Polar Constituents in Crumb Rubber Extracts

Presenter: Patty Wong, Ph.D., OEHHA
Non-Targeted Analysis of Polar Chemicals

• Most of the polar chemicals (especially those with high water solubility) are not suitable for GC/MS analysis

• Polar chemicals can be analyzed using LC/MS
  • LC/MS results will compliment GC/MS results to provide comprehensive analysis of field samples
  • Develop a 2-Tiered Non-Targeted Approach to analyze LC/MS data
  • Applying advanced computational tools may improve the success of identifying candidates for unknowns.
Tier 1: Suspect Screening Analysis – Identify chemicals for testing all fields

- **Suspect Screening Analysis**
  - Identify chemicals for testing all fields

- **Established Databases**
  - US EPA DSSTox Database
  - OEHHA Tire-Related Chemical List

- **LTQ-Orbitrap LC/MS**
  - Verify with Reference Standards

- **Targeted Chemicals**
  - 1,3-Dicyclohexylurea

- **Tentative Chemicals**
  - 1,3-Dicyclohexylurea

- **Compounds**
  - Molecular ion m/z = 225.1967
  - Monoisotopic Mass = 224.1888
  - Truncated MW = 224.18

- **Export Parent Chemicals**
  - that Generate Fragment of m/z=225.1967

- **Purchase**
  - Dicyclohexylurea

- **Verify with Reference Standards**

- **Search ± 5ppm**
  - Monoisotopic Mass = 224.1888

- **Truncated MW**
  - = 224.18
## Tier 1. Suspect Screening Results of Manufacturer Samples – Database Search Using Molecular Mass (from MS1) of LC Peaks

<table>
<thead>
<tr>
<th>Database</th>
<th>Screening Method/Software</th>
<th>No. of Chemicals in the Database</th>
<th>No. of Possible Chemicals</th>
<th>Chemical Purchased</th>
</tr>
</thead>
<tbody>
<tr>
<td>ChemSpider</td>
<td>Compound Discoverer™</td>
<td>72 million chemical structures</td>
<td>743,492</td>
<td>-</td>
</tr>
<tr>
<td>Distributed Structure-Searchable Toxicity (DSSTox)</td>
<td>Web-Based Batch Search</td>
<td>&gt;850,000</td>
<td>&gt;80,000</td>
<td>-</td>
</tr>
<tr>
<td>OEHHA Tire-Related Chemicals List*</td>
<td>Excel</td>
<td>754</td>
<td>250</td>
<td>27</td>
</tr>
</tbody>
</table>

* A collection of tire-related chemicals reported in the literature and federal studies.

3 possible methods of analysis:
1. GC/MS with chemical-specific detection method
2. LC/MS with positive ionization
3. LC/MS with negative mode
18 Tire-Related Targets Confirmed by Reference Standards*

<table>
<thead>
<tr>
<th>Chemical Name</th>
<th>Formula</th>
<th>Chemical Name</th>
<th>Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,3-Benzothiazole-2-thiol</td>
<td>C7H5NS2</td>
<td>13-cis-Retinoic acid</td>
<td>C20H28O2</td>
</tr>
<tr>
<td>2-(Methylthio)benzothiazole</td>
<td>C8H7NS2</td>
<td>Linoleic acid</td>
<td>C18H32O2</td>
</tr>
<tr>
<td>Benzothiazolone</td>
<td>C7H5NOS</td>
<td>Oleic acid</td>
<td>C18H34O2</td>
</tr>
<tr>
<td>Benzothiazole</td>
<td>C7H5NS</td>
<td>3,5-Di-tert-butyl-4-hydroxybenzaldehyde</td>
<td>C15H22O2</td>
</tr>
<tr>
<td>1,3-Diphenylguanidine</td>
<td>C13H13N3</td>
<td>Phenoxyazine</td>
<td>C12H9NO</td>
</tr>
<tr>
<td>N,N-Diethyl-meta-toluamide (DEET)</td>
<td>C12H17NO</td>
<td>1,3-Benzothiazol-2-amine</td>
<td>C7H6N2S</td>
</tr>
<tr>
<td>N,N'-Dicyclohexylurea</td>
<td>C13H24N2O</td>
<td>N-Cyclohexyl-N-methylcyclohexanamine</td>
<td>C13H25N</td>
</tr>
<tr>
<td>N-Cyclohexylformamide</td>
<td>C7H13NO</td>
<td>N,N'-Diphenyl-p-phenylenediamine - 2H</td>
<td>C18H14N2</td>
</tr>
<tr>
<td>Diphenylurea</td>
<td>C13H12N2O</td>
<td>Dicyclohexylamine</td>
<td>C12H23N</td>
</tr>
</tbody>
</table>

*Chemicals were analyzed by LC/MS with positive ionization mode.
Tier 2: Non-Targeted Analysis with the Aid of Cheminformatics
e.g., 1,3-Dicyclohexylurea

Targeted Chemicals:
1. N,N’-Dicyclohexylurea
2. Cuscohygrine

Verify with Reference Standards

Purchase Dicyclohexylurea

Tentative Chemicals:
1. N,N’-Dicyclohexylurea
2. Cuscohygrine

Cheminformatics Tools Prediction

LTQ-Orbitrap LC/MS

Polar Extract

[\text{M+H}]^+ = 225.1961
\text{RT: 31.38 m/z}
\text{Rel Int:}
\begin{align*}
83.2686 & = 4.53 \\
91.0791 & = 0.18 \\
100.1413 & = 100 \\
101.2622 & = 6.19 \\
125.1556 & = 0.23 \\
143.2128 & = 8.19 \\
144.2437 & = 0.72 \\
147.0886 & = 0.35 \\
148.2509 & = 0.17 \\
161.8341 & = 0.29 \\
177.0316 & = 0.39 \\
179.8776 & = 0.16 \\
180.9882 & = 0.20 \\
183.171 & = 0.18 \\
192.5676 & = 0.24 \\
195.2134 & = 0.21 \\
197.2946 & = 0.24 \\
205.1281 & = 0.3 \\
206.8469 & = 0.18 \\
208.0789 & = 0.24
\end{align*}
Cheminformatic Algorithms

• MetFrag (Mass Spectroscopy and Bioinformatics, Bioinformatics Center, Gatersleben-Halle, Germany)

• Global Natural Products Social Molecular Networking (GNPS, UCSD)

• XCMS (The Scripps Research Institute, La Jolla, CA)

• **Compound Discoverer™** (version 3.0, ThermoFisher Scientific, Waltham, MA)

• **Competitive Fragmentation Modeling-ID** (CFM-ID, version 3.0, Wishart Research Group, University of Alberta, Edmonton, Canada). Trained and searched 11 diverse database: CASMI2016, ContaminantDB, DrugBank, FiehnLib, HMDB, KEGG, MassBank, MetaboBASE, NIST, PhytoHub, iTree
Algorithm Validation Using 18 Standard References

Validation Steps:
1. Analysis standard reference using HRAM-LC/MS
2. Extract M2 data
3. Input data into each algorithm for chemical matching
4. Check if the standard chemical is matched as top candidate

<table>
<thead>
<tr>
<th>Algorithm</th>
<th>Position of Correctly Matched Chemical</th>
<th>No. of Correctly Matched Candidate</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFM-ID</td>
<td>Top 1</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Top 2</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Not on the candidate list</td>
<td>5</td>
</tr>
<tr>
<td>MetFrag</td>
<td>Not on the top 10 candidate</td>
<td>0</td>
</tr>
</tbody>
</table>
High-Resolution Accurate-Mass (HRAM)-LC/MS Data Applied to Tier 2 Analysis Using Cheminformatics (CFM-ID) Tool
Manufacturer Sample (MS1 Spectrum)

2D-LC Chromatogram

3D-LC/MS Chromatogram
LC Chromatogram:
Manufacturer Sample vs. Field Composite 1

May 31, 2019
OEHHA Synthetic Turf Scientific Advisory Panel Meeting
3-D LC/MS Chromatogram (MS1)

Field Composite 1

- 3-D chromatogram shows most peaks are resolved
- Drastic difference between field and manufacturer samples
Positive vs. Negative Ionization Mode

- Spectra generated under positive and negative ionization look different!
- Spectra between samples look different!
3-D LC/MS Chromatograms from Positive & Negative Ionization Modes

Field Composite 2
Chemical Analysis Results So Far: Chemicals to be tested in all field samples

<table>
<thead>
<tr>
<th>Chemical Class/Matrix</th>
<th>Instrumental Analysis</th>
<th>No. of Confirmed Chemicals*</th>
<th>No. of Tentatively Identified Chemicals†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polar Extract</td>
<td>LC/MS</td>
<td>18</td>
<td>47 (ongoing)</td>
</tr>
<tr>
<td>Non-Polar Extract</td>
<td>GC/MS</td>
<td>32</td>
<td>182</td>
</tr>
<tr>
<td>PAHs</td>
<td>SIM-GC/MS</td>
<td>20</td>
<td>182</td>
</tr>
<tr>
<td>Aldehydes/Ketones in Field Air</td>
<td>HPLC</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>VOCs in Field Air</td>
<td>GC/MS</td>
<td>67</td>
<td>0</td>
</tr>
<tr>
<td>This cell was purposefully left empty</td>
<td>Total</td>
<td>122 (unique chemicals)</td>
<td>228 (unique chemicals)</td>
</tr>
</tbody>
</table>

*Some of these chemicals were detected in multiple matrices or by different instruments.

#Chemicals tentatively identified in polar extracts analyzed by HR-AM LC/MS under positive ionization mode. Data collected under negative ion mode are being analyzed.

†One chemical was detected in both polar and non-polar extracts of crumb rubber.
Questions for Discussion

1. Do you have any comments or concerns on the general approaches for the non-targeted analysis?

2. Do you agree with the approach of the non-targeted analysis for the Polar Extracts? Do you have any comments or concerns?

3. We are doing the non-targeted analysis in both ionization modes. Do you have any comments on this approach?

4. Do have any comments regarding the following factors to prioritize tentatively identified chemicals? Any additions or suggestions? Do you have any suggestions on how to weigh these factors?

   Factors to Prioritize Chemicals for Confirmation Process:
   - Chemicals with Toxicity Criteria
   - Chemicals associated with Tall Peaks
   - Chemicals known to be Tire Related
   - Chemicals detected in multiple samples (manufacturer’s sample and field composite samples)
   - Others?

5. Any additional comments?