Air Quality Management Training Program

Toxicological Evidence of Air Pollution Health Effects

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* No conflicts of interest to disclose *
Outline

I) Air Pollution and Health

II) Toxicological Evidence
   a. Approaches to study health effects
   b. Effects in the lungs, vasculature, metabolism
   c. New investigational approaches

III) Summary and Perspectives
DALY = YLL (Years of Life Lost) + YLD (Years Lived with Disability)

Lim et al, Lancet 2012; 380: 2224-60
Ambient Air Pollutants

Air Pollution

Gaseous

Particulate

TSP

PM$_{10}$ (< 10 $\mu$m)

Fine (PM$_{2.5}$)

Coarse (PM$_{10-2.5}$)

UFP (PM$_{0.1}$)

Brook et al, Circulation 2004; 109: 2655
Air pollution and total mortality

Dockery & Schwartz, Epidemiol 1995

Samet et al, NEJM 2000
PM$_{2.5}$ and cardiovascular M & M

Total CV deaths

Total CV deaths

Arrhythmic
Heart Failure
Cardiac Arrest

Ischemic

24% increase

76% increase

Pope et al, Circulation 2004; 109: 71

Miller et al, NEJM 2007; 356: 447

Table 3. Estimated Hazard Ratios for the Time to the First Cardiovascular Event or Death Associated with an Exposure Increase of 10 µg per Cubic Meter in the Level of Fine Particulate Matter (PM$_{2.5}$)."
Harvard six cities study

Dockery et al, NEJM 1993

Laden et al, AJRCCM 2006
PM$_{2.5}$ and atherosclerosis

Kunzli et al, EHP 2005

Hoffman et al, Circulation 2007


3% increase in CIMT per each 12 mcg/m$^3$ increase in mean annual PM$_{2.5}$

Multi-Ethnic Study of Atherosclerosis
Increased CV Morbidity & Mortality

Air Pollution

Pulmonary Reflexes

Particle Translocation

Pulmonary Inflammation

Autonomic Nervous System

Oxidative Stress

EC dysfunction

Atherothrombosis

Platelets Coagulation

Atherosclerosis

Systemic Inflammation

CHF CVA PVD

Dysrhythmias

IHD

Increased CV Morbidity & Mortality

Araujo&Brook, In Environmental Cardiology. Issues in Toxicology 2011; 76
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How to Study Health Effects Induced by Air Pollutants?

Associations

Causality

Mechanisms
Mechanistic Models for the involvement of ROS and Inflammation

Air Pollutants

ROS

Inflammation

Health Effects
Assessment of ROS

- **Direct:** $O_2^-$, $OH^-$
- **Indirect:** Colorimetric assays (e.g. NBT), use of probes (e.g. DCF, HE)
- **Oxidative products**
  - Lipids: MDA/TBARS, lipid hydroperoxides, F2-isoprostanes, HETEs, HODEs
  - Proteins: carbonyls
  - DNA: 8-oxodG, 8-oxoGua, M1dG
- **Gene expression:**
  - Prooxidant genes: NADPH oxidase
  - Antioxidant genes: Nrf2, HO-1, SOD, etc.
  determined by qPCR, WBs, IH.
Assessment of Inflammation

- **Histology**
  - Lung infiltration by inflammatory cells: degree, type of cells, localization
  - BALF total cell count/cell differential
  - Vascular infiltration of inflammatory cells, atherosclerosis

- **Chemistry/Biochemistry**
  - Inflammatory mediators: TNF-α, MCP-1, IL-6, IL-8, CAMs (VCAM-1, ICAM-1) …
  - BALF total protein/albumin, LDH

- **Gene expression of inflammatory mediators and signaling pathways by qPCR, WBs or IH.**
Epithelial cells

Li et al, JJ 2002; 169: 4531
Macrophages

Li et al, EHP 2003; 111: 455

Yin et al, JBMT 2013; 27: 172

Li et al, EHP 2003; 111: 455
Endothelial cells

Yin et al, JBMT 2013; 27: 172
Li et al, PFT 2010; 7: 6
PM Exposure, ROS and Inflammation In-Vivo

I.P./I.T. Instillations
- PM
- DEP
- ROFA
- Carbon

Inhalation Exposures
- CAPs
- Reaerosolized particles
- DE, GE

Pulmonary Effects
- BALF
- Lung Tissue

Systemic Effects
- Circulating Blood
- Metabolism
- Vasculature
- Solid Organs: Liver, Pancreas, Kidneys
UFP Induce Pulmonary and Systemic Oxidative Stress

Araujo et al, Part Fibre Toxicol 2009
PM causes endothelial dysfunction and systemic vascular inflammation

Nurkiewicz et al, EHP 2006; 114: 412
Assessment of Atherosclerosis

Libby, Nature 2002; 420: 868
$\text{PM}_{10}$ enhances atherosclerosis in rabbits

PM$_{10}$-Pharingeal 5 mg, 2x/w x 4 w.

42-week → 46-week

Ath Lesions
BM turnover

Suwa et al, JACC 2002; 39: 935
PM$_{2.5}$ Promotes Atherosclerosis in ApoE KO

Table 3. Analysis of Plaque and Immunohistochemical Staining Parameters*

<table>
<thead>
<tr>
<th>Staining</th>
<th>Normal Chow, Mean (SD)</th>
<th>High-Fat Chow, Mean (SD)</th>
<th>P Value†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Filtered Air</td>
<td>PM$_{2.5}$</td>
<td></td>
</tr>
<tr>
<td>Plaque area, %</td>
<td>13.2 (8.1)</td>
<td>19.2 (13.1)</td>
<td>.15</td>
</tr>
<tr>
<td>Oil red-O</td>
<td>10.0 (4.1)</td>
<td>15.3 (11.8)</td>
<td>.13</td>
</tr>
<tr>
<td>CD68</td>
<td>7.0 (2.2)</td>
<td>12.8 (3.7)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>3-Nitrotyrosine</td>
<td>1.1 (0.8)</td>
<td>4.4 (1.5)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Endothelial NOS</td>
<td>0.6 (0.3)</td>
<td>1.1 (0.5)</td>
<td>.06</td>
</tr>
<tr>
<td>Inducible NOS</td>
<td>0.8 (0.5)</td>
<td>3.2 (0.9)</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

Aortic Oxidative Stress

Sun et al, JAMA 2005; 294: 3003
UFP Enhance Atherosclerosis in ApoE null mice

6-week-old male
Chow

Exposures: 5 hours/session
3x/week for 5 weeks

Filtered air (FA)
PM<2.5μm (FP)
PM<0.18μm (UFP)

New Investigational Approaches
Lipid Peroxidation

arachidonic acid

12-HETE
5-HETE
15-HETE

linoleic acid

9-HODE
13-HODE

4-HNE
MDA
PM Enhances Lipid Peroxidation in the Blood

8-week-old male

Exposures: 6 hours/session
DE at ~ 250 µg/m³ PM2.5
5x/w for 2 w +/- FA x 1 w

Diesel Exhaust (DE)

Filtered Air (FA)

Chow

DE followed by FA (DE+FA)

12-HETE

13-HODE

linoleic acid
arachidonic acid
12/15 LO
12-HETE

non-enzymatic
8-isoprostanes

Yin et al, ATVB 2013; 33: 1153
**PM Promotes Lipid Peroxidation in the Liver**

**Hepatic levels of HETEs and HODEs**

<table>
<thead>
<tr>
<th>Liver (ng/ml)</th>
<th>FA</th>
<th>DE</th>
<th>DE+FA</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-HETE</td>
<td>10.6±3.70</td>
<td>56.9±17.0 *</td>
<td>25.3±10.1</td>
</tr>
<tr>
<td>12-HETE</td>
<td>111.1±38.0</td>
<td>119.1±14.0</td>
<td>102.9±13.4</td>
</tr>
<tr>
<td>15-HETE</td>
<td>84.1±8.13</td>
<td>93.7±14.7</td>
<td>108.4±7.05</td>
</tr>
<tr>
<td>9-HODE</td>
<td>1413.7.5±130.1</td>
<td>1304.2±150.3</td>
<td>1365.3±49.8</td>
</tr>
<tr>
<td>13-HODE</td>
<td>1247.5±192.7</td>
<td>989.1±188.7</td>
<td>1357.8±105.8</td>
</tr>
</tbody>
</table>

* p<0.05

![Liver 5-HETE level (fold over FA)](chart.png)
Assessment of Plasma Lipoproteins

DCFH-DA → Hydrolysis → DCFH → Esterase → ROS → DCF

LDL Oxidizability

LDL → oxLDL → ROS → DCFH → DCF

HDL Antioxidant Capacity

LDL → oxLDL → ROS → DCFH → DCF

HDL
PM Increases the Oxidizability of VLDL+LDL Lipoproteins

![LDL diagram with labels: Apoprotein B-100, monolayer of phospholipid & cholesterol, core: cholesteryl esters & some triacylglycerols.]

![Graph showing Relative Fluorescence Units (p<0.05) for FA, DE, DE+FA]
PM Exposures lead to Prooxidative HDL

\[
\text{HDL Oxidant Index} = \frac{\text{FU (+ HDL)}}{\text{FU (- HDL)}}
\]

\[\text{Yin et al, ATVB 2013; 33: 1153}\]
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Summary

✓ Inhalation of air pollutants induce a variety of health effects resulting in increased morbidity and mortality.

✓ Health effects are diverse and affect many organs and systems.

✓ Both gaseous and particulate constituents are toxic.

✓ Most of the mortality is due to cardiovascular and cerebrovascular diseases.

✓ There is need to discover/develop novel biomarkers of exposures and biomarkers of health effects.
Effects of Common Air Pollutants

Respiratory Effects

Symptoms:
- Cough
- Phlegm
- Chest tightness

Increased sickness and premature death from:
- Asthma
- Bronchitis (acute or chronic)
- Emphysema
- Pneumonia

Development of new disease
- Chronic bronchitis
- Premature aging of the lungs

How Pollutants Cause Symptoms

Airway inflammation:
- Infiltrate of white blood cells
- Abnormal mucus production
- Fluid accumulation and swelling (edema)
- Death and shedding of cells that line the airways

Increased Susceptibility to Respiratory Infection

Normal

Lung with respiratory infection

Cardiovascular Effects

Symptoms:
- Chest tightness
- Chest pain (angina)
- Palpitations
- Shortness of breath
- Unusual fatigue

Increased sickness and premature death from:
- Coronary artery disease
- Abnormal heart rhythms
- Congestive heart failure

How Pollutants May Cause Symptoms

Vascular inflammation:
- Increased risk of blood clot formation
- Narrowing of coronary (aorta) arteries
- Increased risk of atherosclerotic plaque rupture

Effects on Cardiovascular Function

Low oxygenation of red blood cells
- Abnormal heart rhythms
- Altered autonomic nervous system control of the heart

Reduce your risk by using the Air Quality Index (AQI) to plan outdoor activities – www.airnow.gov

<table>
<thead>
<tr>
<th>AQI Levels of Health Concern</th>
<th>AQI Values</th>
<th>What Action Should People Take?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Good</td>
<td>0-50</td>
<td>Enjoy Activities</td>
</tr>
<tr>
<td>Moderate</td>
<td>51-100</td>
<td>People unusually sensitive to air pollution: Plan strenuous outside activities when air quality is better</td>
</tr>
<tr>
<td>Unhealthy for Sensitive Groups</td>
<td>101-150</td>
<td>Sensitive Groups: Cut back or reschedule strenuous outside activities People with heart or lung disease (including diabetes), older adults, and children under 18 years. Adults and children with respiratory or heart disease Suffer especially. Active children and adults with asthma. Carbon Monoxide: People with heart disease and possibly others and infants.</td>
</tr>
<tr>
<td>Unhealthy</td>
<td>151-200</td>
<td>Everyone: Cut back or reschedule strenuous outside activities Sensitive groups: Avoid strenuous outside activities</td>
</tr>
<tr>
<td>Very Unhealthy</td>
<td>201-300</td>
<td>Everyone: Significantly cut back on outside physical activities Sensitive groups: Avoid all outside physical activities</td>
</tr>
</tbody>
</table>

This information is based on the EPA’s AirNow.gov site.
Lipid Peroxidation and Biomarkers

Lipid Peroxidation

Dysfunctional HDL
oxLDL

Activation 5-LO

Atherosclerosis

Lipid Peroxidation

Lipid Peroxidation

Lipid Peroxidation

Lipid Peroxidation

Lipid Peroxidation
Air Pollution and Atherosclerosis

Araujo & Rosenfeld, In Air Pollution and Health Effects 2015
Perspectives

- Development of standards
- Policies
- Implementation on susceptible groups

Research

- Basic
  - Pathophysiology
  - Mechanistics
  - Pathways
  - Genetic susceptibility
  - Toxicity

- Epidemiological
  - Metrics
  - Risk assessment
  - Dose-response
  - Genetic susceptibility

Education

- Physicians
- Patients

Regulation & Implementation

- Development of standards
- Policies
- Implementation on susceptible groups