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?

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

What is in Our Dust?

Flame Retardants
BDEs + OP-FRs

Plasticizer,
Phthalates

Parabens +
Biocides

Surfactants +
Cleaning Agents

Pesticides +
Flea Control

Skin Oils

Everything used in and around the homes = lots of “unknowns”

Helpful Databases:

Chemical Inventory of Consumer Products (CPCP): “Walmart Database”


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

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

**Sonication Extraction**
- hexane: acetone
- acetone

**Evaporation and Filtration**

**LC-QTOF-MS**
Agilent 6530
- Zorbax Eclipse Plus C 18
- ESI+: H₂O+FA / ACN+FA
- ESI-: H₂O+NH₄F / 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)

**Surrogates Spike**
- solvent exchange
- ISTD addition
Validation Target Method

- 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

### Method Detection Limits of Targets

<table>
<thead>
<tr>
<th>MDL (ng/g dust)</th>
<th>GC-QTOF-MS</th>
<th>LC-QTOF-MS</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;0.1 - 1</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>&gt;1 - 10</td>
<td>23</td>
<td>30</td>
</tr>
<tr>
<td>&gt;10 - 100</td>
<td>36</td>
<td>10</td>
</tr>
<tr>
<td>&gt;100 - 1000</td>
<td>12</td>
<td>10</td>
</tr>
<tr>
<td>&gt;1000</td>
<td>5</td>
<td>0</td>
</tr>
</tbody>
</table>

Absolute Recovery (blue: GC-MS, orange: LC-MS)
Target Results: # Detected Compounds

N = 18 samples
Suspect Screening

– 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

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

- Example: Tris-butoxyethyl-phosphate

Library spectra (CE 20):

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

Non-Target Screening: LC-QTOF-MS

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*)  


Example: Unknown Feature

Feature m/z 611.2610 @ RT 14.6

Acquired MS/MS spectrum (CE 20)

Chemspider Top Hit: Candesartan cilexetil
MSC score 85.5
6 main fragments plausible
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**

**Example:** Cannabinol

- Library fragments match score: 94.5
- RI NIST: 2582, RI measured: 2591
Summary NORMAN dust sample

- GC Non-Target (115)
- LC Non-Target (59)
- LC Target (30)
- GC Target (31)
- LC Suspect (65)
- LC Homologues (130)
Non-Target Chemical Fingerprinting

- 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

- 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 \(\rightarrow\) prioritization strategies needed (e.g. cases vs. control)
- large number of contaminants in dust \(\rightarrow\) potential health concern
- study will show if a linkage between chemical fingerprint and health output of children can be done
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Target Results: PCA of Compounds

- PCA shows grouping of household sources in 18 household samples
- More samples needed to strengthen this hypothesis
Non-Target Screening: LC-QTOF-MS

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/