Analytical tools for comprehensive micropollutant analysis

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Workshop on Constituents of Emerging Concern
May 2017

Study Setup – Storm Driven Sampling

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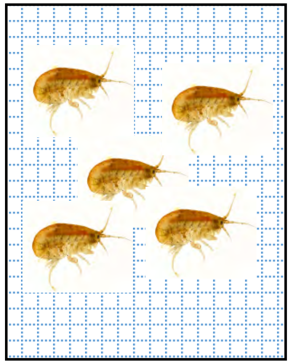
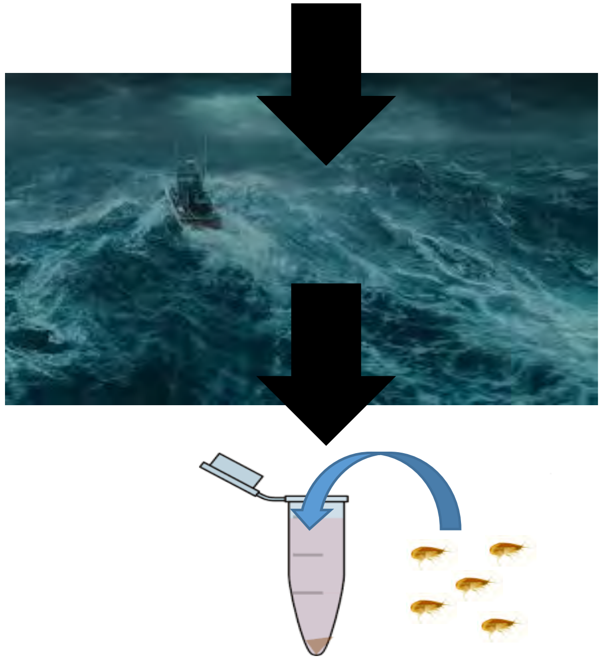
Chemcatcher® Passive Sampler

Hyallela azteca

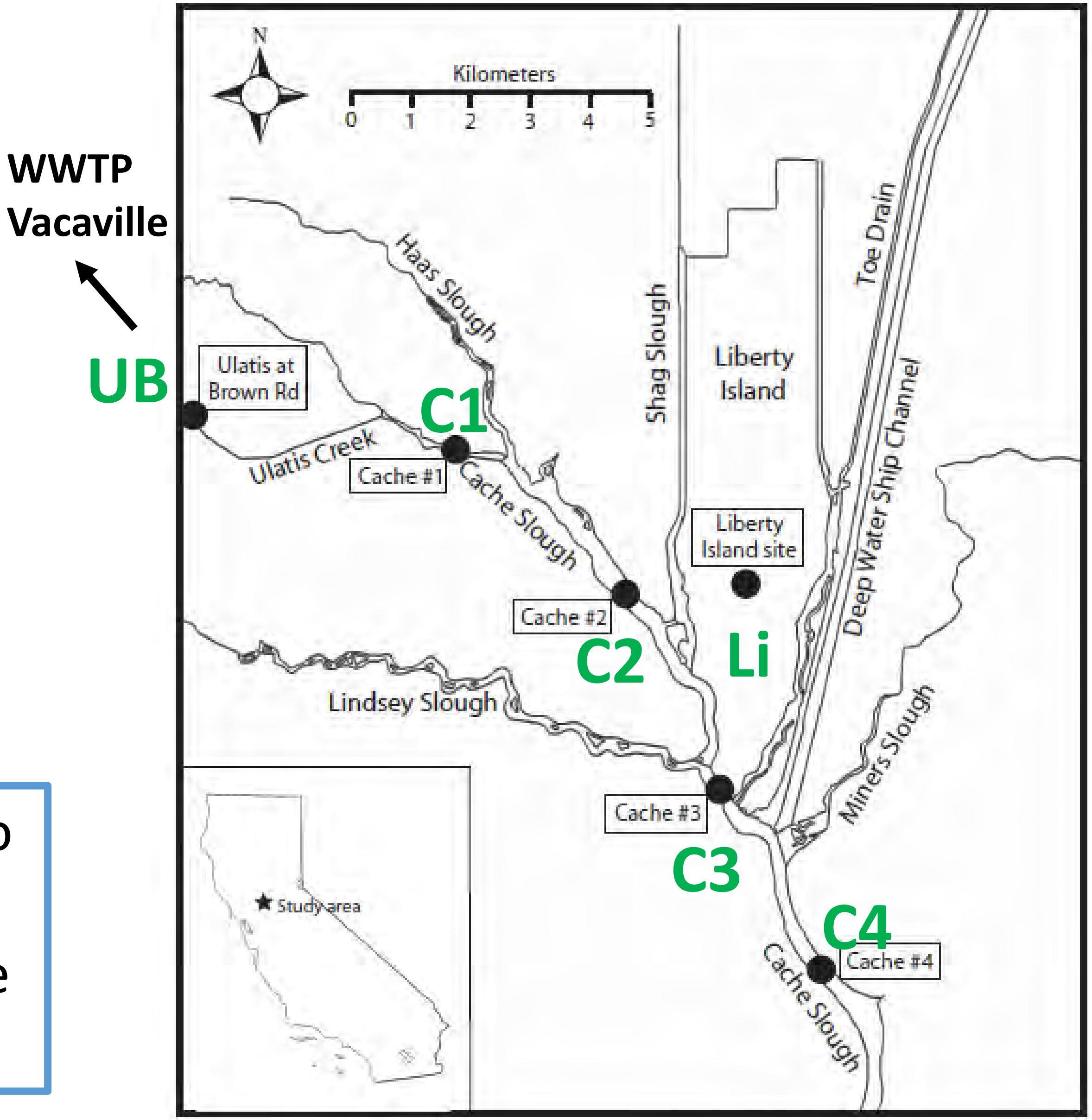




- rain event Jan 2016
- rain event March 2016

- grab samples for chemical analysis every day

Research Question: Do organic contaminants contribute to a decline in fish-prey?



Comparing Target and Nontarget Analysis

- Target Analysis (e.g., LC-MS/MS/MS)
  - Select target constituents and best ions to monitor (one parent ion and multiple ions produced in collision cell)
  - Advantages: selective, sensitive, good quantitation
  - Disadvantages: only find what you know to look for—possible to miss key constituents, especially byproducts
- Nontarget Analysis (e.g., LC-QTOF-MS)
  - Use high resolution capability of TOF-MS to determine accurate mass of ions (<5 ppm=±0.001 amu @ m/z=200) to produce short list of possible molecular formulas
  - Further narrow identification using MS/MS and databases
  - Advantages: can find unknown unknowns
  - Disadvantages: recovery and detection of non-target constituents uncertain; definitive compound identification challenging without standards

Overview of Hybrid Approach

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27 targets LC-QTOF
21 targets GC-QTOF

- polar chemicals

Filtration: only water analysis

SPE: multilayer cartridge (Oasis, anion & cation exchanger)

adapted from Eawag, Switzerland

Analysis: Agilent LC-QTOF-MS/MS

All-Ions ESI pos, ESI neg

- non-polar chemicals

Filtration: separate analysis water and filter

Water: SPE Oasis

Filter: sonication extraction

adapted from USGS, CA

Analysis: Agilent GC-QTOF-MS

NCI mode, RT-locked EI mode

www.agilent.com

Suspect Screening LC-QTOF

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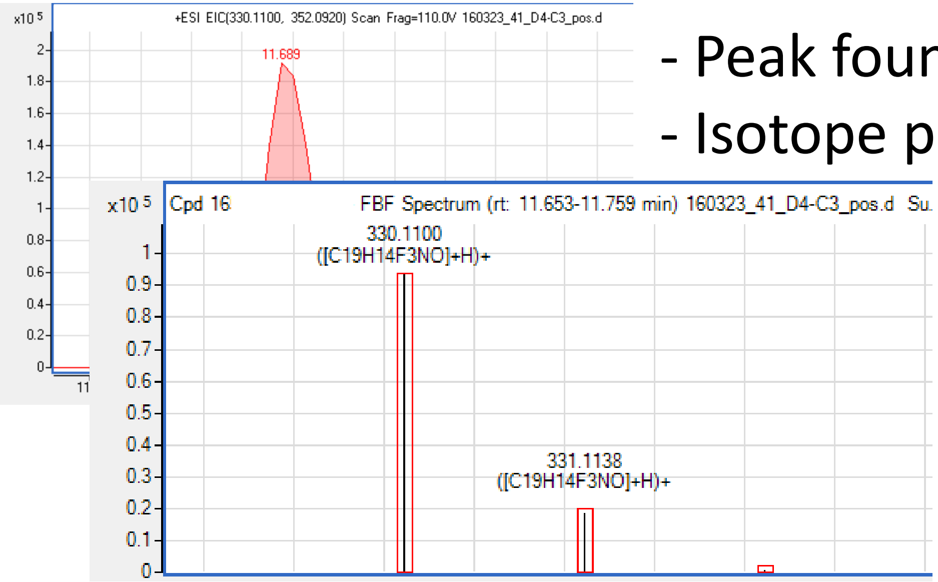
A) Search for exact masses in Chromatogram

→ library containing 1600 pesticides and transformation products

- Example



- Peak found for mass 330.1100

- Isotope pattern match $C_{19}H_{14}F_3NO$ (score 98)

→ 1 database match: Fluridone

→ confirmation of fragments with library spectra

Suspect Confirmation LC-QTOF

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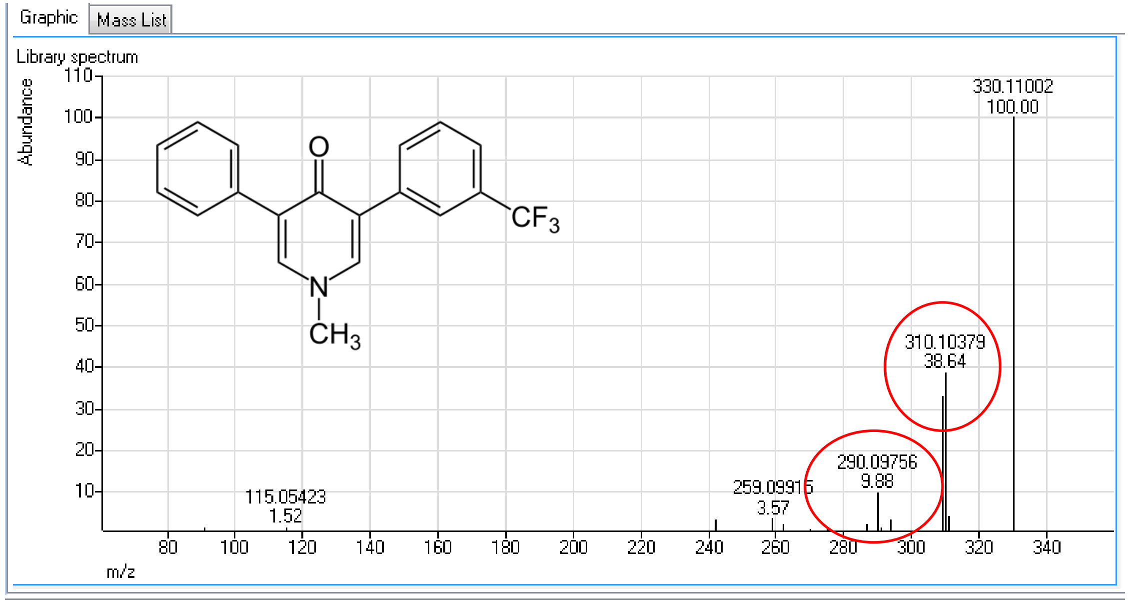
- Example Herbicide Fluridone

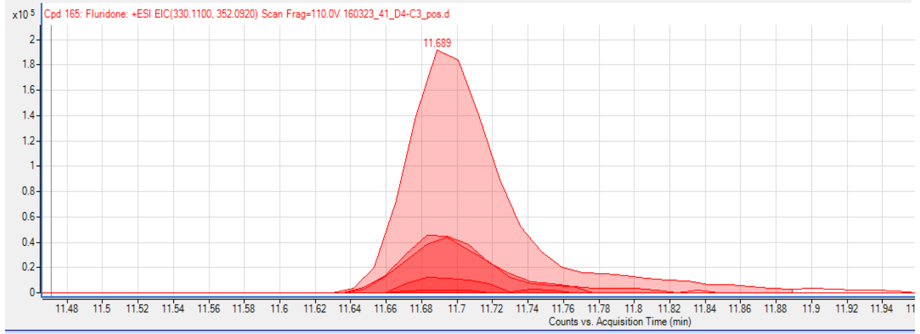
→ Library spectra



→ 4 fragments confirmed

Best	Name	Formula	m/z	Mass	Mass (Tgt)	Dif
	Fluridone	C19 H14 F3 N O	330.1100	329.1028	329.1028	

Coelution Score	CE	Flags(Fls)	FV	Height	m/z
		EIC with zero abund			259.0992
97.3	40	Qualified		12179	290.0976
86	40	Qualified		2334.1	294.0725
97.4	40	Qualified		45604.4	309.096
98.8	40	Qualified		43756.8	310.1038

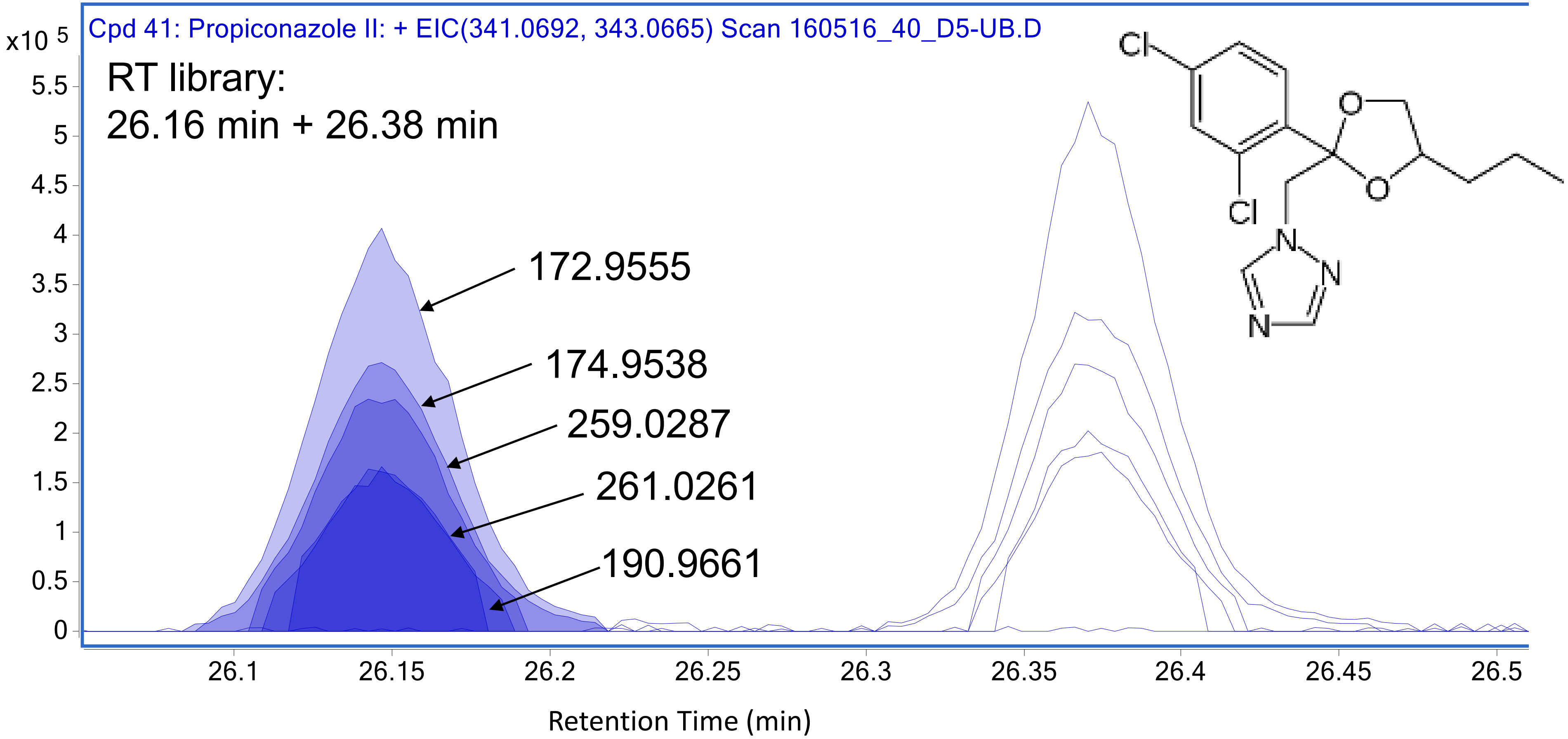


Detected in all samples of March event, no samples in Jan event

→ Confirmed by reference standard

Suspect Screening GC-QTOF-MS

- Retention time locked Agilent GC-EI accurate mass pesticide library
- cis- and trans-propiconazole (Fungicide) identified



Target and Suspect Results

Analytical Method	Targets Detected	Suspects Detected
LC-QTOF-MS	21	57*
GC-QTOF-MS	16	43
Total	37	90**

* 18 of 21 confirmed with reference standard
** 10 in both GC-MS and LC-MS, 25 not confirmed with MS/MS

→ **15-25 targets in every sample**

GC-QTOF-MS Targets
7 Pyrethroids, e.g. Cyhalothrin, Bifenthrin, Cypermethrin,
Chlorpyrifos
Fipronil and degradates

GC-QTOF-MS Suspects
Dacthal, 2,6-Dichlorobenzamide (BAM), Bromacil, Oxadizone, Propiconazole, Kinoprene, Diazinone

LC-QTOF-MS Targets
Insecticides: e.g. Methoxyfenozide, Imidacloprid, Dimethoate
Fungicides: e.g. Azoxystrobin, Boscalid, Cyprodinil
Herbicides: e.g. Diuron, 2,4-D, Hexazinone
Biocides: e.g. Triclosan, DEET

LC-QTOF-MS Suspects
Propiconazole, Norflurazone, Triclopyr, Fluridone, Quinclorac, Diethofencarb

Overview of Spatial/Temporal Trends

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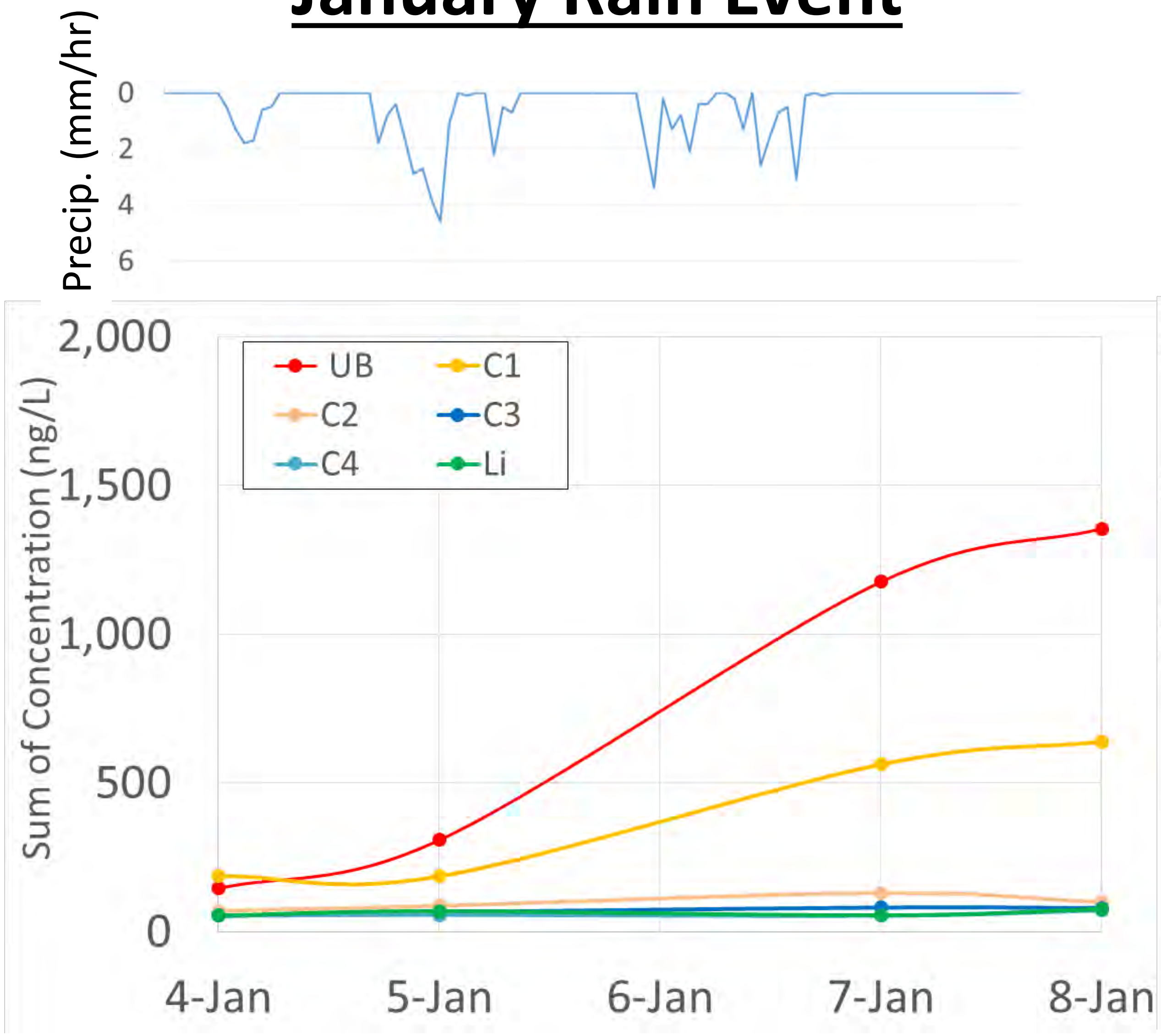
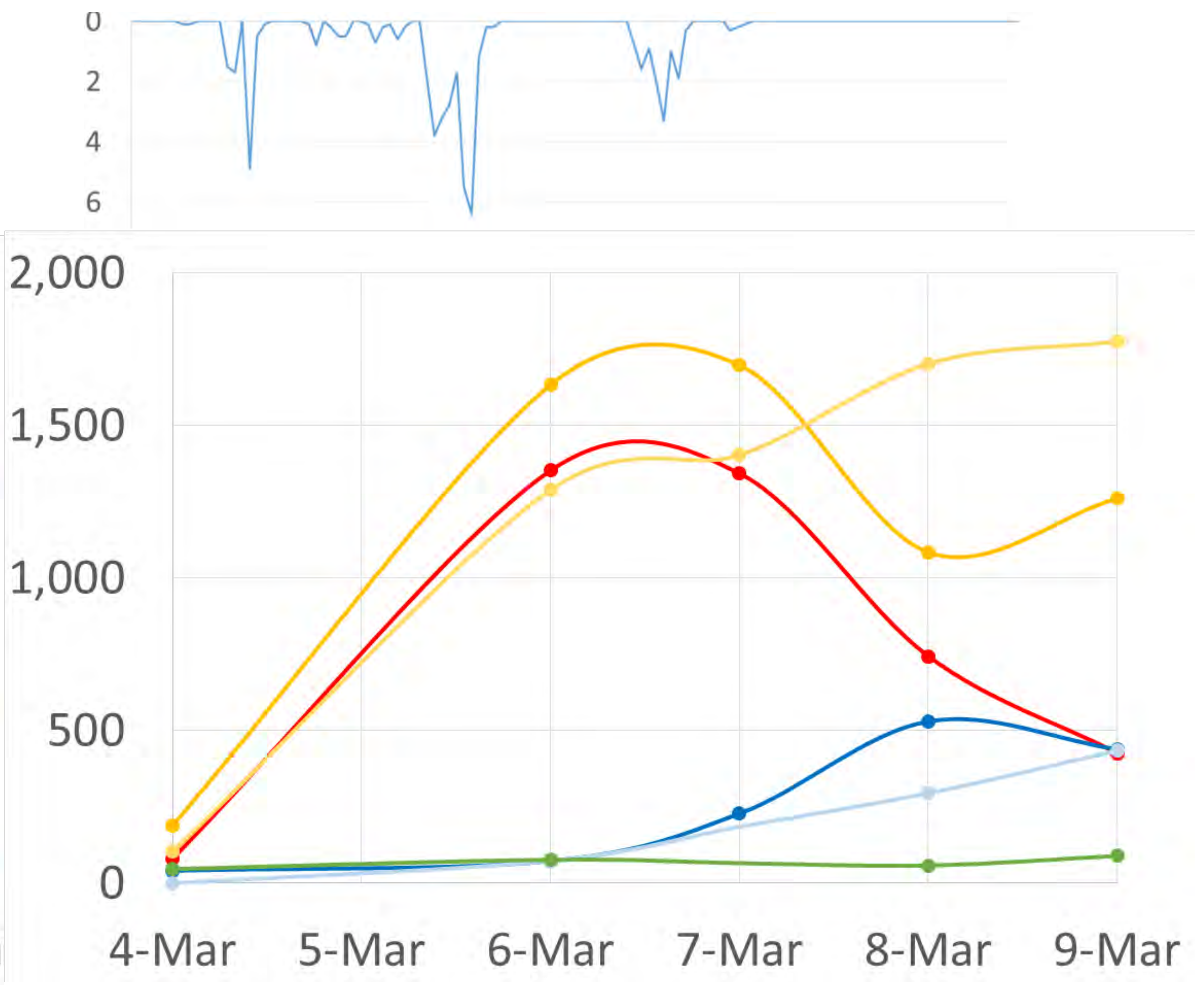
January Rain Event

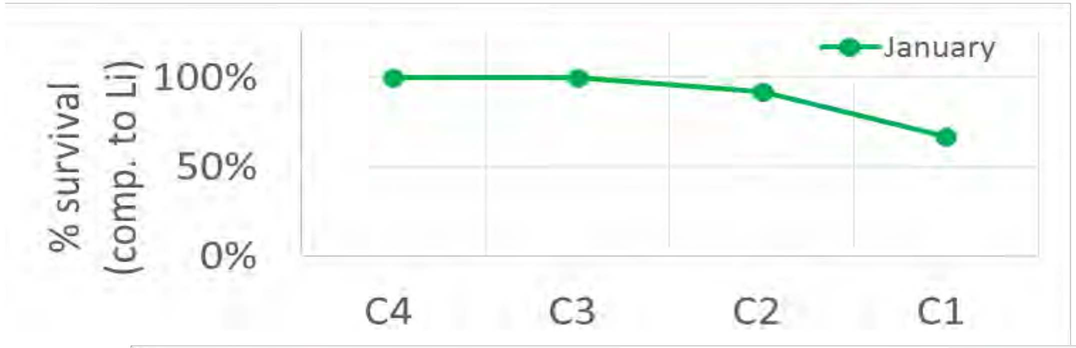


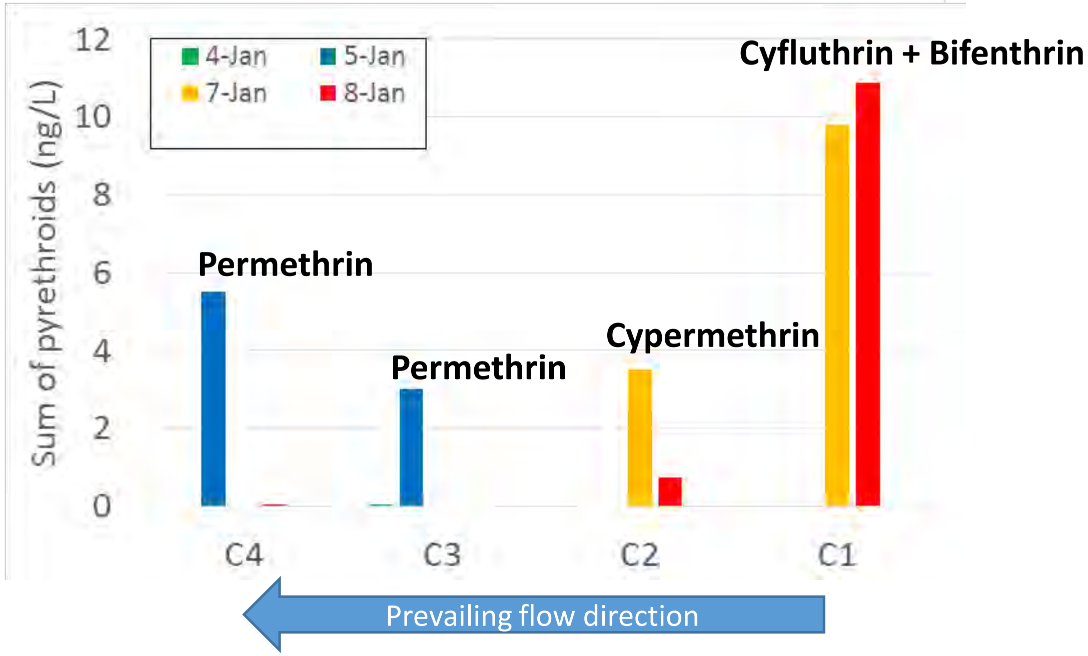
March Rain Event



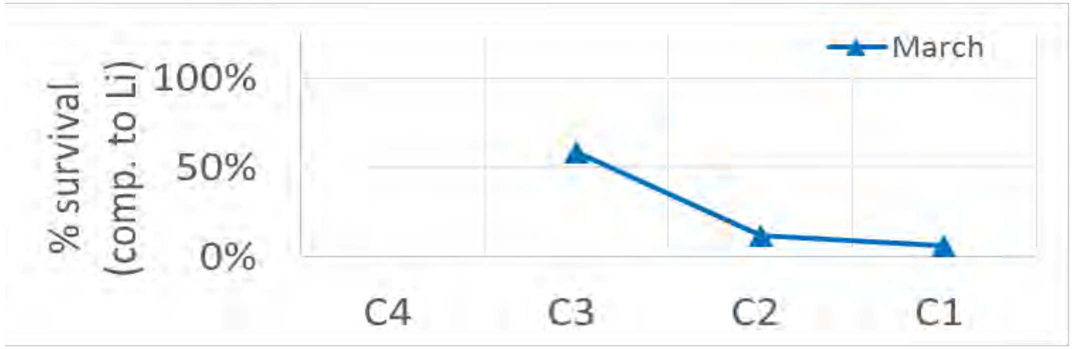
Acute Toxicity and Pyrethroids

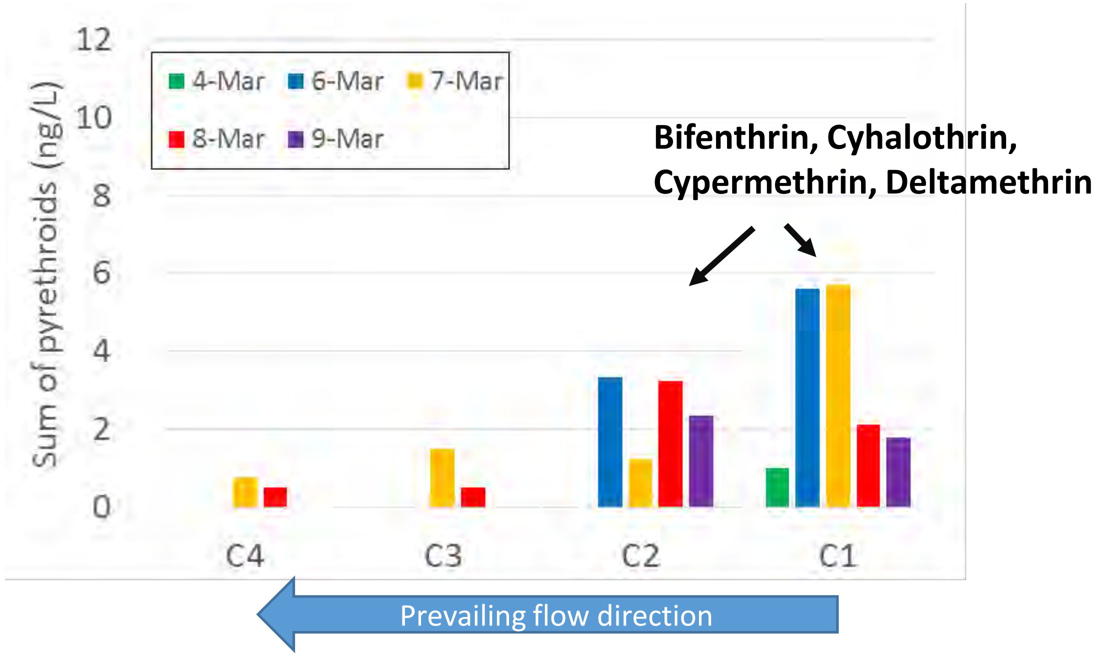
January Event





March Event





Finding Transformation Products

- Used EAWAG-PPS to predict 1409 transformation products (3 generations) for 76 detected pesticides
- Theoretically ionizable TPs (1338) entered into database
- All 51 samples screened for TPs using LC-QTOF-MS in ESI+/ESI- with MassHunter Qual Find-by-Formula
- Manual screening of all compounds with score >70 and >5 detections
- Plausible candidates re-run in targeted MS/MS mode
- MS/MS spectra predicted using Molecular Structure Correlator (Agilent) and CFM-ID (http://cfmid.wishartlab.com/predict)
- Further prioritization based on comparing spatial/temporal similarity of TP to parent compound

Nontarget TP Detection Example

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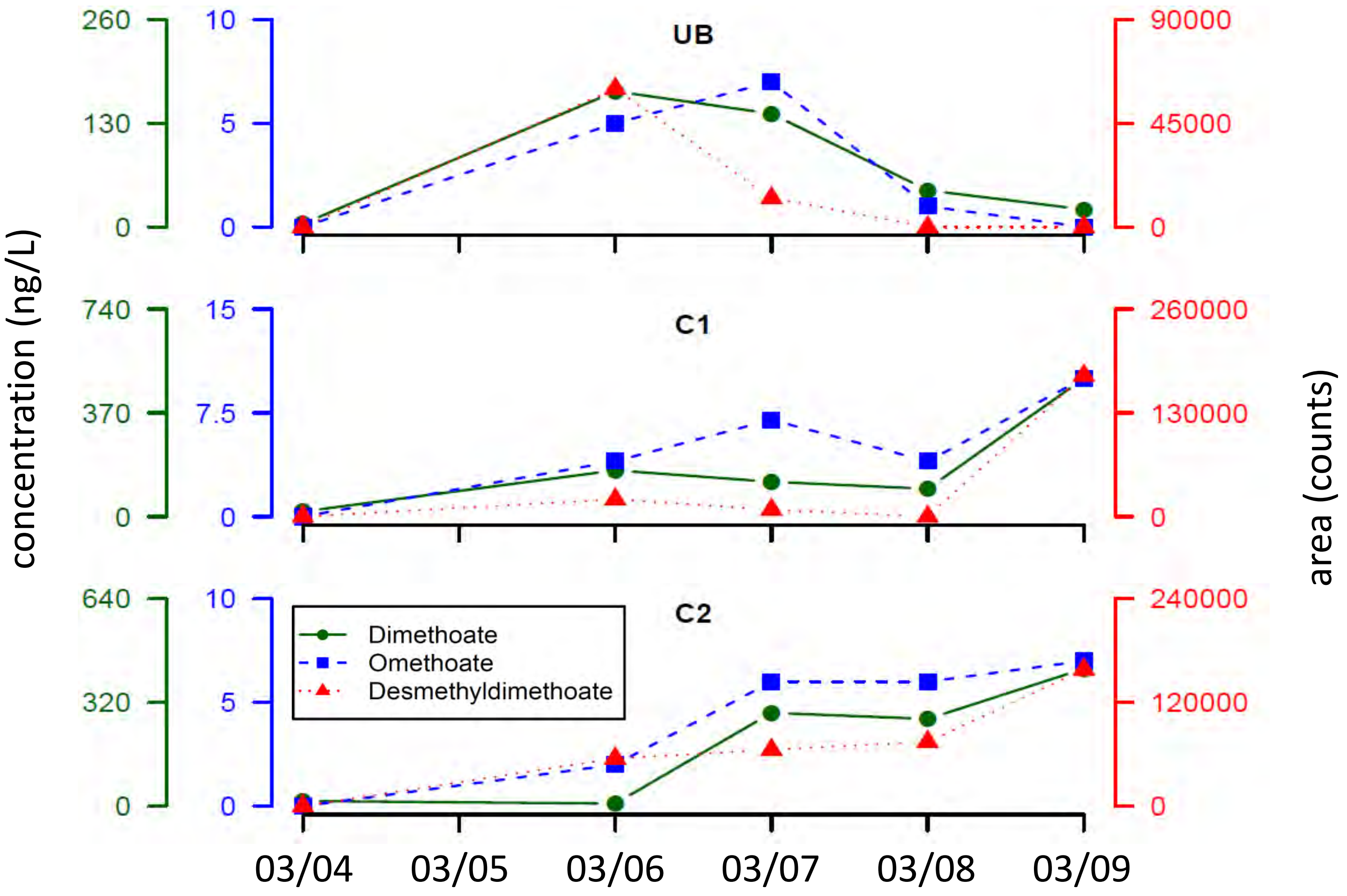
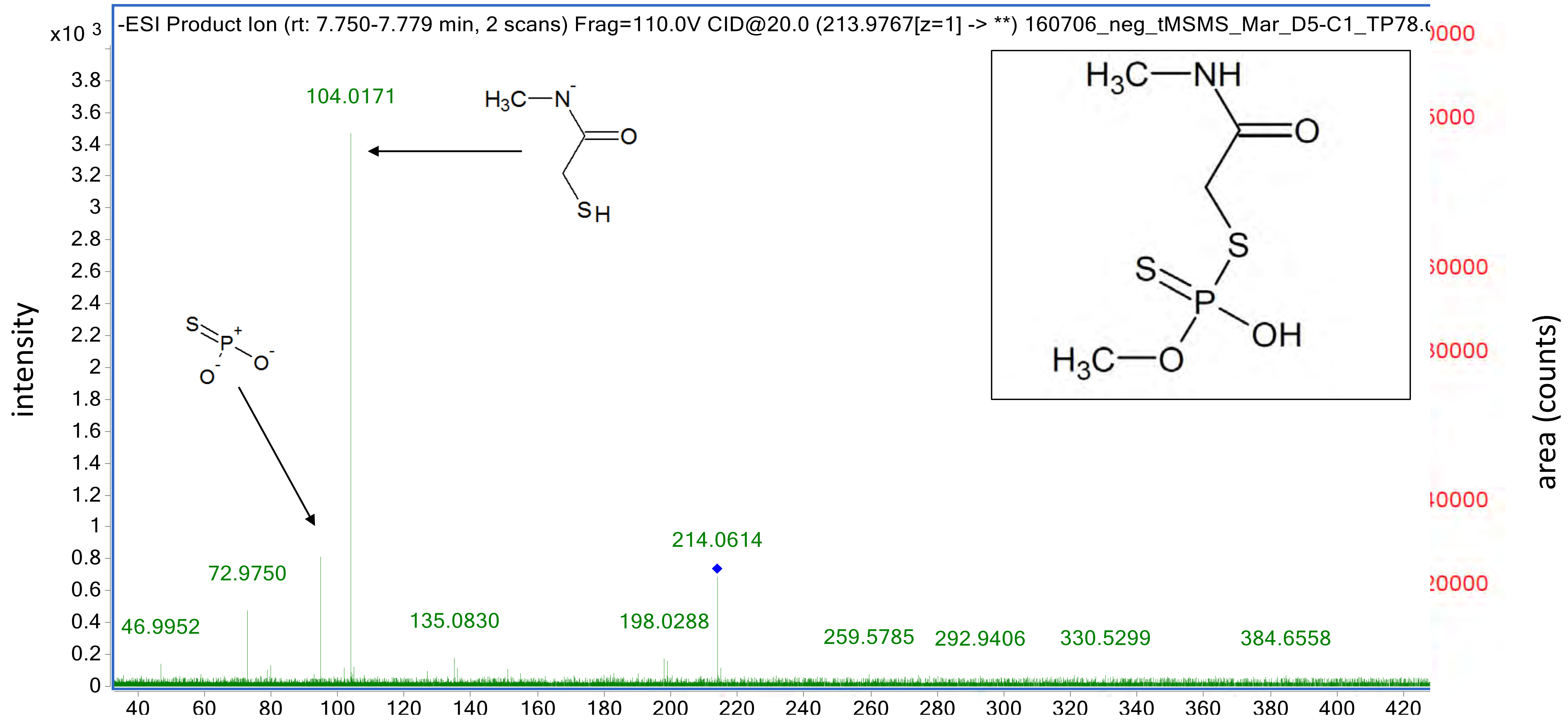
- Insecticide Dimethoate and two TPs
  - Omethoate found in All-Ions Workflow and confirmed with standard
  - O-desmethyl dimethoate- no reference standard available but plausible MS/MS fragments
- 7 TPs detected via this workflow



Nontarget TP Detection Example



Nontarget Analysis Supports Source ID

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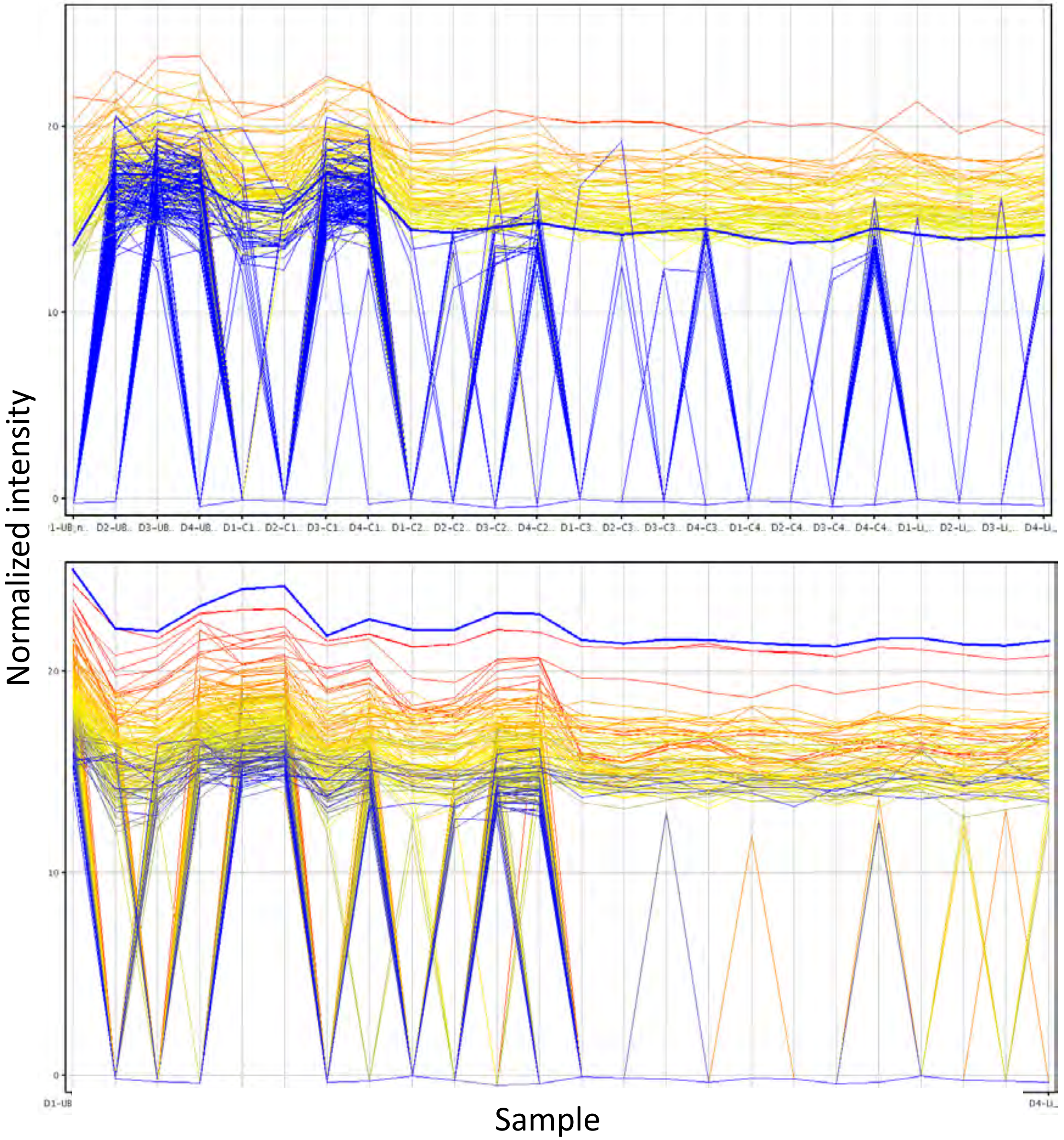
212 patterns like
<u>2,4-D</u>
similarity > 0.75

235 patterns like
<u>sucralose</u>
similarity > 0.75

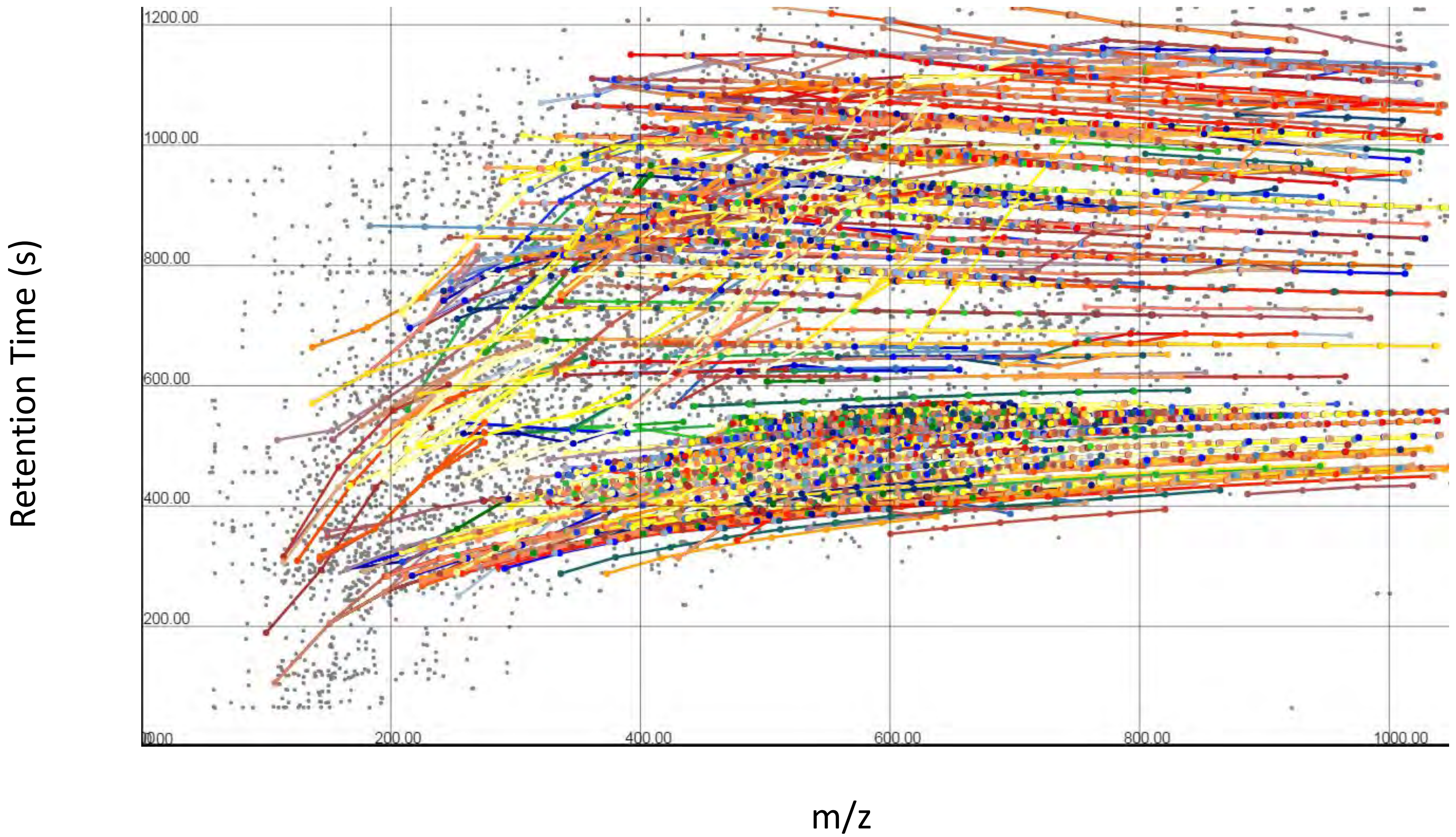


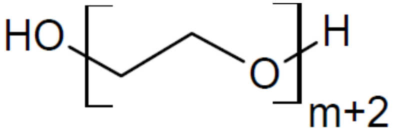
indicator of
diffuse source

indicator of
point source

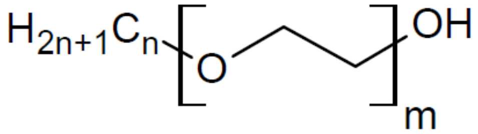
* using Agilent MPP software

Nontarget: Ethoxylated Surfactants

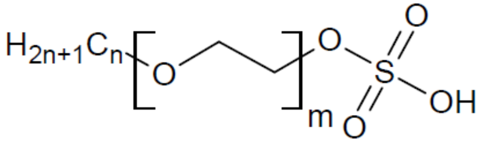




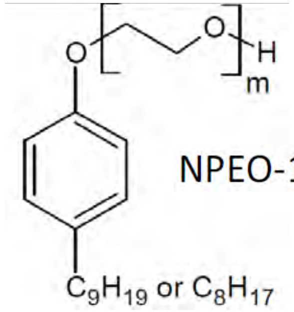
PEG-2EO to PEG-14EO



C10-AEO-1 to C18-AEO-20



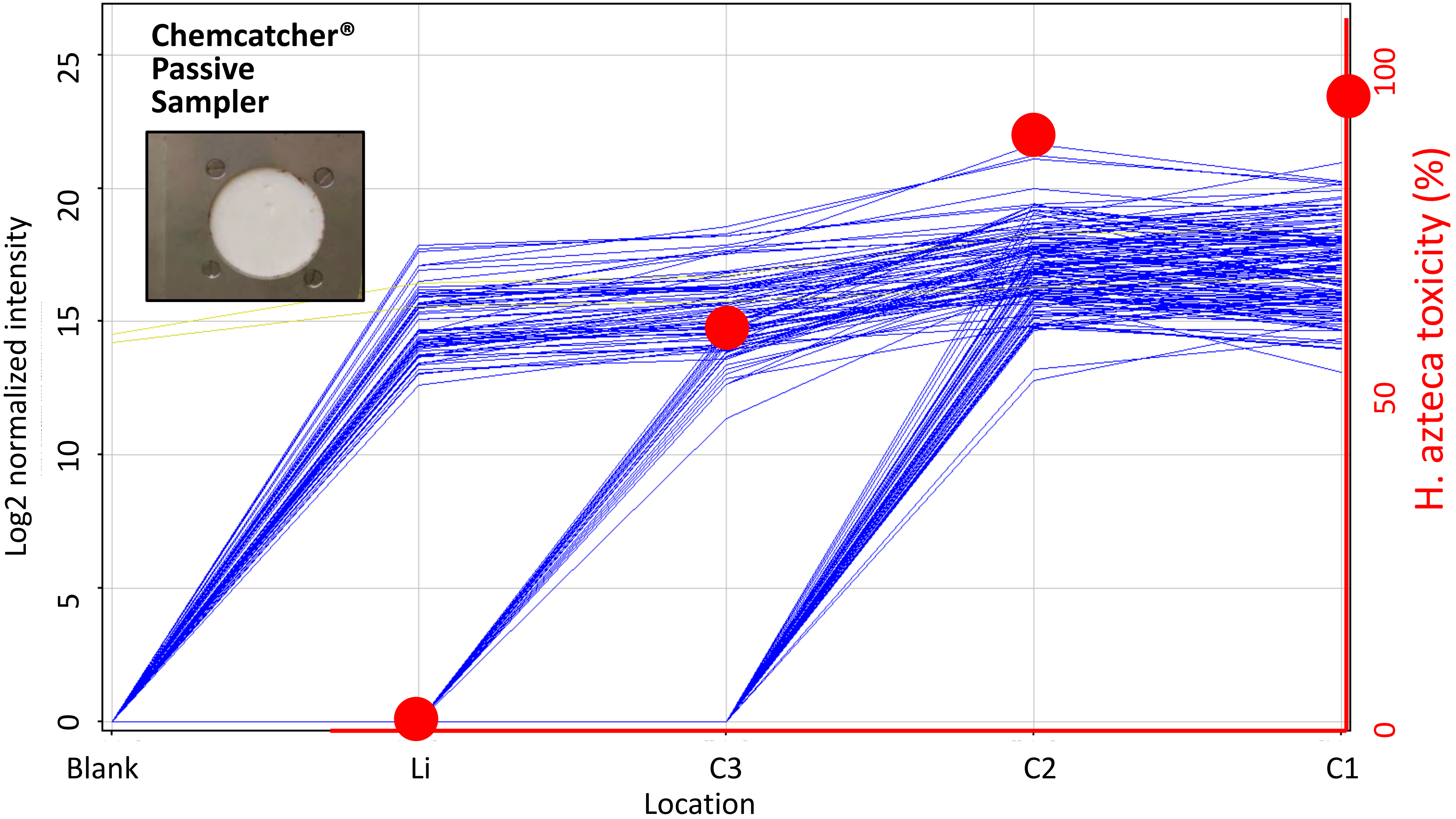
C12-AE1S to C16-AE12S



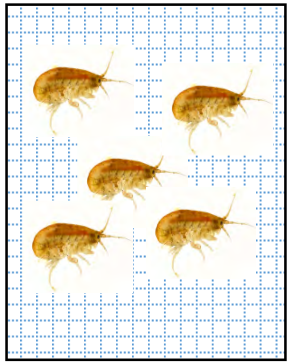
NPEO-1 to NPEO-20

Procedure: LC QTOF ESI+, EnviHomolog, Repeat Unit Range: 44.0242-44.0262, mztol=5 ppm

Hyallela Toxicity vs. Passive Sampling



Hyallela azteca



Assessing Significance

Compound Name	Compound Class	Work -flow	Instrument	Max RQ	Max MEC	# Det.
Cypermethrin	Insecticide	T	GC	**16**	33	6
Cyfluthrin	Insecticide	T	GC	**2.5**	29	18
Bifenthrin	Insecticide	T	GC	**0.6**	5.4	20
Cyhalothrin	Insecticide	T	GC	**0.5**	6.3	23
Malathion	Insecticide	S	LC+GC	**0.4**	236	4
Dimethoate	Insecticide	T+S	LC+GC	**0.2**	493	27
Diazinon	Insecticide	S	GC	**0.2**	60	4
Esfenvalerate	Insecticide	T	GC	**0.2**	1.9	6
Deltamethrin	Insecticide	T	GC	**0.2**	1.0	13
Permethrin	Insecticide	T	GC	**0.1**	5.5	2

Assessing Significance

Compound Name	Compound Class	Work-flow	Instrument	Max RQ	Max MEC	# Det.
Sucralose	Food additive	S	LC	-	**>5000**	**51**
Iohexol	PPCP	S	LC	-	**>5000**	**51**
Metformin	PPCP	S	LC	9E-05	**>5000**	39
2,4-dichlorophenol	Herbicide TP	S	LC	-	**>1000**	22
Triclopyr	Herbicide	S	LC	4E-04	**>1000**	44
2,4-Dinitrophenol	different uses	S	LC	0.003	**>1000**	1
Tolyltriazole	Corrosion inhibitor	S	LC	-	**>1000**	45
9-Octadecenamide	Endogenous	S	LC	-	**940**	26
TCPP	Flame Retardant	S	LC	-	**930**	40
TDCPP	Flame Retardant	S	LC	-	**890**	**51**

Assessing Significance

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Compound Name	Compound Class	Work-flow	Instrument	Max RQ	Max MEC	# Det.
2,4-D	Herbicide	T	LC	5E-05	778	**51**
Metoprolol	PPCP	S	LC	7E-05	487	**51**
Boscalid	Fungicide	T+S	LC+GC	3E-04	368	**51**
Diuron	Herbicide	T	LC	0.08	199	**51**
Fluxapyroxad	Fungicide	S	LC	3E-05	76	**51**
DEET	Insect repellent	T+S	LC+GC	7E-07	53	**51**
fipronil	Insecticide	T	LC+GC	0.01	14	**51**
Fipronil amide	Insecticide TP	T	GC	-	13	**51**
Fipronil-sulfone	Insecticide TP	T	LC+GC	4E-04	9.0	**51**
Fipronil-desulfinyl	Insecticide TP	T	LC+GC	9E-05	4.5	**51**
PFHxS	PFCs	S	LC	-	4.2	**51**
Chlorthal-dimethyl	Herbicide	S	GC	5E-07	3.1	**51**
Dichlobenil	Herbicide	S	GC	-	-	**51**
Dithiopyr TP	Herbicide TP	S	LC	-	-	**51**

Conclusion and Outlook

- Toxicity towards *H. azteca* → pesticide exposure possible cause for decline of fish prey
- With over 100 detected pesticides from varied classes, mixture toxicity likely important
- Broad scope suspect/non-target screening finds many more compounds than those on a typical target list
- Nontarget workflow finds ubiquitous pesticide TPs
- Statistical analysis can group molecular features to provide information regarding contaminant sources, similar fate processes—currently coupling results with hydrologic models
- Significance of non-target analytes being confirmed by toxicity correlations and genomic profiling (S. Hasenbein and H. Poynton)

Acknowledgements

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