Average Mass: 406.56 g/mol

Monoisotopic Mass: 405.949565 g/mol

Structural Identifiers

Record Information

Chemical Properties | External Links | Synonyms | Product Composition | ToxCast in Vitro Data | Exposure | PubChem | Comments

Summary

Octanol-Water Partition Coefficient (LogP)

Water Solubility

Melting Point

Download as: CSV | Excel | SDF

Property	Average (Exp.)	Median (Exp.)	Range (Exp.)	Average (Pred.)	Median (Pred.)	Range (Pred.)	Result Unit
Octanol-Water Partition Coefficient (LogP)	-	-	-	2.85 (1)	2.85	-	-
Water Solubility	-	-	-	6.99e-06 (1)	6.99e-06	-	mol/L
Melting Point	-	-	-	183 (1)	183	-	°C

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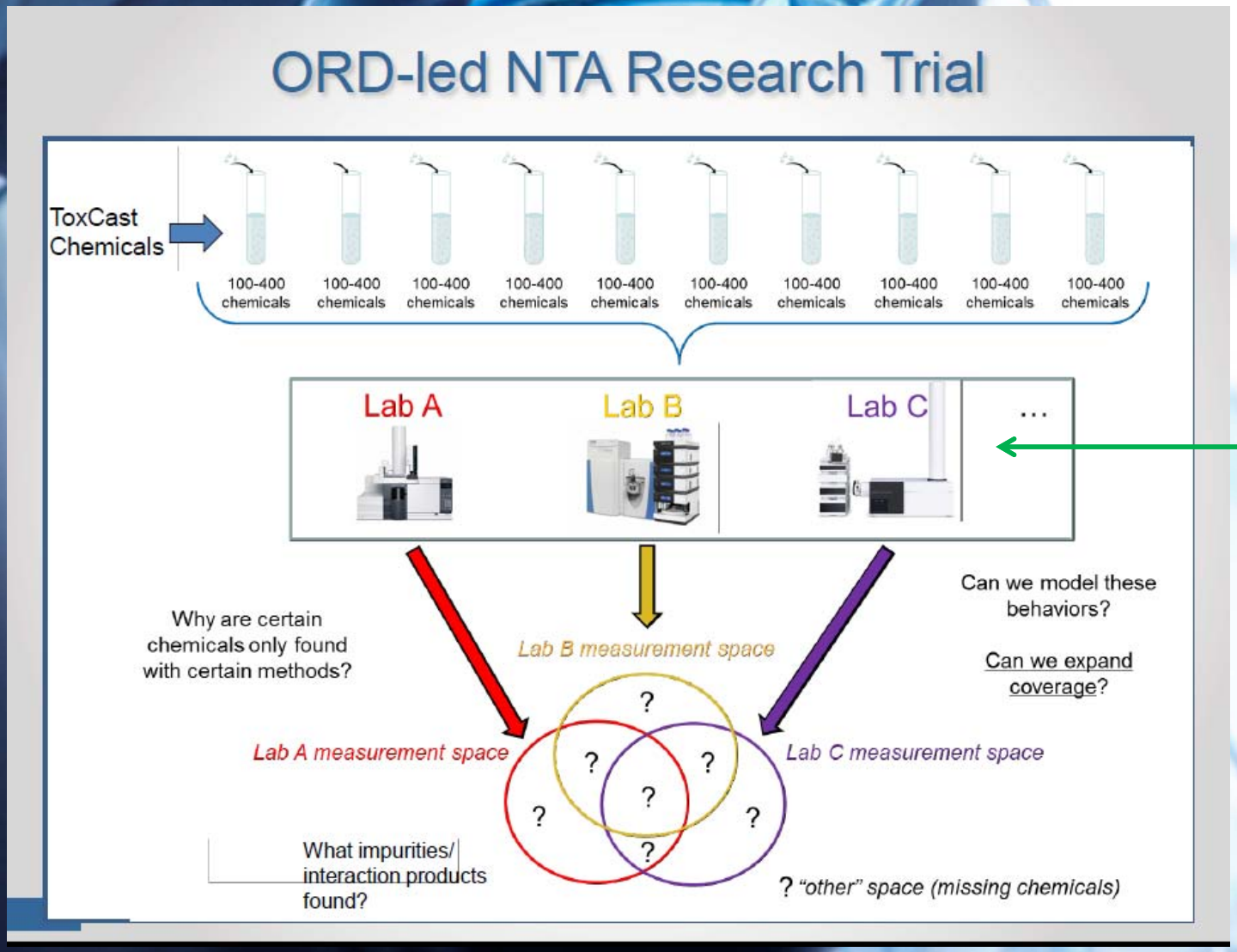
Ask me anything | ENG | 6:58 AM 9/27/2016

Cat Exposome Screening Summary

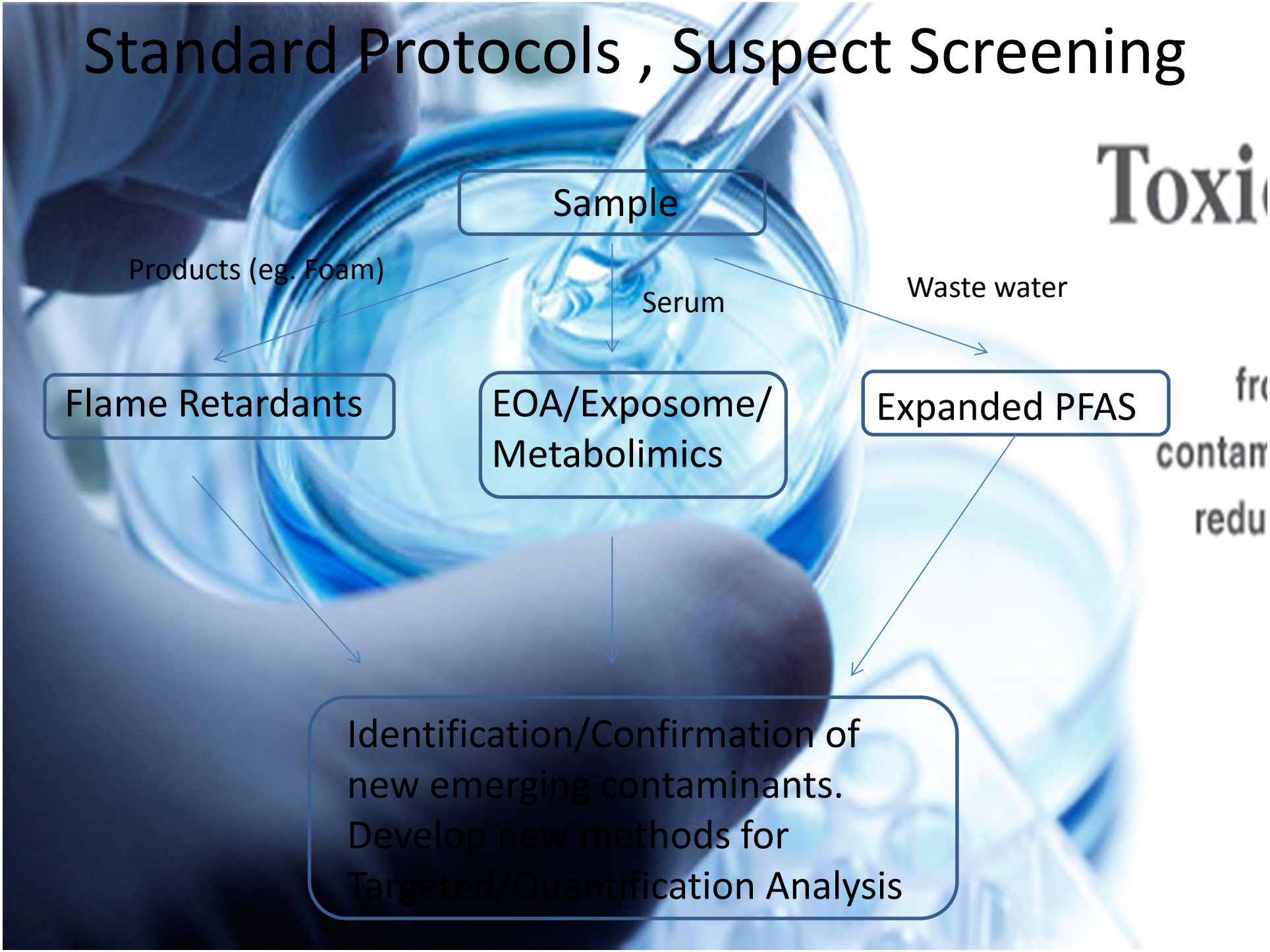
- Established method for biological sample prep and screening. The biological exposome includes: various PFASs, PCPs, pesticides, pharmaceuticals, FRs
- Exploratory project on profiles comparison: procedures, criteria's
- SOP in process

What's Next?

Slide from Jon Sobus presentation, US EPA



Standard Protocols , Suspect Screening



Acknowledgements

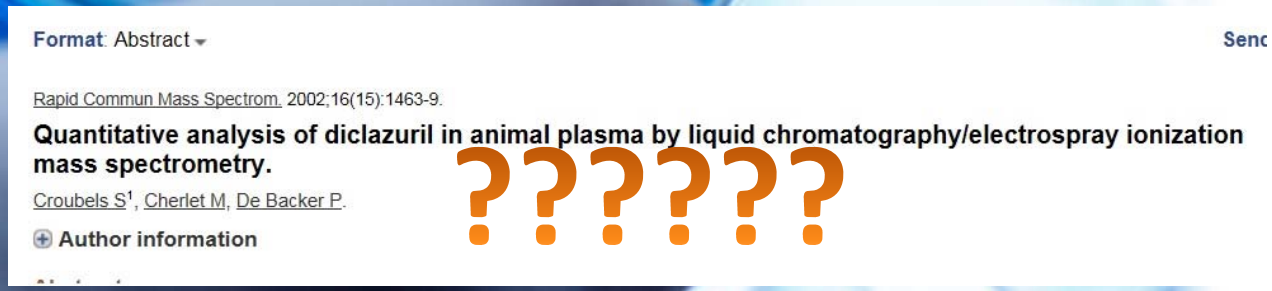
B2 Group
HEMS Group
Dr. Steve Garner, Albany Animal Hospital, Albany, California, USA
William Zolan, Supervising Chemist, Mel Leong Treatment Plant
Dr. Bruce Labelle, ECL Chief

Questions?

Pubmed Search

Diclazuril is used in cats to treat Isospora species

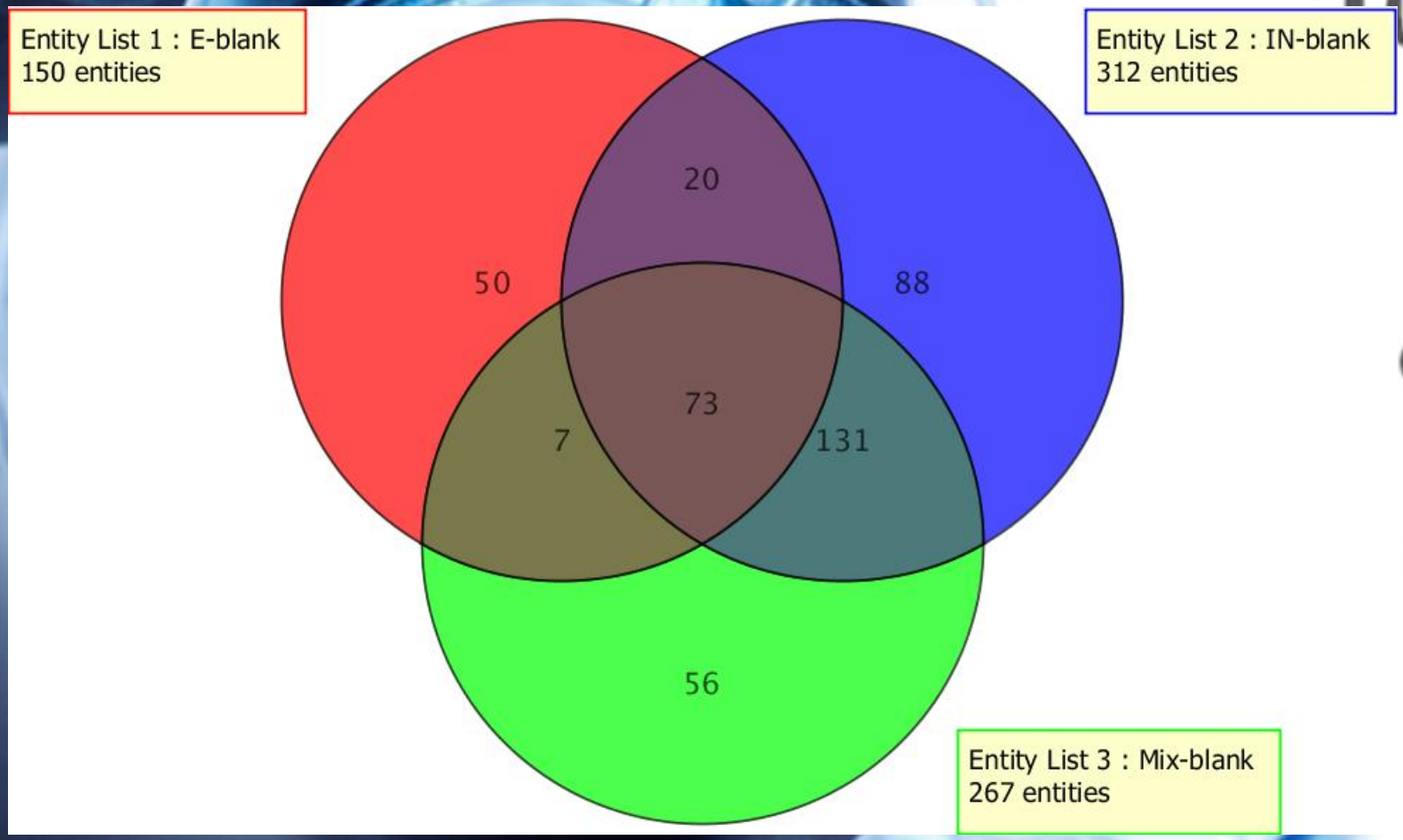
$C_{17}H_9Cl_3N_4O_2$



???????

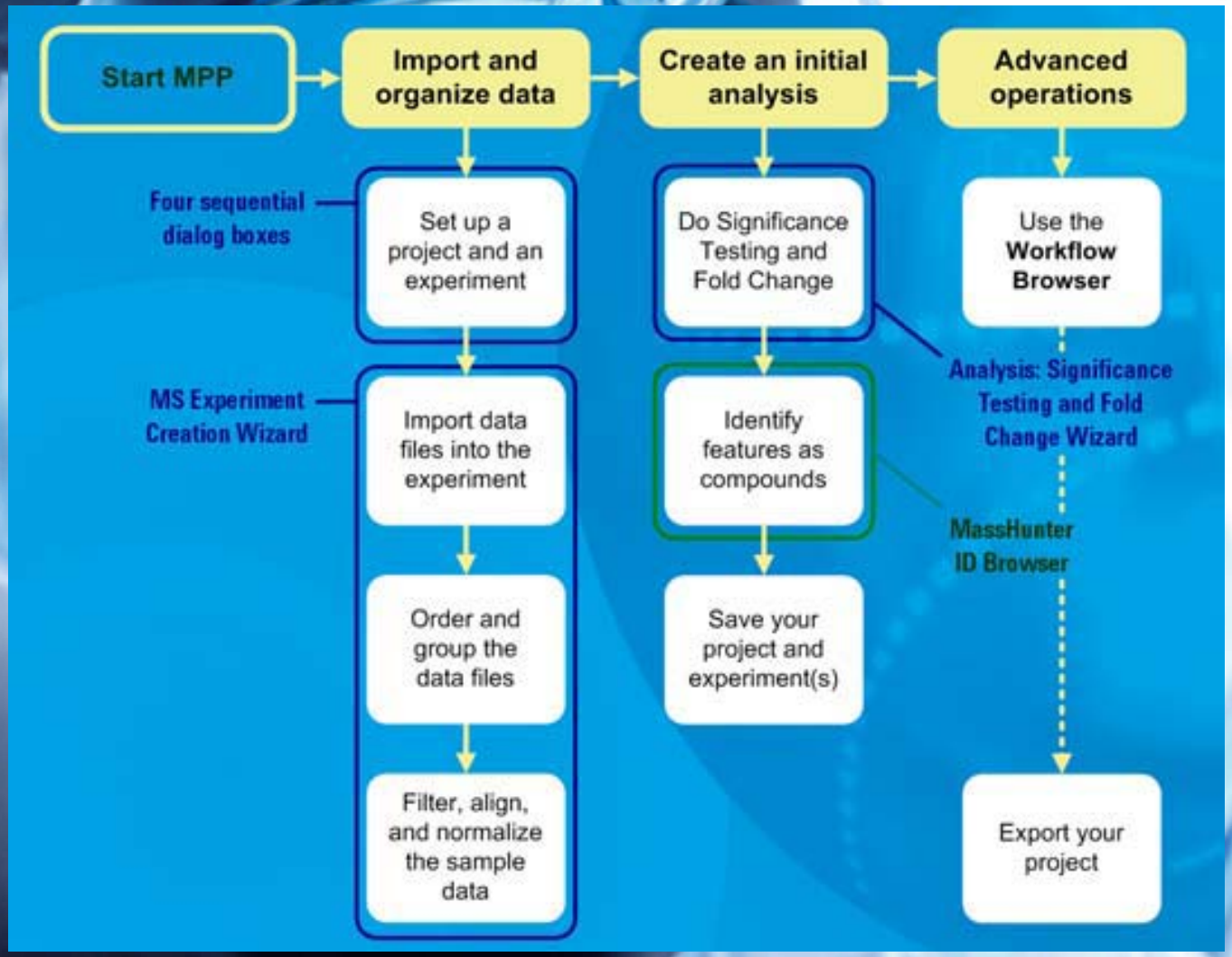
Mass error ~0.03 da

Venn diagram

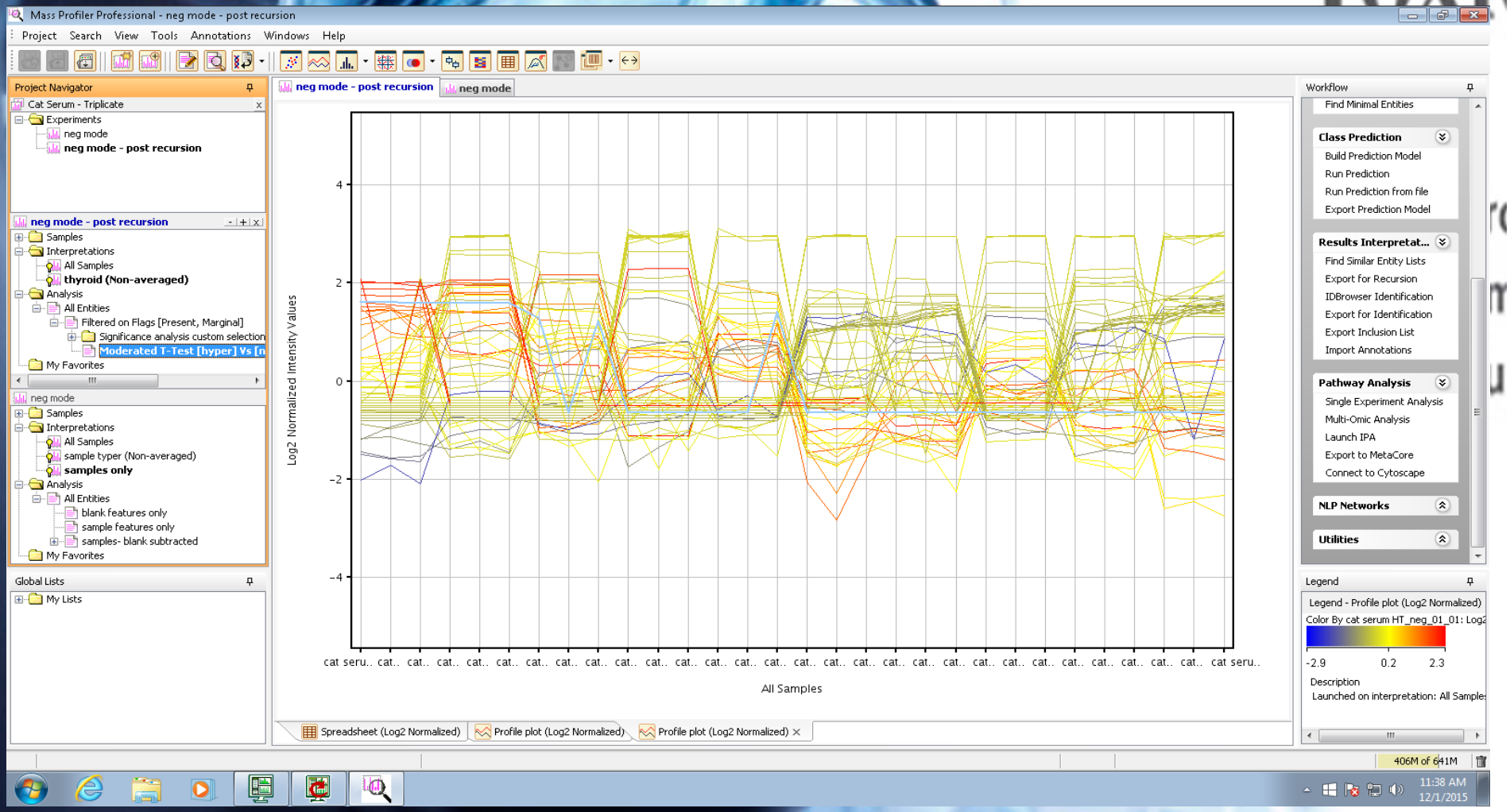


Targeted vs. non targeted SFO WW

MPP Data processing:
non targeted workflow, recursive analysis



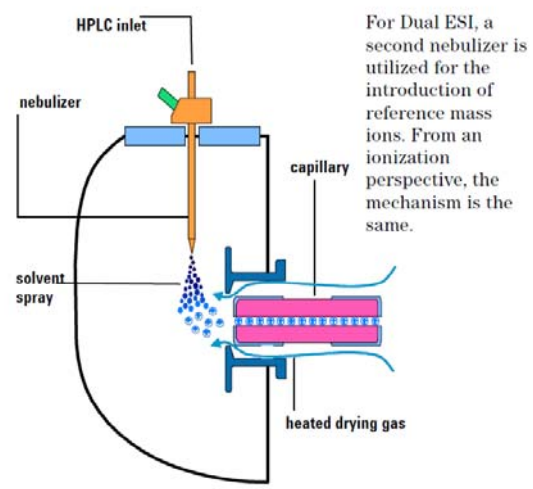
Profile plots, 131 features: 76 up regulated, 55 down regulated



System Overview: Analysis method

HPLC system: Agilent UPLC 1290

- Agilent extended C18 column, 1.8 um, 2.1X50 mm
- Electrospray Ionization (ESI) , negative/positive ion polarity full scan (m/z 100-1700)
- Two sets of Gradients for positive/negative ionization
- System Calibration before each run using Agilent Tuning mix (ESI-L Low Concentration Tuning Mix)
- References used during the sample run



For Dual ESI, a second nebulizer is utilized for the introduction of reference mass ions. From an ionization perspective, the mechanism is the same.

Figure 1 Electrospray ion source

Electrospray ionization (ESI) consists of four steps: