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Chemical Characterization of Indoor Dust by Comprehensive Target and Non-Target Screening Using GC- and LC-QTOF-MS/MS

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Motivation – Why Indoor Dust?

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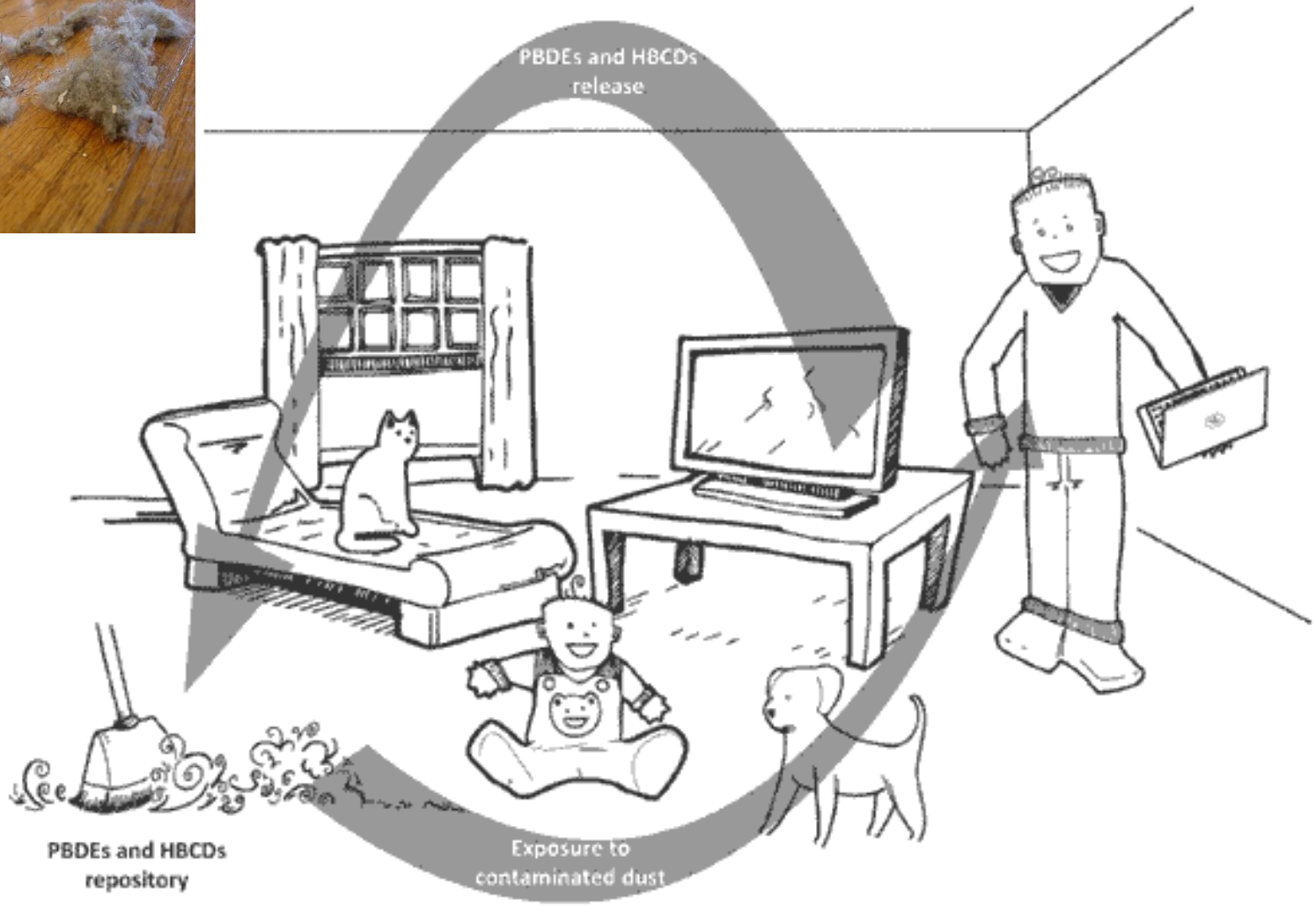

- Human Exposure
  - Inhalation
  - Ingestion (toddlers)
- Human Health Impacts?

Pictures: http://benthamscience.com, https://www.acs.org, http://money.aol.co.uk

What is in Our Dust?

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Flame Retardants
BDEs + OP-FRs

Plasticizer,
Phthalates

Parabens +
Biocides

Surfactants +
Cleaning Agents

Pesticides +
Flea Control

Skin Oils

→Everything used in and around the homes **= <u>lots of "unknowns"</u>**

<u>Helpful Databases:</u>

Chemical Inventory of Consumer Products (CPCP): "Walmart Database"

→ Goldsmith et al., Food Chem Toxicol, 2014. 65: p. 269-279

EPA DSS Tox Database (>100,000 chemicals) → www.epa.gov

Pictures: www.tierneycyanne.com, www.wiu.edu, www.agein.com, www.weiku.com, http://slsfree.net, http://healthyhints.com.au, http://unique-cleaning-tips.blogspot.com, www.bunnings.com.au, www.lawn-care-academy.com

Goals and Approach

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Goals:

- determine known and unknown chemicals linked to different household sources
- compare chemical fingerprint of different household groups

Approach

- Combined Target, Suspect and Non-Target Screening using high-resolution mass spectrometry
- Comprehensive analysis of 10-15 samples of each group of households
  - kids with autism
  - kids with asthma
  - healthy kids

Developed Analytical Method

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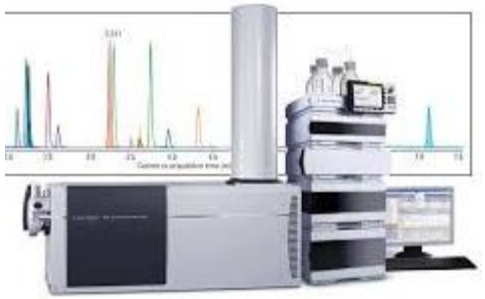
Sonication Extraction
- hexane: acetone
- acetone

surrogates spike

↓

Evaporation and Filtration



solvent exchange
ISTD addition ↙

ISTD addition ↘

LC-QTOF-MS

Agilent 6530

Zorbax Eclipse Plus C 18
ESI+: H_2O+FA / ACN+FA
ESI-: H_2O+NH_4F / ACN
Data Acquisition:
All-Ion CE 0, 10, 20, 40 (target + suspect)
Full scan MS (non-target)

GC-QTOF-MS

Agilent 7200B

HP-5MS (30m x 0.25mm, 025µm)
Temp gradient: linear increase 35-325°C in 80 min
Data Acquisition:
EI (target + non-target)

Pictures: www.agilent.com

Validation Target Method

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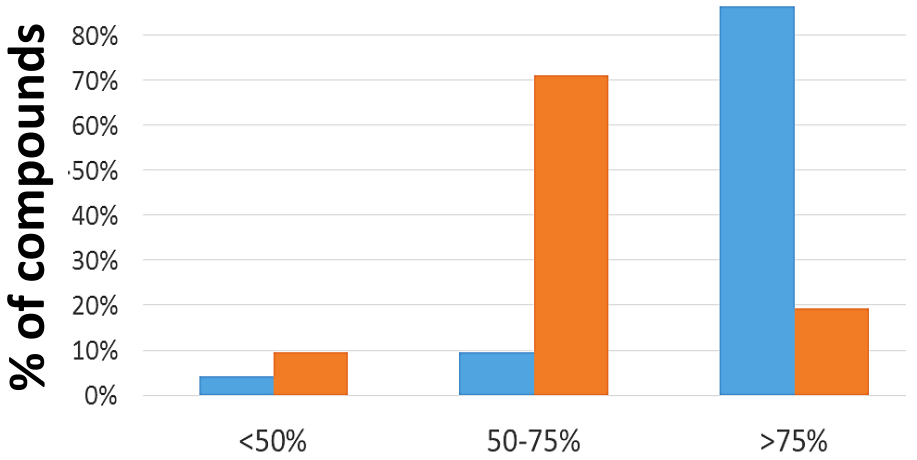
- 77 targets GC-TOF-MS
  - e.g. BDEs, phthalates, PAHs, pyrethroids
- 56 targets LC-TOF-MS
  - e.g. parabens, PFCs, surfactants

- Quality Control:
  - GC-TOF-MS: 9 surrogates, 1 ISTD
  - LC-TOF-MS: 1 surrogate, 9 ISTDs
  - Method-Blanks ("Min-U-Sil"), triplicate per sequence
  - NIST SRM 2585, triplicate per sequence → 11 out of 14 compounds within 25% accuracy
  - Spike recovery experiments, triplicate per sequence
  - Precision of triplicates → 95% of compounds with <20% coefficient of variation



Absolute Recovery (blue: GC-MS, orange: LC-MS)

Method Detection Limits of Targets		
MDL (ng/g dust)	**GC-QTOF-MS**	**LC-QTOF-MS**
>0.1 - 1	1	6
>1 - 10	23	30
>10 - 100	36	10
>100 - 1000	12	10
>1000	5	0

Target Results: # Detected Compounds

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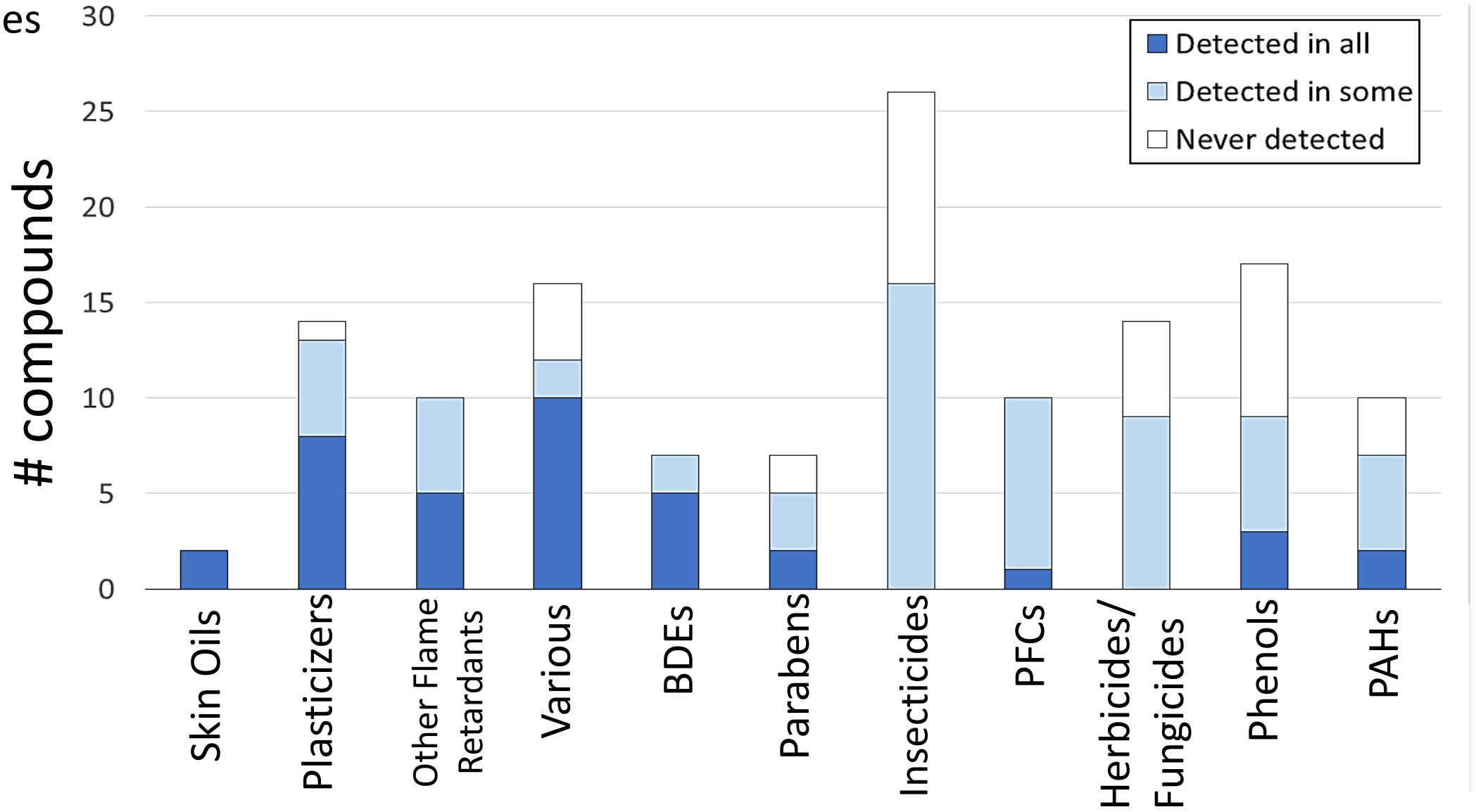
N= 18 samples



Suspect Screening

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– Example NORMAN Collaborative Trial

- NORMAN: European platform for information exchange and harmonization of analysis of emerging pollutants (www.norman-network.net)

- Non-Target Dust Collaborative Trial:
  - One dust sample sent out in January 2016 to roughly 30 research institutes (mainly Europe)
  - Extraction, analysis by LC-TOF-MS and GC-TOF-MS, results reported in June 2016 (http://www.normandata.eu/?q=node/27)

Suspect Screening: LC-QTOF-MS

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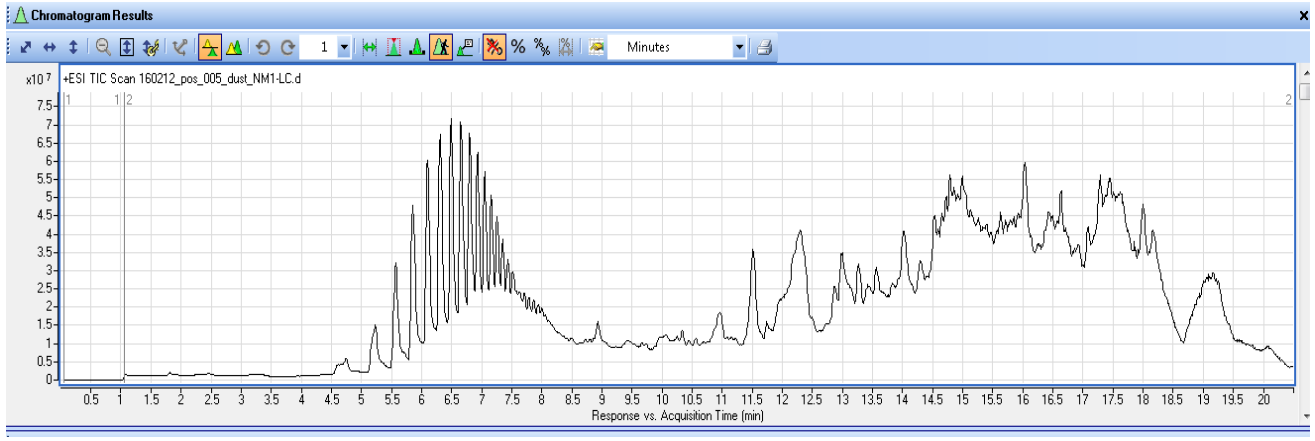


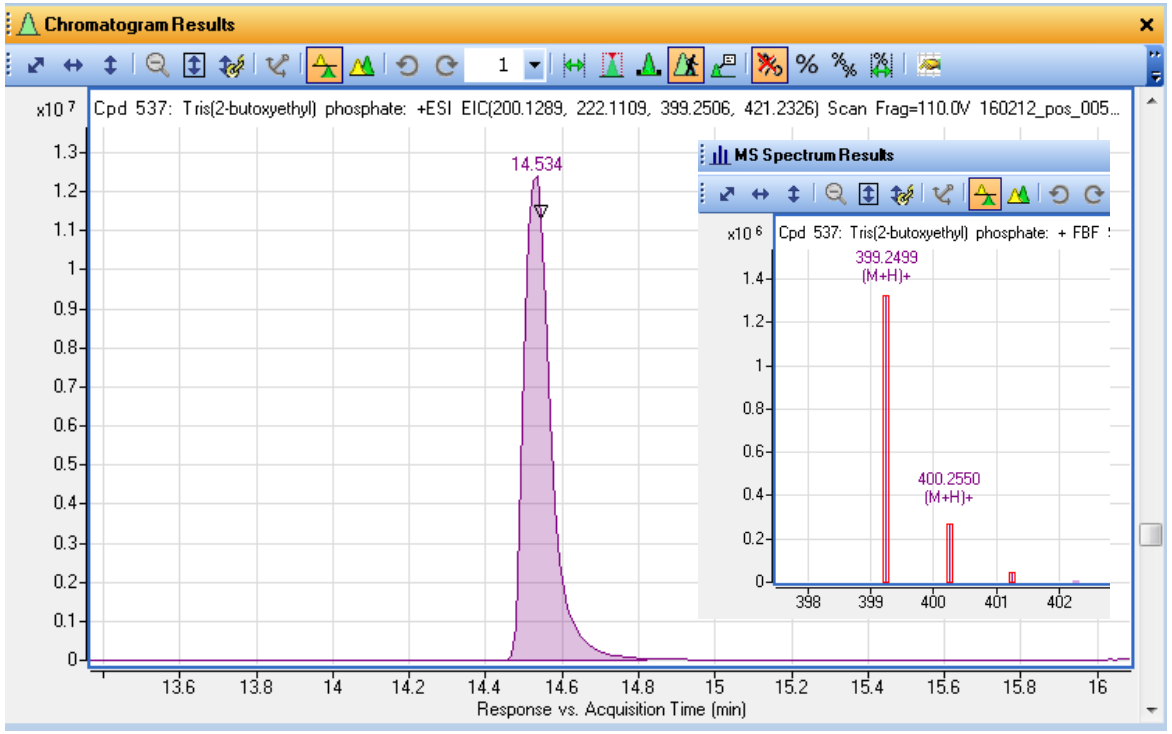
Total Ion Chromatogram of NORMAN dust sample (pos)

A) Use of Agilent MS/MS libraries:

- Forensic Tox PCDL (8000 compounds)
- Pesticide PCDL (1700 compounds)
- NIST PCDL (5600 compounds)

B) Find by Formula (MassHunter Qual) with Fragment Confirmation

- Example: Tris-butoxyethyl-phosphate
  - peak found: m/z 399.2506 @ RT 14.5 min
  - isotope pattern match (score 98) for formula $C_{18}H_{39}O_7P$



Suspect Screening: LC-QTOF-MS

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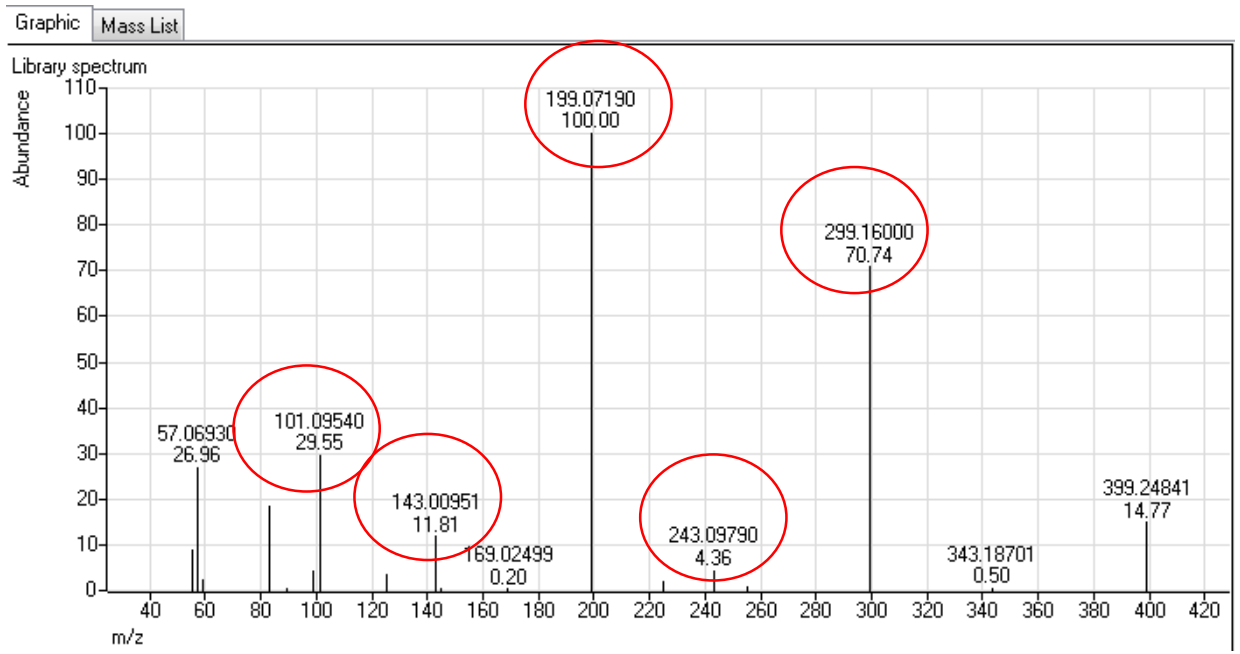
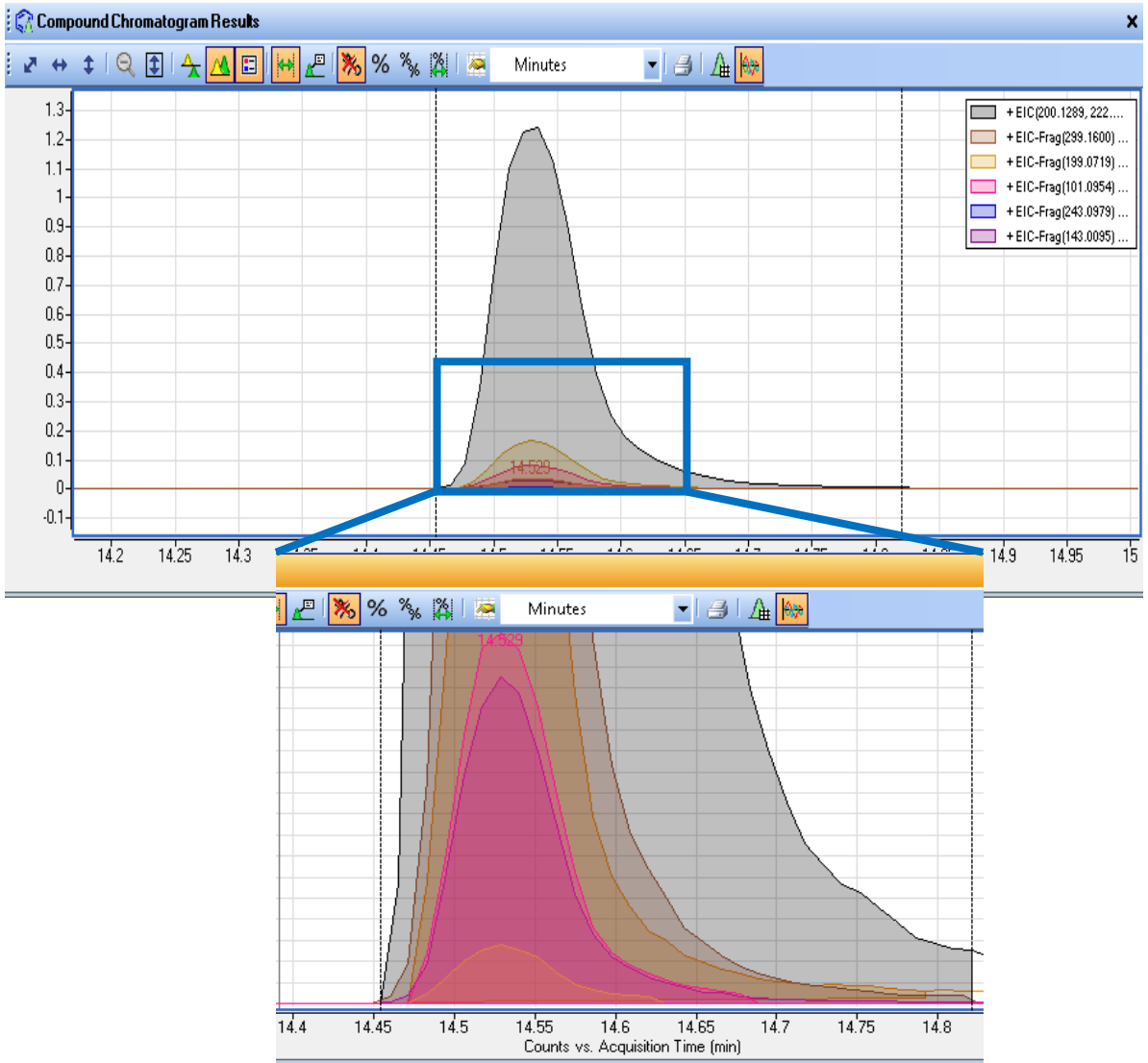
- Example: Tris-butoxyethyl-phosphate

Library spectra (CE 20):





→ 5 fragments confirmed by co-eluting peaks (level 2A*)

* Schymanski et al. (2014) Environ Sci Technol **48**(4): 2097-2098.

Non-Target Screening: LC-QTOF-MS

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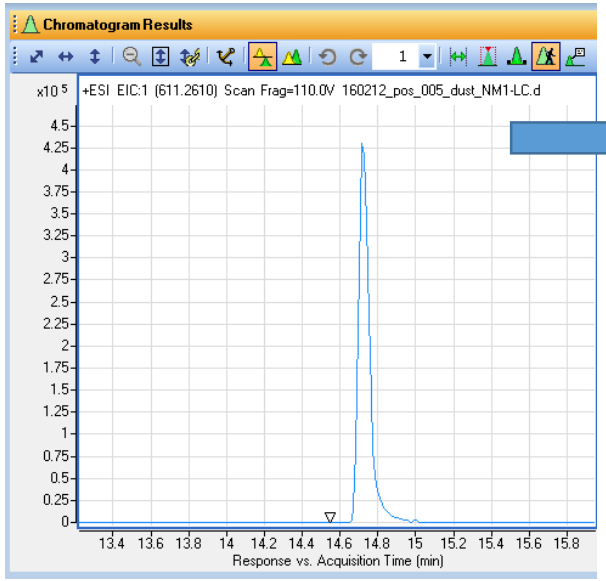
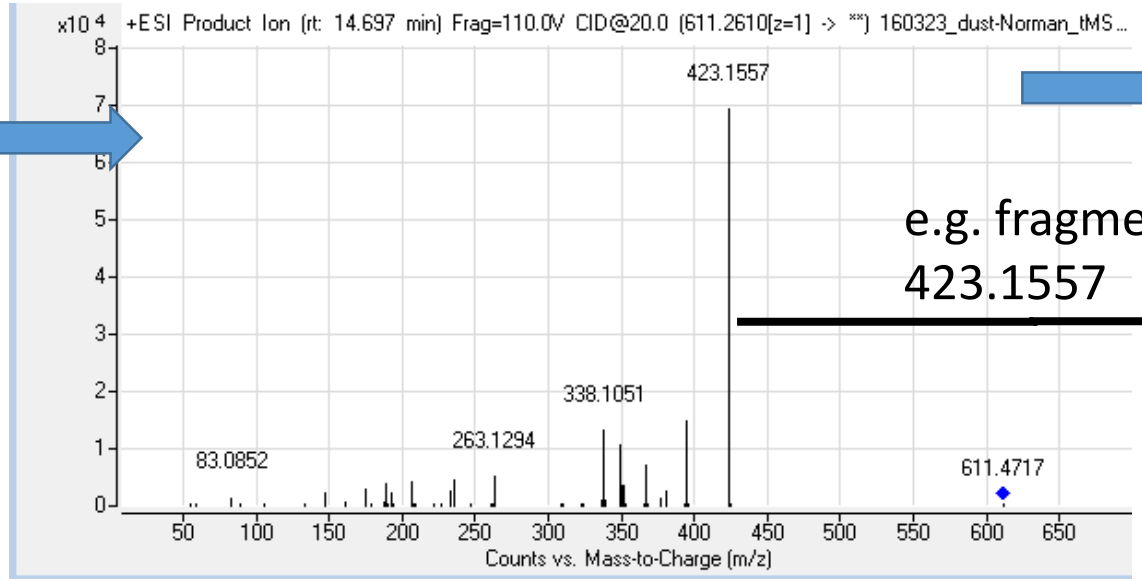
1. Recursive Feature Extraction (Agilent Profinder)
2. Blank Subtraction (Agilent MPP software) → 2300 true features remaining (pos and neg)
3. Re-run sample in targeted MS/MS mode → most intense features in inclusion list
4. Compare MS/MS spectra with in-silico fragmentation software, e.g. Agilent MSC or MetFrag (level 3 confirmation*) * Schymanski et al. (2014) Environ Sci Technol **48**(4): 2097-2098.

Example: Unknown Feature



Feature m/z 611.2610
@ RT 14.6

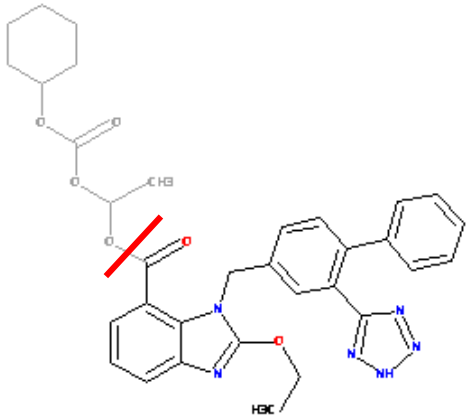


Acquired MS/MS
spectrum (CE 20)

e.g. fragment
423.1557

Chemspider Top Hit:
Candesartan cilexetil

MSC score 85.5
6 main fragments plausible



Non-Target Screening: GC-TOF-MS

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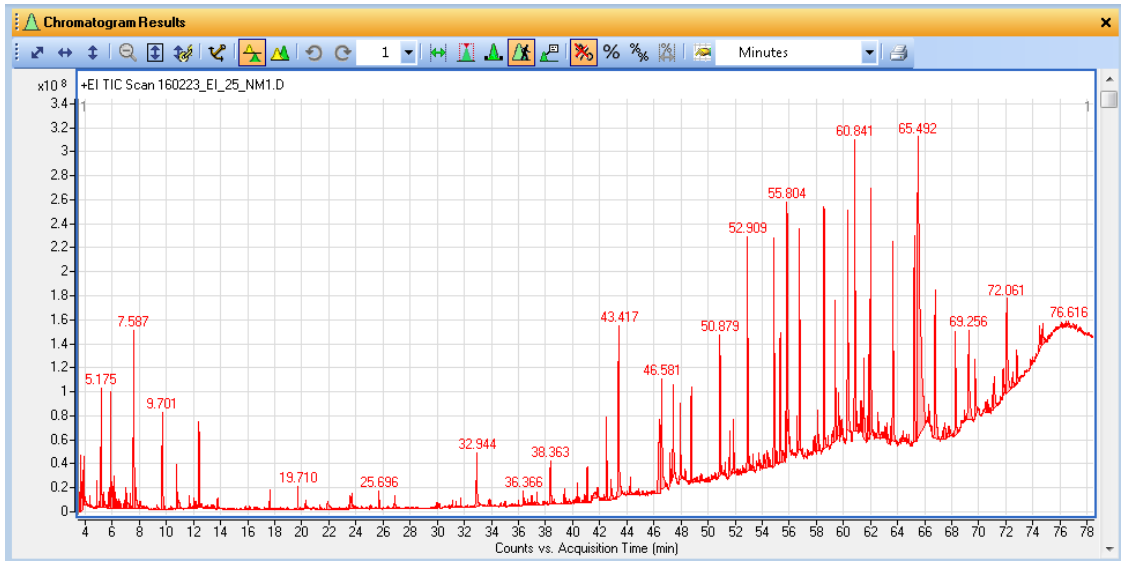
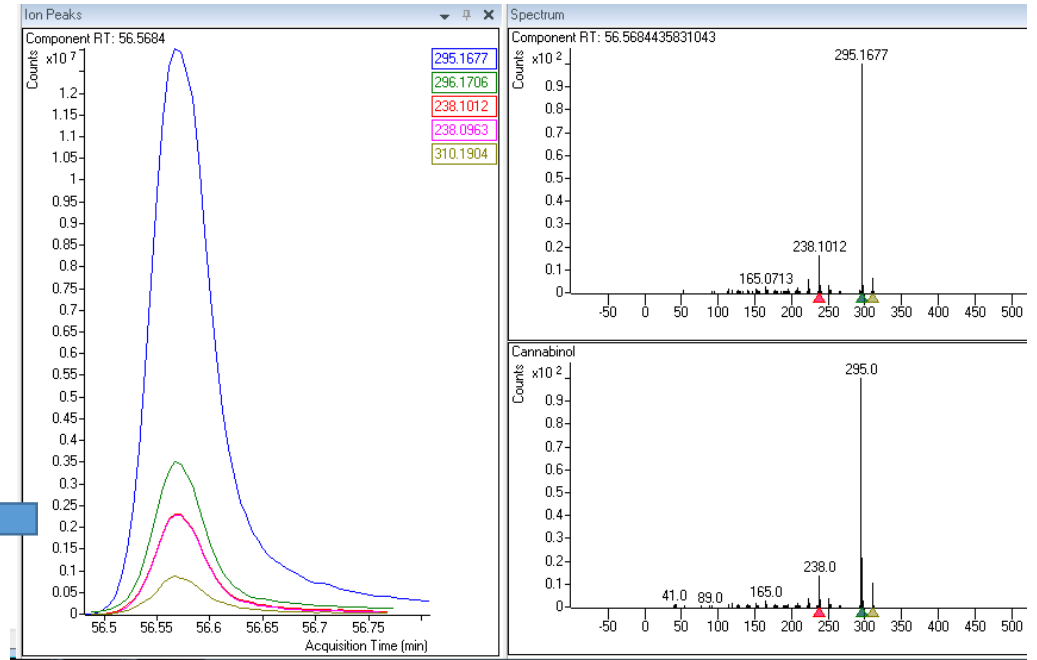
1. A) Agilent MassHunter Qual: *Find by Integration* (background subtraction)
   B) NIST library search
   C) Comparison of retention indexes (RI)
   → to get the big peaks out of TIC



2. A) *Agilent Unknown Analysis* → Spectral Deconvolution, Blank Subtraction
   B+C) NIST library search + RI comparison

<u>Example:</u> Cannabinol

- Library fragments match score: 94.5
- RI NIST: 2582, RI measured: 2591



Summary NORMAN dust sample

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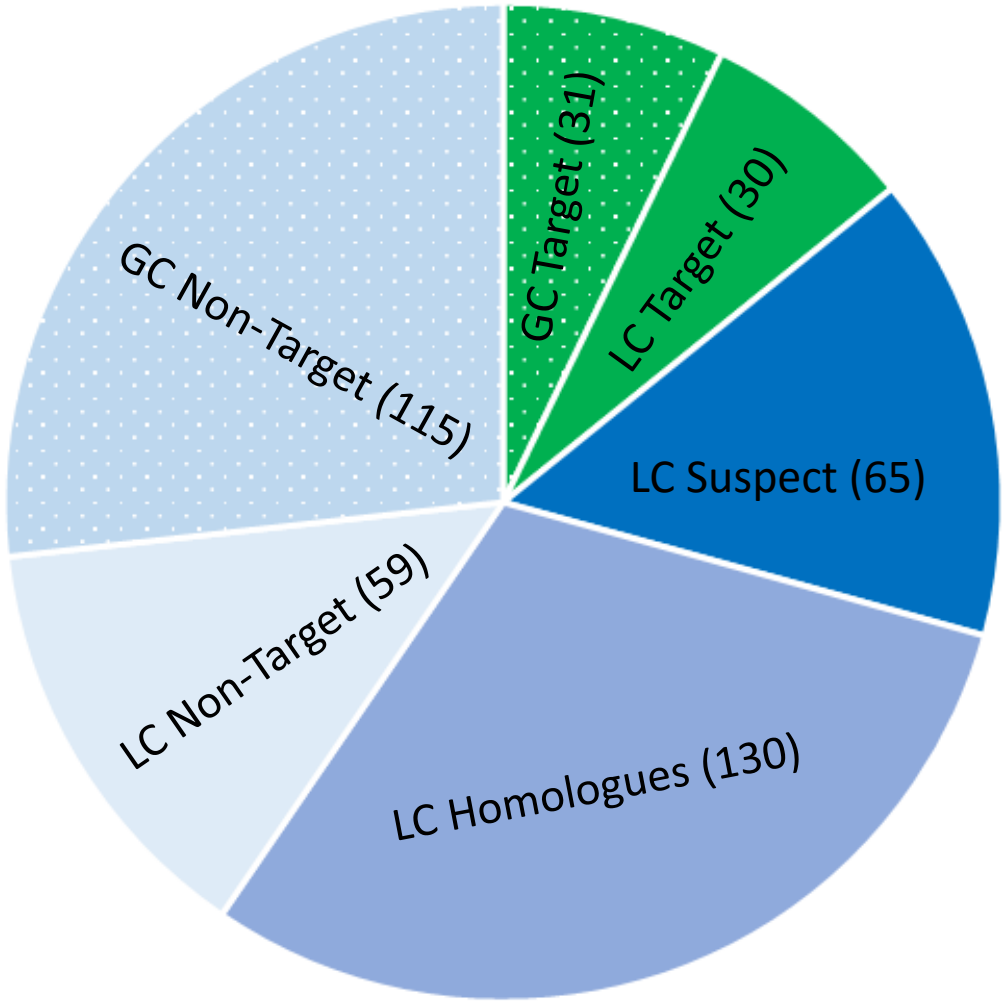

Non-Target Chemical Fingerprinting

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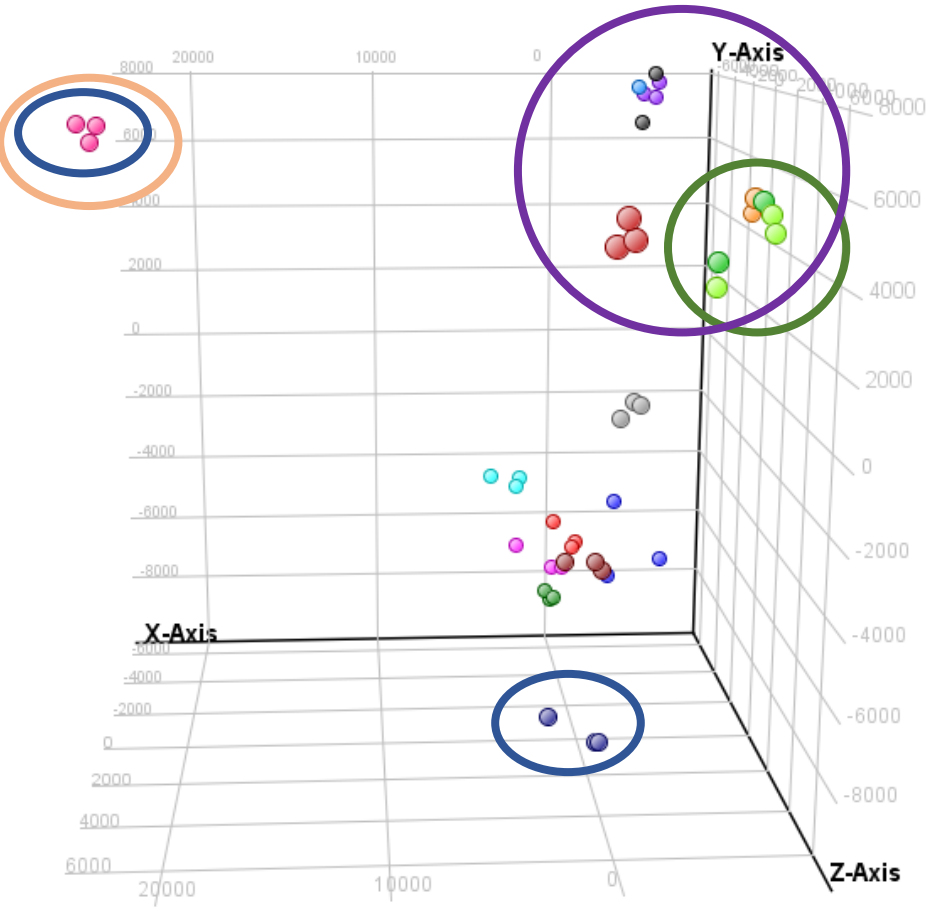
- Goal: find features that are different between household groups
- Approach: statistical evaluation of non-target features (Agilent MPP)

Principle Component Analysis (PCA)



Triplicate injection

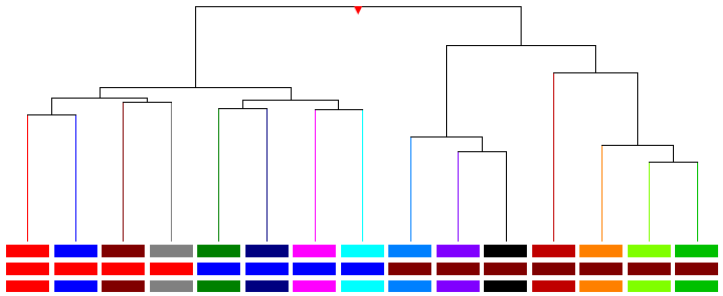
Extraction Replicate

Method blank

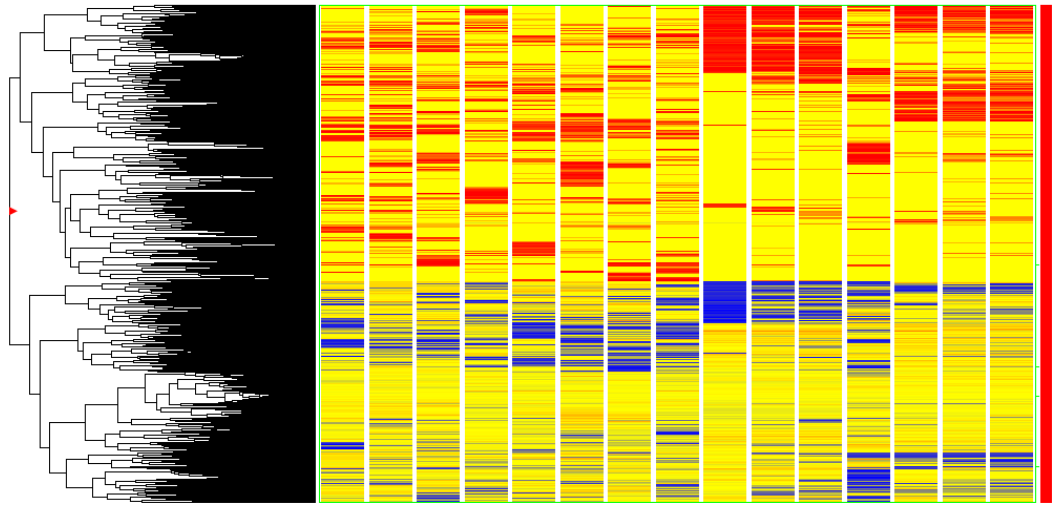
Similar Samples

Hierarchical Clustering

Samples that cluster together



Features that cluster together



Conclusions and Outlook

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- developed analytical method fast and reliable to detect hundreds of targets in household dust
- LC-QTOF-MS “All-Ions” workflow and spectral libraries very helpful for efficient identification of suspects
- non-target feature extraction and identification of features is labor intensive → prioritization strategies needed (e.g. cases vs. control)

- large number of contaminants in dust → potential health concern
- study will show if a linkage between chemical fingerprint and health output of children can be done

Acknowledgement

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- Prof. Deborah Bennett (PI, together with Prof. Thomas Young) and Dr. Hyeong-Moo Shin (co-PI), UC Davis Department of Public Health, for study design and development, Rebecca Moran for sample collection

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Target Results: PCA of Compounds

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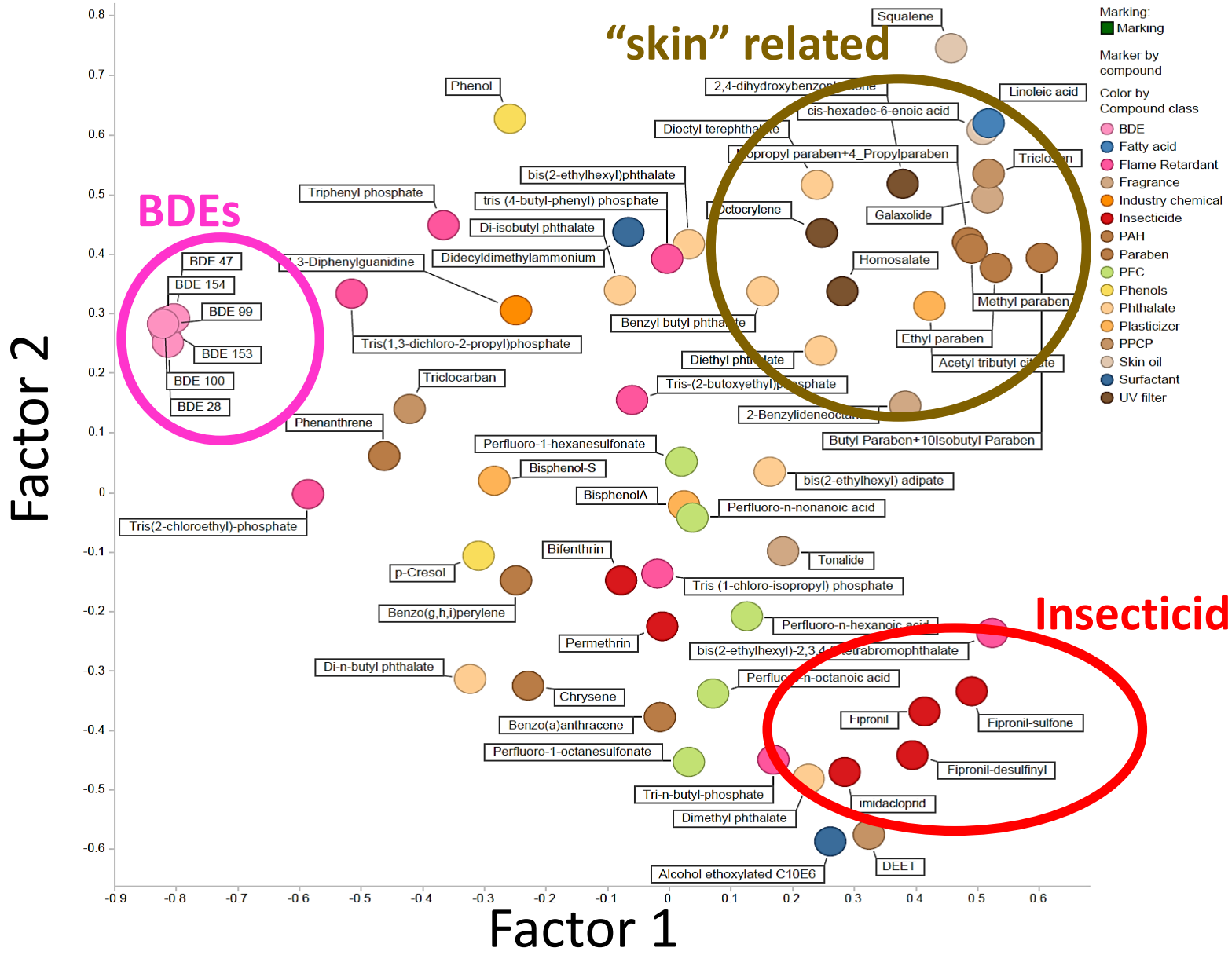

- PCA shows grouping of household sources in 18 household samples
- more samples needed to strengthen this hypothesis

Non-Target Screening: LC-QTOF-MS

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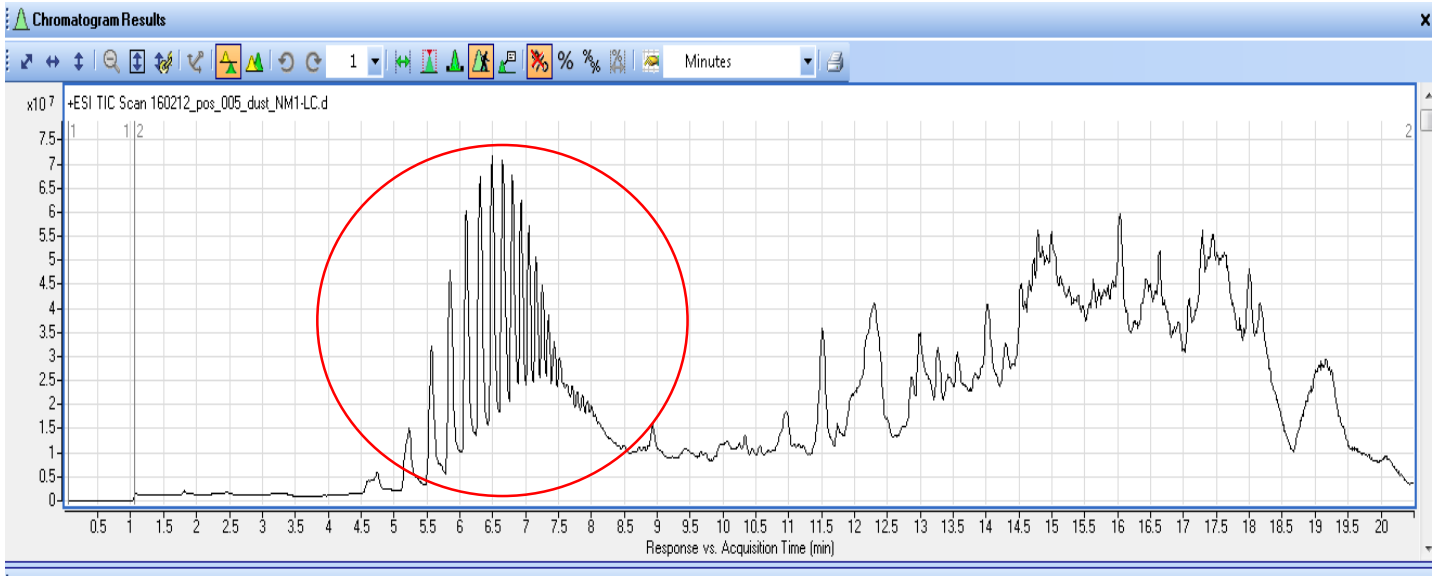
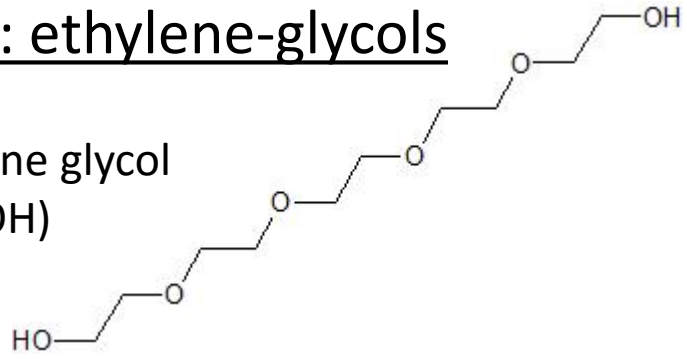


Homologues Series:

- polyethylene-glycols (PEGs)
- alcohol ethoxylates (AEOs)
- linear alkyl benzene sulfonates (LAS)
- alcohol ethoxysulfates (AES)
- ……

Example: ethylene-glycols

Pentaethylene glycol
(HO-PEG5-OH)



Open source software tool available:
http://www.envihomolog.eawag.ch/