Comparative Toxicity of Biomass Smoke from Different Fuels and Combustion Conditions

M Ian Gilmour Ph.D., DABT
Environmental Public Health Division
National Health and Environmental Effects Research Laboratory

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Sept. 1, 2017, Air quality in Seeley Lake had hourly pollution readings classified as hazardous in 26 days in August. (Rion Sanders/AP)
Wildfire Smoke Crosses the U.S. on Jet Stream

- Burned area in the US, 2017: ~9.8 million acres (~6.7 million acres: 2000-2016)
- Wildfires are increasing in frequency, size, and intensity in the US.
- Wildfire (biomass) smoke exposure is associated with increased cardiopulmonary disease.
Where There is Smoke There is Illness

Wildfire smoke

Health effects

Wildfires

Inhaled particle deposition in the lung

- Extrathoracic region
  - Nasal Cavity
  - Larynx

- Tracheobronchial region
  - Trachea
  - Bronchi

- Alveolar region
  - Alveoli

Wildfires

Inorganic species

Volatile liquids

Particles

Organic species

Reactive metals

Gases

Health effects

0.1 – 2.5 µm (Fine PM)

< 0.1 µm (Ultrafine PM)

2.5 – 10 µm (Coarse PM)

> 10 µm
# Emissions by Chemical Class for Particle and Vapor Constituents in Woodsmoke

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Particle-phase (mg/kg wood burned)</th>
<th>Vapor-phase (mg/kg wood burned)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon monoxide</td>
<td>---</td>
<td>130,000</td>
</tr>
<tr>
<td>Alkanes (C2-C7)</td>
<td>0.47 - 570</td>
<td>1.01 - 300</td>
</tr>
<tr>
<td>Alkenes (C2-C7)</td>
<td>0.58 - 280</td>
<td>92 - 1300</td>
</tr>
<tr>
<td>Polycyclic aromatic hydrocarbons (PAHs) and substituted PAHs</td>
<td>5.1 - 32,000</td>
<td>43.4 - 355</td>
</tr>
<tr>
<td>Methane</td>
<td>---</td>
<td>4100</td>
</tr>
<tr>
<td>Total nonmethane hydrocarbons C2-C7</td>
<td>[Included in vapor phase]</td>
<td>390 - 4000</td>
</tr>
<tr>
<td>Unresolved complex mixture (UCM)</td>
<td>300 - 1,130,000</td>
<td>---</td>
</tr>
<tr>
<td>Alkanols</td>
<td>0.24 - 5400</td>
<td>120 - 9200</td>
</tr>
<tr>
<td>Carboxylic acids</td>
<td>6200 - 755,000</td>
<td>2.4</td>
</tr>
<tr>
<td>Aldehydes and ketones</td>
<td>[Included in vapor phase]</td>
<td>0.94 - 4450*</td>
</tr>
<tr>
<td>Alkyl esters</td>
<td>0.37 - 4450</td>
<td>---</td>
</tr>
<tr>
<td>Methoxylated phenolic compounds</td>
<td>28 - 1000</td>
<td>1200 - 1500</td>
</tr>
<tr>
<td>Other substituted aromatic compounds</td>
<td>5.0 - 120,000</td>
<td>110 - 3600</td>
</tr>
<tr>
<td>Sugar derivatives</td>
<td>1.4 - 12600</td>
<td>---</td>
</tr>
<tr>
<td>Coumarins and flavonoids</td>
<td>0.71 - 12</td>
<td>---</td>
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<tr>
<td>Phytosteroids</td>
<td>1.7 - 34.0</td>
<td>---</td>
</tr>
<tr>
<td>Resin acids and terpenoids</td>
<td>1.7 - 41,000</td>
<td>21 - 430</td>
</tr>
<tr>
<td>Unresolved compounds</td>
<td>1.2 - 120</td>
<td>20 - 600</td>
</tr>
</tbody>
</table>

* Only aldehydes reported.

L.P. Naeher et al, Inhalation Toxicology 2007
Mechanisms of Acute Lung Injury

Adapted from Bakowitz et al. SJTREM. 2012
Mechanisms of PM-induced Cardiovascular Effects

Blood
- PM or constituents in the circulation
  - UFP, soluble metals
  - Organic compounds
- Acute or Chronic?
- PM and/or constituents transmitted into blood
- Pulmonary oxidative stress & inflammation
- Sub-acute or Chronic
- "Systemic spill-over"

Vasculature
- Vasoconstriction
- Endothelial dysfunction
- PM-mediated ROS
- BP
- Atherosclerosis
- Blood
  - Platelet aggregation

Blood
- Activated or Inflamed Fat
  - Activated or Inflamed Liver

PM
- Bronchioles/Alveoli
  - Activation of lung ANS reflex arcs

ANS
- ANS imbalance
  - ↑SNS / ↓PSNS

Systemic Oxidative Stress and Inflammation
- Cellular inflammatory response
  - Activated WBCs, platelets, MPO
  - Cytokine expression/levels
  - ET, histamine, cell microparticles, oxidized lipids; anti-oxidants

Vasculature
- Endothelial cell dysfunction/vasoconstriction, ↑ROS
- Atherosclerosis progression/plaque vulnerability
- Thrombogenicity (e.g., tissue factor)

Metabolism
- Insulin resistance, dyslipidemia, impaired HDL function

Blood
- Coagulation, thrombosis; fibrinolysis (e.g., PAI-1)

Brook et al. 2010
Source Emissions Toxicology Program

**Source**
- Residual Oil
- Diesel Exhaust
- Coal
- Biofuels
- Nano-Catalysts
- Biomass

**Effect**
- Biological Assays
  - Pulmonary inflammation
  - Allergic potential
  - Viral infectivity
  - Cardiopulmonary function
  - Cardiac/pulmonary gene expression
  - Epigenetics
  - Fetal immune polarization
  - Trans-generational effects
  - Mutagenicity

**Physicochemical Analyses**
- PM size, mass, and morphology
- Inorganic elements
  - XRF, ICP-OES
- Organic components
  - Gravimetric analysis, GC-MS
- Carbonaceous components
  - Elemental and organic carbon, black carbon
- Reactive Oxygen Species
  - EPR, reduction-oxidation potential
- Real-time PM analysis
  - Aerosol Time-Of-Flight Mass Spectrometer (ATOF-MS)
- Continuous emission monitoring for gaseous components

**Size, Chemistry**
- Photo-chemical aging
Health Effects of Smoke from Wildfires in Rural Eastern North Carolina

TIMELINE

- **June 1 2008** - lightening strike initiates smoldering fire in peat soil; drought, low humidity and high temperature contribute to rapid spreading

- **June 13** - peak of fire; burns through 41,000 acres, including 24,000 acres of Pocosin Lakes National Wildlife Refuge

- **June 15** - first rainfall
Hospital Admissions from a 2008 Wildland Peat Fire in North Carolina

Percent change in cumulative RR by discharge diagnosis category for exposed and referent counties in NC during 3-day period of high exposure compared with the entire 6-week study period.
Location of Chemvol PM Sampler and Monitoring Data for (ENCF-1 and ENCF-4)

Wildfire occurred on 6/1/2008 and was 100% contained on 9/24/2008

The wind rose diagram and PM monitoring data were obtained from the State Climate Office of North Carolina and the U.S. EPA Air Data, respectively.
Coarse ENCF-1 and ENCF-4 had greater endotoxin content than the fine and ultrafine PM.

Coarse ENCF-1 had greater endotoxin content than ENCF-4.

Fine and Ultrafine ENCF-1 had a far greater abundance of organic material than ENCF-4.

Kim et al Particle, Fiber Tox, 16, 2014
Exposure to the coarse PM significantly increased the levels of cytokines (IL-6, TNF-α, and MIP-2) and neutrophils in BALF of mice and this was higher with ENCF-1.

Exposure to the ultrafine PM from ENCF-1 significantly decreased cardiac function (left ventricular developed pressure) in mice compared to all other samples.
Cardiac Toxicity of Peat Wildfire PM

Cardiac function in mice at 24 h post-exposure

- Recovery of left ventricular developed pressure (LVDP) and infarct size measured at 1 hr and 2 hrs of reflow after 20 min of ischemia, respectively.
- Exposure to only ultrafine ENCF-1 markedly decreased LVDP and increased the infarct size.

Kim et al. Part. Fibre Toxicol. 2014
Conclusions

These findings confirm that the coarse particles during ENCF-1 may have contributed to exacerbation of asthma while ultrafine particles from this period had more severe cardiac effects.
Different Types of Wildfire Smoke

- Peat wildfire
- Forest wildfire
- Smoldering
- Flaming

Fuel type
Combustion phase
Particulate Matter (PM)

Ultrafine
Fine
Coarse

NHEERL / EPHD / CIB
U.S. EPA
# 2011 National Emission Inventory Estimates (in tons)

<table>
<thead>
<tr>
<th></th>
<th>NH3</th>
<th>CO</th>
<th>NOx</th>
<th>PM10</th>
<th>PM2.5</th>
<th>SO2</th>
<th>VOCs</th>
<th>BC (EC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fires - Agricultural Field Burning</td>
<td>3,469</td>
<td>965,662</td>
<td>43,173</td>
<td>142,641</td>
<td>95,728</td>
<td>16,402</td>
<td>75,542</td>
<td>15,500</td>
</tr>
<tr>
<td>Fires - Prescribed Fires</td>
<td>162,420</td>
<td>10,091,996</td>
<td>168,204</td>
<td>1,063,159</td>
<td>903,062</td>
<td>83,255</td>
<td>2,320,330</td>
<td>102,000</td>
</tr>
<tr>
<td>Fires - Wildfires</td>
<td>203,252</td>
<td>12,701,426</td>
<td>184,802</td>
<td>1,325,991</td>
<td>1,125,176</td>
<td>95,837</td>
<td>2,891,271</td>
<td>119,000</td>
</tr>
<tr>
<td>Fuel Comb - Residential - Wood</td>
<td>19,740</td>
<td>2,524,960</td>
<td>34,547</td>
<td>382,509</td>
<td>382,283</td>
<td>8,972</td>
<td>443,966</td>
<td>22,000</td>
</tr>
</tbody>
</table>

|                          | 2011 NEI v2 total emissions (all sectors but biogenics and soil emissions) | 4,257,000 | 75,202,000 | 16,189,000 | 20,835,000 | 6,175,000 | 6,857,000 | 18,301,000 | 555,750 |
| Percentage of Total Emissions due to Ag, Wild and Prescribed Fires | 9% | 35% | 3% | 14% | 41% | 3% | 31% | 47% |
Current Approaches For Assessing Toxicity of Wildfire Smoke

➢ Size fractionated sampling of wildfire smoke with subsequent extraction, toxicity testing and comparison with ambient PM.

➢ Laboratory generated smoke collection and analysis with subsequent toxicity testing

➢ Real time exposures to “natural” or laboratory generated smoke

➢ Photochemical aging of smoke with real time or subsequent health impact testing.

➢ Note: each of these approaches can be performed in healthy and at risk (susceptible) sub-populations under various types of disease conditions.
Laboratory Generated Smoke Collection and Analysis with Subsequent Toxicity Testing

Objectives:
1. Compare the relative cardiopulmonary toxicity and mutagenicity of coarse and fine/ultrafine emissions from four distinct fuel types (pine, oak, peat, and a mixed pine and deciduous Southeastern forest biomass) obtained from both smoldering and active flame phases.

2. Provide a potency ranking and compare effects to size-fractionated ambient PM samples collected from urban and rural sites.

Combustion

- Fire enclosure (28 m³) Mixed fuels

Collection/Analysis

- Cyclone/Cryotrap system
- Particle and gas phase chemistry and mass balance

Toxicity Test

- WT and K/O mice
- Toxicity Ranking matrix
  - Cells, Tissue slices

JFSP FY 15-17 Project #14-1-04
The tube furnace is able to sustain stable flaming or smoldering phase consistently for 60 min.

The cryotrap system is able to collect PM and semi-volatile compounds.
Biomass Fuels and Their Distribution in the United States
Chemical Components in Biomass Smoke Condensate

Major chemical compounds

Semi-volatile organic compounds

- Chemical properties of biomass smoke varied depending on fuel types and combustion phases.
- Inorganic elements (e.g., heavy metals) and PAHs were higher in the flaming smoke PM.
Lung Toxicity of the Biomass Smoke Condensates Based on Equal Mass

Lung toxicity
BAL fluid of CD-1 mice at 24 h exposure

Kim et al. EHP (126:1), 2018
Mutagenicity of the Biomass Smoke Condensates Based on Equal Mass

Mutagenicity
Salmonella strain TA98 +S9

Flaming emissions
Smoldering emissions

\[ \text{ revertants / g PM extract} \]

Kim et al. EHP (126:1), 2018
Comparison of Mutagenicity Emission Factors (EFs) of Various Combustion Emissions

The mutagenicity EFs (rev/kg fuel; Figure 6C) were converted to rev/MJ_{th} using the values for the heat energy of each fuel (MJ_{th}/kg fuel). The mutagenicity EFs for wood burning cookstove emissions were 0.2, 1.2, and 2.4×10^5 rev/MJ_{th} for the force-draft stove, natural-draft stove, and three-stone fire, respectively, and the data were obtained from a previous report (Mutlu et al. 2016). The mutagenicity EFs for non-wood burning emissions were 0.4, 0.4, 2.5, and 22.7×10^5 rev/MJ_{th} for the municipal waste, diesel exhaust, agricultural plastic, and tire, respectively, and the data were obtained from previous reports (DeMarini et al. 1994; Linak et al. 1989; Mutlu et al. 2015; Watts et al. 1992).

Kim et al. EHP (126:1), 2018
Creating Stable Biomass Emissions in a Computer-Controlled Inhalation Exposure System
Stable Atmospheres of Biomass Smoke Created Through a Feedback Control Loop
- Total carbon accounted for ~50 – 60% of PM from the smoke under any combustion conditions.
- Inorganic elements and ions were higher in the flaming smoke PM.
- Acetaldehyde, formaldehyde, and acrolein were found to be a major component of VOCs.
- The flaming samples (peat and eucalyptus) were more toxic than the smoldering samples at ten times less particle mass concentration.
Lung Function Responses (*In Vivo*)

Mouse Ventilatory Function Analysis
Ventilatory function parameters were measured at 9 min intervals in the 30 min before exposure (baseline), right after exposures (Day 1 and Day 2), and 24 h after the second day exposure (Post), respectively.

- A significant increase in airflow obstruction (as measured by Penh) was observed in mice exposed to flaming peat (*), and eucalyptus (*), and smoldering eucalyptus ($) smoke immediately after each day of exposure.
Conclusions

Peat and eucalyptus smoke produces equivalent toxicity between flaming and smoldering conditions despite tenfold lower PM concentrations for flaming.

Smoke from oak combustion is less toxic than that from peat or eucalyptus.

Smoke inhalation has significant effects on pulmonary function depending on fuel type and combustion conditions that sometimes do not recover after 24 hours.

Acute exposure to smoke has greater physiological effects than cumulatively similar exposures over a more prolonged period but other endpoints need to be studied.
What About Atmospheric Aging of Wildfire Smoke?

Satellite images acquired on Sept. 3, 2017
(Source: RAMMB/CIRA)
Dichloromethane extracts of PM were 3X more mutagenic in TA98 (+/- S9) with UV lights on (reactants) than when lights were off (products).

Gas-phase emissions from combustion of burning oak produced 36 revertants/h in TA100 (same + or – S9) with UV lights on. No mutagenicity was observed with lights off.
Toxicological Comparisons of Complex Air Pollution Mixtures Using the (Canadian) Air Quality Health Index

(M Ian Gilmour, Mehdi Hazari, Steve Gavett, David DeMarini Jonathan Krug, Mike Lewandowski, et al  ES&T 2018)
Simulated Atmosphere Characteristics

SA-PM – High PM / Low Ozone

• Initial Conditions
  • 27-31 ppmC Total Hydrocarbon
    • 23 ppmC Gasoline #10
    • 5.4 ppmC α-pinene
  • 529 ppb NO
  • 3 µg/m³ (NH₄)₂SO₄
  • 35% RH @ 21.1°C

SA-O₃ – Low PM / High Ozone

• Initial Conditions
  – 12-14 ppmC Total Hydrocarbon
    • 8 ppmC Gasoline #10
    • 5.3 ppmC Isoprene
  – 874 ppb NO
  – 2 µg/m³ (NH₄)₂SO₄
  – 35% RH @ 21.1°C
### SA-PM and SA-O₃: AQI and AQHI

<table>
<thead>
<tr>
<th></th>
<th>O₃</th>
<th>NO₂</th>
<th>PM₂.₅</th>
<th>O₃-1 h</th>
<th>O₃-8 h</th>
<th>NO₂-1 h</th>
<th>PM₂.₅-24 h</th>
<th>Calculated AQHI</th>
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</thead>
<tbody>
<tr>
<td><strong>SA-PM</strong></td>
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<td></td>
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<td></td>
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<tr>
<td>MRC</td>
<td>141.3</td>
<td>326.2</td>
<td>976.7</td>
<td>121</td>
<td>238</td>
<td>144</td>
<td>691</td>
<td>97.7</td>
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<tr>
<td>Exp Chambers</td>
<td>100.9</td>
<td>253.8</td>
<td>1029.6</td>
<td>71.8</td>
<td>187</td>
<td>130</td>
<td>706</td>
<td>92.5</td>
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<tr>
<td><strong>SA-O₃</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>MRC</td>
<td>430.1</td>
<td>643.1</td>
<td>54.5</td>
<td>326</td>
<td>542</td>
<td>199</td>
<td>148</td>
<td>99.8</td>
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<tr>
<td>Exp Chambers</td>
<td>376.2</td>
<td>616.5</td>
<td>254.6</td>
<td>286</td>
<td>485</td>
<td>194</td>
<td>305</td>
<td>102.6</td>
</tr>
</tbody>
</table>
Mutagenicity Results

- +UV: mutagenicity from secondary reaction products
- Direct-acting mutagens (no metabolism required)
- SA-O₃ 2.5x more mutagenic than SA-PM (3x when corrected for gas-phase carbon), reaches 2x threshold ~3x faster
- Mutation spectra not significantly different:
  - Average for SA-PM, SA-O₃: 54% C → T and 46% C → A

Gilmour et al  ES&T 2018
Effects in Susceptible Diabetic Rats

- **Model:** Goto Kyoto (GK) rats – non-obese Type II diabetic
- **Exposure duration:** 4h/day x 5 consecutive days
- **Lung, serum markers:** No consistent effects of exposure
- **Ventilatory function** (whole body plethysmography)

![Graphs showing PenH over days post-exposure for SA-PM and SA-O3 with air and smog conditions, indicating statistically significant differences at specific days with * p<0.05.](Gilmour et al. ES&T 2018)
Cardiac Arrhythmia in Mice During Exposures
SA-O$_3$ > SA-PM

SA Node Dysfunction:
- SA node pause
- Non-conducted p-wave

Gilmour et al. ES&T 2018
Photochemical Aging of Smoke with Real-Time or Subsequent Health Impact Testing.
Where do we go from here?

Continue exploring effects of acute, intermittent and chronic exposures from various scenarios on health endpoints.

Validate and apply higher throughput in vitro systems to:

Determine relative potency of fresh and “aged” biomass smoke (using integrated AQHIs) to compare against other sources: e.g. mobile sources, power plant emissions and urban “smog”.

Assess health impact in more vulnerable populations such as asthma, COPD, hypertension, diabetes, developing fetus.

Utilize toxicological approaches in tandem with exposure assessment to identify most effective mitigation practices (e.g. filtration).
**Smoke Toxicology**
Ian Gilmour, NHEERL
David DeMarini, NHEERL
Andy Ghio, NHEERL

**Smoke Exposure (Monitors/Sensors)**
Matt Landis, NERL
Amara Holder, NRMRL
Gayle Hagler, NERL

**Smoke Emissions and AQ Impacts Modeling**
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Tom Pierce, NERL
Kirk Baker, OAR-OAQPS

**Smoke Epidemiology**
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Wayne Cascio, NHEERL
Susan Stone, OAQPS

**Public Health**

**Biomass Emissions Factors & Speciation**
Brian Gullett, NRMRL
Mike Hays, NRMRL
Amara Holder, NRMRL
Venkatesh Rao, OAR-OAQPS

**In Vivo Test**
WILDFIRE PM (100 µg)

- OROPHARYNGEL ASPIRATION
- BALF Analysis
- Lung injury
- Lung inflammation
- Cardiac function

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**FASME Initiative w/ OAR-OAQPS**

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