Comparison of biomarkers for use in assessing woodsmoke exposure among wildland firefighters

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Exposure and health effects

• 70-80,000 workers involved in wildland firefighting annually \cite{Harrison}
• Woodsmoke is complex mixture
  – Hundreds of chemicals (PAHs, aldehydes, etc.)
• Possible respiratory health effects \cite{Naeher}
  – Asthma, infections, lung cancer, COPD
• 40\% of firefighter medical problems during ‘88 Yellowstone fires respiratory \cite{Naeher}
Woodsmoke exposure assessment

• Difficult among wildland firefighters
  – Highly transient
    • Area level personal exposure
  – Spatial/temporal variation
  – Irregular shifts, conditions

• Previous air measurements
  – Particulate matter ($PM_{10}$, $PM_{3.5}$) (Reinhardt and Ottmar, 2004)
  – Carbon monoxide (CO) (Reinhardt and Ottmar, 2004)
  – Methoxyphenols (MPs) (Dills et al, 2006; Dills et al, 2001)
  – Levoglucosan (LG) (Simpson et al, 2004; Lee et al, 2005)
Levoglucosan and methoxyphenols

• LG is pyrolysis product of wood polymer cellulose; particulate in air
  – Most abundant organic compound in wildland woodsmoke particles (Lee et al, 2005)
  – More specific for woodsmoke than PM, CO?
    • Less likely to come from non-woodsmoke sources

• PM$_{2.5}$, LG levels related to urinary MPs in controlled exposures (Dills et al, 2005)
  – Pyrolysis products of wood lignin
  – Vapor and particulate in air
Selected markers for biomass combustion

MP level proportions, presence vary depending on type of wood

Guaiacols = single methoxy group on ring, syringols = two; other groups at position 4
Biological monitoring review

• Advantages
  – Measure of internal dose
  – Integrates exposure from multiple routes (e.g. ingestion, inhalation)
  – PPE, personal activities accounted for in dose

• Disadvantages
  – Potential for confounding from other sources
  – Requires additional subject-specific info
  – Inter-subject variability in uptake, metabolism
Current study

- Biomarkers may be more accurate measure of woodsmoke exposure than air samples
  - Evaluate relationships between PM$_{2.5}$, CO, and LG woodsmoke exposure and urinary MPs

- Hypotheses:
  1. PM$_{2.5}$, CO, LG levels will be highly correlated
  2. PM$_{2.5}$, CO, LG concentrations will be highly correlated with cross-shift urinary MP changes
Study data

• 20 shifts worked by 13 firefighters
  – Part of dataset collected by UGA, CDC
  – Chosen to cover range of PM$_{2.5}$ exposures

• Personal TWA levels of CO, PM$_{2.5}$, LG + qxr
  – CO measured via datalogging monitor
  – PM$_{2.5}$, LG from single filter
  – Smoked/grilled foods, smoking

• Pre-/post-shift urine samples
  – 22 MPs + creatinine
Methods

• Problem:
  – Urinary MP levels represent full-shift exposure
  – CO monitors all ran for full-shift
  – PM/LG sample pumps often failed during shift
    • Failure times, later exposure recorded in field notes

• Solution: data divided into three subsets
  – Full-shift exposure measurements
  – Measurement length >60% of full-shift
  – All measurements
Methods

• Invalid or non-detect (ND) data for 22 MPs
  – Some compounds available in all measures; some absent in nearly all measures
    • Mean 19.7% ND, 22.5% invalid for any reason
    • Invalid/ND data excluded unless noted

• Questionnaire results (shift + 48 hrs prior):
  – MPs not sig. different after smoking; no subjects removed from analysis
  – Syringol MPs sig. different after smoked/grilled food; 2 subjects removed from syringol analyses
Methods

• CO and LG levels normally distributed
• PM$_{2.5}$ levels log-normally distributed
  – Not transformed; non-parametric analyses
  – 2 PM$_{2.5}$ samples with very low (<0.2%) LG:PM$_{2.5}$ ratio and low CO:PM$_{2.5}$ ratio removed
• Urinary MP levels
  – Pre-/post-shift MP levels normalized to creatinine levels before analysis (Dills et al, 2005)
    • Correct for temporal variations in urine concentration
Significant creatinine-adjusted urinary MP correlations

• Most abundant MPs; all vapor phase
  – Four guaiacol-type MPs
    • Guaiacol, methylguaiacol, ethylguaiacol and propylguaiacol (Pearson r >0.6, p<0.01)
  – Three syringol-type MPs
    • Syringol, methylsyringol, and ethylsyringol (Pearson r >0.6, p<0.01)

• Levels for these MPs combined into summed guaiacol and syringol variables
  – Summed variables only: ND assigned LOD/2
Correlations: PM$_{2.5}$, CO, and LG exposures

Full-shift exposure data only (n=9)
Correlations: *full-shift* exposures vs. creatinine-adjusted guaiacols

<table>
<thead>
<tr>
<th>Agent</th>
<th>Parameter</th>
<th>Guaiacol</th>
<th>Methyl-guaiacol</th>
<th>Ethyl-guaiacol</th>
<th>Propyl-guaiacol</th>
<th>Summed guaiacols</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO</td>
<td>r</td>
<td>0.781</td>
<td>0.879</td>
<td>0.614</td>
<td>0.734</td>
<td>0.794</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>0.0001</td>
<td>&lt;0.0001</td>
<td>0.004</td>
<td>0.001</td>
<td>0.0004</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>19</td>
<td>19</td>
<td>20</td>
<td>16</td>
<td>15</td>
</tr>
<tr>
<td>LG</td>
<td>r</td>
<td>0.231</td>
<td>0.277</td>
<td>0.339</td>
<td>0.290</td>
<td>0.160</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>0.550</td>
<td>0.506</td>
<td>0.372</td>
<td>0.449</td>
<td>0.704</td>
</tr>
<tr>
<td></td>
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<td>9</td>
<td>9</td>
<td>9</td>
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<td>9</td>
</tr>
<tr>
<td>PM$_{2.5}$</td>
<td>r</td>
<td>0.033</td>
<td>0.000</td>
<td>0.150</td>
<td>-0.050</td>
<td>-0.228</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>0.932</td>
<td>1</td>
<td>0.700</td>
<td>0.898</td>
<td>0.587</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>9</td>
<td>9</td>
<td>9</td>
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</tr>
</tbody>
</table>

Syringols not significantly correlated with full-shift PM$_{2.5}$, CO, or LG
Correlations: *all* exposures vs. creatinine-adjusted guaiacols

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<tr>
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<th>Propylguaiacol</th>
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<td>0.879</td>
<td>0.614</td>
<td>0.734</td>
<td>0.794</td>
</tr>
<tr>
<td>(n=20)</td>
<td>p</td>
<td></td>
<td>0.0001</td>
<td>&lt;0.0001</td>
<td>0.004</td>
<td>0.001</td>
<td>0.0004</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td></td>
<td>19</td>
<td>19</td>
<td>20</td>
<td>16</td>
<td>15</td>
</tr>
<tr>
<td>LG</td>
<td>r</td>
<td></td>
<td>0.423</td>
<td>0.718</td>
<td>0.702</td>
<td>0.605</td>
<td>0.334</td>
</tr>
<tr>
<td>(n=19)</td>
<td>p</td>
<td></td>
<td>0.116</td>
<td>0.003</td>
<td>0.003</td>
<td>0.022</td>
<td>0.289</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td></td>
<td>15</td>
<td>15</td>
<td>16</td>
<td>14</td>
<td>12</td>
</tr>
<tr>
<td>PM&lt;sub&gt;2.5&lt;/sub&gt;</td>
<td>r</td>
<td></td>
<td>0.278</td>
<td>0.443</td>
<td>0.409</td>
<td>0.303</td>
<td>0.131</td>
</tr>
<tr>
<td>(n=16)</td>
<td>p</td>
<td></td>
<td>0.315</td>
<td>0.098</td>
<td>0.116</td>
<td>0.293</td>
<td>0.686</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td></td>
<td>15</td>
<td>15</td>
<td>16</td>
<td>14</td>
<td>12</td>
</tr>
</tbody>
</table>

Syringols not significantly correlated with PM<sub>2.5</sub>, CO, or LG
CO vs. change in creatinine-adjusted summed guaiacols

CO (ppm) vs Summed Guaiacols (ug/mg creatinine)

\[ y = 0.283x - 0.051 \]

\[ p = 0.002 \]

\[ r^2 = 0.63 \]

Cross-Shift difference in summed guaiacol concentration (ug/mg creatinine)

Other model \( r^2 \): LG vs guaiacols, 0.03; CO+LG vs. guaiacols, 0.79
Conclusions: exposure levels (hypothesis 1)

• LG and PM$_{2.5}$ significantly correlated
• LG and CO variably correlated
  – Insignificant correlation among full-shift samples
  – Significant correlation among all samples
• PM$_{2.5}$ and CO correlation poor to negligible
  – Literature generally shows strong correlation between PM$_{2.5}$ and CO for firefighters
  – Lack of correlation here possibly due to small n
Conclusions: urinary MPs vs. exposure levels (hypothesis 2)

• Significant cross-shift changes in 14 of 22 urinary MPs

• Exposures vs. MPs
  – Individual and summed creatinine-adjusted guaiacols highly associated with CO levels
  – Smaller association with LG; none with PM\textsubscript{2.5}
  – CO explains most variance in MPs
Future directions and acknowledgements

- Examine exposure and urinary MP relationship in full UGA/CDC dataset
  - Focus on urinary MPs from current study
  - Stratify analysis by forest type, activity, etc
- Thanks to:
  - Chris Simpson and Mike Paulsen, UW
  - Luke Naeher, UGA
  - Kevin Dunn, Alison Stock, Dana Barr, CDC
  - Participating firefighters
- Funded in part by:
  - Northwest Center for Particulate Air Pollution and Health (U.S. EPA grant #CR827355) and NIOSH (#R03-OH007656)
Additional: key features for a woodsmoke biomarker

- Relatively abundant in woodsmoke
- Ideally, should be particle-associated
- Should be stable in the atmosphere
- Should not be appreciably metabolized
- Must have low abundance in urine from subjects with low exposures to woodsmoke
Additional: abundance of molecular markers in smoke from oak combustion

<table>
<thead>
<tr>
<th>Marker</th>
<th>Concentration (mg/kg fuel)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Levoglucosan</td>
<td>750</td>
</tr>
<tr>
<td>Guaiacol</td>
<td>200</td>
</tr>
<tr>
<td>4-methyl-guaiacol</td>
<td>100</td>
</tr>
<tr>
<td>4-ethyl-guaiacol</td>
<td>200</td>
</tr>
<tr>
<td>Syringol</td>
<td>400</td>
</tr>
<tr>
<td>Eugenol</td>
<td>600</td>
</tr>
<tr>
<td>Vanillin</td>
<td>300</td>
</tr>
<tr>
<td>cis-Isoeugenol</td>
<td>100</td>
</tr>
<tr>
<td>trans-Isoeugenol</td>
<td>200</td>
</tr>
<tr>
<td>4-Aceto-vanillone</td>
<td>300</td>
</tr>
<tr>
<td>4-Propyl-syringol</td>
<td>400</td>
</tr>
<tr>
<td>4-Propyl-syringol</td>
<td>500</td>
</tr>
<tr>
<td>Syring-aldehyde</td>
<td>600</td>
</tr>
<tr>
<td>Syring-aldehyde</td>
<td>700</td>
</tr>
<tr>
<td>Coniferyl aldehyde</td>
<td>800</td>
</tr>
<tr>
<td>Coniferyl aldehyde</td>
<td>900</td>
</tr>
<tr>
<td>4-Aceto-syringol</td>
<td>100</td>
</tr>
<tr>
<td>Guaiacyl acetaldehyde</td>
<td>200</td>
</tr>
<tr>
<td>Coniferyl aldehyde</td>
<td>300</td>
</tr>
<tr>
<td>Propenyl syringol</td>
<td>400</td>
</tr>
<tr>
<td>Acetyl syringol</td>
<td>500</td>
</tr>
</tbody>
</table>

LG exists only in particle phase; MPs primarily in vapor phase

(GC elution order →)

(Schauer et al, 2000)
Additional: MP analysis

- MPs with simple alkyl substituents at 4 position on ring primarily excreted as phase 2 conjugates
  - Conjugates of glucuronide and sulfate
  - Guaiacol, methyl guaiacol, ethyl guaiacol, etc.
- Analysis: conjugates hydrolyzed, reform original MP
- Compounds with complex sidechains undergo more extensive metabolism (usually side chain oxidation)
  - Eugenol, vanillin, etc.
- Only tried to measure parent compounds (not oxidized metabolites)
  - So don't see much of a biomarker signal for complex MPs
Additional results: regression models, full-shift exposure vs. creatinine-adjusted guaiacols

<table>
<thead>
<tr>
<th>Model</th>
<th>Model variables</th>
<th>N</th>
<th>Coeff.</th>
<th>95% CI</th>
<th>Coeff. P-value</th>
<th>Model R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CO (ppm)</td>
<td>15</td>
<td>0.283</td>
<td>0.167 - 0.400</td>
<td>0.0002</td>
<td>0.63</td>
</tr>
<tr>
<td>2</td>
<td>LG (ug/m³)</td>
<td>9</td>
<td>0.003</td>
<td>-0.018 - 0.024</td>
<td>0.701</td>
<td>0.03</td>
</tr>
<tr>
<td>3</td>
<td>CO (ppm)</td>
<td>9</td>
<td>0.425</td>
<td>0.221 - 0.629</td>
<td>0.002</td>
<td>0.79</td>
</tr>
<tr>
<td></td>
<td>LG (ug/m³)</td>
<td></td>
<td>-0.008</td>
<td>-0.021 - 0.004</td>
<td>0.151</td>
<td></td>
</tr>
</tbody>
</table>

- Models for other data subsets displayed similar trends