Non-targeted analysis approaches: A perspective from the USA

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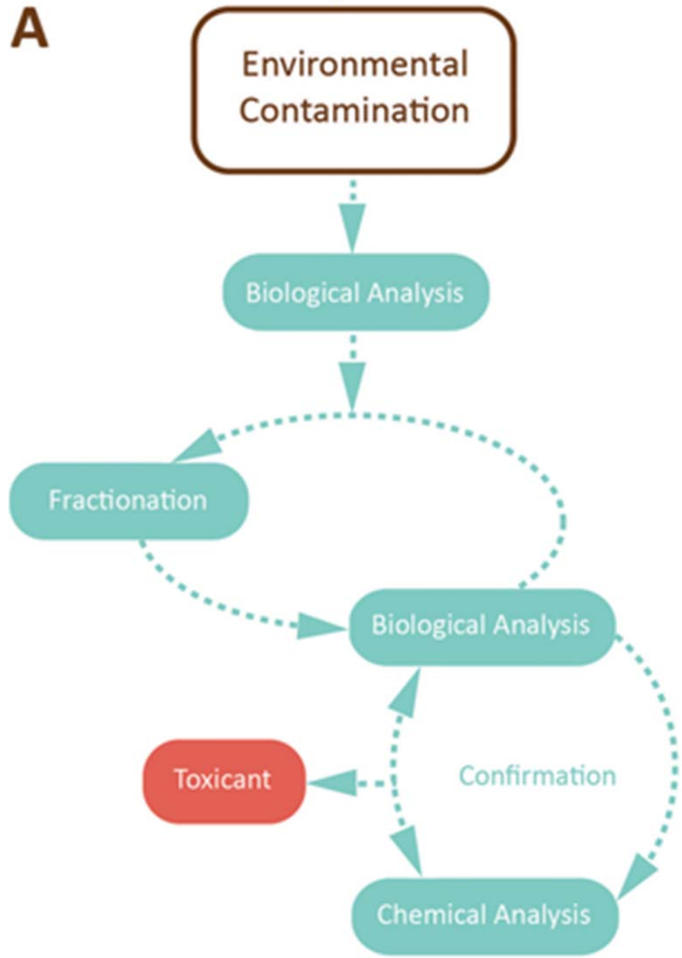
Evolution of non-targeted analysis approaches within the US scientific community

- Toxicity Identification and Evaluation (TIE) – Early attempt at EDA
- Identification of “causative stressors” driven by observed sediment (usually) toxicity.
- Recent attempts at *in silico* emerging contaminant “prioritization” provide a roadmap (e.g. Howard and Muir).
- No systematic US framework (e.g. NORMAN) exists for non-targeted analysis.



R. M. Burgess, K. T. Ho, W. Brack, M. Lamoree, *Environ. Toxicol. Chem.* 2013, *32*. 1935-1945

What have we learned from sediment TIE experiments over the years?

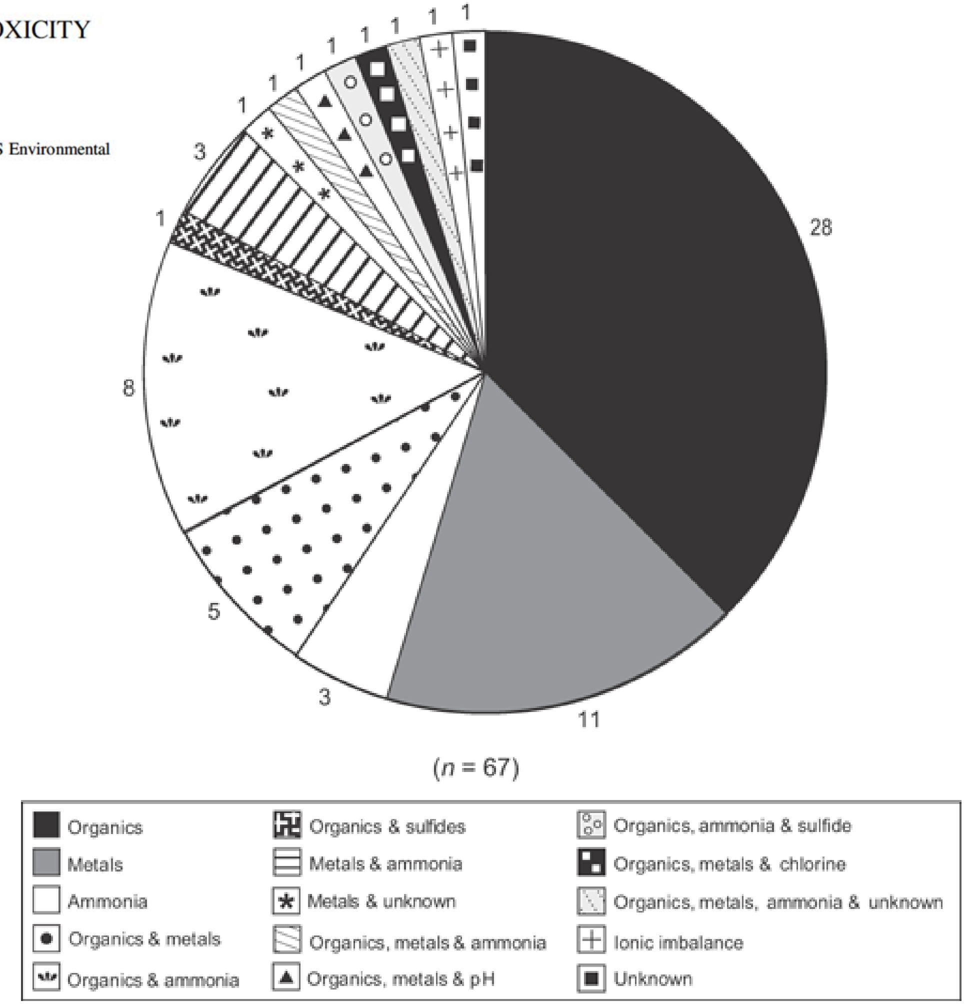
Environmental Toxicology and Chemistry, Vol. 32, No. 11, pp. 2424–2432, 2013

Critical Review

WHAT'S CAUSING TOXICITY IN SEDIMENTS? RESULTS OF 20 YEARS OF TOXICITY IDENTIFICATION AND EVALUATIONS

KAY T. HO* and ROBERT M. BURGESS
National Health and Environmental Effects Research Laboratory, Atlantic Ecology Division, Office of Research and Development, US Environmental Protection Agency, Narragansett, Rhode Island, USA

- Nonionic organics accounted for the largest fraction of observed toxicity.
- Whole-sediment TIE implicated nonionic organics in 90% of cases.
- "Molecular" identity of causative stressor was not always determined.

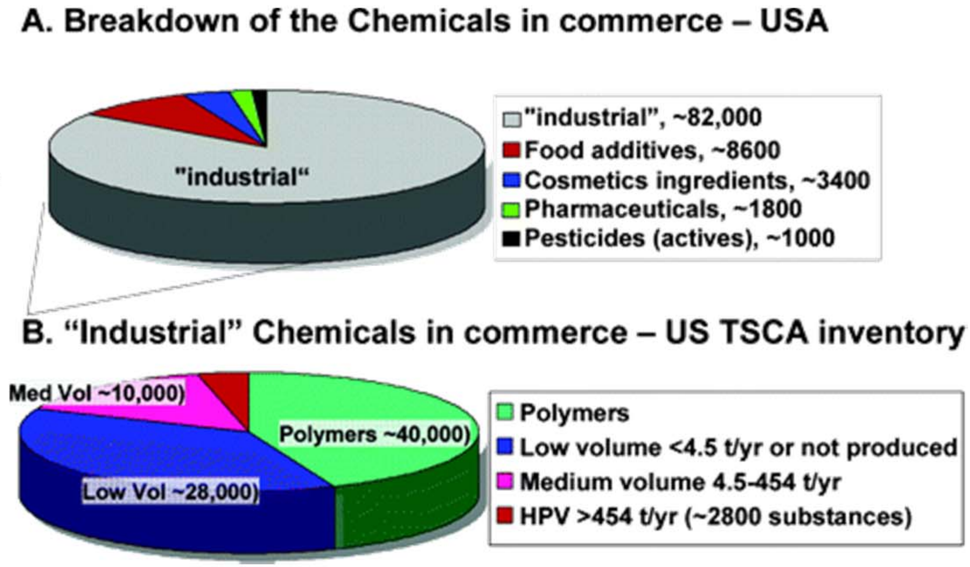


The "Howard and Muir" studies: Providing a roadmap for rational non-targeted analysis

Environ. Sci. Technol. **2006**, *40*, 7157–7166

Are There Other Persistent Organic Pollutants? A Challenge for Environmental Chemists†

DEREK C. G. MUIR*,‡ AND PHILIP H. HOWARD§
Water Science and Technology Directorate, Environment Canada, Burlington, Ontario, Canada, and Syracuse Research Corporation, Environmental Science Center, North Syracuse, New York



Environ. Sci. Technol. **2010**, *44*, 2277–2285

Identifying New Persistent and Bioaccumulative Organics Among Chemicals in Commerce

PHILIP H. HOWARD*,† AND DEREK C. G. MUIR‡
SRC, Environmental Science Center, 6502 Round Pond Road, North Syracuse, New York, and Aquatic Ecosystem Protection Research Division, Environment Canada, 867 Lakeshore Road, Burlington, Ontario

Identifying New Persistent and Bioaccumulative Organics Among Chemicals in Commerce II: Pharmaceuticals

Philip H. Howard*,† and Derek C. G. Muir‡
†SRC, Inc. Chemical, Biological, and Environmental Center (CBEC), 7502 Round Pond Road, North Syracuse, New York, and ‡Aquatic Ecosystem Protection Research Division, Environment Canada, 867 Lakeshore Road, Burlington, Ontario
dx.doi.org/10.1021/es201196x | *Environ. Sci. Technol.* 2011, 45, 6938–6946

Identifying New Persistent and Bioaccumulative Organics Among Chemicals in Commerce. III: Byproducts, Impurities, and Transformation Products

Philip H. Howard*,† and Derek C. G. Muir‡
†SRC, Inc. Defense and Environmental Solutions (DES), 7502 Round Pond Road, North Syracuse, New York 13212, United States
‡Environment Canada, Aquatic Contaminants Research Division, 867 Lakeshore Road, Burlington, Ontario, Canada
dx.doi.org/10.1021/es4004075 | *Environ. Sci. Technol.* 2013, 47, 5259–5266

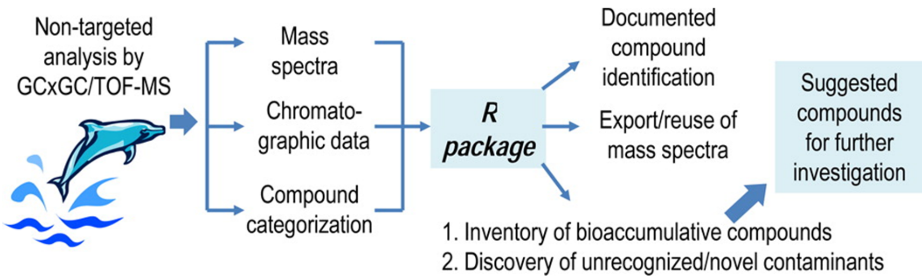
Putting this roadmap to the test: Non-targeted analysis of POPs in dolphins

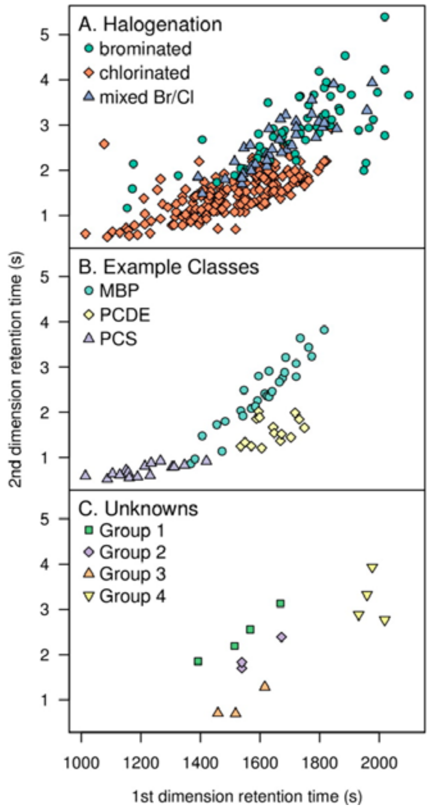
Environmental Science & Technology
Article
pubs.acs.org/est

Nontargeted Comprehensive Two-Dimensional Gas Chromatography/Time-of-Flight Mass Spectrometry Method and Software for Inventorying Persistent and Bioaccumulative Contaminants in Marine Environments

Eunha Hoh,*,† Nathan G. Dodder,*,‡ Steven J. Lehotay,§ Kristin C. Pangallo,∥ Christopher M. Reddy,⊥ and Keith A. Maruya‡

dx.doi.org/10.1021/es301139q | *Environ. Sci. Technol.* 2012, 46, 8001−8008





- 271 Unique compounds were identified, all but one were halogenated.
- 2D GC separation allowed clustering by “compound class”.
- Many compounds were likely natural products (e.g. methylbipyrroles).
- Halogenated natural products were present at concentrations in blubber similar to PBDE congeners.

Table 3. Concentrations (ng/g of lipid mass) of Selected Halogenated Natural Products Compared to the Six Major PBDE Congeners

compd	concn	compd	concn
MBP-Cl_7	85	2,2′-diMeO-BB-80	12.8
MBP-HBr_5Cl	1110	PBHD (3Br)	18
MBP-HBR_6	478	PBHD (4Br)	246
MBP-Br_6Cl	1.62	BDE-28	8.6
MBP-Br_7	0.504	BDE-47	727
DMBP-Br_4Cl_2	124	BDE-100	241
DMBP-Br_5Cl	16.5	BDE-99	123
DMBP-Br_6	30.9	BDE-154	103
2′-MeO-BDE68	47	BDE-153	51.3
6-MeO-BDE47	103		

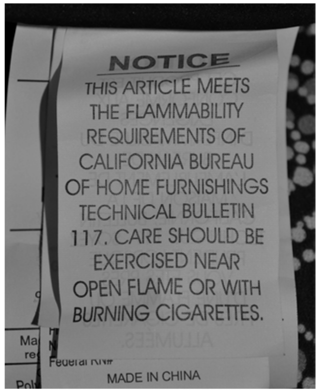
What can you do when the compound is not present in any chemical registry?

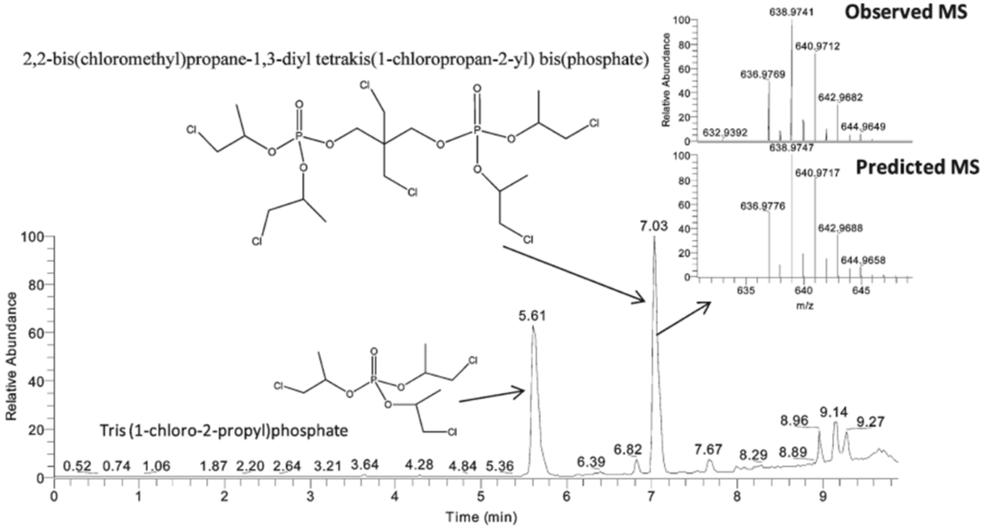
Environmental Science & Technology
ARTICLE
pubs.acs.org/est

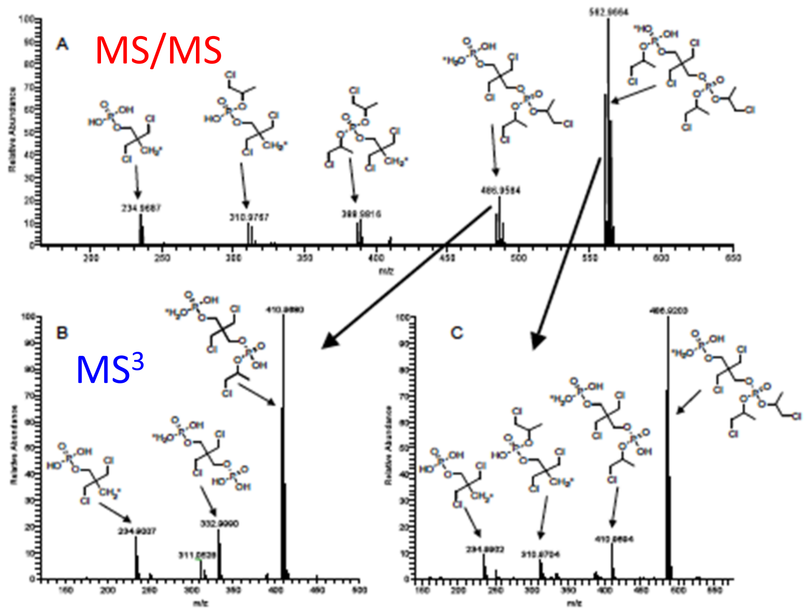
Identification of Flame Retardants in Polyurethane Foam Collected from Baby Products

Heather M. Stapleton,*[†] Susan Klosterhaus,[‡] Alex Keller,[†] P. Lee Ferguson,[†] Saskia van Bergen,[§] Ellen Cooper,[†] Thomas F. Webster,[‖] and Arlene Blum[⊥]

dx.doi.org/10.1021/es2007462 | *Environ. Sci. Technol.* 2011, 45, 5323–5331







Example approaches from my laboratory:

1. **<u>Effects-directed analysis:</u>** Identifying toxic components of aircraft deicing/anti-icing fluids (ADAF)

2. **<u>Activity-directed analysis:</u>** Receptor affinity extraction for identifying estrogenic compounds associated with water reuse

3. **<u>Fate-directed analysis:</u>** Non-targeted analysis of micropollutant fate in wastewater treatment

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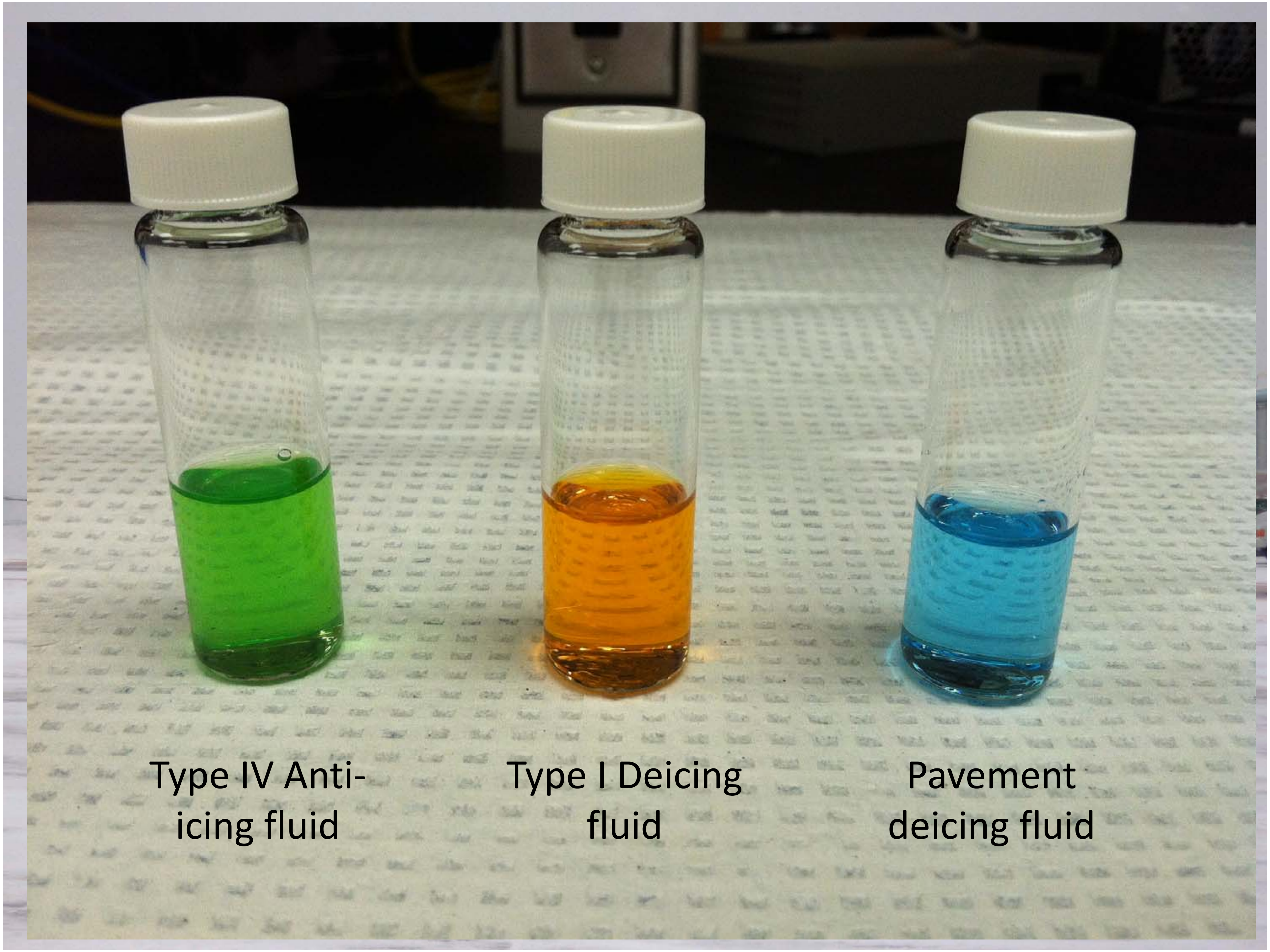

Type IV Anti-icing fluid
Type I Deicing fluid
Pavement deicing fluid

Aircraft deicer and anti-icer fluid (ADAF) formulations: What are they?

ADAF =

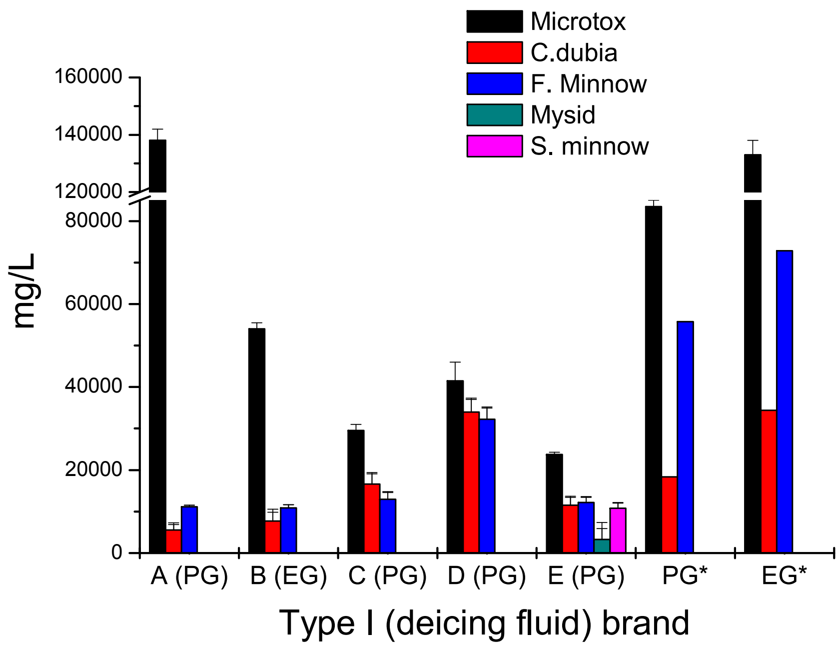
50-88% → Glycol
- -Propylene
- -Ethylene
- -Diethylene

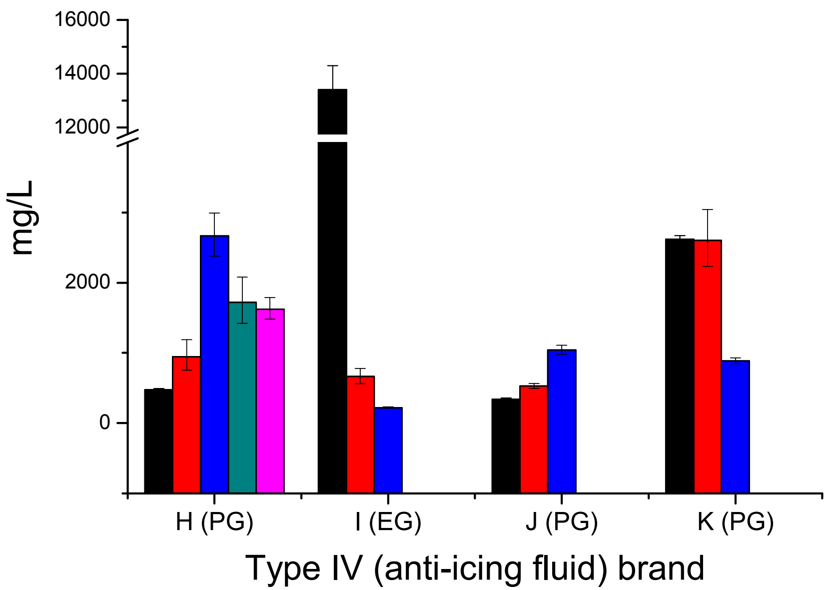
11-49% → Water

1->10% → AdPack
- -Corrosion inhibitors
- -Surfactants
- -Anti-foaming
- -Thickeners
- -Dyes
- -Flame retardants
- -other additives

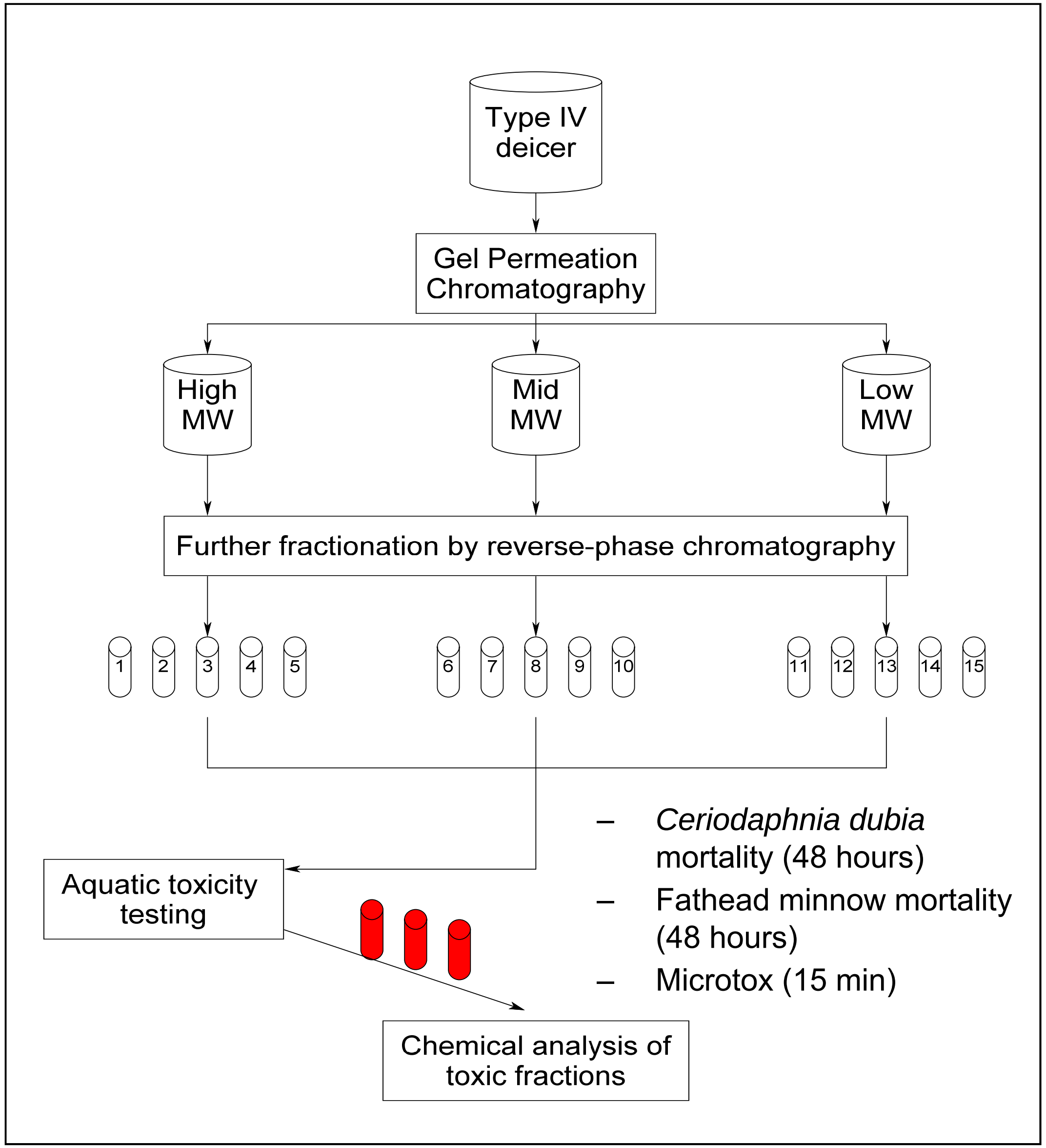
Aircraft anti-icing fluids are more toxic to aquatic organisms than deicing fluids

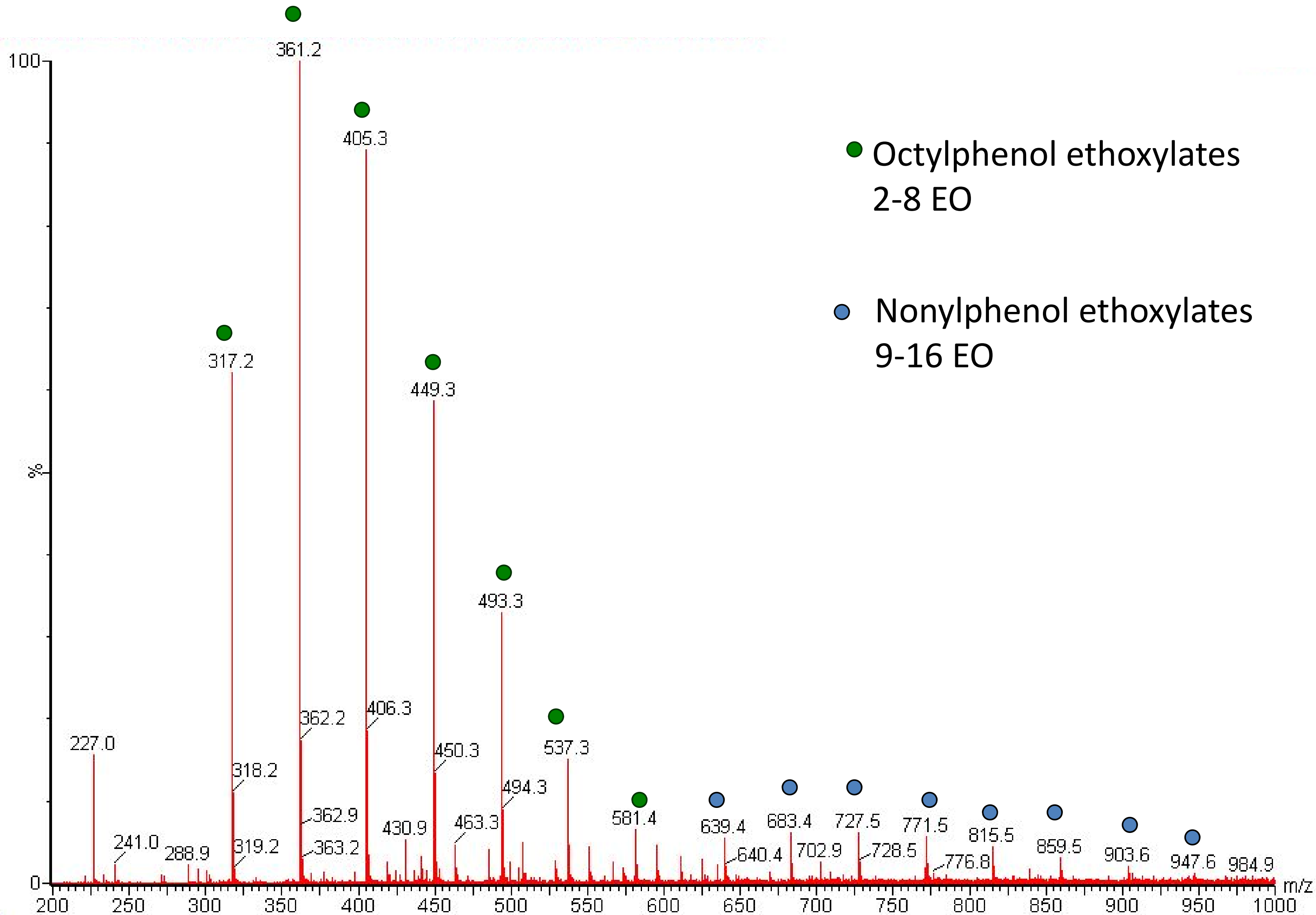
Toxicities varied within classes, and freezing point depressants did not account for the majority of toxicity in most cases.





Anti-Icer (Type IV) EDA Approach





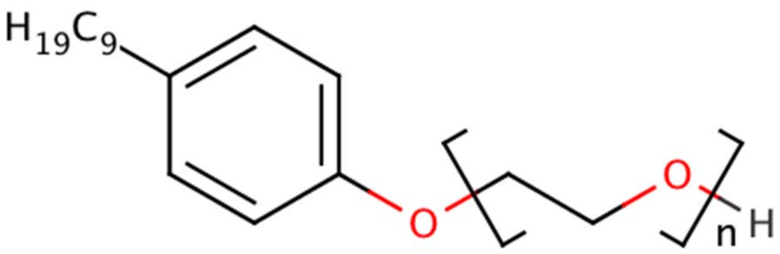
Octylphenol ethoxylates
2-8 EO
Nonylphenol ethoxylates
9-16 EO
100
%
0
m/z
200
250
300
350
400
450
500
550
600
650
700
750
800
850
900
950
1000
361.2
405.3
317.2
449.3
493.3
537.3
581.4
639.4
683.4
727.5
771.5
815.5
859.5
903.6
947.6
984.9
227.0
241.0
288.9
318.2
319.2
362.2
362.9
363.2
406.3
430.9
450.3
463.3
494.3
640.4
702.9
728.5
776.8

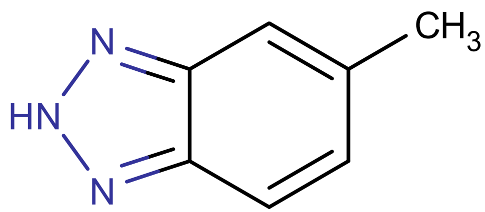
Summary of EDA findings for Type IV ADAF

- Ethoxylated surfactants were identified in toxic fractions from all 4 fluids
- Tolyltriazole was identified in 1 toxic fraction

Deicer	Fraction #	Compounds Identified in Fraction
Product K	5	OPEO 2-9* NPEO 4-24* C_{16}EO 3-6*
	8	methyl-1H-benzotriazole*
	10	OPEO 2-5
Product J	5	C_{10}EO 5-16* C_{12}EO 2-19* C_{14}EO 2-17*
Product I	5	NPEO 2-18* C_{13}EO 15-19* C_{16}EO 3-13*
Product H	5	C_{12}EO 5-19 C_{14}EO 4-18
	10	C_{12}EO 2-6

*Compounds identified with high mass accuracy(<5ppm)

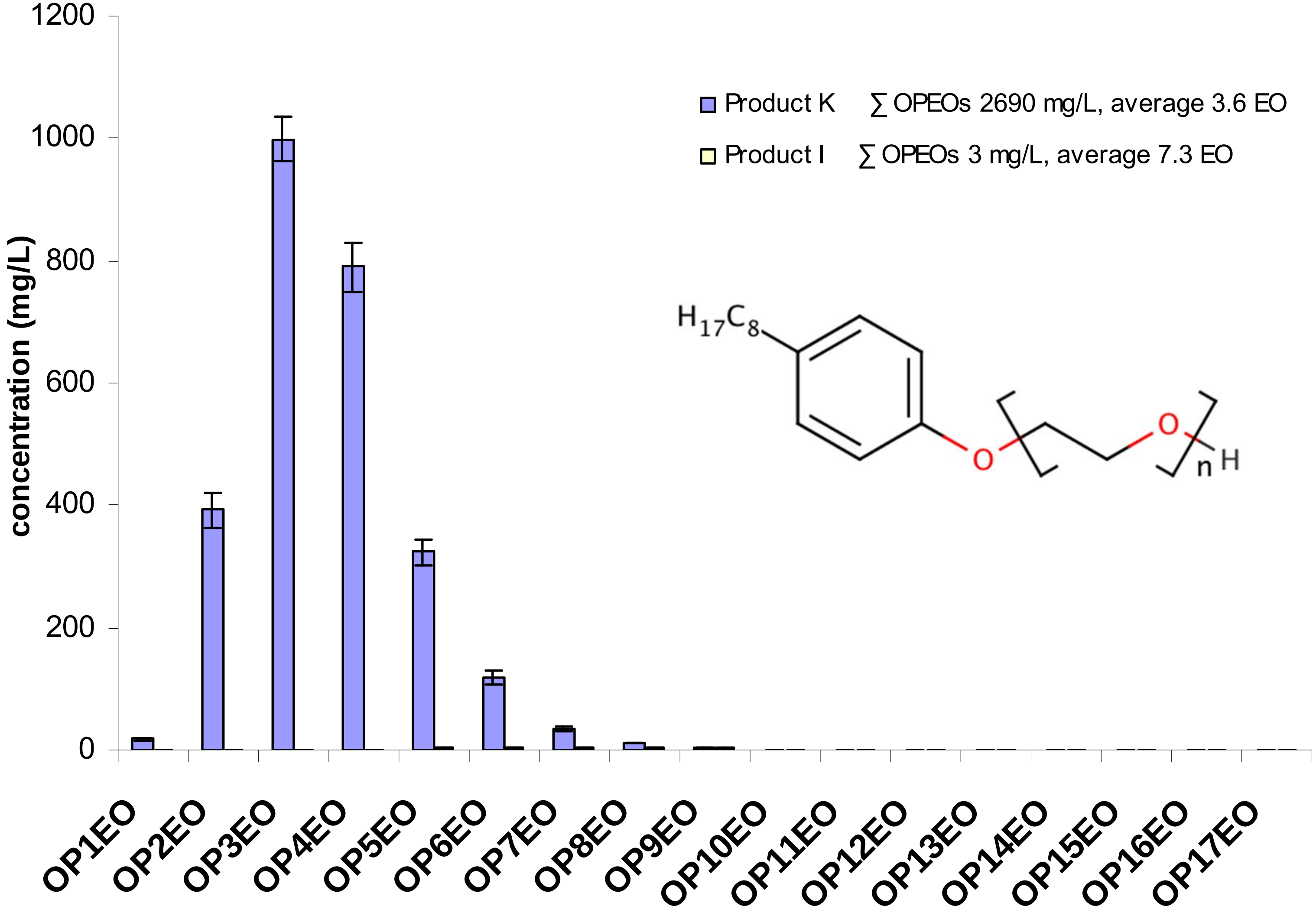




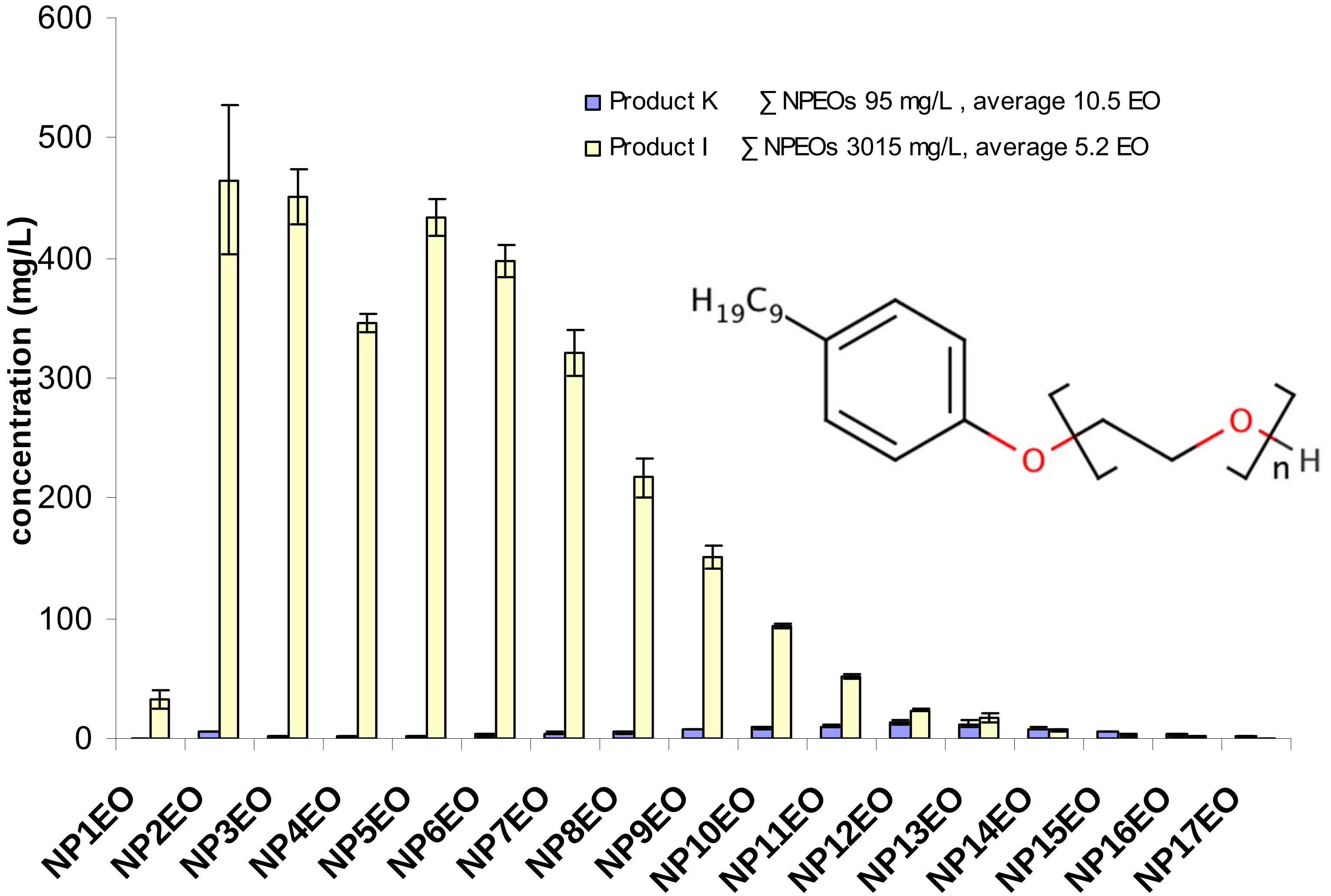
Targeted analysis revealed ethoxylated surfactants in all Type IV deicer fluids

Anti-icing fluid	Surfactants Identified	Estimated average EO number	Relative spectral abundance
Product H	C_{10}EO 2-19	6.13	2
	C_{11}EO 2-18	8.13	1
	C_{13}EO 1-17	6.08	3
	C_{15}EO 1-17	9.53	5
	C_{16}EO 1-5	2.85	1
Product I	OPEO 5-13	7.33	1
	C_{13}EO 1-19*	7.69	0.3
	C_{16}EO 1-16*	7.16	2
Product J	C_{10}EO 2-16*	7.07	2
	C_{11}EO 4-18	9.20	1
	C_{15}EO 1-17	9.49	3
	C_{16}EO 1-9	3.23	1
Product K	NPEO 4-24*	10.48	7
	C_{10}EO 3-18	9.86	1
	C_{12}EO 1-17	5.73	1
	C_{15}EO 1-17	6.61	3
	C_{16}EO 1-8*	4.58	0.1

Octylphenol ethoxylate concentrations in Product K and Product I



Nonylphenol ethoxylate concentrations in Product K and Product I



EDA validation: reformulated "mock" Product I

Formulation	Water	Ethylene Glycol (59%)	Polyacrylic Acid Thickener (2.7 g/L)	NPEO Surfactant (3.0 g/L)	4,5 MeBT Corrosion Inhibitor (0.163 g/L)	EC_{50} and LC_{50} Values (mg/L)		
						Microtox	***C. dubia***	**Fathead Minnow**
Product I	✓	✓	✓	✓	✓	**14,063**	**506**	**253**
Mock #1	✓	✓	✓	—	—	>51,500	>3,000	>3,000
Mock #2	✓	✓	✓	✓	—	29,167	1,576	1,237
Mock #3	✓	✓	✓	—	✓	36,750	>3,140	>3,140
Mock #4	✓	✓	✓	✓	✓	**7,737**	**725**	**290**

Checkmark indicates presence of component in mock formulation.

EDA validation: reformulated "mock" Product K

Formulation	Water	Propylene Glycol (46.6%)	Polyacrylic Acid Thickener (2.37 g/L)	OPEO Surfactant (3.0 g/L)	4,5 MeBT Corrosion Inhibitor (1.16 g/L)	EC$_{50}$ and LC$_{50}$ Values (mg/L)		
						Microtox	*C. dubia*	Fathead Minnow
Product K	✓	✓	✓	✓	✓	**2,620**	**2,600**	**888**
Mock #1	✓	✓	✓	—	—	>11,160	>11,160	>11,160
Mock #2	✓	✓	✓	✓	—	2,807	1,452	1,140
Mock #3	✓	✓	✓	—	✓	4,313	>11,500	>11,500
Mock #4	✓	✓	✓	✓	✓	**2,250**	**1,061**	**576**

Checkmark indicates presence of component in mock formulation.

Deicer toxicity: After the dust settled…

- Type IV aircraft anti-icing fluids were much more toxic to aquatic organisms than were Type I deicers.
- Toxic fractions identified through EDA of Type IV deicer fluids contained polyethoxylated surfactants.
- Quantitative analysis of Type IV deicers revealed:
  - Alkylphenol ethoxylate surfactants (3.0 g/L)
  - Alcohol ethoxylate surfactants (0.3 – 0.8 g/L)
  - Benzotriazole-based corrosion inhibitors (~0.1 – 0.8 g/L)
- Toxicity testing of reformulated “mock” Type IV deicer fluids validated EDA results
- “Green” Deicer fluids will require re-examination of surfactant components.

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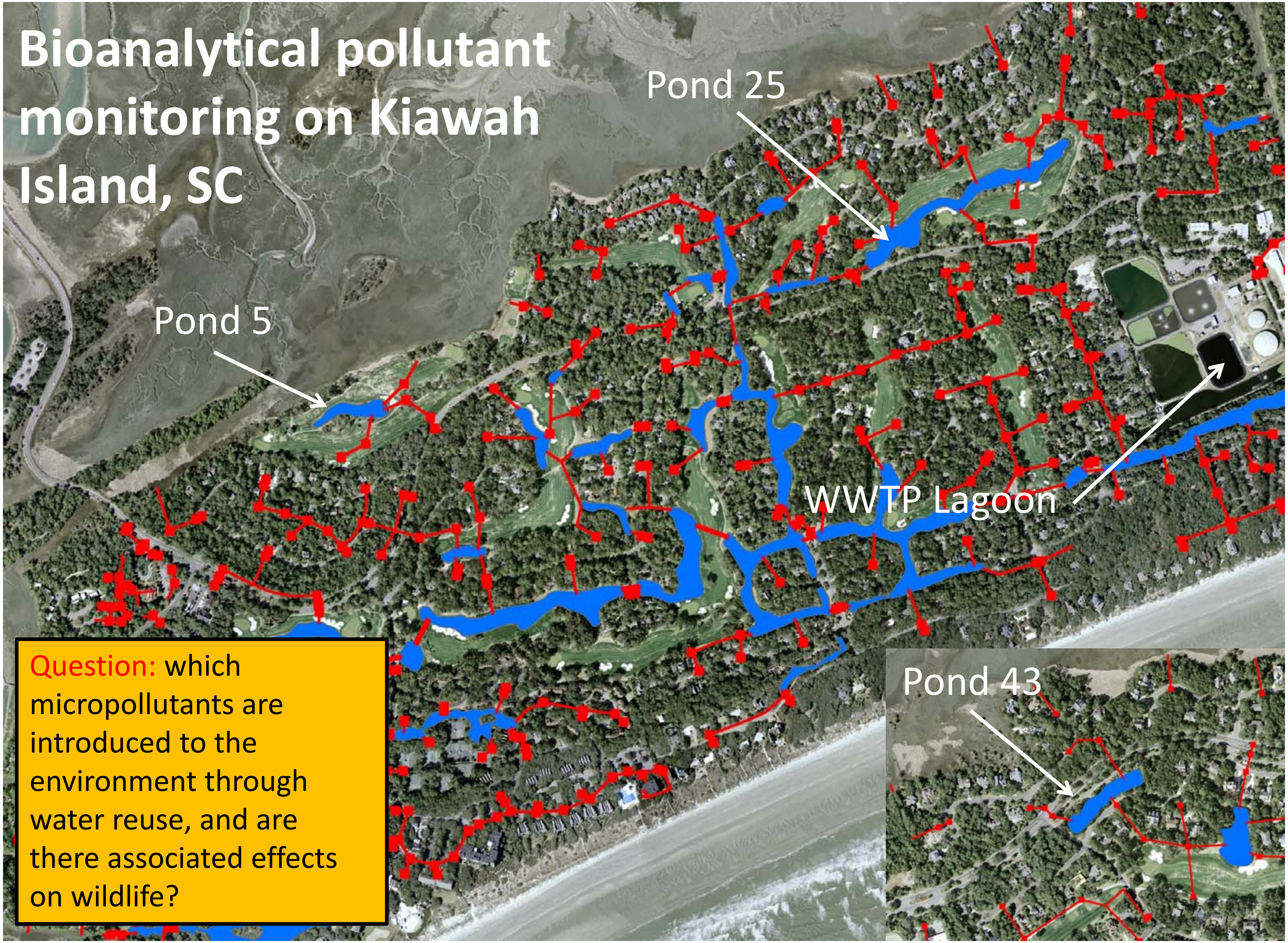
Water reuse in turf-management: wastewater treatment or contaminant source?

Bioanalytical pollutant monitoring on Kiawah Island, SC

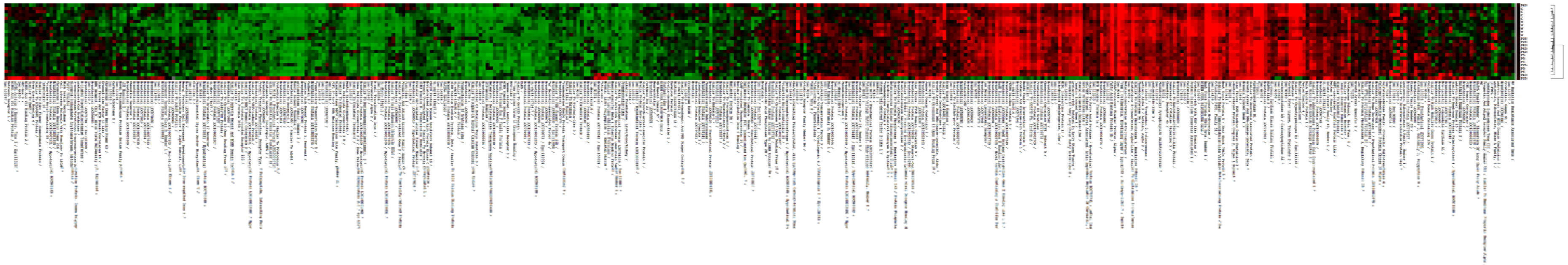


Question: which micropollutants are introduced to the environment through water reuse, and are there associated effects on wildlife?

Male Fathead Minnow Exposures

Mini Mobile Units for "safer" in-situ fish exposure (Alan Kolok)

Microarray analysis of hepatic gene expression in Male Fathead Minnows After 1 Week Exposure



15K fathead minnow microarray

~ 3700 genes significantly expressed
differently between ponds

~ 400 genes significantly expressed
differently between ponds > 2X

Upregulated

Downregulated

Microarray analysis of hepatic gene expression in Male Fathead Minnows After 1 Week Exposure

Number of genes significantly up- and down-regulated ≥ 2X in fish from each pond (n=4-5)

	WWTP	P43	P25	P5
Vitellogenin 1				
II-FBPL				
Vitellogenin 3				
Hy... Protein LOC799792				
[illegible]				
...LOC79...				
...36				
[illegible]				
[illegible]				
...glutaryl-...				
...EGF-like Do...				
Trans...brane Protein 4				
Chromosome 1 Open Reading Frame 216				
Zgc:135871				
Protein Disulfide Isomerase Associated 4				
Zgc:136890				
Chaperonin Containing TCP1, Subunit 5				
Similar To Chromosome 15 Open Reading Frame 24				
Hypothetical Protein LOC100000298				
DnaJ Homolog, Subfamily B, Member 1				

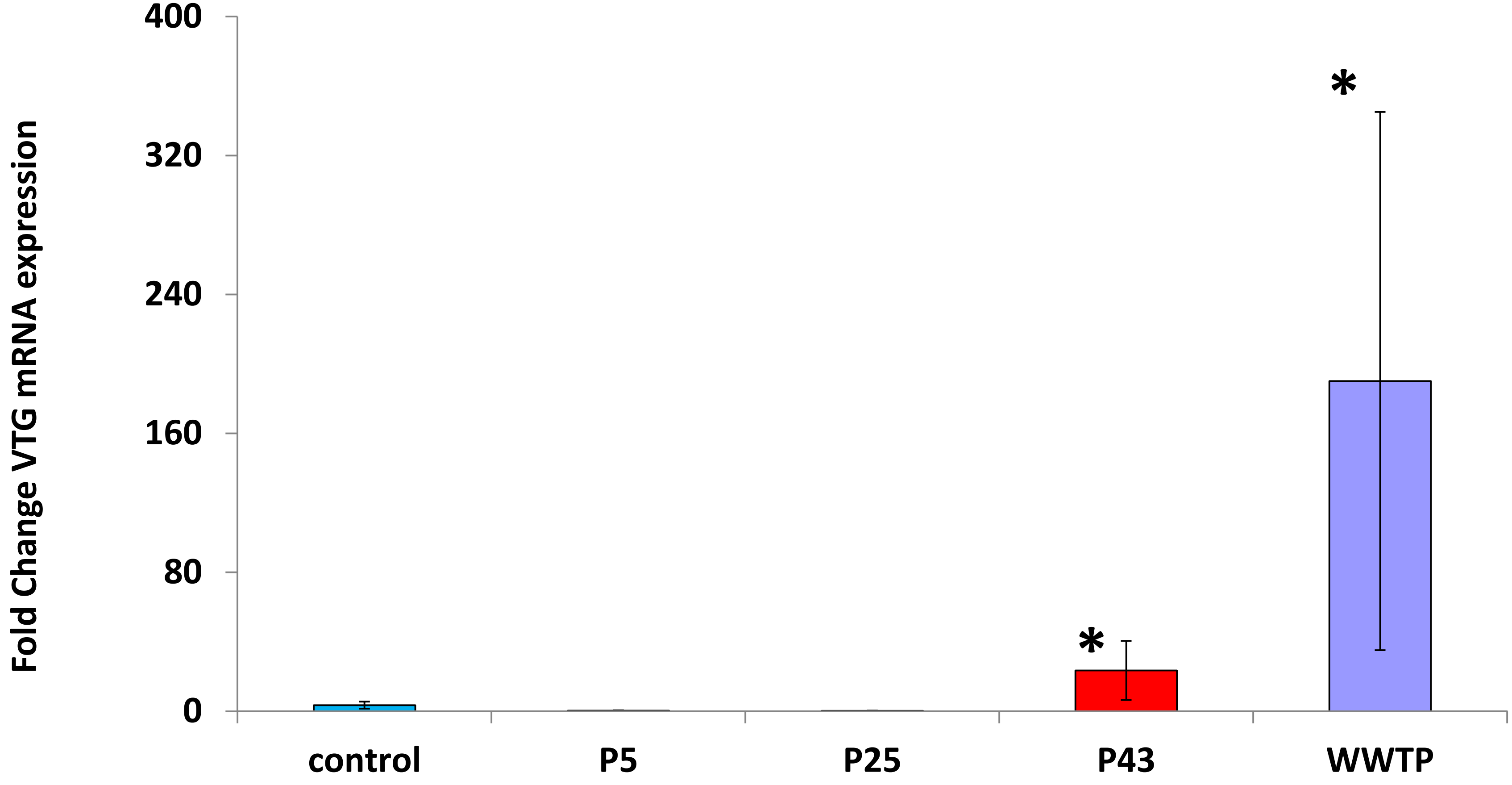
59 ↑
53 ↓
P5

106 ↑
47 ↓
P25

103 ↑
59 ↓
P43

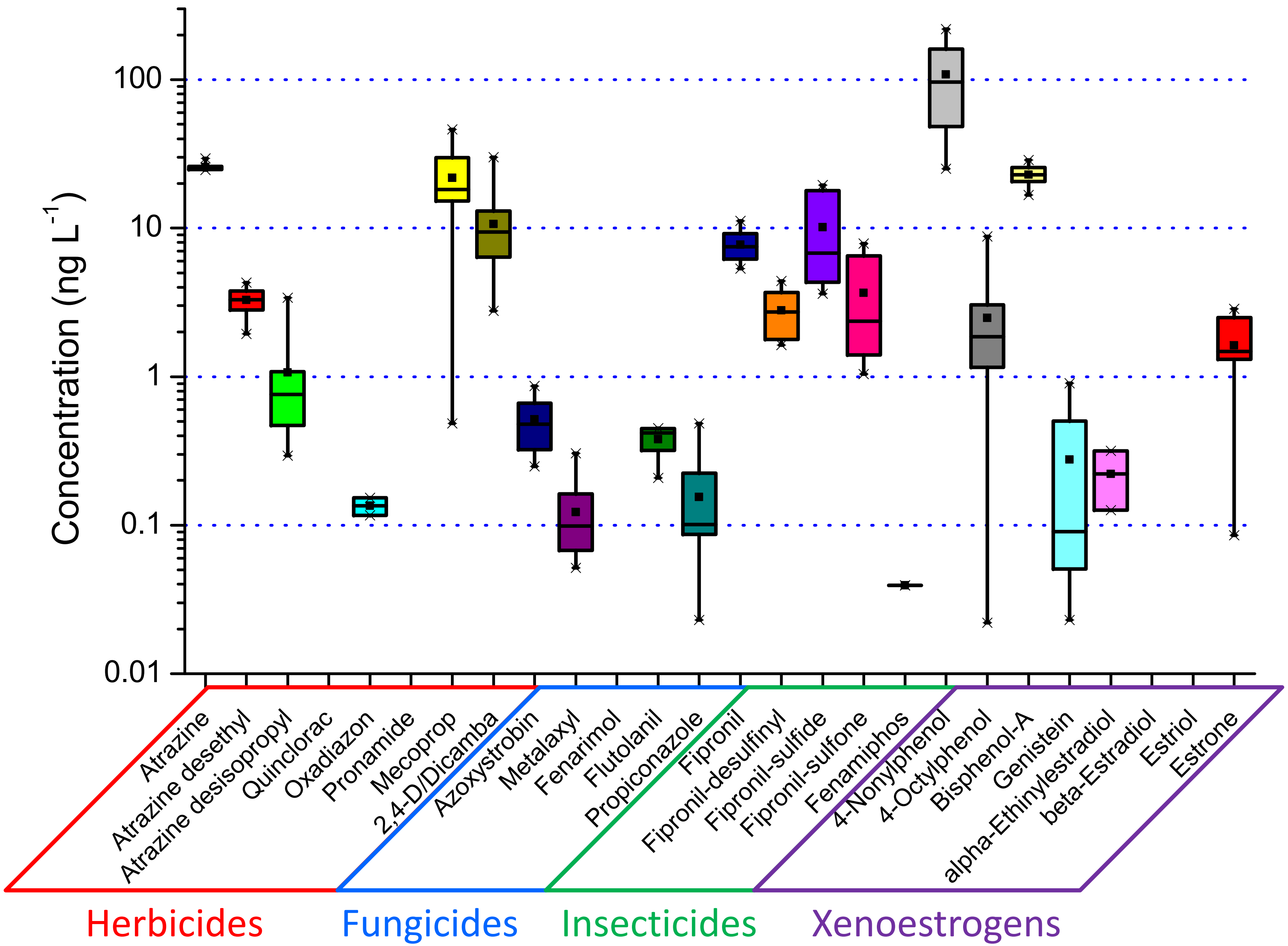
76 ↑
83 ↓
WWTP

Hepatic Vitellogenin mRNA Expression in Male Fathead Minnows After 1 Week Exposure



* Indicate statistically significant values compared to control using one-way ANOVA ($p < 0.05$)

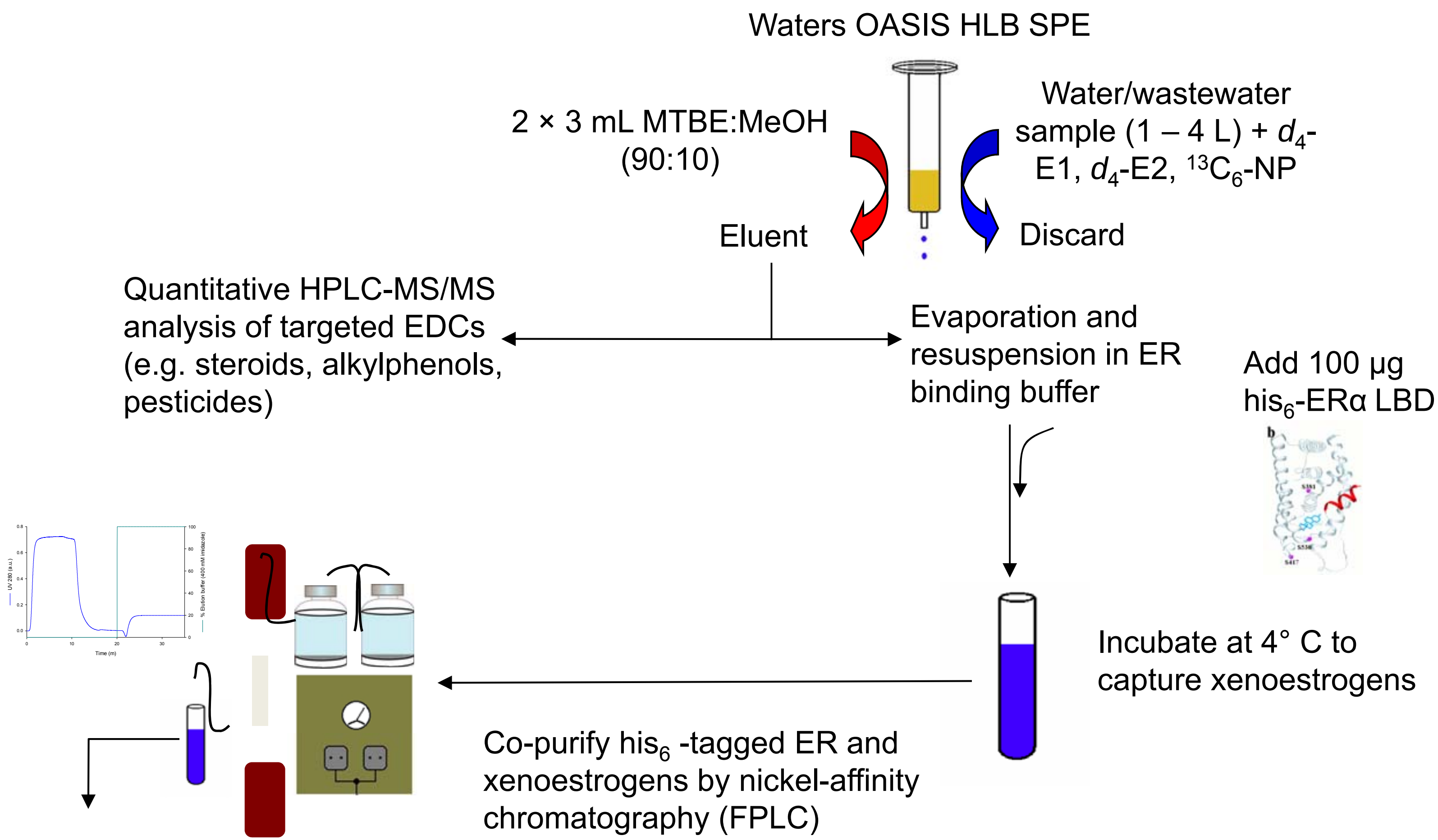
Micropollutants in WWTP Lagoon



Yes, but what ELSE might be contributing to estrogenicity in the reclaimed water??

- **Receptor affinity extraction:** Similar in concept to immunoaffinity chromatography – relies on high specificity/selectivity molecular interaction to isolate target analytes from a mixture prior to analysis
- Recombinant protein engineering:
  - ERα ligand binding domain triple Cys → Ser mutant
  - Fusion of thioredoxin to ER enhances solubility
  - His_6 tag allows subsequent purification
- Proteins are cloned, expressed in bacterial vectors, and purified chromatographically

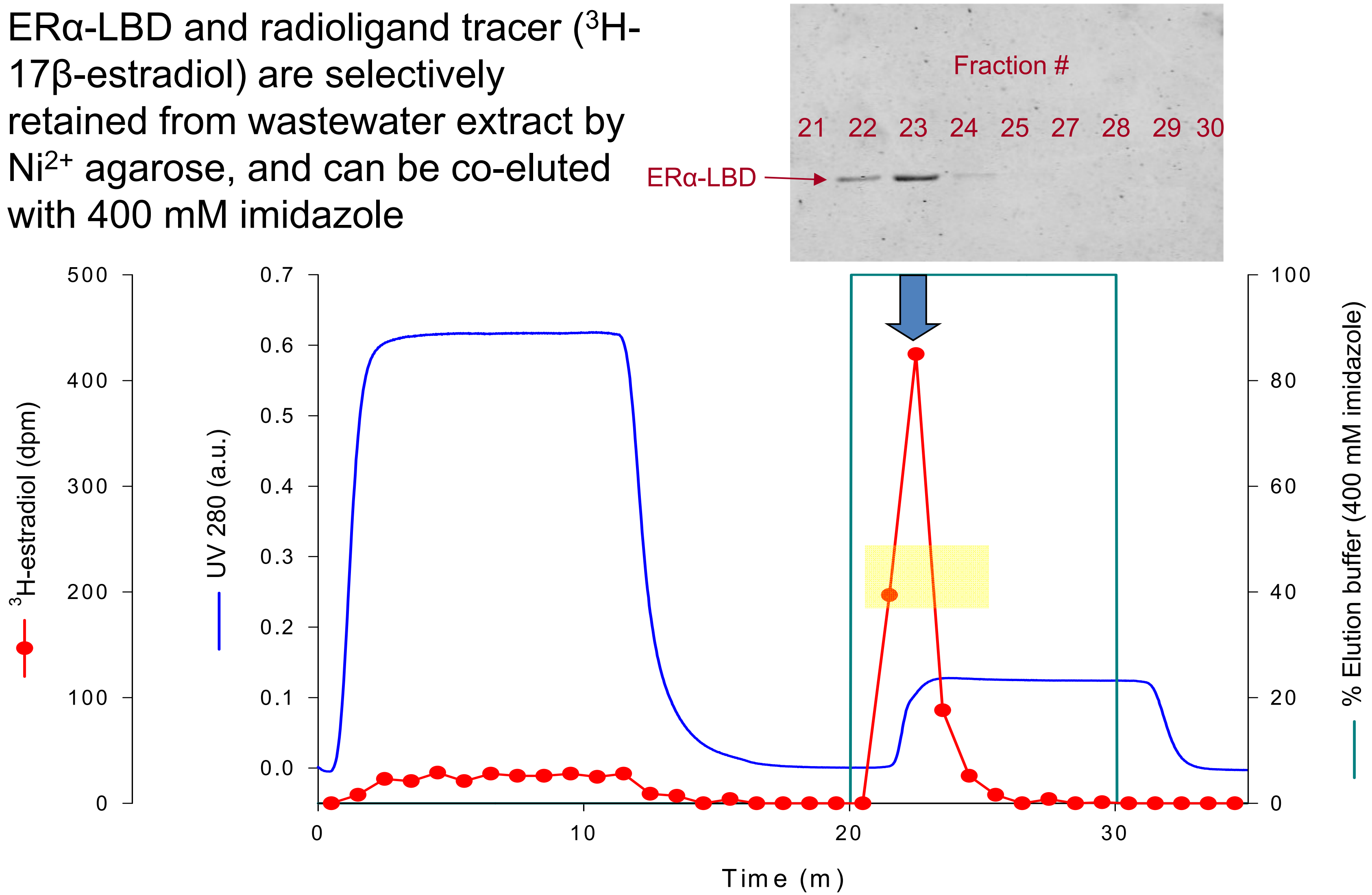
Estrogen receptor-affinity isolation for activity-directed analysis



Analysis of receptor-active EDCs in eluent by UHPLC-Orbitrap MS/MS or triple-quadrupole MS

Purification of ER-bound xenoestrogens from wastewater

ERα-LBD and radioligand tracer (^{3}H-17β-estradiol) are selectively retained from wastewater extract by Ni^{2+} agarose, and can be co-eluted with 400 mM imidazole



HRMS-enabled screening using published databases and curated/literature MS/MS data

Question: which compounds within a curated list are present in this sample?

Three databases

Environmental Science & Technology

ARTICLE

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‡Aquatic Ecosystem Protection Research Division, Environment Canada, 867 Lakeshore Road, Burlington, Ontario

1. 745 High production volume pharmaceuticals predicted to be persistent and or bioaccumulative (Howard & Muir, 2011)
2. 610 High production volume chemicals in commerce predicted to be persistent and or bioaccumulative (Howard & Muir, 2010)
3. 1006 pesticides & pharmaceuticals (ThermoFisher Scientific Environmental & Food Safety database)

Identifying New Persistent and Bioaccumulative Organics Among Chemicals in Commerce

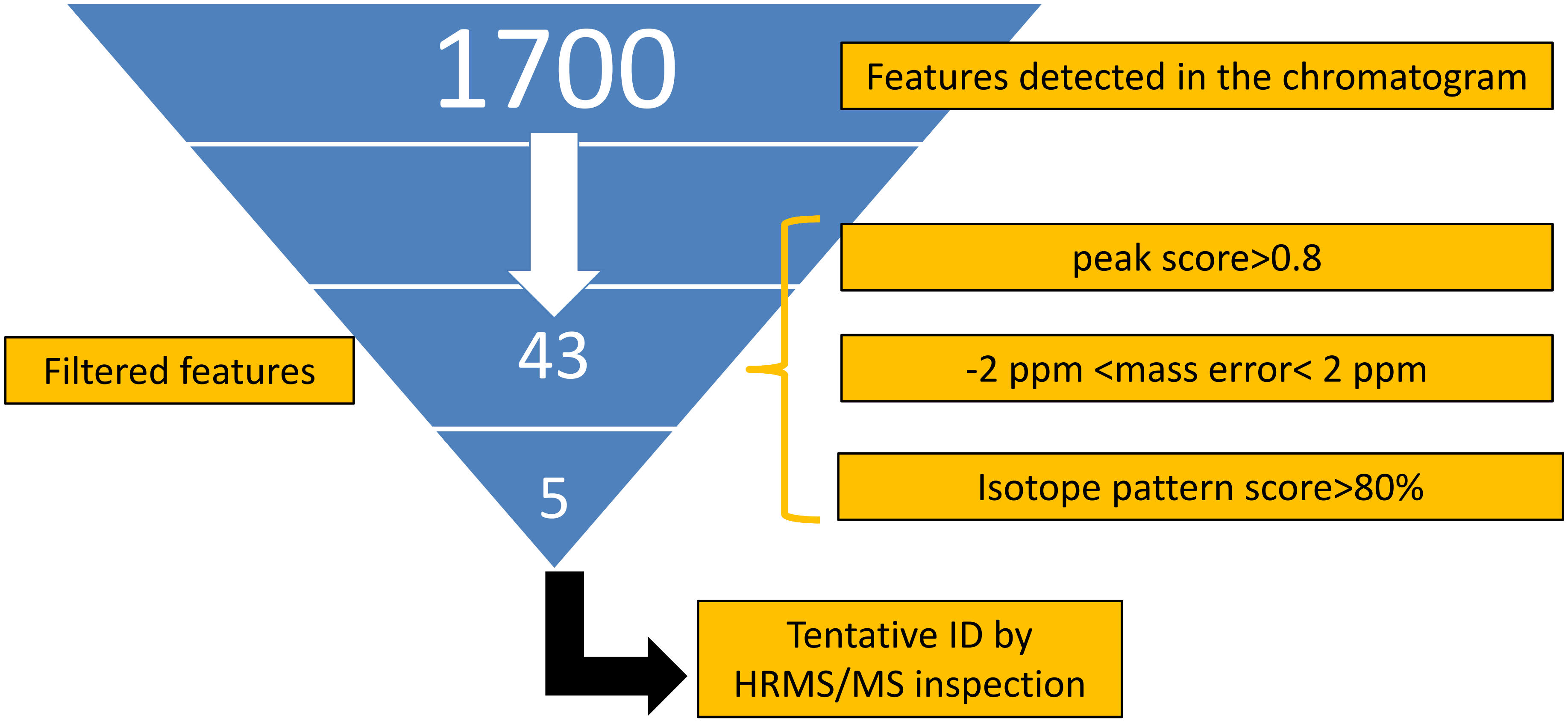
PHILIP H. HOWARD*,† AND DEREK C. G. MUIR‡

SRC, Environmental Science Center, 6502 Round Pond Road, North Syracuse, New York, and Aquatic Ecosystem Protection Research Division, Environment Canada, 867 Lakeshore Road, Burlington, Ontario

Received November 6, 2009. Revised manuscript received January 19, 2010. Accepted January 22, 2010.

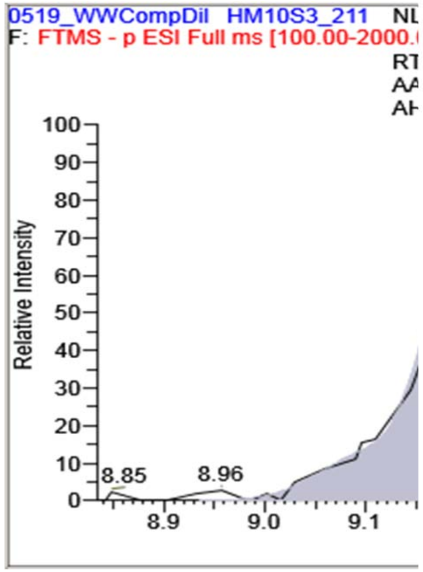
Thermo SCIENTIFIC

Data filtering and tentative compound identification



Exam /MS

Peak ID	core
Peak 211(-412.96681 9.21)	



TOXICOLOGICAL SCIENCES 120(1), 42–58 (2011)
doi:10.1093/toxsci/kfq379
Advance Access publication December 16, 2010

Estrogen-Like Activity of Perfluoroalkyl Acids *In Vivo* and Interaction with Human and Rainbow Trout Estrogen Receptors *In Vitro*

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Received August 26, 2010; accepted December 06, 2010

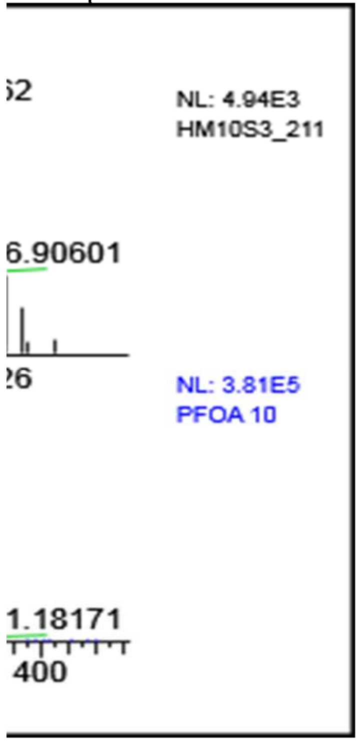
The objectives of this study were to determine the structural characteristics of perfluoroalkyl acids (PFAAs) that confer estrogen-like activity *in vivo* using juvenile rainbow trout (*Oncorhynchus mykiss*) as an animal model and to determine whether these chemicals interact directly with the estrogen receptor (ER) using *in vitro* and *in silico* species comparison approaches. Perfluorooctanoic (PFOA), perfluorononanoic (PFNA), perfluorodecanoic (PFDA), and perfluoroundecanoic (PFUnDA) acids were all potent inducers of the estrogen-responsive biomarker protein vitellogenin (Vtg) *in vivo*, although at fairly high dietary exposures. A structure-activity relationship for PFAAs was observed, where eight to ten fluorinated carbons and a carboxylic acid end group were optimal for maximal Vtg induction. These *in vivo* findings were corroborated by *in vitro* mechanistic assays for trout and human ER. All PFAAs tested weakly bound to trout liver ER with half maximal inhibitory concentration (IC_{50}) values of 15.2–289μM. Additionally, PFOA, PFNA, PFDA, PFUnDA, and perfluorooctane sulfonate (PFOS) significantly enhanced human ERα-dependent transcriptional activation at concentrations ranging from 10–1000nM. Finally, we employed an *in silico* computational model based upon the crystal structure for the human ERα ligand-binding domain complexed with E2 to structurally investigate binding of these putative ligands to human, mouse, and trout ERα. PFOA, PFNA, PFDA, and PFOS all efficiently docked with ERα from different species and formed a hydrogen bond at residue Arg394/398/407 (human/mouse/trout) in a manner similar to the environmental estrogens bisphenol A and nonylphenol. Overall, these data support the contention that several PFAAs are weak environmental xenoestrogens of potential concern.

Key Words: perfluoroalkyl acid; estrogen; perfluorooctanoic acid; perfluorooctane sulfonate; vitellogenin; molecular docking.

The widespread industrial and commercial use of polyfluorinated chemicals (PFCs) as surfactants and surface protectors for paper and textile coatings, polishes, food packaging, and fire-retardant foams has led to the pervasive presence of these chemicals in the environment, wildlife, and humans (see reviews by Calafat *et al.*, 2007; Houde *et al.*, 2006). The general structure of PFCs resembles that of fatty acids in that each compound has a hydrophobic polyfluorinated carbon tail of varying length and a functional end group, which provides the basis for classification (Fig. 1). Perfluoroalkyl acids (PFAAs) and fluorotelomers comprise the two major structural groups of PFCs. Human blood levels of perfluorooctanoic acid (PFOA) and perlfuorooctane sulfonate (PFOS), the two most commonly studied PFCs, are about 4 and 20 ppb, respectively, although national survey data suggest that these levels have decreased in recent years (Calafat *et al.*, 2007). Levels of PFOS and PFOA in wildlife tend to be higher, in the range of tens to thousands ppb (Kannan *et al.*, 2002). Other PFCs commonly detected in biological samples include perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), and perfluorododecanoic acid (PFDoDA).

The toxicology and toxicokinetics of PFOA, as an example PFC, have been thoroughly reviewed by Kennedy *et al.* (2004). PFOA does not accumulate in fatty tissues because of its dual lipophobic and hydrophobic chemical properties but instead binds to blood proteins and is distributed primarily to liver, plasma, and kidney. The measured biological half-life of PFOA varies among species ranging from hours in female rat to days in dogs or rainbow trout (Hanhijarvi *et al.*, 1988; Martin *et al.*, 2003). The estimated half-life of PFOA in humans is nearly 4 years, pointing to a lower capacity for elimination of the compound compared with other species (Olsen *et al.*, 2007). Finally, PFOA is not metabolized or defluorinated *in vivo*, although some fluorotelomer compounds may be metabolized to PFAAs in rodents (Martin *et al.*, 2005; Nabb *et al.*, 2007).

PFOA and other PFCs are members of a large group of chemicals called peroxisome proliferators (PPs), which



Identification a perfluoroocanc acid (PFOA)

Xenoestro… …tor affinity extracts … …screening

Name	Class	… Pattern …ore	Confirmed & Quantified?
17β-estradiol	Endoge… steroid	…00	0.70 ng/L
Estrone	Endoge… steroid	…95	11.5 ng/L
Celecoxib	**NSAID Pharma…**	**…00**	**no**
PFOA	Fluorina… surfacta…	…00	no
Efavirens	**Antivira… pharma…**	**…00**	**no**

DOI: 10.1111/j.1468-1293.2010.00831.x
HIV Medicine (2010), 11, 603–607

SHORT COMMUNICATION

Efavirenz directly modulates the oestrogen receptor and induces breast cancer cell growth

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Objectives
Efavirenz-based HIV therapy is associated with breast hypertrophy and gynaecomastia. Here, we tested the hypothesis that efavirenz induces gynaecomastia through direct binding and modulation of the oestrogen receptor (ER).

Methods
To determine the effect of efavirenz on growth, the oestrogen-dependent, ER-positive breast cancer cell lines MCF-7, T47D and ZR-75-1 were treated with efavirenz under oestrogen-free conditions in the presence or absence of the anti-oestrogen ICI 182,780. Cells treated with 17β-oestradiol in the absence or presence of ICI 182,780 served as positive and negative controls, respectively. Cellular growth was assayed using the crystal violet staining method and an *in vitro* receptor binding assay was used to measure the ER binding affinity of efavirenz.

Results
Efavirenz induced growth in MCF-7 cells with an estimated effective concentration for half-maximal growth (EC_{50}) of 15.7 μM. This growth was reversed by ICI 182,780. Further, efavirenz binds directly to the ER [inhibitory concentration for half maximal binding (IC_{50}) of ~ 52 μM] at a roughly 1000-fold higher concentration than observed with 17β-oestradiol.

Conclusions
Our data suggest that efavirenz-induced gynaecomastia may be caused, at least in part, by drug-induced ER activation in breast tissues.

Keywords: efavirenz, gynaecomastia, highly active antiretroviral therapy, oestrogen receptor, oestrogens

Accepted 12 January 2010

Introduction

The introduction of highly active antiretroviral therapy (HAART) multi-drug combination regimens has considerably improved the prognosis of patients infected with HIV by reducing AIDS-related morbidity and mortality [1]. However, chronic treatment with these regimens is associated with multiple adverse effects, nonadherence and eventually therapy failure [2]. Treatment regimens containing the nonnucleoside reverse transcriptase inhibitor efavirenz are preferred in treatment-naïve patients and are widely used in other settings [3]. While efavirenz is generally well tolerated, concentration-dependent side effects that impact drug adherence and promote resistance have been documented [4]. Common adverse effects of efavirenz include central nervous system symptoms, occurring in up to 50% of patients [5], but other less common adverse effects have also been reported. An increasing number of reports suggest that the use of HAART, in particular efavirenz-based therapy, is associated with breast hypertrophy or gynaecomastia [6–11]. While mechanisms underlying efavirenz-induced gynaecomastia are not well understood, a number of hypotheses exist, including a direct oestrogenic effect, induction of an immune response, or altered steroid hormone metabolism by cytochrome P450 enzymes. To our knowledge, none of

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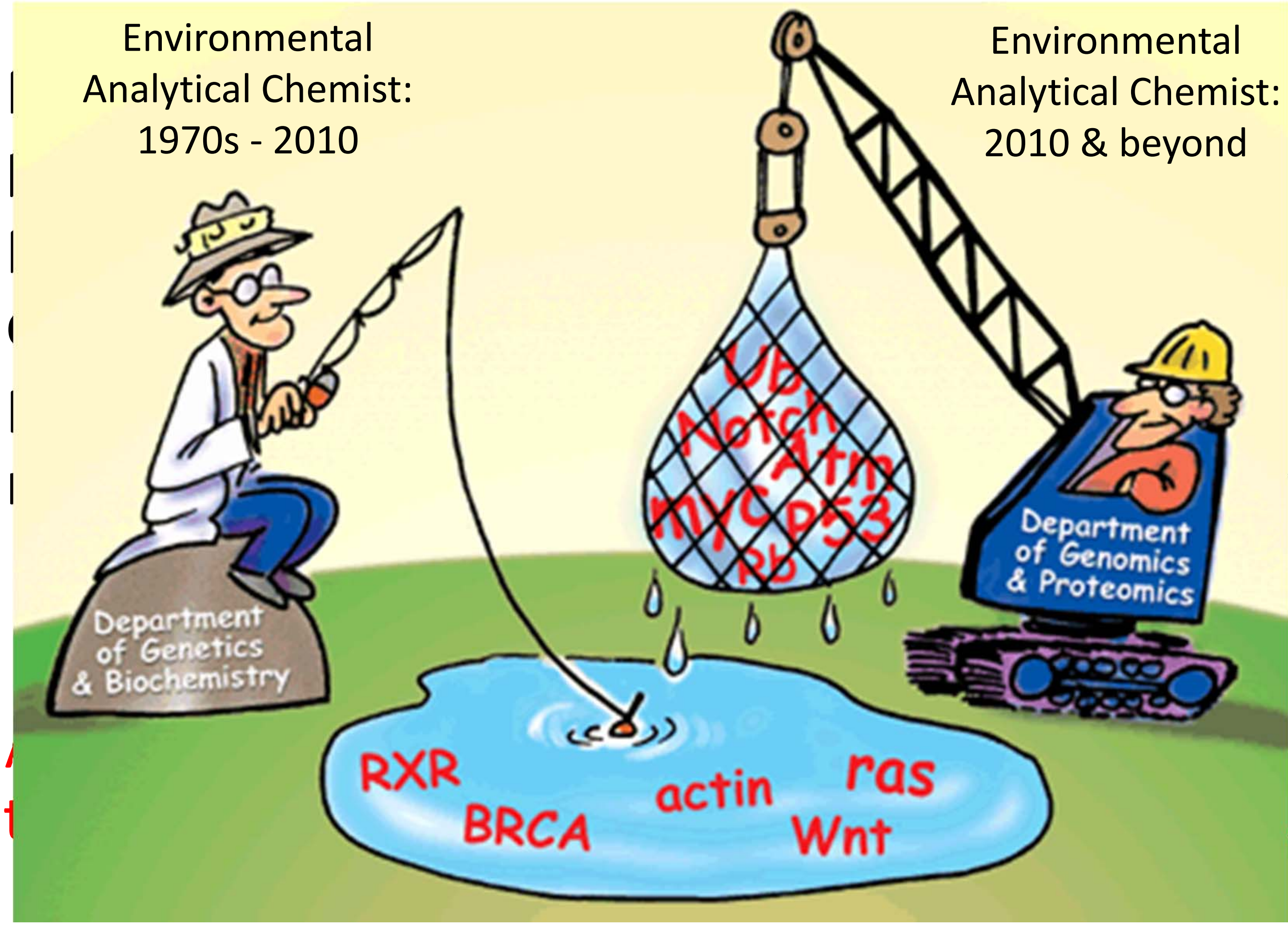
Example approaches from my laboratory:

1. **Effects-directed analysis:** Identifying toxic components of aircraft deicing/anti-icing fluids (ADAF)

2. **Activity-directed analysis:** Receptor affinity extraction for identifying estrogenic compounds associated with water reuse

3. **Fate-directed analysis:** Non-targeted analysis of micropollutant fate in wastewater treatment

Wastewater is a significant source of emerging contaminants to the aquatic environment



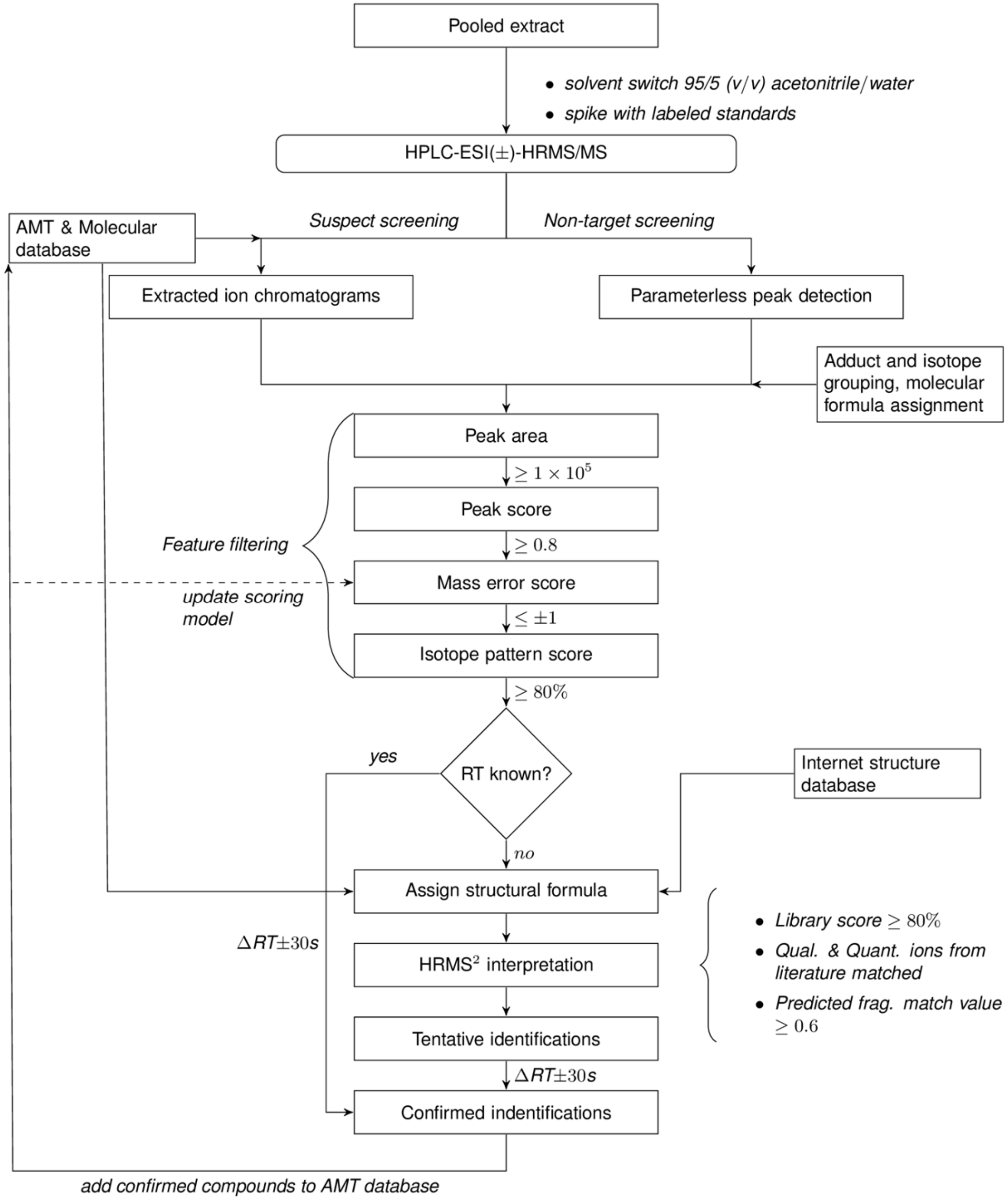
Science 16 February 2001: vol. 291 no. 5507 1221-1224

Study site and sampling

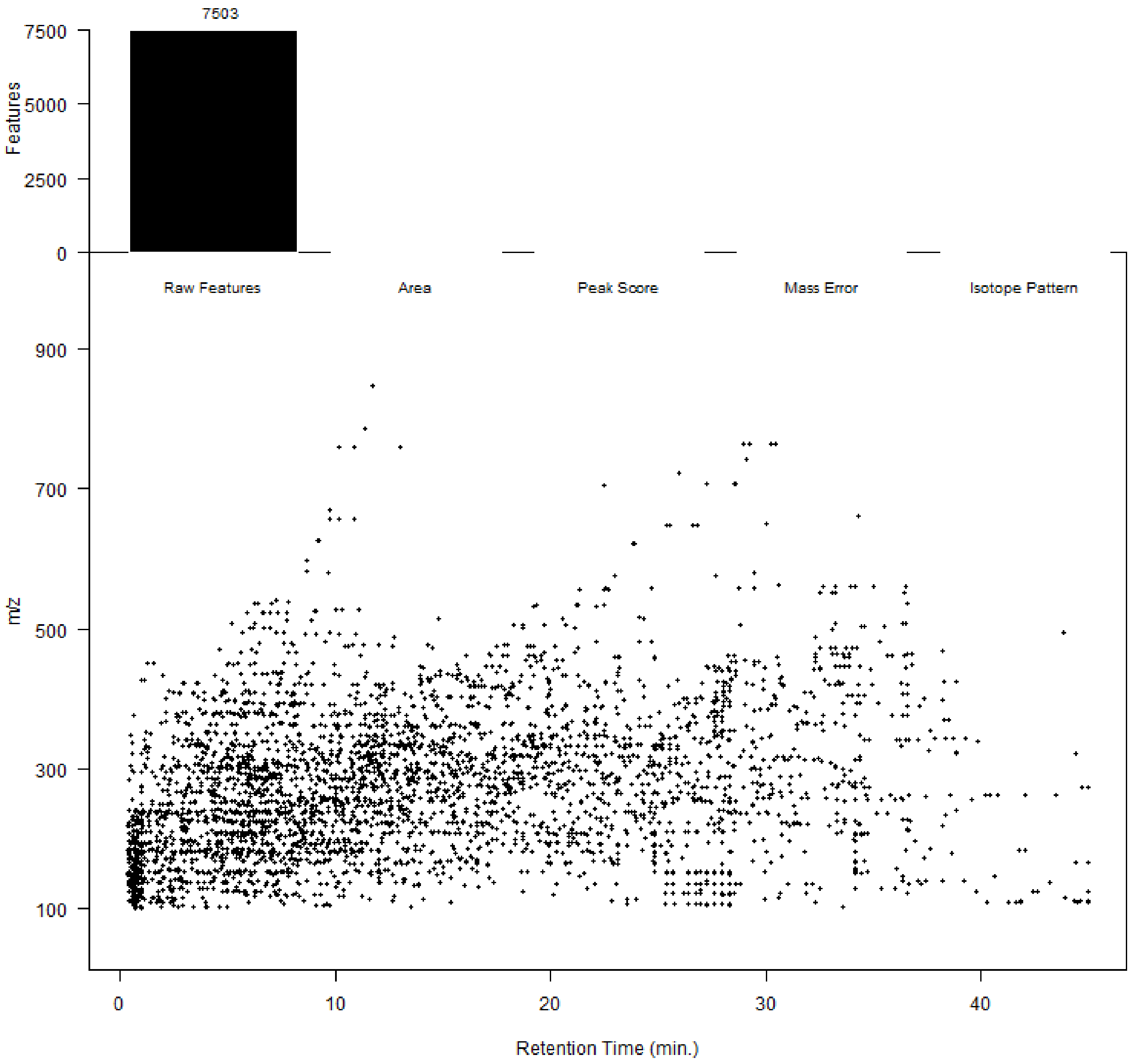
Data analysis workflow

Two suspect databases:

1. Thermo EFS database (1,004 substances with MS/MS library)
2. Compiled from literature including PBT, occurrence, and pharm. analytics (4,475 substances)



Feature filtering: Results



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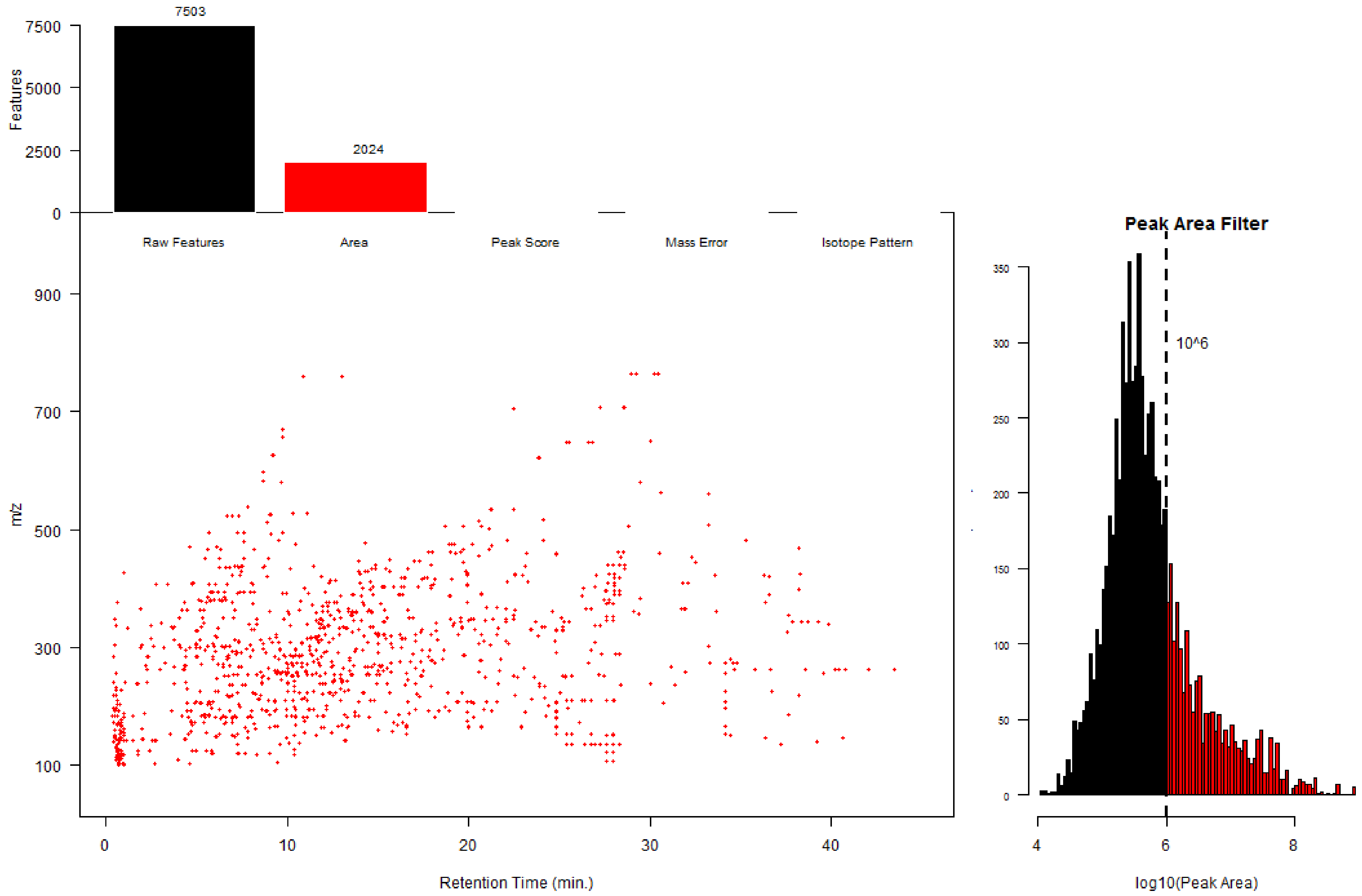

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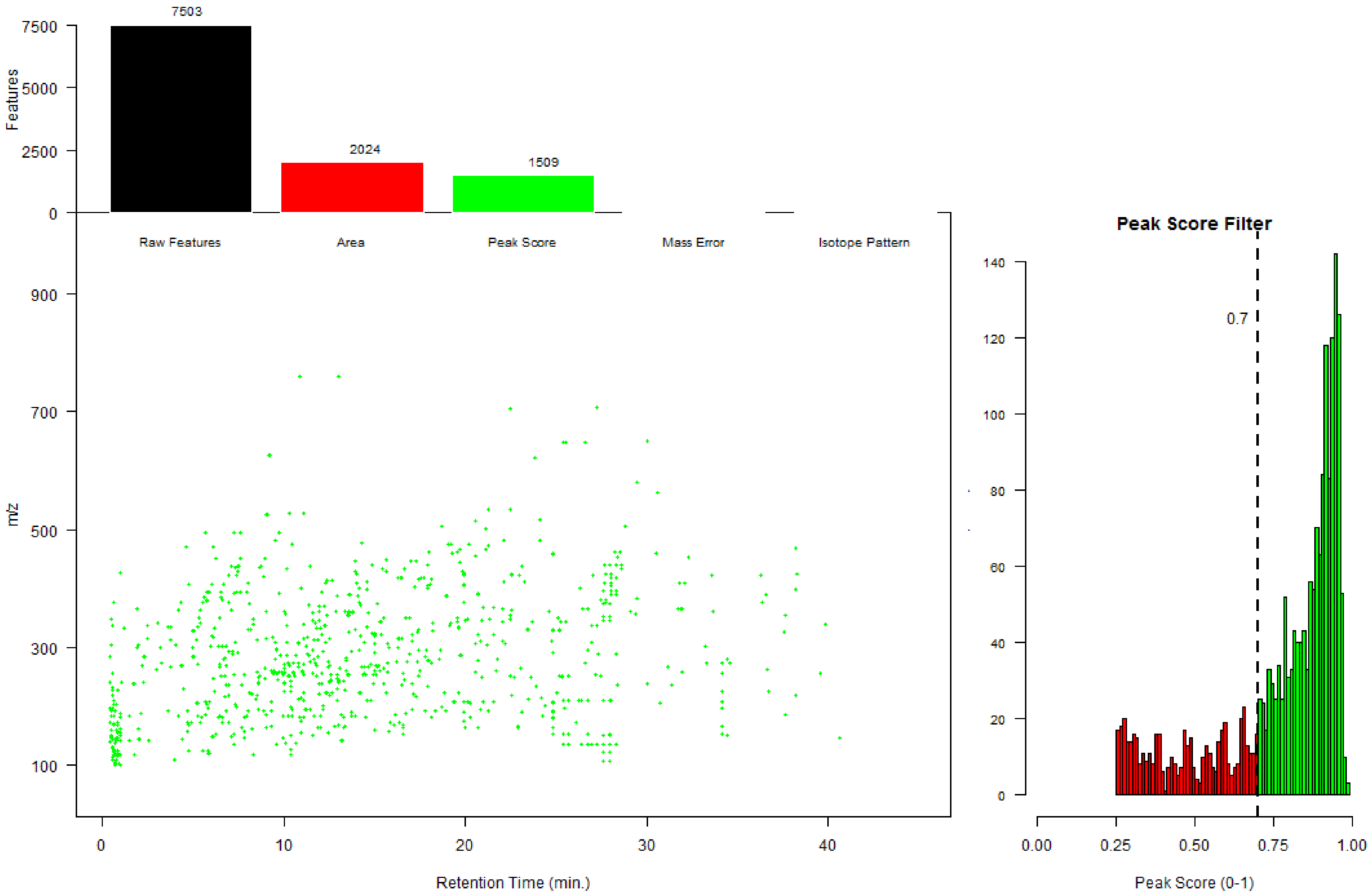

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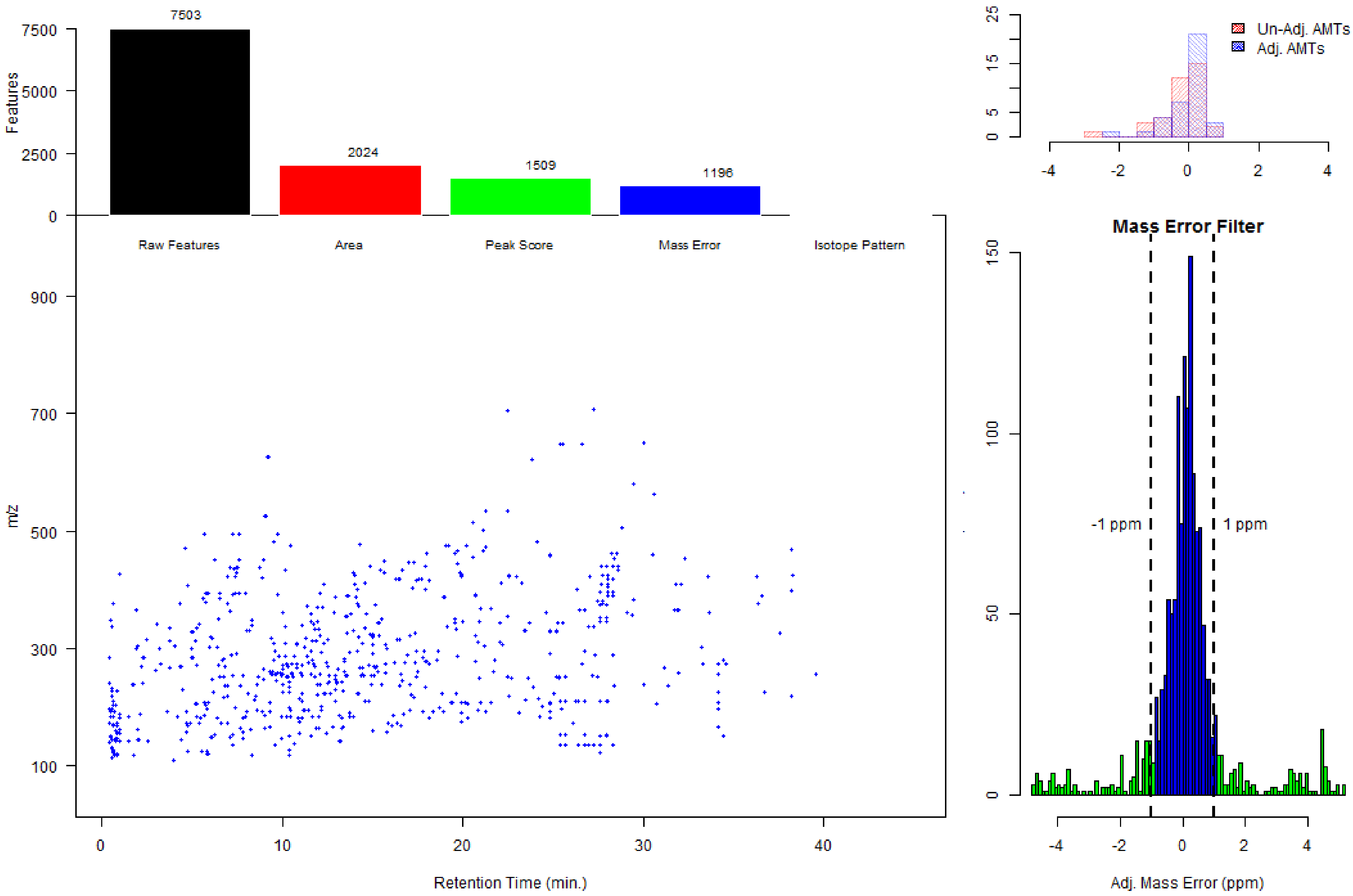

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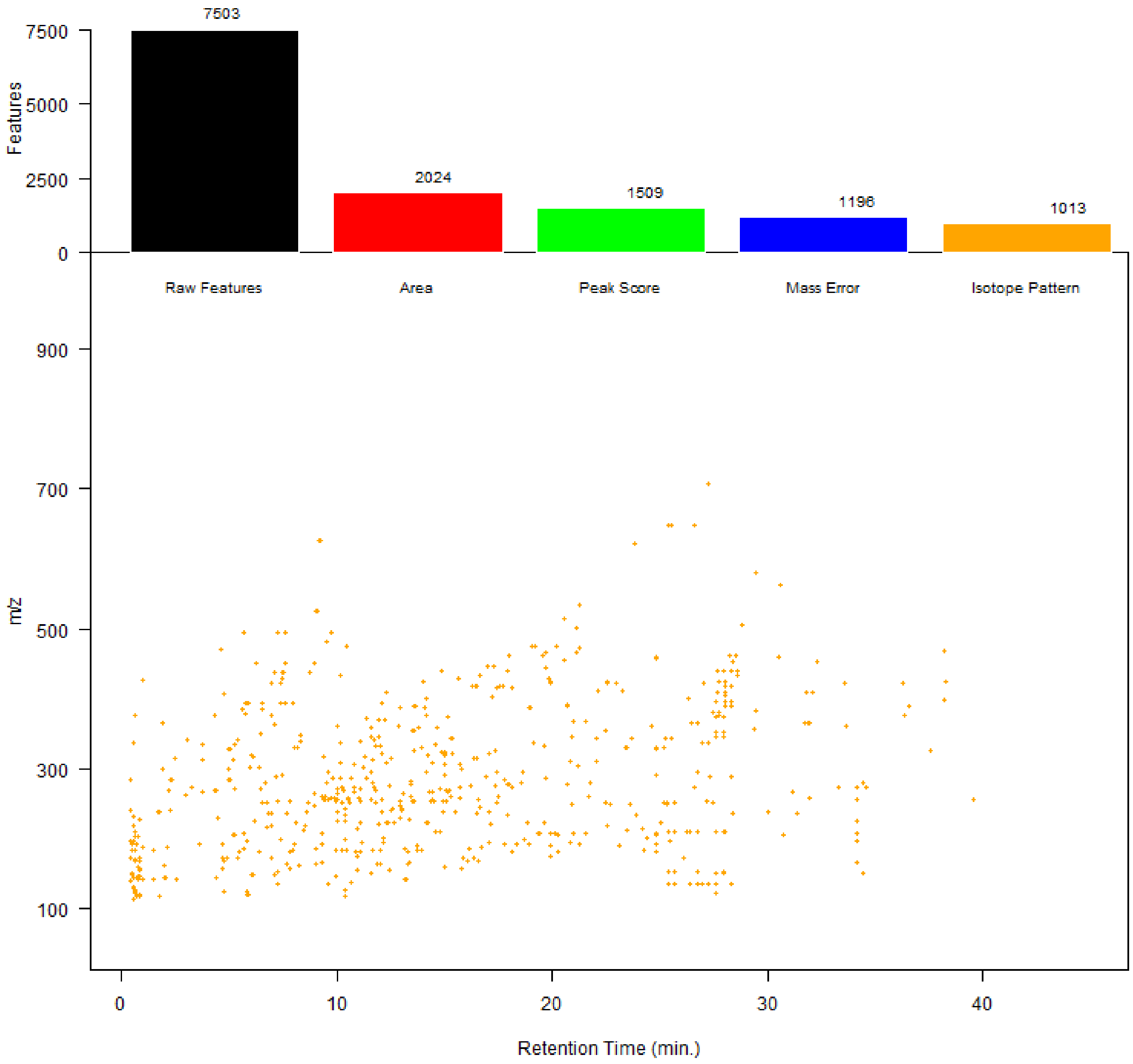
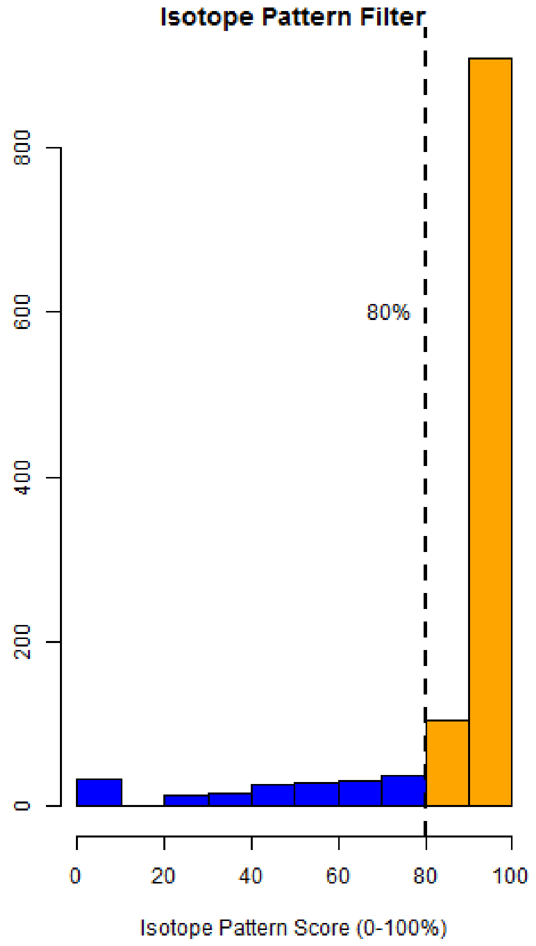
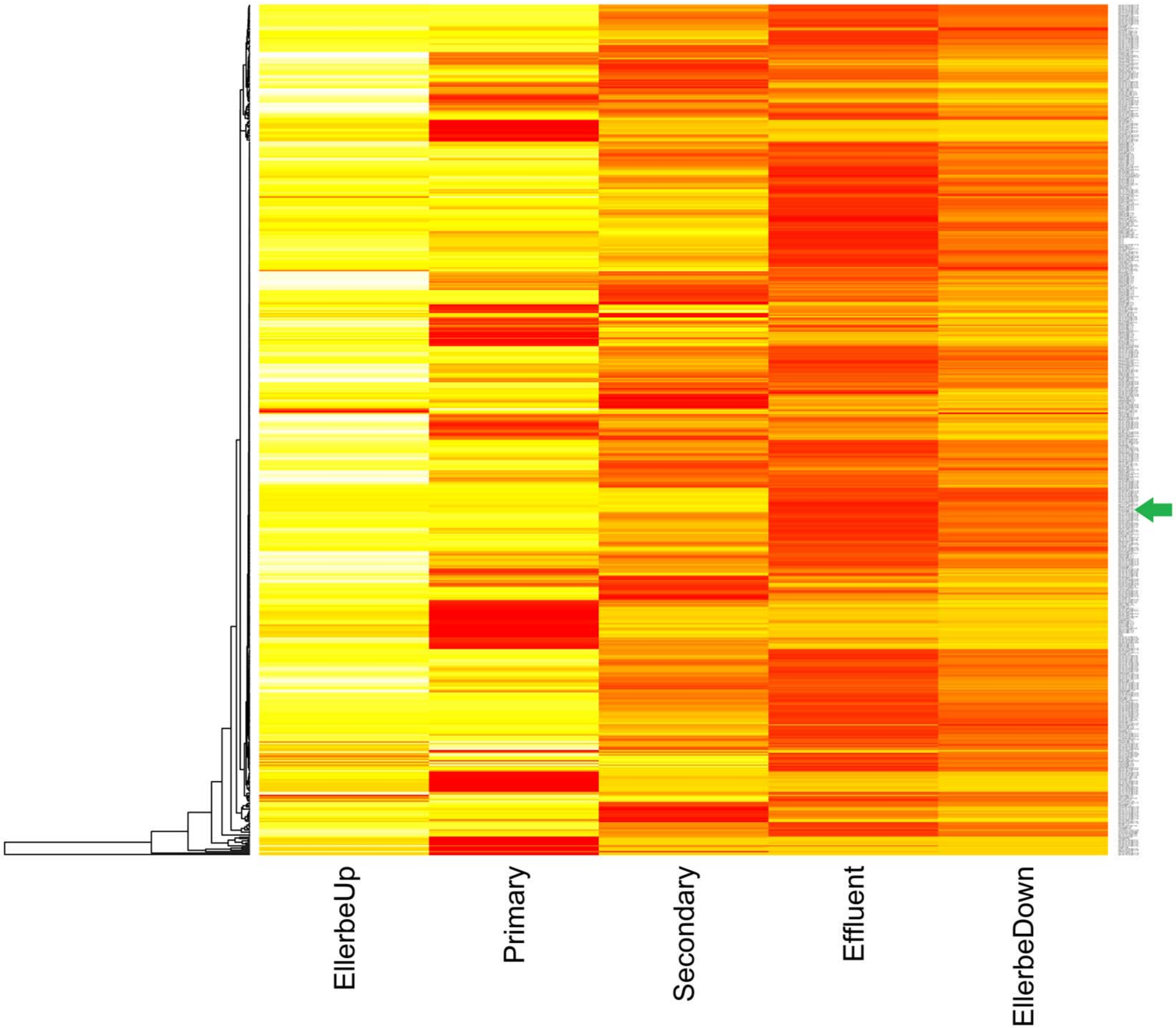


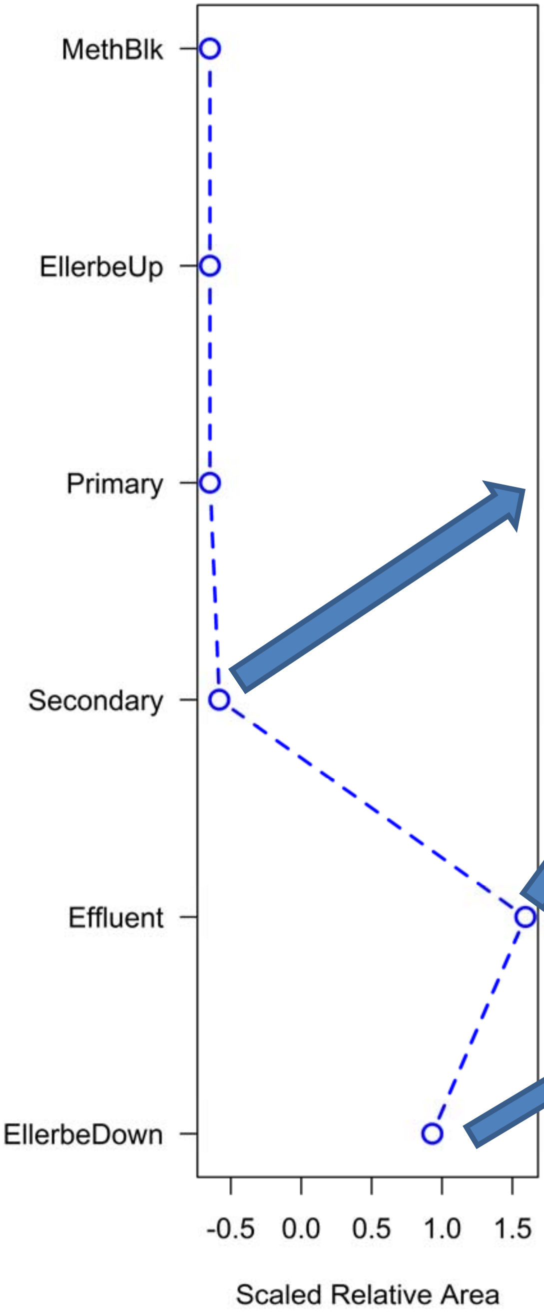


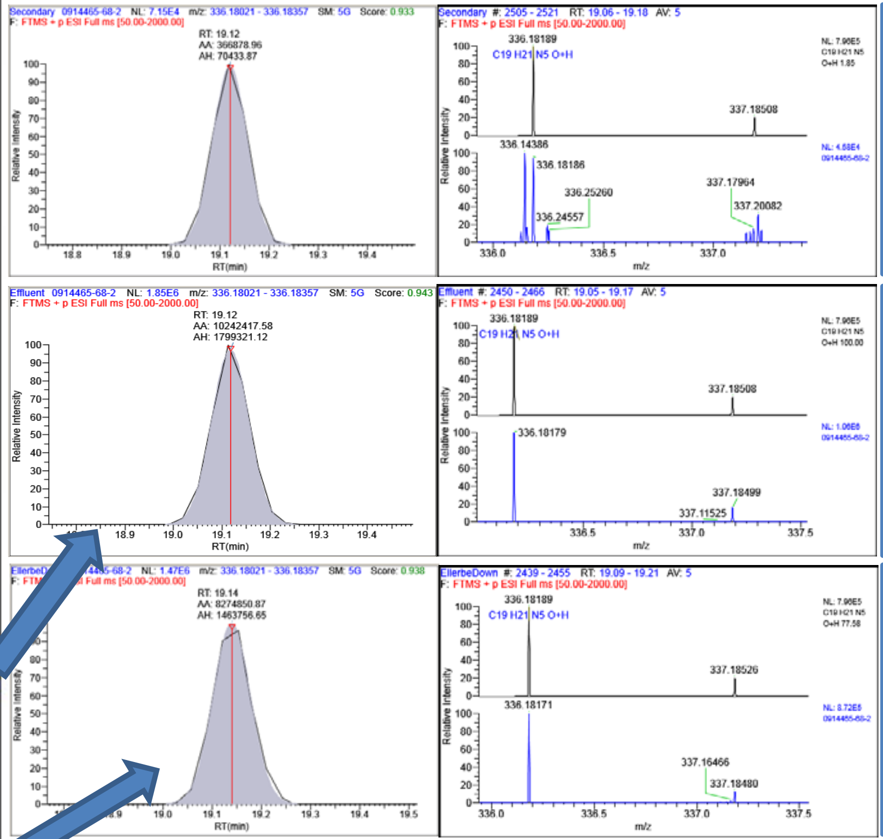
Clustering of suspect hits enables pattern-dependent analysis



Example of suspect with apparent increase after treatment

Detailed inspection reveals strong increase after UV disinfection





Secondary

- RT : 19.12 min
- Mass error: -0.37
- Peak area: 3.6e5

Effluent

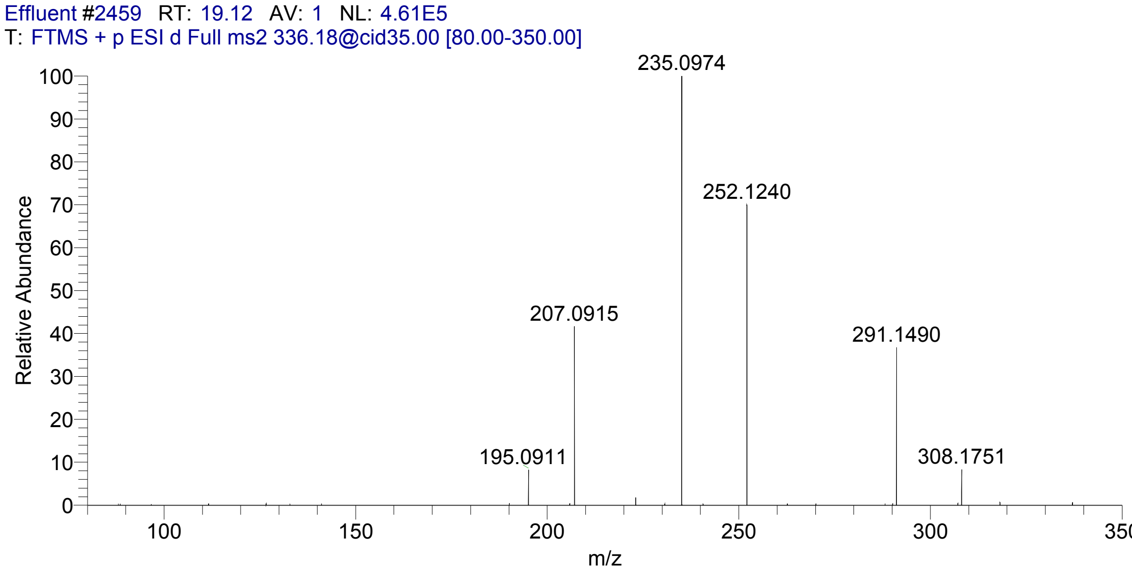
- RT : 19.12 min
- Mass error: -0.19
- Peak area: 1.0e7

Ellerbe Down

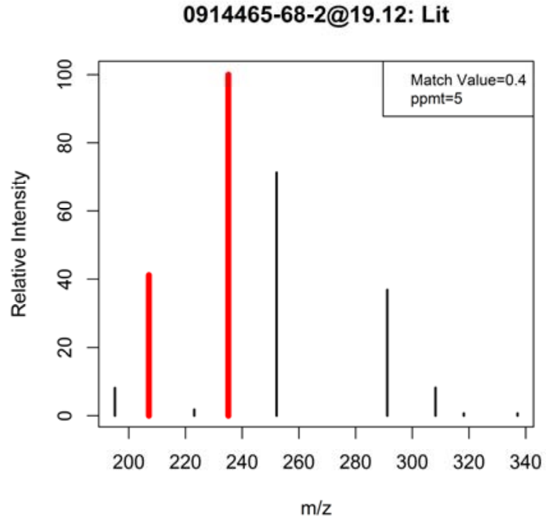
- RT : 19.14 min
- Mass error: -0.64
- Peak area: 8.3e6

Tentative ID: Valsartan transformation product

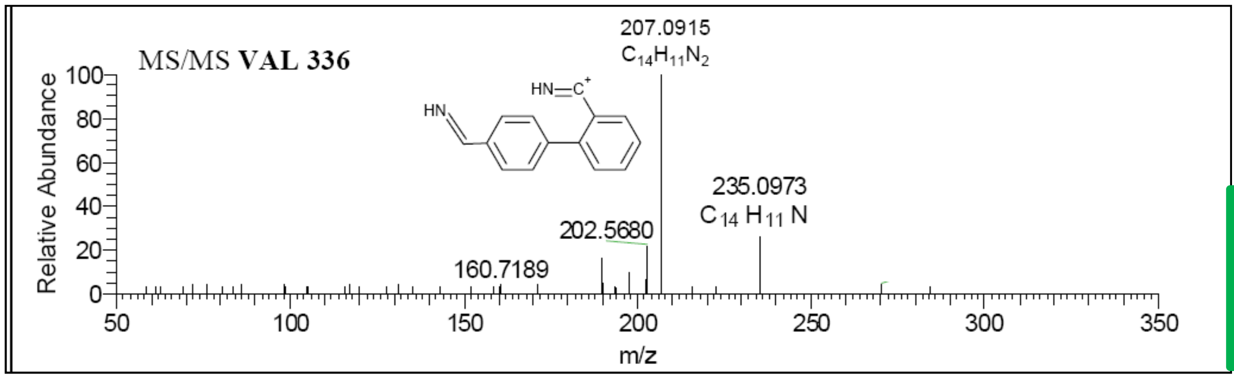
Observed HR/AM MS^2:

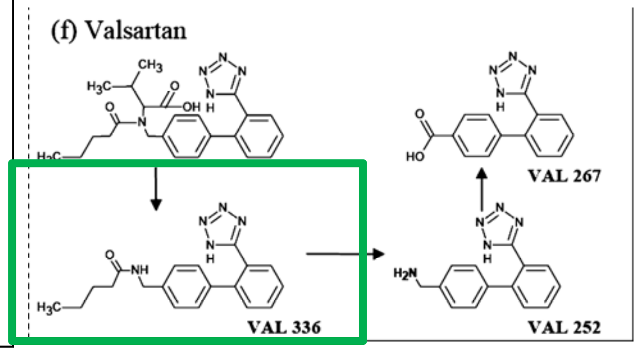


Match Value:



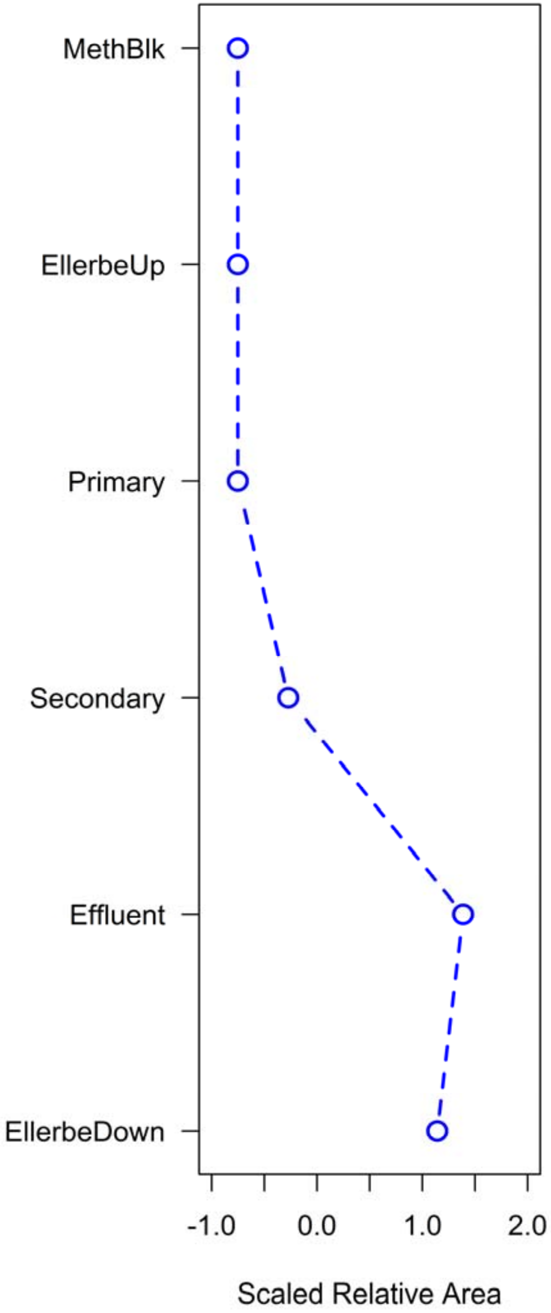
Literature HR/AM MS^2:

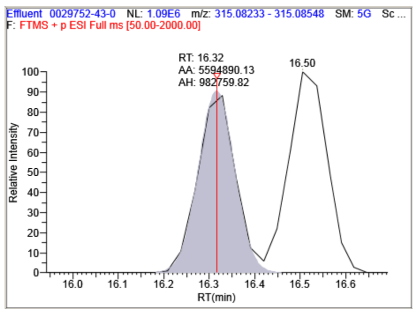


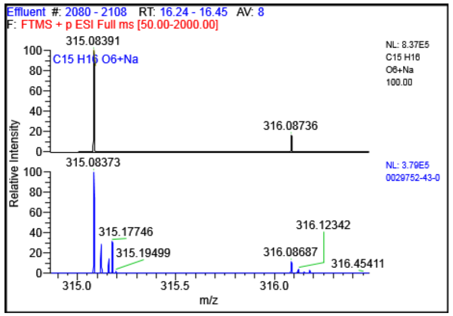


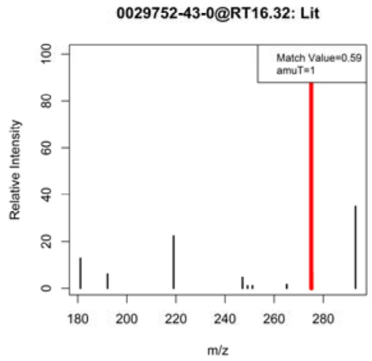
Helbling, et. al., *Environ. Sci. Technol.* **2010**, 44, 6621-6627

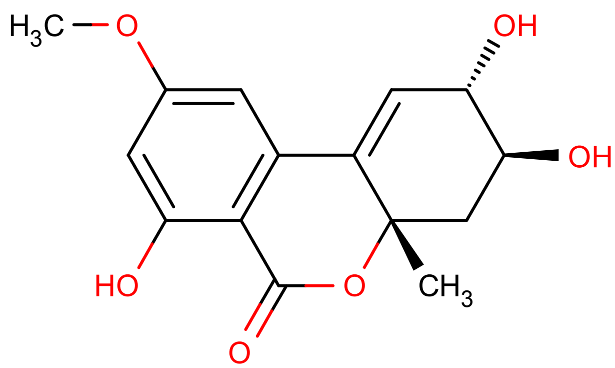
Example of a compound appearing after treatment: altenuene





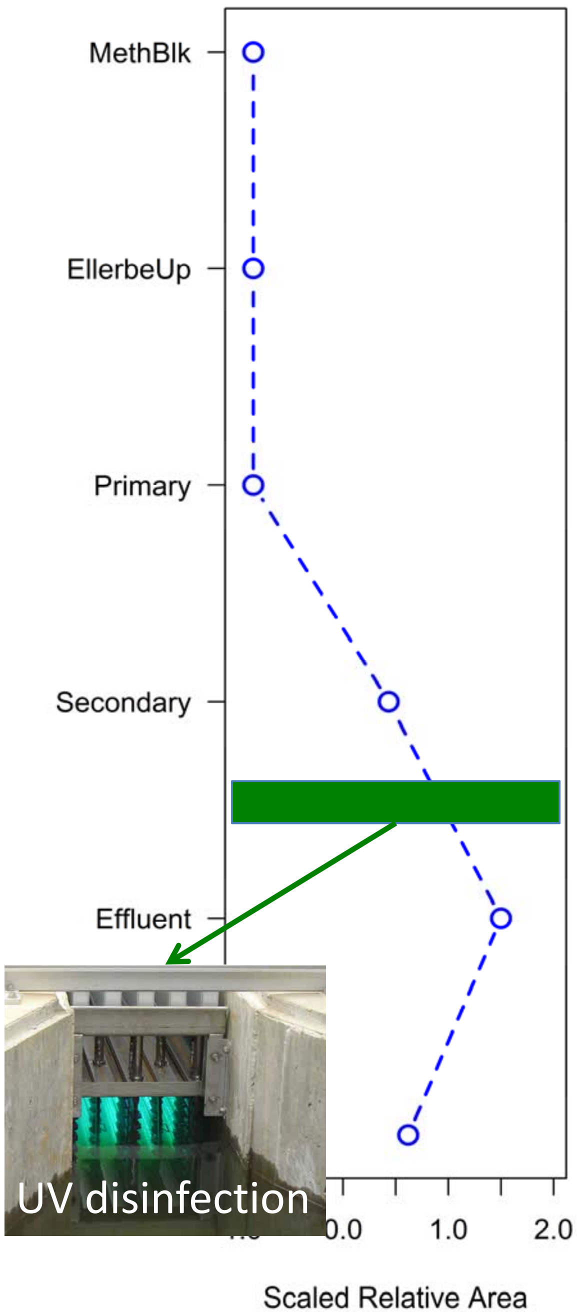


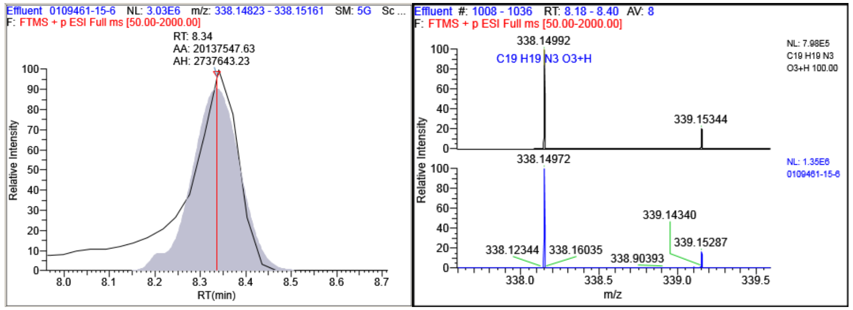


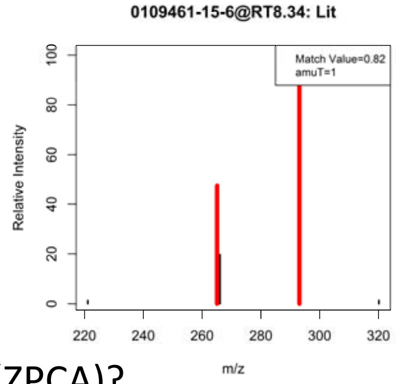


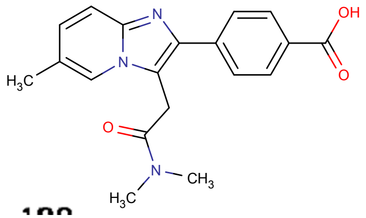
What is its source? Mycotoxin detected in foodstuffs…

Identification of a novel TP in the environment







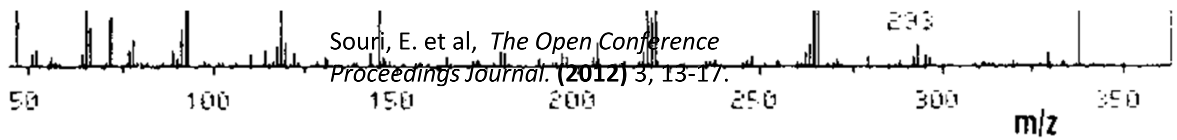


Zolpidem carboxylic acid (ZPCA)?

Possible photodegradation product?

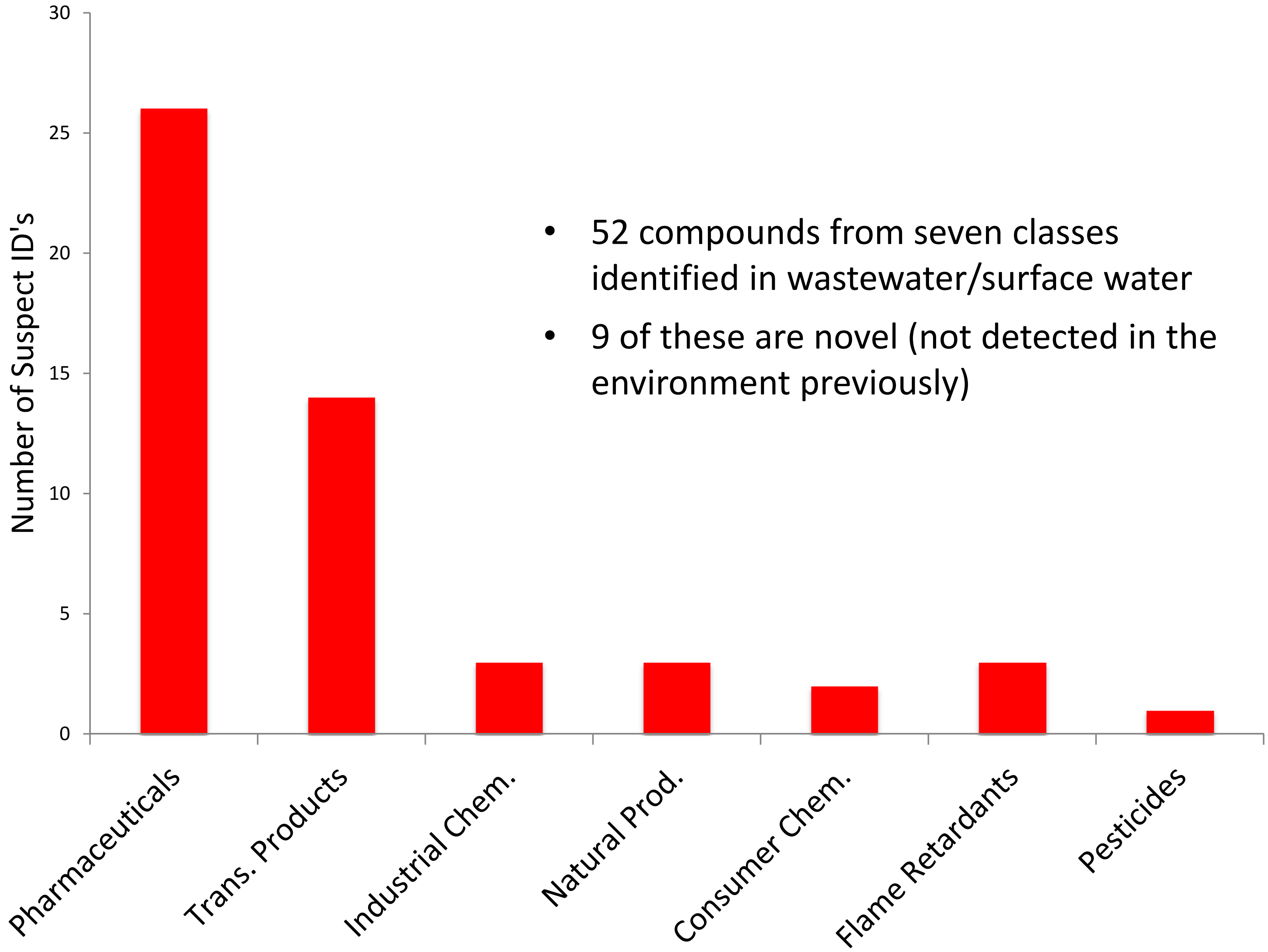
Table 4. The Results of the Stress Degradation Tests Using Different Conditions

Stress Test Condition	Solvent	Temperature	Time	% of Zolpidem
Acidic	1 M HCl	95 °C	4 h	90.2
	2 M HCl	70 °C	3 h	97.0
	2 M HCl	95 °C	3 h	75.7
	5 MHCl	95 °C	3 h	62.9
Alkaline	1 M NaOH	95 °C	1 h	35.1
Oxidative	3% H_2O_2	70 °C	1 h	54.6
	10% H_2O_2	70 °C	1 h	37.6
Photolytic				
UV light	Solid form	Room temperature	5 days	90.2
UV light	Water	Room temperature	5 days	45.0
Visible light	Solid form	Room temperature	5 days	84.6
Visible light	Water	Room temperature	5 days	83.0
Heat	Solid form	80 °C	5 days	85.0
	Water	80 °C	5 days	85.7



Souri, E. et al, *The Open Conference Proceedings Journal.* **(2012)** 3, 13-17.

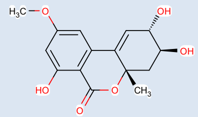
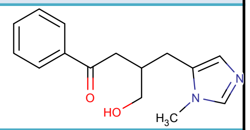
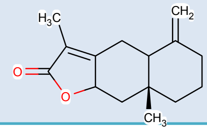
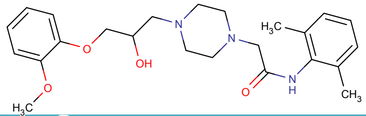
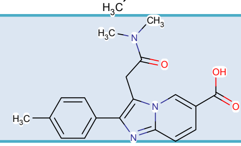
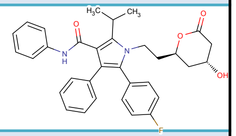
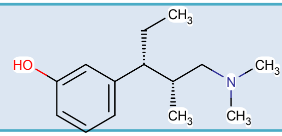
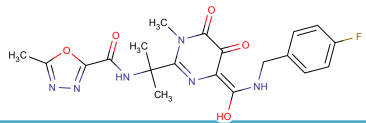
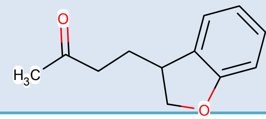
Results: Suspect compounds tentatively identified



- 52 compounds from seven classes identified in wastewater/surface water
- 9 of these are novel (not detected in the environment previously)

LF12

Tentative Novel Compound Identifications

Compound	Class	Structure
Altenuene	Mycotoxin	
1-Butanone, 3-(hydroxymethyl)-4-(1-methyl-1*H*-imidazol-5-yl)-1-phenyl-	Transformation product of pilocarpine (glaucoma treatment)	
Atractylenolide II	Sesquiterpene natural product	
Ranolazine	Antianginal	
ZPCA	Transformation product of zolpidem (Ambien)	
Atorvastatin lactone	Transformation product of atorvastatin (Lipitor)	
Tapentadol	Analgesic	
Raltegravir	Antiretroviral HIV treatment	
4-(2,3)-dihydro-3-benzofuranyl)-2-butanone	Transformation product of butylphthalide (celery oil comp.)	

Slide 51

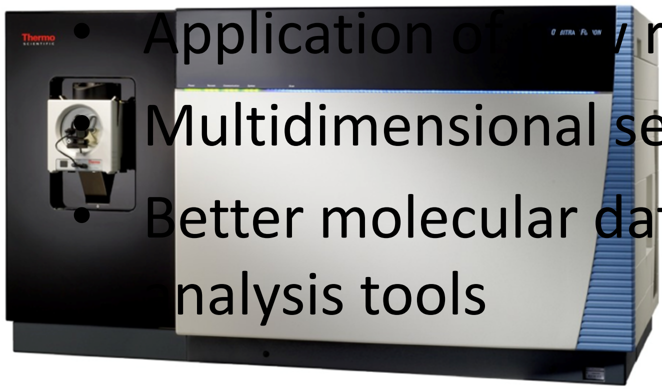
LF12 Lee Ferguson, 07.06.2013

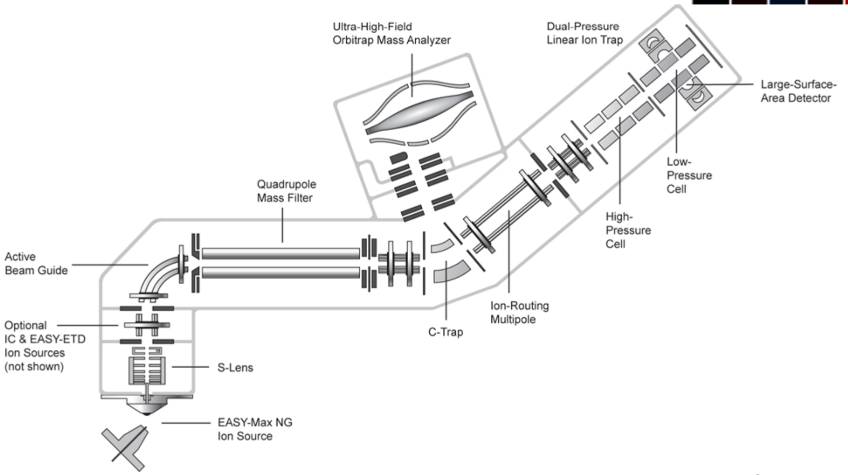
Conclusions

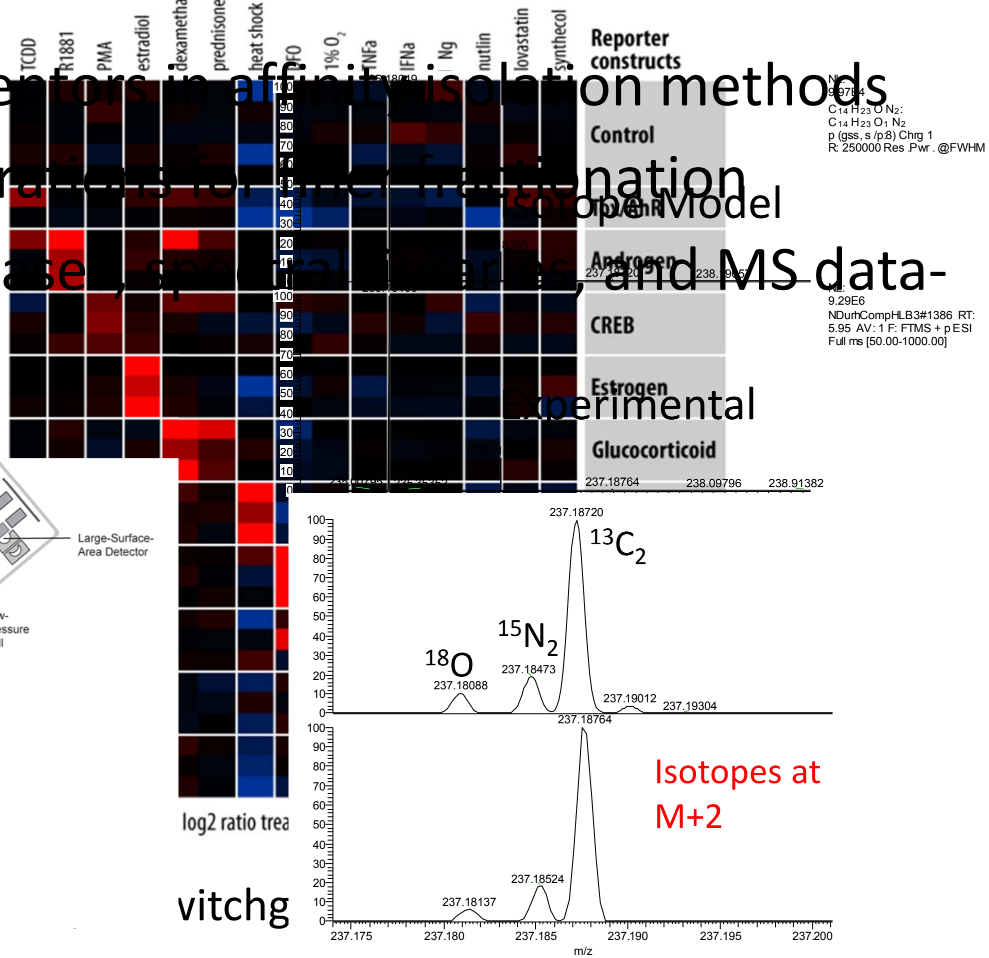
- Systematic effects-directed analysis approaches can solve practical problems in environmental contaminant science.
- Affinity purification approaches based on mode-of-action-specific molecular interactions of toxicants with receptors are well-suited for coupling with analytical detection methods.
- Process-dependent analysis is a powerful tool in combination with non-targeted or suspect screening for prioritizing the most relevant micropollutants in a contaminant mixture.
- New generation non-targeted analysis relies CRITICALLY on both analytical technologies (e.g. mass spectrometry) and high-content bioassays.

What’s next for non-targeted analysis??

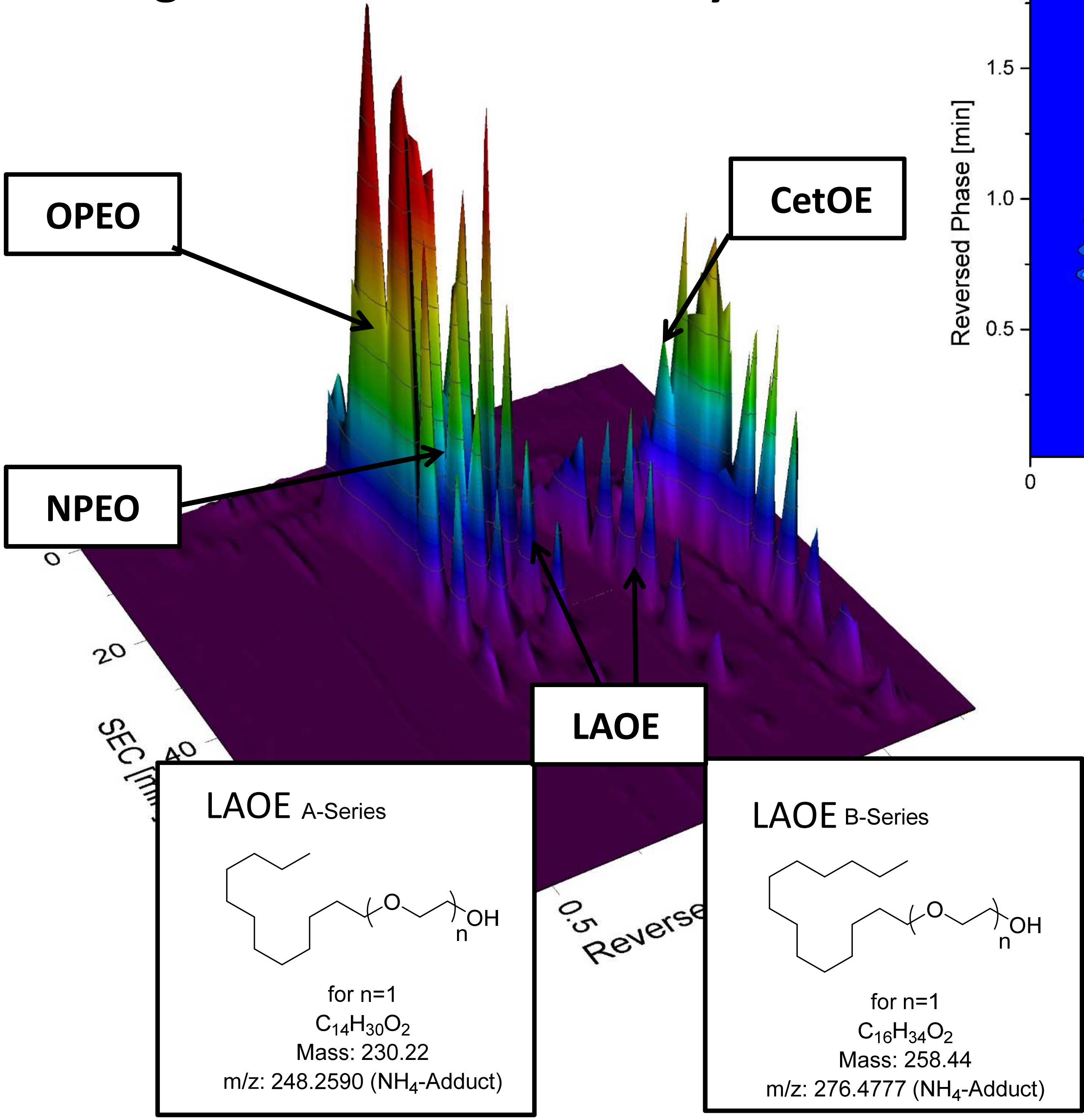
- New screening assays for determining effects:
  – High throughput fish embryo assays (*in vivo*)
  – Multi-target reporter assays (*in vitro*)
- Ultra-high resolution mass spectrometry
- Application of new receptors in affinity isolation methods
- Multidimensional separations for finer fractionation
- Better molecular databases, spectral libraries, and MS data-analysis tools

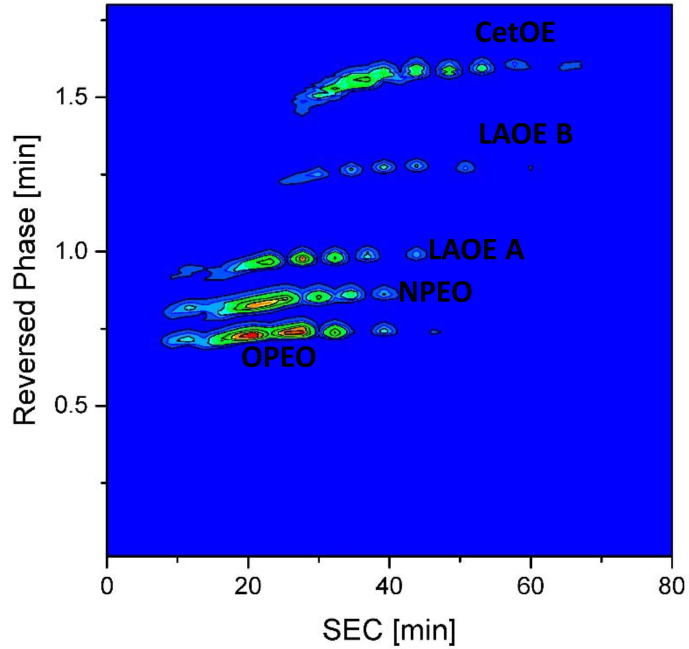






Resolving nonionic Surfactants by 2D-HPLC





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Devon Cancilla (Western Washington U.)

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