Comprehensive Organic Contaminant Assessment: Nontargeted Analysis

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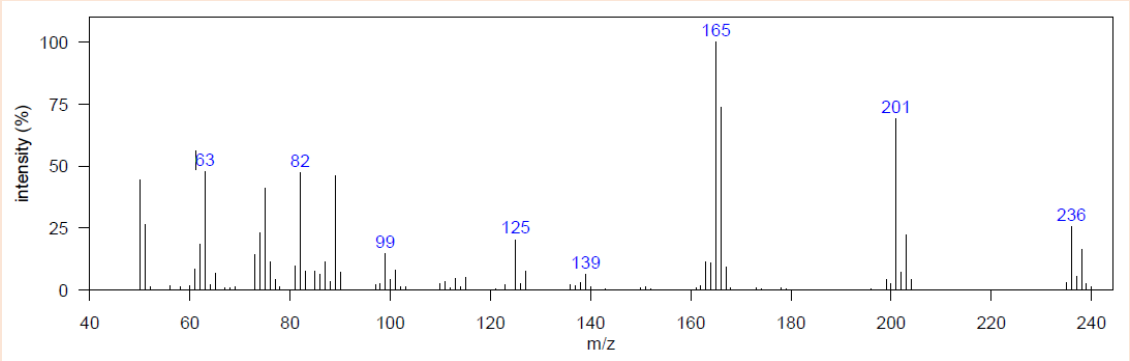
1. Non-targeted Analysis: Identification of Contaminants in Southern California Sentinel Marine Mammals

2. Non-targeted Analysis: Identification of Bioaccumulative Chemicals in Smoked Cigarette Leachate

Objectives of Non-Targeted Analysis

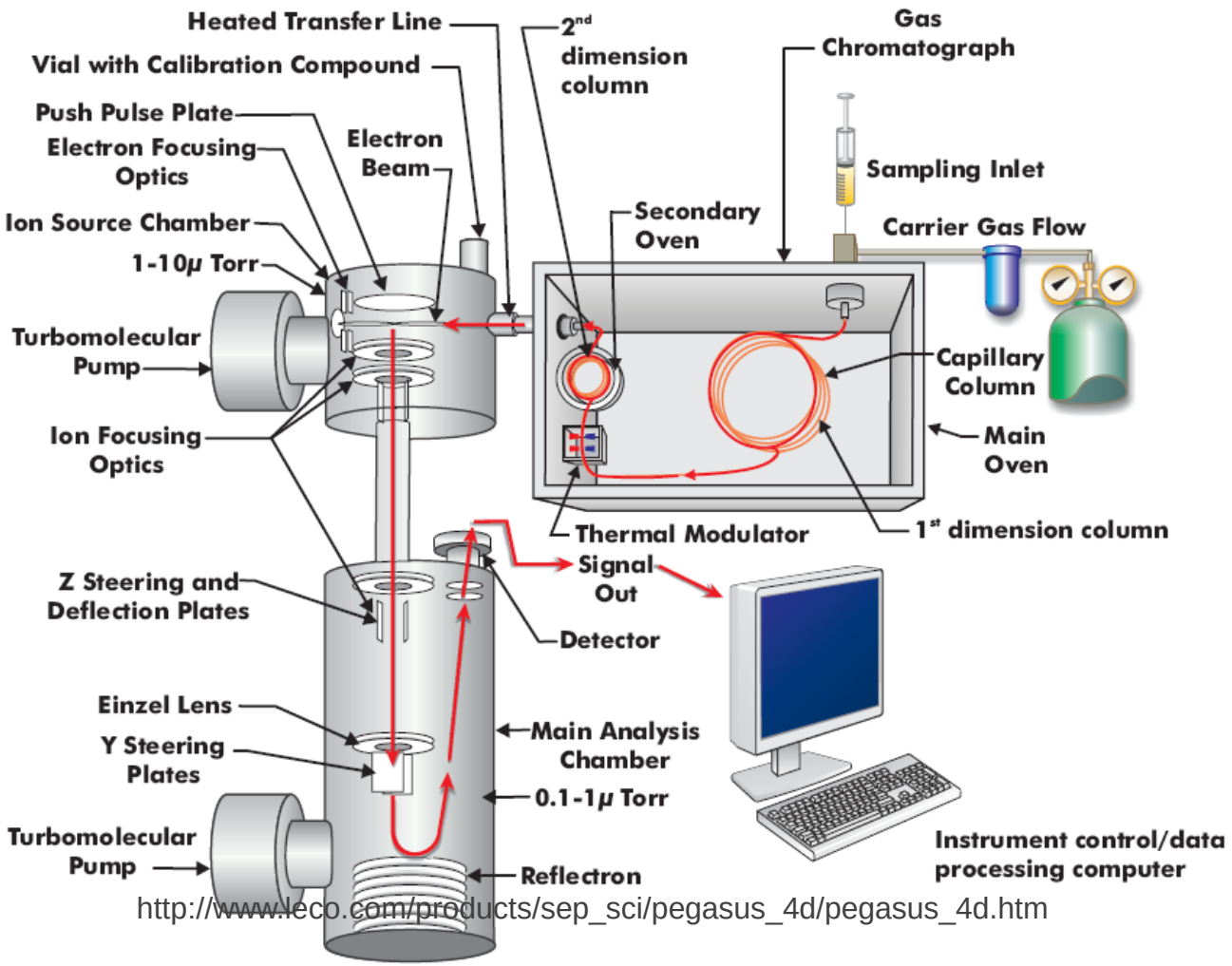
Non-targeted analysis is a systematic method of identifying both expected and unexpected contaminants.



Full scan mass spectrometry

- Examines the “total” contaminant load and allows profile comparisons.
- Identifies contaminants missed by targeted analysis.
- May be used to direct toxicity/risk studies of new contaminants.
- May be used to investigate causes of observed toxicity.

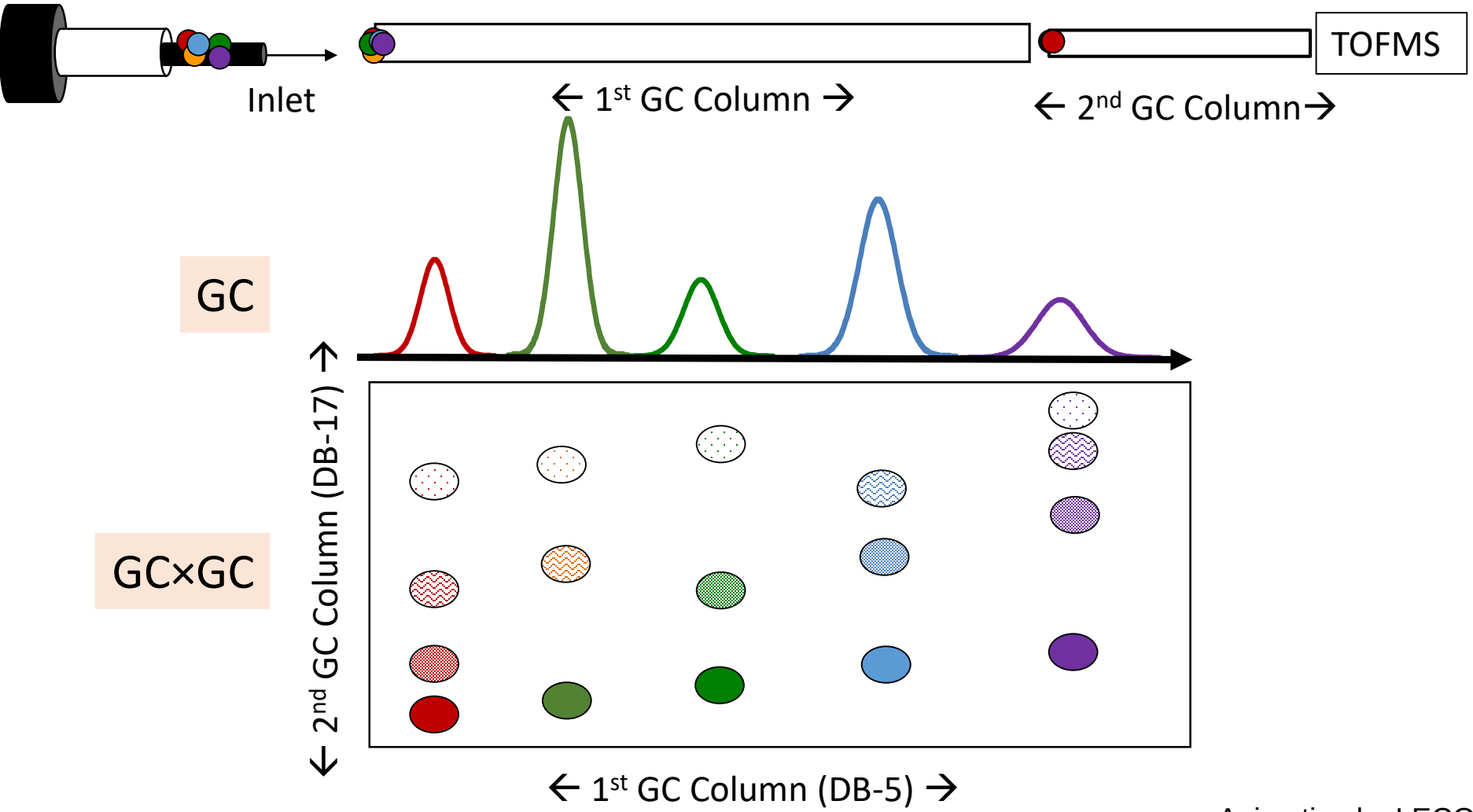
GCxGC/TOF-MS



http://www.leco.com/products/sep_sci/pegasus_4d/pegasus_4d.htm

GC×GC-TOF Instrumentation

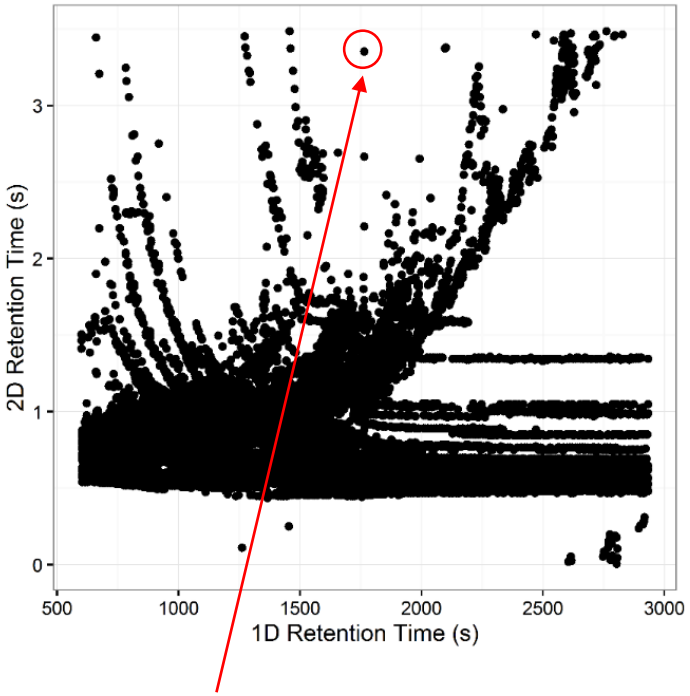
Increased number of resolved chromatographic peaks



Animation by LECO

Automated Data Reduction

Raw 2D Chromatogram, Dolphin Blubber Extract

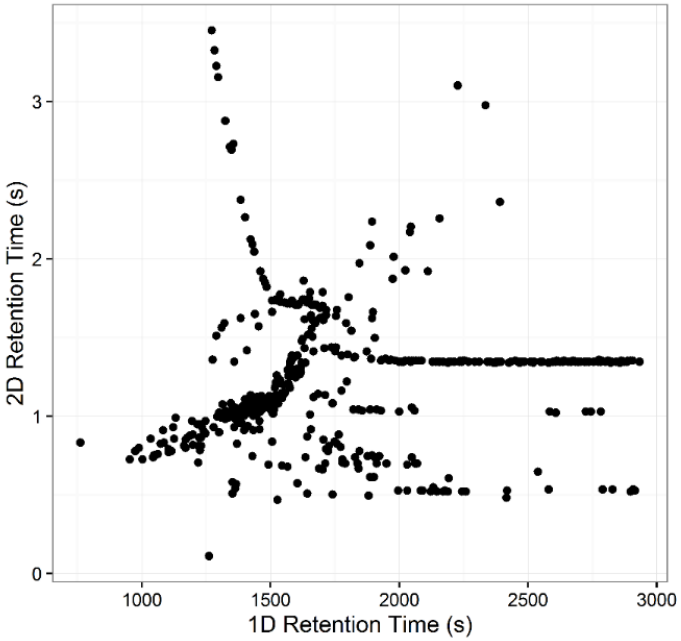


Chromatographic "feature" with associated mass spectrum

Approx. 9000 chromatographic features/sample

Data reduction algorithm that identifies halogenation patterns in the mass spectra

Filtered 2D Chromatogram

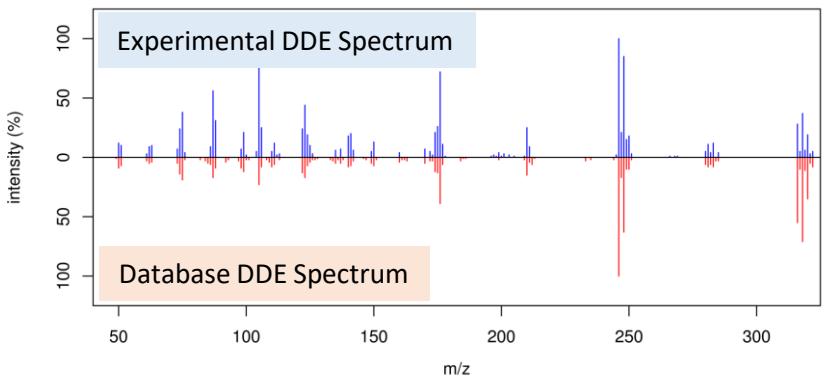


Approx. 400 compounds identified as halogenated

Approx. 8 hours/sample to manually verify

Compound Identification

Identification of Halogenated Compounds



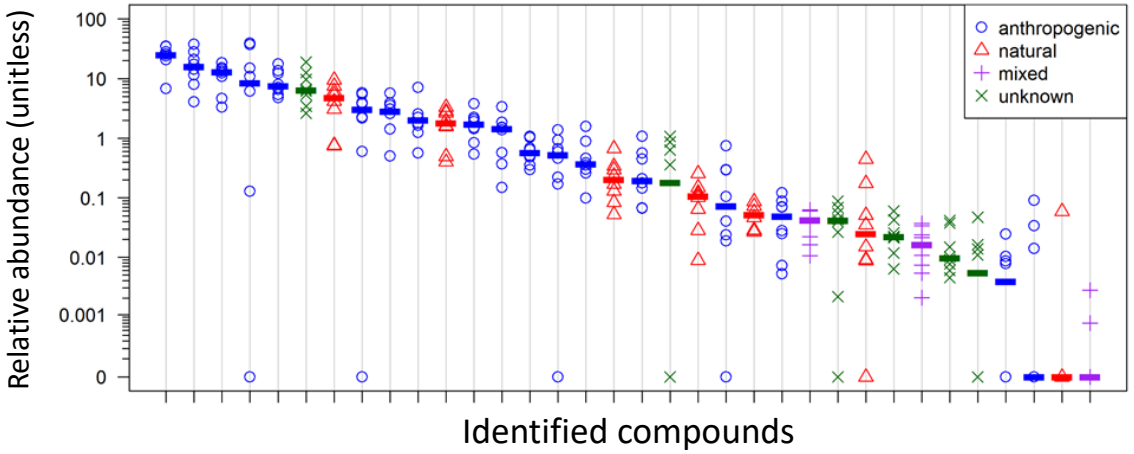
Identifications based on:

1. Confirmation with authentic standards
2. Database match
3. Manual interpretation
4. Classified as unknown

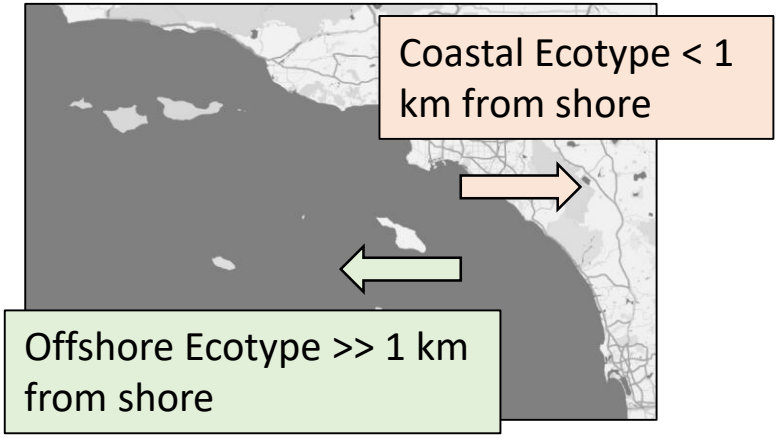
Decreasing confidence

Contaminant Profiles

Internal standards are used to determine relative abundances of all compounds

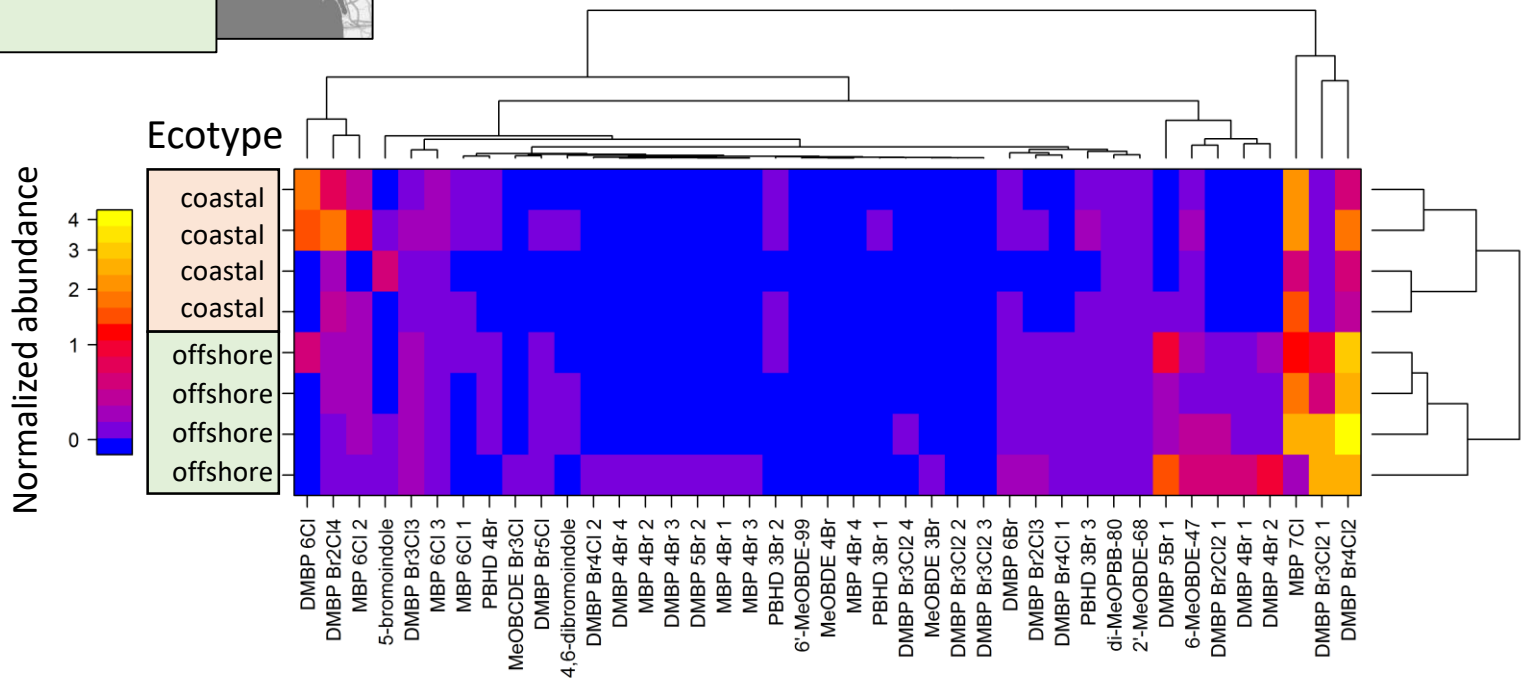


Nearby Regional Differences



Hierarchical clustering analysis of Southern California bottlenose ecotypes:

- Natural product profiles were different
- Anthropogenic profiles were the same



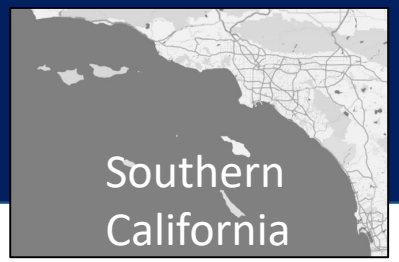
Halogenated natural products

Shaul et al., Environ Sci Technol., 2015.

Differences in Species from the Same Region

In which species do we have the best chance of detecting emerging contaminants?

Number of Contaminants and Natural Products Identified

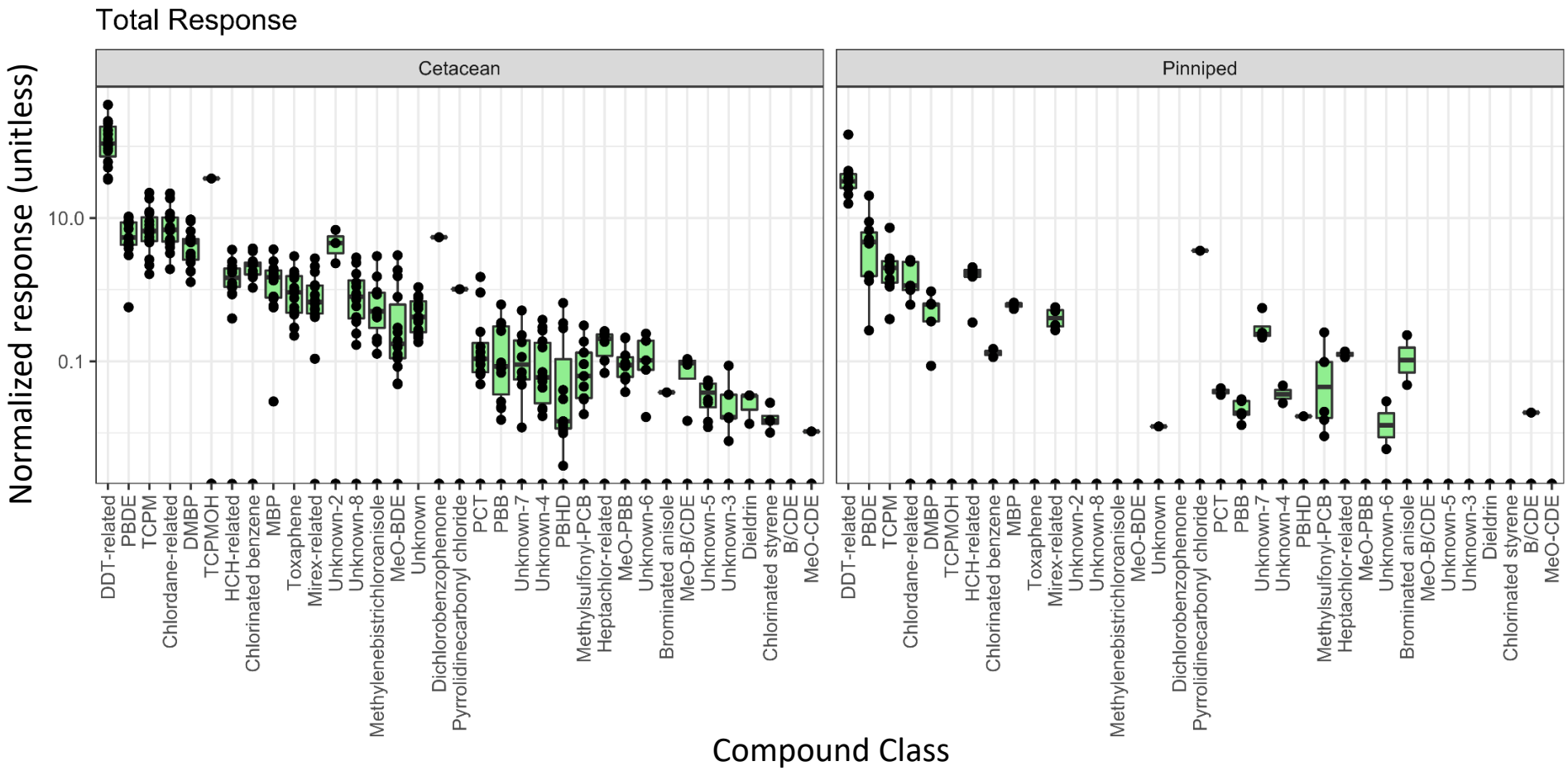


Clade	Species	n	Total Halogenated Compounds, Avg. (Range)	Spectral Filtering Method	Specimen Availability
Cetacean	Bottlenose common dolphin	8	261 237-308	Manual	Low
Cetacean	Long-beaked common dolphin	5	133 (120–128)	Automated	Medium
Cetacean	Short-beaked common dolphin	5	128 (113–144)	Automated	High
Cetacean	Risso's dolphin	5	124 (106–152)	Automated	Low
Pinniped	California sea lion	5	53 (29–94)	Automated	High
Pinniped	Harbor seal	5	40 (25–57)	Automated	Medium

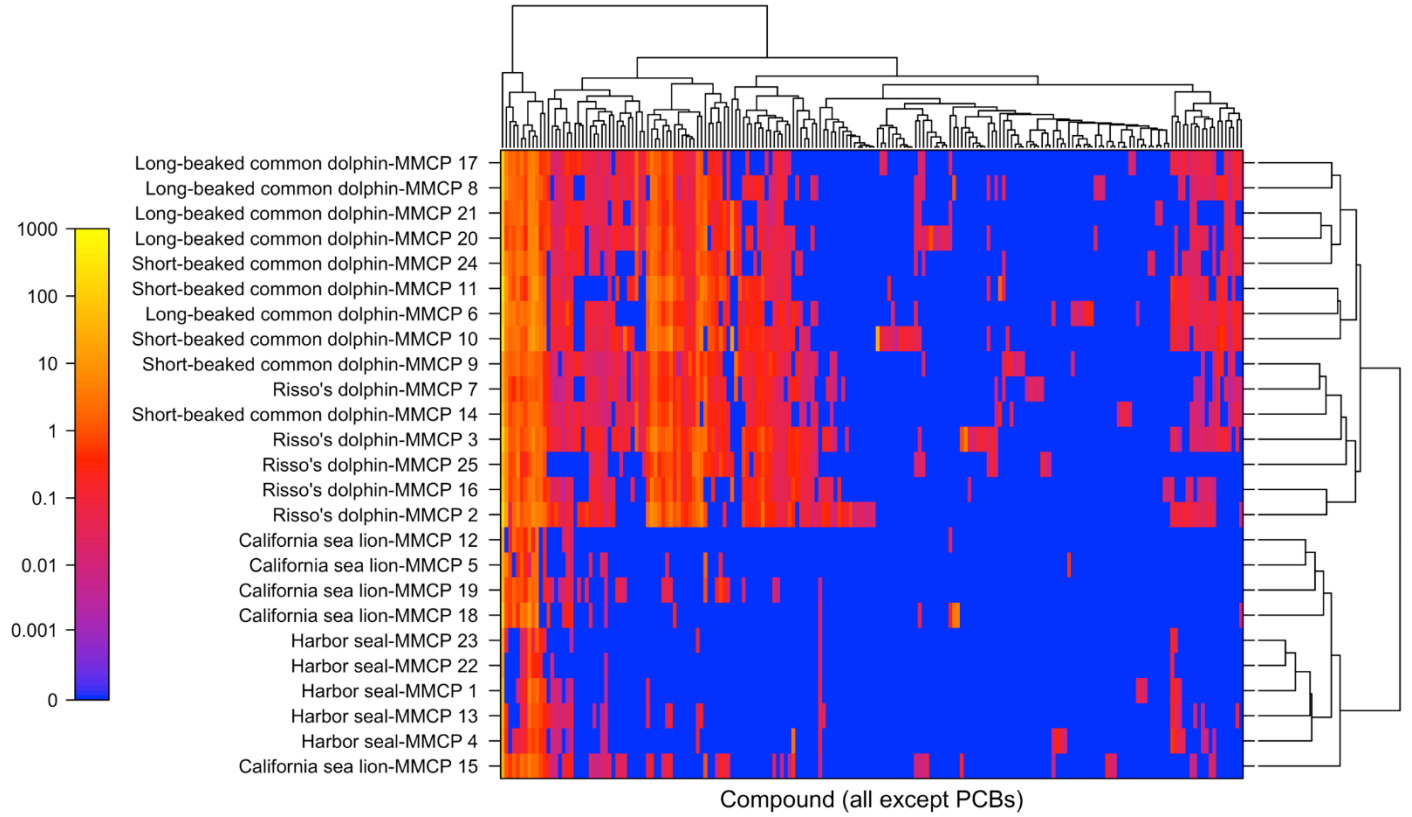
Cossaboon et al., under review

Cetacean vs Pinniped Profiles

Cossaboon et al. Chemosphere. 2019, 221, 656-664.



Example: Research Projects Using Non-Targeted Analysis



Novel Contaminants

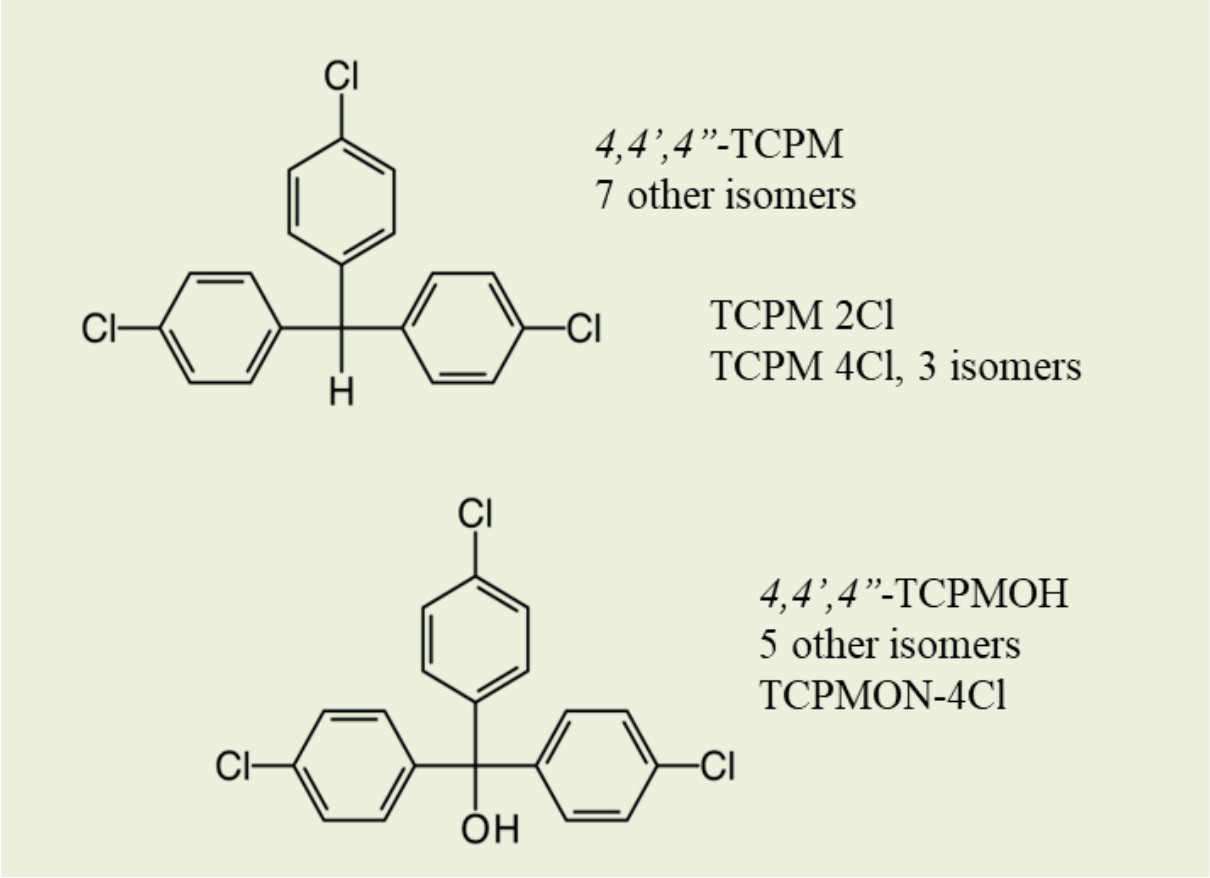
One of the goals is to determine if there are abundant contaminants that are not typically monitored.

What did we find?

Neglecting Important DDT-related Compounds?

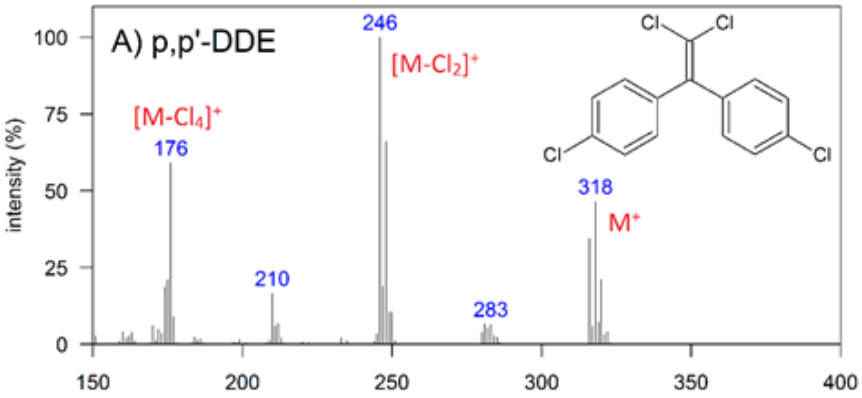
Mackintosh et al. Environ. Sci. Technol. 2016, 50, 12129-12137.

Class	No. Cmpds.	Source	No. Not Monitored
DDT-related	23	Anthropogenic	15-17
Tris(chlorophenyl)methane (TCPM)	12	Anthropogenic	12
TCPMOH	7	Anthropogenic	7
Hexa to octa-chlorinated diphenylethylene	8	Unknown	8

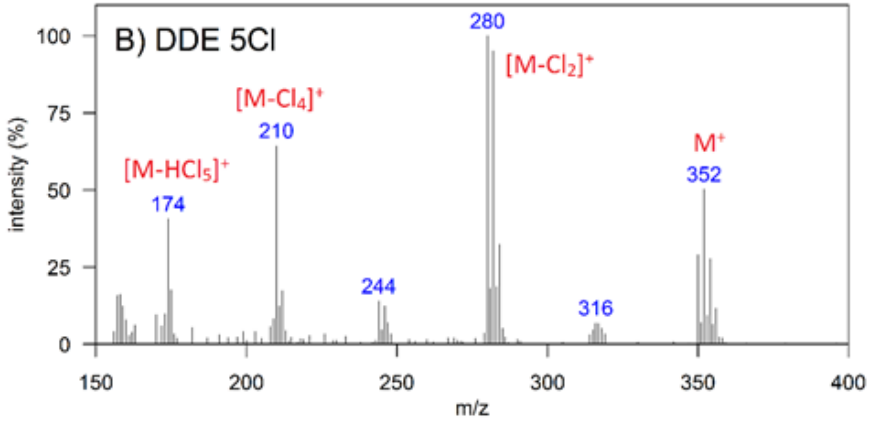


Example: Identification of unknown compounds

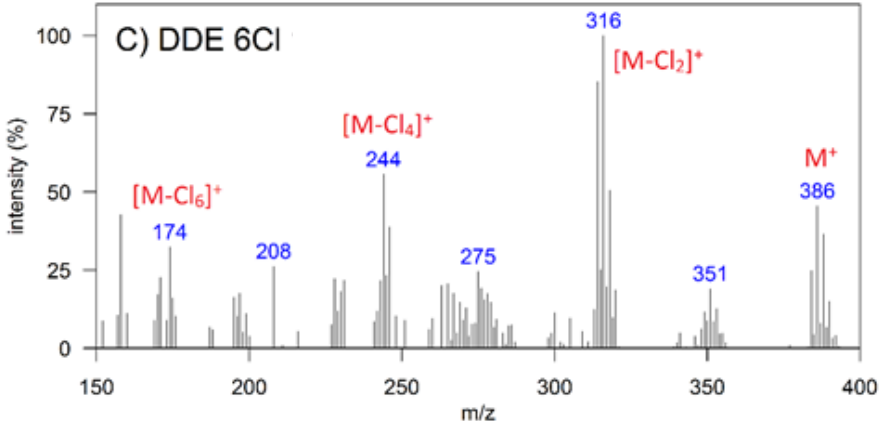
DDE Standard (4 chlorines)



Penta-chlorinated diphenylethene observed in DDT technical mixture



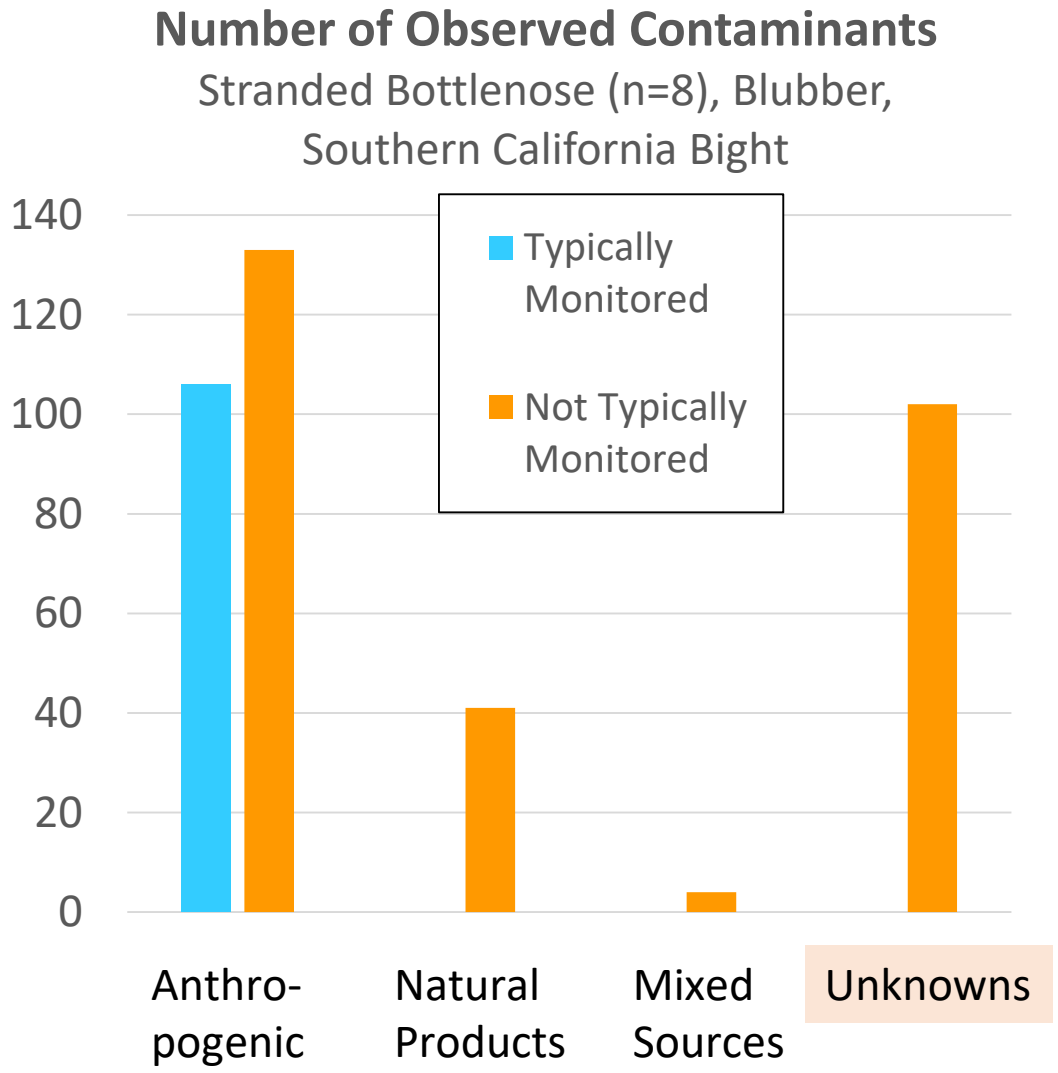
Potential hexa- to octa-chlorinated diphenylethenes observed in southern California bottlenose dolphin blubber



Mackintosh et al., Environ Sci Technol., 2016.

Halogenated Unknowns

- 86% not typically monitored
- 61% not in standard mass spectral databases
- We need to keep track of halogenated unknowns



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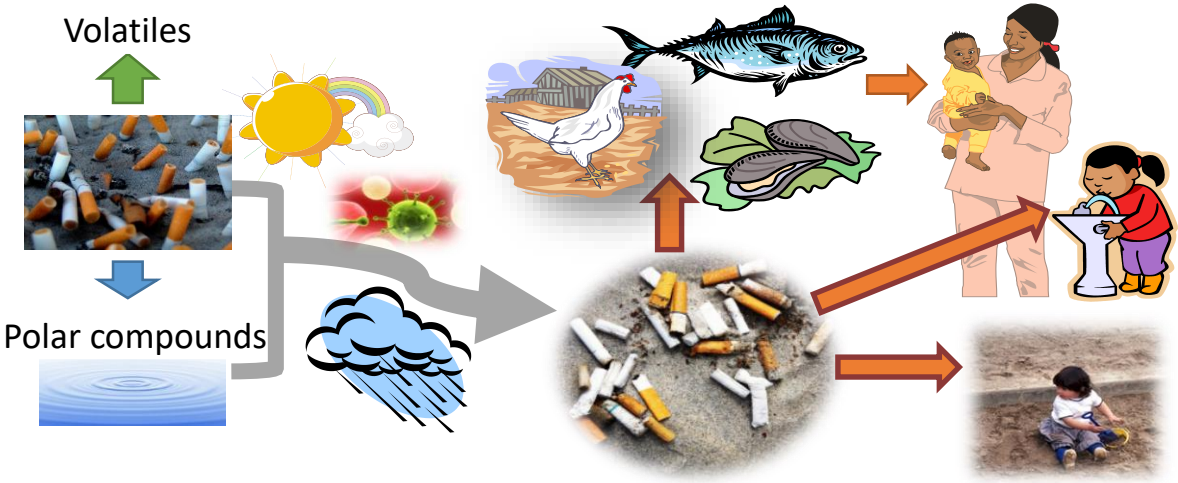
Smoked Cigarette Waste

- Despite the intensive studies on tobacco and tobacco smoke, not many studies conduct the experiment on the littler of cigarette
- In 2016, 5.5 trillion cigarettes were sold globally; 246 billion cigarettes were sold in the US alone
- The U.S. alone collected 1,030,640 cigarette butts in 2016 coastal cleanup and cigarette butts accounted for 37.7% of visible litter in the U.S. during the coastal cleanup event
  - 55.7% of smoker admitted disposing of cigarette butts on the ground or waterway in the past months
  - About 76% of cigarettes smoked in public urban area were littered and then possible transported by the urban runoff to the nearby water bodies

Risks are not recognized

- About 1.4~2.2 mg of chemical compounds were released during one session of cigarette
- When cigarette filters soaking in the water, 50% of elution happened in the first 27 mins and could release up to 7.3 mg/g of nicotine relate compounds
- Cigarette filters showed a really slow decomposition rate
- It can continuously release chemical compounds into the area where its not regularly removed. The quality of the water is question in those area and fate of the release chemicals in the aquatic environment is unknown.



Rainbow Trout Bioaccumulation Bioassay

Rainbow Trout 28-Day Cigarette Butt Leachate Bioaccumulation Definitive Test

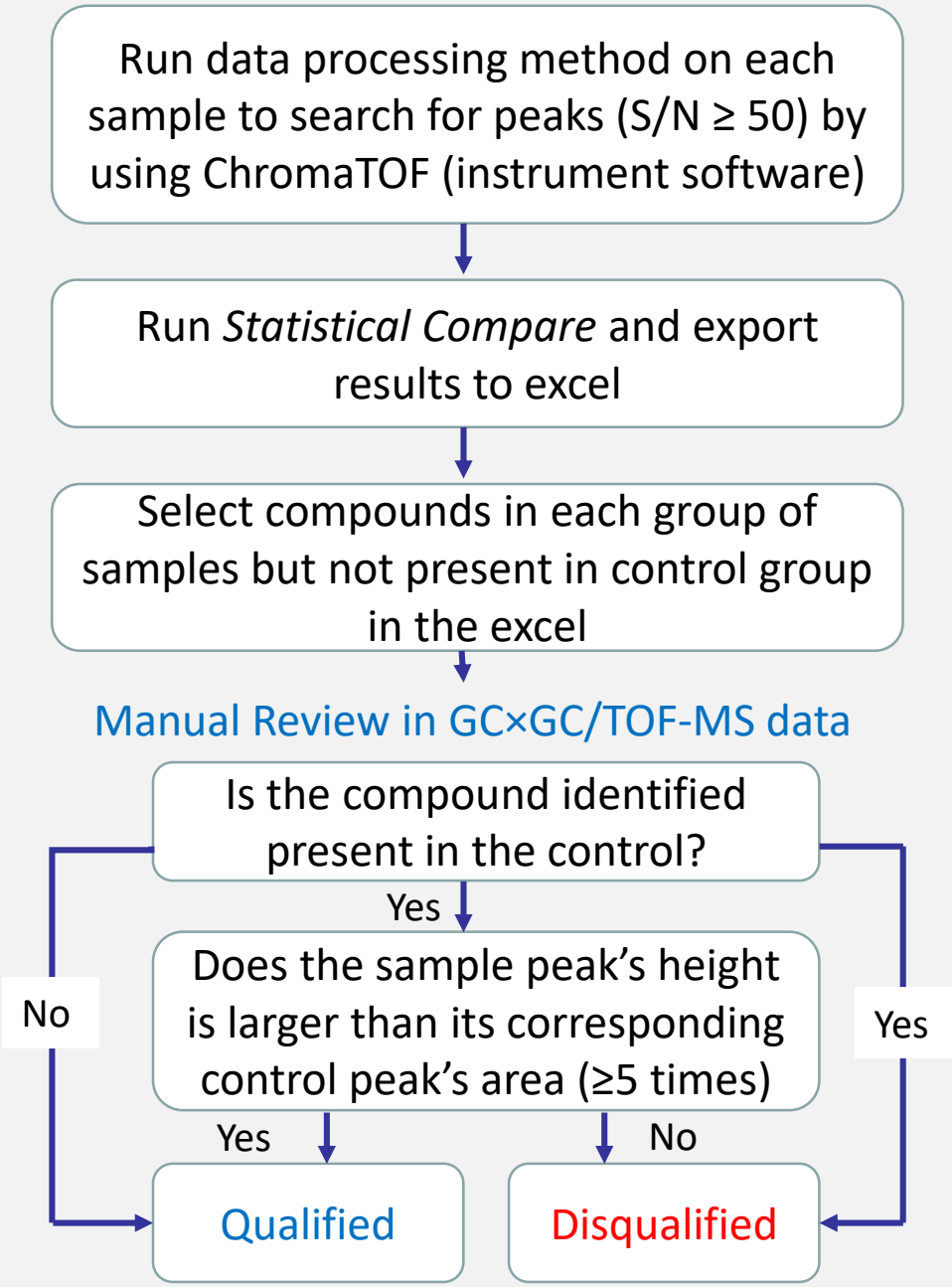
Leachate Concentration (CB/L)	Replicate	Number of 28-day Surviving Organisms	Number of Surviving Organisms (Post-depuration)	Total Wet Weight (g)	Individual Wet Weight (g)	Average Individual Wet Weight (g)	Standard Deviation
0 (Lab Control)	A	13	13	12.1	0.931		
	B	14	14	12.7	0.909	0.880	0.069
	C	15	15	12.0	0.801		
0.5	A	14	14	10.6	0.754		
	B	15	15	11.3	0.754	0.745	0.016
	C	14	14	10.2	0.727		

5 g of homogenized fish tissues in each tank was extracted by ethyl acetate, and then followed by gel permeation chromatography (GPC) to remove lipids. Chemical analysis is done by non-targeted analysis based on GC×GC/TOF-MS.

Non-Targeted Analysis: Data Analysis

Statistical Compare is a post data processing software package for sets of complex GC×GC/TOF-MS data that facilitates data mining of peak tables through generation and comparison of statistical information between analytes from different classes containing multiple samples (analyte alignment - GC×GC retention times and mass spectral match across samples)

Samples	Control
Fish exposure to 0.5 CB/L (n=3)	Fish no exposure to cigarette butt leachate (n=3)



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