Subchronic CAPs Exposures in Mice: Biological Endpoints and Exposure Assessment

Lung Chi Chen, Ph.D.
And
Morton Lippmann, Ph.D.

Department of Environmental Medicine
NYU School of Medicine, Tuxedo, NY
Issues Addressed:

- Does long term exposure to PM$_{2.5}$ cause cumulative damage to cardiovascular, pulmonary and other tissues?

- What is the relationship between the temporal variations in CAPs concentrations/composition and acute changes in cardiac function?
Normal and compromised mice are exposed, whole body, to CAPs or filtered air for 6 hours/day, 5 days per week, for up to 6 months.

CAPs @ ~10x ambient Northeastern U.S. background aerosol concentrations

- Cyclone Inlet was used to remove PM larger than 2.5 \( \mu \text{m} \)

Filtered air exposure controls

- PM is removed prior to the virtual impactor concentrator system.
An identical system with a HEPA filter at the inlet (to remove ambient particles) is used for sham exposure.
## Exposure Atmosphere Sampling and Methods of Analyses

### Exposure Atmospheres

**PM$_{2.5}$ Concentrations**
- Ambient (post cyclone)
- Exposure Chambers
  - Gravimetric
  - DataRAM (MIE)

**PM Compositions**
- XRF (Jordan Valley) for elements
- IC (Dionex) for ions
- Single Particle Mass Spec (RSMS III)

**Humidifier Air**
- Temperature and dew point

**Exposure Chamber**
- Temperature, Relative Humidity
- Particle Size and Mass Concentration
  - DataRAM (MIE), SMPS (TSI)
- Particle Number Concentration
  - CNC (TSI)

### Ambient Air
- Temperature
- Relative Humidity
- PM$_{2.5}$ - TEOM
- EC - Aethalometer
- EC/OC - R&P 5400
- VOC - PUFF-GC/MS
- Ozone
- NOx
- SO$_2$
Experimental Animals

- **C57BL/6**: “Normal mice”
- **C57BL/6J ApoE^{-/-}**
  - Hyperlipidemia, progressive atherosclerosis on a high fat diet
- **B6;129 ApoE^{-/-} LDLr^{-/-}**
  - Hyperlipidemia, progressive atherosclerosis and coronary artery disease on a high fat diet.
  - Myocardial ischemia within 6 months.
  - Males have more severe disease than females.
  - A model of humans with severe atherosclerosis and coronary artery disease.
Exposure Parameters

- **Average Concentration During Exposure**
  - $110 \pm 79 \text{ ug/m}^3$
  - equivalent to $20 \text{ ug/m}^3$ annually

- **Median Concentration**
  - $91 \text{ ug/m}^3$ (1.2 - 426 ug/m$^3$)

- **Particle Size**
  - $0.43 \pm .13 \mu\text{m}$ (SMPS, Volume distribution)

- **Concentrating factor**
  - $9.6 \pm 3.8$
Subchronic Effects of CAPs on the Lungs

- No change in lavage cell counts, differentials, LDH, protein
- No histological change
- No change in immunological endpoints

No change in serum troponin and IL-6 levels.
The time of day that significant effects of CAPs at the end of exposure occurred was found using the “Fishing License” method, based on the averages of the 5 post exposure days.
Subchronic Effects of CAPs on Heart Rate and Core Body Temperature

- The time of day that significant effects of CAPs at the end of exposure occurred was found using the “Fishing License” method, based on the averages of the 5 post exposure days.

- The differences between CAPs and control at that time were used to estimate the daily crude effects of CAPs using a time-varying model.

\[ y_{ijt} = \gamma_0 + \gamma_1 I(i \in ApoE) + \gamma_2 I(j \in PM) + \gamma_3 I(i \in ApoE \text{ and } j \in PM) \]
\[ + \sum_{l=1}^{t-h} \phi_{il}(y_{ij,t-l} - \hat{y}_{ij,t-l}) + e_{ijt} \]

- The daily crude effects were used to estimate the chronic trend of CAPs exposure using a Bayesian inference model.

\[ \theta_t = \delta + \alpha \times [1 - e^{-\lambda \times \max(t-\omega, 0)}] + \eta \times I(C_t > 0) + \beta \times I(C_t > \psi) + \phi \times I(t \in [a,b]) + \epsilon_t \]
\[ = \mu_t(\delta, \alpha, \lambda, \omega, \eta, \beta, \psi, \phi) + \epsilon_t \]
Subchronic Effects - ApoE^{-/-}

1:30-4:30 AM

Body Core Temperature (°C)  Heart Rate (beats/min)

![Graph showing subchronic effects of ApoE^{-/-} on body core temperature and heart rate. The graphs display data from dates 4/12 to 9/06, with a decline in body temperature and an increase in heart rate over time.]
Subchronic Effects - C57

1:30-4:30 AM

Body Core Temperature (°C)  Heart Rate (beats/min)

<table>
<thead>
<tr>
<th>Date</th>
<th>Effect (°C)</th>
<th>Date</th>
<th>Effect (beats/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4/12</td>
<td></td>
<td>4/12</td>
<td></td>
</tr>
<tr>
<td>5/03</td>
<td></td>
<td>5/03</td>
<td></td>
</tr>
<tr>
<td>5/14</td>
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<td>5/14</td>
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<tr>
<td>7/05</td>
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<td>7/05</td>
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<tr>
<td>7/16</td>
<td></td>
<td>7/16</td>
<td></td>
</tr>
<tr>
<td>8/16</td>
<td></td>
<td>8/16</td>
<td></td>
</tr>
<tr>
<td>9/06</td>
<td></td>
<td>9/06</td>
<td></td>
</tr>
</tbody>
</table>

4/12 5/24 7/05 8/16 5/03 6/14 7/26 9/06
Trend of Daily Acute Effects - ApoE^{-/-}

11:00 AM - 1:30 PM

Body Core Temperature (°C)

Heart Rate (beats/min)
### Summary Effects of CAPs on Core Temperature and Heart Rate

<table>
<thead>
<tr>
<th></th>
<th>Effects of CAPs (1:30 am - 4:30 am)</th>
<th>Effects of CAPs (11:00 am - 1:00 pm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Core temp (°C)</td>
<td>Heart rate (beats/min)</td>
</tr>
<tr>
<td></td>
<td>ApoE</td>
<td>C57</td>
</tr>
<tr>
<td>Onset of the chronic effects calendar days into exposure</td>
<td>26.3 (9.1, 37.1)</td>
<td>76.7 (31.8, 106)</td>
</tr>
<tr>
<td>Magnitude of change at the end of exposure</td>
<td>-0.86 (-0.73, -1.02)</td>
<td>0.3 (0.15, 0.45)</td>
</tr>
<tr>
<td>Average acute effect of CAP</td>
<td>0.1 (-0.15, 0.28)</td>
<td>-0.01 (-0.8, 0.17)</td>
</tr>
</tbody>
</table>
Effects of CAPs on Heart Rate Variability

Changes of log SDNN for ApoE−/− mice during (a) 16:00 – 18:00 and (b) 1:30 – 4:30 obtained from the Bayesian model in the 2nd stage.
Effects of CAPs on Heart Rate Variance ApoE\(^{-/-}\)

- Fast Fourier transform with Daniell smoother was used to estimate the power spectral densities for the 5-min average HR collected on the last non-exposed days (weekends).
- Fishing license method was used to estimate the frequency band during which the mean log\(_{10}\) transformed powers differed most significantly between CAPs and air.
- Two Stage method was used to estimate the effects of CAPs on heart rate variance over the 22 weekends.

No change in Heart Rate Variance in C57
Subchronic effects of CAPs on Heart Rate Variance-ApoE\textsuperscript{-/-}

Low Frequency (3.6-6.7 hr)

High Frequency (16 minutes)
Effects of CAPs on aorta plaque size

Lesion area of longitudinal sections

* P = 0.03

ApoE Male
DK Female
DK Male
Effects of CAPs on aorta plaque cellularity

* P = 0.01
Longevity of the ApoE<sup>-/-</sup> LDLr<sup>-/-</sup> double knockout mice reduced by CAPs
Effects of CAPs on substantia nigra of ApoE-/- Mice

Tyrosine Hyrolase Stained Cells (number of pixels)

GFAP (glial fibrillary acidic protein) Stained Cells (number of pixels)
Effects of CAPs on gene expression in lung and heart

- Affymetrix GeneChips
- 3 animals/strain/exposure group/tissue
- “Heart” genes altered by subchronic CAPs exposure
  - Pyruvate dehydrogenase kinase
    - Energy regulation
  - 6 ion channels for $K^+$, $Na^+$, and $Ca^{2+}$
    - Ion balance
  - Myosin heavy chain
    - muscle
  - Albumin D-element binding protein
    - Circadian rhythm
  - Heat shock proteins
    - Protection from injury
Average contribution of individual sources to CAPs mass concentration

- Resuspended soil: 23%
- Pb/Br/Zn-rich source: 1%
- Other sources: 1%
- Regional secondary sulfate: 72%
- Oil combustion: 3%
Time series plot of each source mass contribution ($\mu$g/m$^3$)
Correlation coefficients (r) of the cellular response NF-kB with the sources identified by factor analysis

<table>
<thead>
<tr>
<th>Source</th>
<th>NF-kB</th>
<th>Secondary sulfate</th>
<th>Resuspended soil</th>
<th>Oil combustion</th>
<th>Pb/Br/Zn-rich source</th>
<th>Other sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>NF-kB</td>
<td>1</td>
<td>-0.179</td>
<td>0.003</td>
<td><strong>0.326</strong></td>
<td>-0.044</td>
<td>0.039</td>
</tr>
<tr>
<td>Coal combustion</td>
<td></td>
<td></td>
<td>-0.026</td>
<td>-0.003</td>
<td>0.096</td>
<td>-0.059</td>
</tr>
<tr>
<td>Resuspended soil</td>
<td></td>
<td></td>
<td>-0.005</td>
<td>-0.056</td>
<td></td>
<td>-0.011</td>
</tr>
<tr>
<td>Oil combustion</td>
<td></td>
<td></td>
<td>1</td>
<td>-0.137</td>
<td></td>
<td>0.015</td>
</tr>
<tr>
<td>Pb/Br/Zn-rich source</td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td></td>
<td>-0.135</td>
</tr>
</tbody>
</table>
Correlation between NF-kB and CAPs Components

(Stepwise Regression)

|       | Coefficient | t    | Pr(|t|) |
|-------|-------------|------|--------|
| (Intercept) | 11.83      | 5.29 | 0.000  |
| V     | 0.24        | 2.25 | 0.027  |
| Ni    | 0.44        | 2.86 | 0.005  |
| Cu    | 0.50        | 2.89 | 0.006  |
| As    | 0.29        | 2.07 | 0.041  |

N=99, $R^2=0.3343$
There were significant associations between HRV parameters with NFκB

<table>
<thead>
<tr>
<th>Strain</th>
<th>Time Interval</th>
<th>HRV Measure</th>
<th>Mean (95% C.I.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ApoE⁻/-</td>
<td>1600-1800</td>
<td>Log SDNN</td>
<td>-0.0190 (-0.0353, -0.0014)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Log RMSSD</td>
<td>-0.0271 (-0.0449, -0.0089)</td>
</tr>
<tr>
<td></td>
<td>0130-0430</td>
<td>Log SDNN</td>
<td>-0.0051 (-0.0183, 0.0083)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Log RMSSD</td>
<td>-0.0031 (-0.0203, 0.0129)</td>
</tr>
<tr>
<td>C57</td>
<td>1600-1800</td>
<td>Log SDNN</td>
<td>-0.0149 (-0.0342, 0.0048)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Log RMSSD</td>
<td>-0.0253 (-0.0471, -0.0025)</td>
</tr>
<tr>
<td></td>
<td>0130-0430</td>
<td>Log SDNN</td>
<td>-0.0105 (-0.0258, 0.0046)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Log RMSSD</td>
<td>-0.0145 (-0.0320, 0.0037)</td>
</tr>
</tbody>
</table>
Summary of the Subchronic CAPs Exposure Studies

- Cumulative changes in body core temperature, HR, and HRV.
- Animals prone to develop atherosclerosis appeared to be more sensitive to CAPs exposure than normal mice.
- Marked acceleration of plaque development and affected plaque characteristics.
- Alterations in the brain that are consistent with Parkinson’s disease.
- Alterations in gene expression in heart and lung tissue.
- PM derived from oil combustion sources most closely associated with NF-kB response *in vitro* and depressed HRV in animals.
Acknowledgement

Collaborators:

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  - Affymetrix GeneChip analysis
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- Michael Kleinman, Ph.D., UC Irvine
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