Generation of Hydrogen Peroxide by Urban Aerosols and Behavior of Hydrogen Peroxide in the VACES

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Outline

• Introduction: reactive oxygen species
• Ambient particles generate hydrogen peroxide
• A few related measurements
• Working out the mechanism
• Gas phase hydrogen peroxide in the virtual aerosol concentration enrichment system (VACES)
Reactive Oxygen Species

$O_2 \rightarrow \rightarrow O_2^\cdot \rightarrow \rightarrow HO_2 \rightarrow \rightarrow$

$H_2O_2 \rightarrow \rightarrow OH\cdot \rightarrow \rightarrow H_2O$

Other ROS: ClO$^-$, O($^1$D)
Reactive Oxygen Species (ROS) May Play a Role in Particle Toxicity

• ROS are generated by lung tissues in response to foreign material, but sometimes this process gets out of control, resulting in a state of oxidative stress and inflammation.

• ROS have been implicated in respiratory diseases such as asthma, pulmonary and circulatory morbidity and mortality and in carcinogenesis.
Hydrogen peroxide, like other gasses, follows Henry’s Law:

\[ [X]_{aq} = H_x P_x \text{(atm)} \]

- \([X]_{aq}\) = concentration of \(X\) in liquid phase
- \(H_x\) = The Henry’s Law Coefficient for \(X\);  
- \(H(\text{hydrogen peroxide}) = 10^5 \text{ M/atmosphere}\)
- \(P_x\) = The gas phase concentration of \(X\), in atmospheres.
Particles Provide a Way to Deliver \( \text{H}_2\text{O}_2 \) to the Lungs

- Most gas-phase \( \text{H}_2\text{O}_2 \) should be absorbed in the upper airways, but particles can deliver peroxide to the lungs.
- Due to ppb-levels of hydrogen peroxide in air, peroxides in airborne water should be \( \sim 0.1 \text{ mM} \).
- Our measurements indicate ambient particles generate \( \text{H}_2\text{O}_2 \) in aqueous solution, producing levels equivalent to \( >50 \text{ mM} \) in aerosol liquid water.
Does H$_2$O$_2$ Contribute to Particle Associated Health Effects? *In Vitro and in Vivo Results*

- Exposure of lung epithelial cells to 20 pM - 1 µM hydrogen peroxide solutions results in significant cell damage.$^1$

- Morio et al. (2001) exposed rats to ammonium sulfate aerosols (100-200 × ambient), and H$_2$O$_2$ (10 - 100 × ambient) alone or in combination, for 2 hours. These aerosols contained 10-180 ng/m$^3$ H$_2$O$_2$, according to our measurements & consistent with Henry’s law.

- Ambient aerosols generate H$_2$O$_2$ while ammonium sulfate aerosols do not. Ambient aerosols have 10-~120 ng/m$^3$ H$_2$O$_2$ associated with them.
In Vivo data are Consistent with Significant Effects from Aerosol-Generated ROS at Levels Common in Urban Areas

- All but the most minor effects were observed only when rats were exposed to $\text{H}_2\text{O}_2$ and particles in combination.

- Effects:
  - Increased numbers of neutrophils in pulmonary capillaries.
  - Production of tumor necrosis factor $\alpha$ by alveolar macrophages.
  - Increased production of superoxide by alveolar macrophages.
  - Increased expression of antioxidant heme oxygenase-1 by stimulated alveolar macrophages.
A role in aerosol aging?

The quantity of $\text{H}_2\text{O}_2$ produced by ambient particles is comparable (larger) than the flux of OH radicals to the surface of fine mode particles.
Field Measurements of Particle-Associated Hydrogen Peroxide
Measurement of Peroxides Associated with Aerosols

Collect particles on Teflon filters, add stripping solution (0.1 mM Na$_2$EDTA, various pHs, 4 mL), extract for two hours with gentle agitation.

Monitor H$_2$O$_2$ using HPLC/fluorescence via the reaction of H$_2$O$_2$ with horseradish peroxidase and p-hydroxyphenylacetic acid.
Validation of Aerosol Sampling Method

- Aqueous H$_2$O$_2$ aerosols were generated with a Collison nebulizer, diluted, collected on filters and extracted. Final [H$_2$O$_2$] were within 20% of the expected values, indicating that H$_2$O$_2$ solutions do not decompose appreciably on Teflon filters.

- Gas Phase H$_2$O$_2$ is not collected on second filter

PEROXIDE SAMPLING: GAS PHASE

- Gas phase peroxides are partitioned into the aqueous phase
- Collection efficiency >95%

Source: Hartkamp & Bachhausen *Atmos. Environ.*, 1987, 21, 2207
Virtual Impactor Sample Collection

**Ambient Air**

Virtual Impactor
Size Cut 1.8/2.5 μm

Ambient Air

Major Flow (105/55 lpm)  
Fine Mode

Minor Flow (5/2.5 lpm)  
Coarse Mode

FLOW METERS  
VIRTUAL IMPACTORS  
FINE MODE (105/55 lpm)  
COARSE MODE (52.5 lpm)  
FILTER HOLDERS (47mm)(637,818),(743,895)
H$_2$O$_2$ Generation by Aerosols Varies by Site

Fine (blue), Coarse (yellow) Hydrogen Peroxide
H$_2$O$_2$ Generation by Aerosols Normalized to Mass Varies by Site

**Fine** (blue), **Coarse** (yellow)

Hydrogen Peroxide

**Graph:**
- **110 Freeway**
- **UCLA**

**Axes:**
- Y-axis: $\text{H}_2\text{O}_2$/particle mass (ng/µg)
- X-axis: Sampling Date

**Legend:**
- Fine Mode
- Coarse Mode
Hydrogen Peroxide is Generated in Aqueous Solution
Peroxides exceed levels predicted by Henry’s law by a factor of ~700.

Henry’s Law Expectation (red): $[\text{H}_2\text{O}_2] = P_{\text{H}_2\text{O}_2} \text{ (measured)} \times H_{\text{H}_2\text{O}_2} \text{ (known)}$

Equivalent measured aerosol $\text{H}_2\text{O}_2$ "concentration" = measured aerosol phase $\text{H}_2\text{O}_2$ ÷ aerosol liquid water content (which is calculated from the measured aerosol mass × f(RH)*)

*LWC dependence on RH from Slone and Wolff, 1985.
More Field Data
2006 Annually Averaged Fine Particles ($\mu g/m^3$); Standard = 15 ($\mu g/m^3$)
# Summary of Multi-Day Averages

<table>
<thead>
<tr>
<th></th>
<th>UCLA 05</th>
<th>110 Fwy</th>
<th>UCLA 09 fine Riv. 1 crse</th>
<th>Riv. 2 (Orange Grove)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ultrafines (PM 0.15 to 0.18)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mass (µg/m³)</td>
<td>~0.8</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>H₂O₂ (ng/m³)</td>
<td>~0.4</td>
<td>--</td>
<td>--</td>
<td>0.9 ±0.35</td>
</tr>
<tr>
<td>H₂O₂/mass</td>
<td>~0.5</td>
<td>--</td>
<td>--</td>
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</tr>
<tr>
<td><strong>PM 2.5</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mass (µg/m³)</td>
<td>13 ± 10</td>
<td>23 ± 8</td>
<td>18 ± 7 (UCLA)</td>
<td>19 ± 6</td>
</tr>
<tr>
<td>H₂O₂ (ng/m³)</td>
<td>5.4 ± 6</td>
<td>12 ± 9</td>
<td>1.8 ± 1.4</td>
<td>8 ± 8</td>
</tr>
<tr>
<td>H₂O₂/mass**</td>
<td>0.42 ± 0.3*</td>
<td>0.58 ± 0.3</td>
<td>0.11 ± 0.08</td>
<td>0.5 ± 0.6*</td>
</tr>
<tr>
<td><strong>PM &gt; 2.5</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mass (µg/m³)</td>
<td>26 ± 15</td>
<td>27 ± 33</td>
<td>98 ± 26 (Riv)</td>
<td>50 ± 21</td>
</tr>
<tr>
<td>H₂O₂ (ng/m³)</td>
<td>13 ± 10</td>
<td>20 ± 9</td>
<td>33 ± 13</td>
<td>17 ± 8</td>
</tr>
<tr>
<td>H₂O₂/mass**</td>
<td>0.58 ± 0.3*</td>
<td>1.05 ± 0.3</td>
<td>0.37 ± 0.2*</td>
<td>0.48 ± 0.32</td>
</tr>
</tbody>
</table>

*Correlation between H₂O₂ and particle mass not significant

** in ng/µg
Extraction in Simulated Lung Fluid, Normalized to Extraction at pH 7.4

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Gamble’s</th>
<th>Ringer’s</th>
</tr>
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<tbody>
<tr>
<td>NaCl</td>
<td>116</td>
<td>114</td>
</tr>
<tr>
<td>NH₄Cl</td>
<td>10</td>
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<tr>
<td>NaHCO₃</td>
<td>27</td>
<td>31</td>
</tr>
<tr>
<td>Na₂HPO₄</td>
<td>1.2</td>
<td>14.6</td>
</tr>
<tr>
<td>NaH₂PO₄</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>Na₃Citrate</td>
<td>0.2</td>
<td>-</td>
</tr>
<tr>
<td>Glycine</td>
<td>6</td>
<td>-</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>0.2</td>
<td>-</td>
</tr>
<tr>
<td>pH</td>
<td>7.4</td>
<td>7.4</td>
</tr>
<tr>
<td>Ionic Strength</td>
<td>160</td>
<td>190</td>
</tr>
</tbody>
</table>

Chemical Molar Concentration (mM)
Closely Related Measurements
Dichlorofluorescein Assay for ROS

- Used to monitor ROS in cells since at least the early 1990’s
- Due to its indiscriminate nature to various free radicals, DCF can be useful in quantifying overall oxidative stress in cells
- Fluorescence signal is linear with some oxidants (including H$_2$O$_2$) and nonlinear with others
- The assay has been applied to ambient aerosols since the late 1990’s.

Sources: MEDICINA INTERNA E TERAPIA MEDICA, H. WANG & J. A. JOSEPH, 1999
H$_2$O$_2$ and ROS generation by ambient coarse mode aerosols at several locations

<table>
<thead>
<tr>
<th>Site</th>
<th>PM Mass (µg/m$^3$)</th>
<th>PM ROS or H$_2$O$_2$ (ng/m$^3$)</th>
<th>PM ROS or H$_2$O$_2$ /Mass (ng/µg)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Riverside, CA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>upwind</td>
<td>46 ± 22</td>
<td>17 ± 8</td>
<td>0.48 ± 0.32</td>
<td>UCLA</td>
</tr>
<tr>
<td>downwind</td>
<td>97 ± 27</td>
<td>34 ± 14</td>
<td>0.37 ± 0.18</td>
<td></td>
</tr>
<tr>
<td>Los Angeles, CA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UCLA campus</td>
<td>26 ± 15</td>
<td>14 ± 10</td>
<td>0.58 ± 0.3</td>
<td>UCLA</td>
</tr>
<tr>
<td>110 freeway</td>
<td>27 ± 33</td>
<td>20 ± 9</td>
<td>1.05 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>Taipei, Taiwan</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sidewalk</td>
<td>8 ± 3</td>
<td>2 ± 1</td>
<td>0.28 ± 0.11</td>
<td>Hung and Wang 2001</td>
</tr>
<tr>
<td>underpass</td>
<td>14 ± 8</td>
<td>6</td>
<td>0.43 ± 0.15</td>
<td></td>
</tr>
<tr>
<td>Rubidoux, CA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>urban</td>
<td>-</td>
<td>26</td>
<td>-</td>
<td>Venkatachari et al 2005</td>
</tr>
<tr>
<td>Flushing, NY</td>
<td></td>
<td></td>
<td></td>
<td>“ 2007</td>
</tr>
<tr>
<td>campus</td>
<td>-</td>
<td>5</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

White = H$_2$O$_2$  Orange = ROS by dichlorofluorescin
<table>
<thead>
<tr>
<th>Site</th>
<th>PM Mass (µg/m³)</th>
<th>PM H₂O₂ (ng/m³)</th>
<th>PM H₂O₂/Mass (ng/µg)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Riverside, CA</td>
<td></td>
<td></td>
<td></td>
<td>UCLA Wang et al</td>
</tr>
<tr>
<td>(orange grv)</td>
<td>19 ± 6</td>
<td>8 ± 7</td>
<td>0.49 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>Los Angeles, CA</td>
<td></td>
<td></td>
<td></td>
<td>UCLA</td>
</tr>
<tr>
<td>UCLA 2005</td>
<td>15 ± 17</td>
<td>4 ± 2</td>
<td>0.50 ± 0.28</td>
<td>Arellanes et al.,</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Wang et al.</td>
</tr>
<tr>
<td>UCLA 2009</td>
<td>18 ± 7</td>
<td>1.8 ± 1.4</td>
<td>0.11 ± 0.08</td>
<td></td>
</tr>
<tr>
<td>110 freeway</td>
<td>27 ± 22</td>
<td>8 ± 6</td>
<td>0.45 ± 0.25</td>
<td></td>
</tr>
<tr>
<td>Taipei, China</td>
<td></td>
<td></td>
<td></td>
<td>Hung &amp; Wang</td>
</tr>
<tr>
<td>sidewalk</td>
<td>16 ± 8</td>
<td>11 ± 8</td>
<td>0.57 ± 0.16</td>
<td></td>
</tr>
<tr>
<td>underpass</td>
<td>99 ± 27</td>
<td>37</td>
<td>0.37 ± 0.18</td>
<td></td>
</tr>
<tr>
<td>Singapore</td>
<td></td>
<td></td>
<td></td>
<td>See et al.</td>
</tr>
<tr>
<td>campus</td>
<td>19 ± 2</td>
<td>194 ± 70</td>
<td>10 ± 3</td>
<td></td>
</tr>
<tr>
<td>curbside</td>
<td>33 ± 6</td>
<td>513 ± 25</td>
<td>16 ± 2</td>
<td></td>
</tr>
<tr>
<td>Rubidoux, CA</td>
<td>urban</td>
<td></td>
<td></td>
<td>Venkatachari et al</td>
</tr>
<tr>
<td></td>
<td>193</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flushing, NY</td>
<td>campus</td>
<td>29</td>
<td></td>
<td>&quot;</td>
</tr>
</tbody>
</table>

White = H₂O₂  Orange = ROS by dichlorofluorescin
OH radical assays: usually add and electron donor or $\text{H}_2\text{O}_2$, detect w/ scavenger technique.

- Electron spin resonance
  - e.g., Shi et al. 2003, others
- 2-Deoxyribose + OH → Malondialdehyde
  - e.g., Ball et al. 2000
- Benzoate + OH → p-Hydroxybenzoate
  - e.g., Vidrio et al. 2008

Methods are mostly sensitive to transition metals;
e.g., $\text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \text{OH}^\cdot + \text{OH}^\cdot$
Transition metal mediated OH formation with added H$_2$O$_2$ is larger in coarse particles

Hydroxyl radical generation by serial dilutions of suspensions of coarse and fine PM

(Shi et al. 2003)
Metal-mediated OH generation with added ascorbate or H₂O₂ (large excess)

Filled symbols: left axis, study from Ball et al. (2000)
Open symbols: right axis, study from Vidrio et al. (2008)
What Generates the Oxidants?
What is the Mechanism?

• **Redox chemistry.** Quinones and metals (such as Fe$^{2+/3+}$, Cu$^{+/2+}$) mediate redox chemistry that could generate H$_2$O$_2$.

• Several other sources, many likely minor: decomposition of larger hydroperoxides and other complexes, high ionic strength-induced enhancements to gas-liquid partitioning, and photochemistry.

• Data suggest different mechanisms for the fine and coarse modes.
**H$_2$O$_2$ Generation past t = 2 hours, normalized to the 2 hour level**

- **Initial H$_2$O$_2$ generation rate:**
  - **Coarse mode**, $7.8 \pm 5.7 \times 10^{-8}$ M min$^{-1}$
  - **Fine mode**, $5.9 \pm 2.8 \times 10^{-9}$ M min$^{-1}$
  - **Quinones**, $5 \times 10^{-9}$-$1 \times 10^{-7}$ M min$^{-1}$
  - **Iron/copper/zinc solution (OH radical with added H$_2$O$_2$)** $(0.14-20) \times 10^{-8}$ M min$^{-1}$ (with added H$_2$O$_2$)
Signal Persistence Over Days to Weeks

H$_2$O$_2$ activity relative to t = 0 (%)

Time (days)

Fine

Coarse
Coarse Particles: Transition Metals
Evidence for metal-mediated H₂O₂ production in the Coarse mode

<table>
<thead>
<tr>
<th></th>
<th>UCR, 2005</th>
<th>CRCAES, 2008</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R</td>
<td>p</td>
</tr>
<tr>
<td>Fe</td>
<td>0.66**</td>
<td>0.00</td>
</tr>
<tr>
<td>Zn</td>
<td>0.51</td>
<td>0.08</td>
</tr>
<tr>
<td>Cu</td>
<td>0.40</td>
<td>0.06</td>
</tr>
<tr>
<td>Al</td>
<td>.</td>
<td>.</td>
</tr>
<tr>
<td>Si</td>
<td>.</td>
<td>.</td>
</tr>
</tbody>
</table>
The pH Dependence is also consistent with a role for metals in Coarse mode H$_2$O$_2$ production.

---

Copper

Iron

Deguillaume et al 2005
The Metals and H$_2$O$_2$ are About the Same Concentration, Especially When We Consider the (Unknown) Speciation/Availability of Metals

<table>
<thead>
<tr>
<th>Type</th>
<th>(nmol/m$^3$)</th>
<th>H$_2$O$_2$</th>
<th>Fe</th>
<th>Cu</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>2005 Coarse Mean</td>
<td>1.00</td>
<td>2.05</td>
<td>0.90</td>
<td>0.56</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>0.97</td>
<td>1.60</td>
<td>0.16</td>
<td>0.52</td>
<td></td>
</tr>
<tr>
<td>2008 Coarse Mean</td>
<td>0.50</td>
<td>1.58</td>
<td>0.42</td>
<td>0.68</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>0.44</td>
<td>1.44</td>
<td>0.22</td>
<td>0.42</td>
<td></td>
</tr>
</tbody>
</table>
Fine Particles: Mixed Source
H$_2$O$_2$ Generation Varies by Source Aerosol

- Diesel Load
- Diesel Idle
- Biodiesel Load
- Biodiesel Idle
- Toluene SOA
- β-pinene SOA
- a-pinene SOA
- NIST
- Ammonium Sulfate
- Riverside_Coarse
- Riverside_Fine
- UCLA_Fine_2009
- 110 Freeway_Fine
- UCLA_Fine_2006

20 hr values

- Diesel
- Biodiesel
- SOA
- Ambient Coarse
- Ambient Fine

H$_2$O$_2$ Generation Varies by Source Aerosol

Per extracted mass

H$_2$O$_2$ (ng/µg)
The pH dependence is very different for the fine mode. It appears to be consistent with a contribution from organics.
Quinone Chemistry? Needs and Electron Donor—
Aerosol Quinone Concentrations are Small.

Source: A. Hasson
Evidence for the Contribution of Quinones in the Fine Mode: Increased Activity in the presence of Dithiothreitol

Ratio of \( \frac{[H_2O_2]_{DTT}}{[H_2O_2]_{H_2O}} \) in \( 10^{-6} \) M DTT to \( H_2O \)

- Toluene SOA
- Fine
- Coarse
- a-pinene SOA
- \( \beta \)-pinene SOA
- Biodiesel Idle
- Biodiesel load
- Diesel idle
- Diesel load

\[ \begin{align*}
Q + DTT & \rightarrow Q^- + DTT-thiyl \\
Q + DTT-thiyl & \rightarrow Q^- + DTT-disulfide \\
Q^- + O_2 & \rightarrow Q + O_2^- \\
O_2^- + H^+ & \rightarrow HO_2 \rightarrow H_2O_2
\end{align*} \]
Conclusions

- Particles generate high concentrations of \( \text{H}_2\text{O}_2 \) for many hours after they are removed from the atmosphere.

- *In vitro* and *in vivo* results point to significant cell damage from aerosol borne/generated \( \text{H}_2\text{O}_2 \).

- Aerosol borne ROS is variable day-to-day and location to location; source seems to be metal-mediated redox activity for larger particles, possibly organics, including quinones, for smaller particles.
Behavior of Hydrogen Peroxide in the VACES
Virtual Aerosol Concentration Enrichment System Operation

1. Ambient air is filtered through a HEPA filter array to produce particle-free air.
2. The particle-free air is directed to the VACES (Virtual Aerosol Concentration Enrichment System) chamber.
3. H2O2 vapor is added to the air stream in the VACES chamber.
4. The mixture is then passed through a cooler at -8 °C to condense the H2O2 vapor into liquid H2O2.
5. The cooled mixture is directed to a heated DI water bath to raise the temperature to 28~32 °C.
6. The heated mixture is then directed to a pump.
7. The system can be operated in coarse or fine mode, with the choice determined by the application.

Special gas generator
Particle generator

Heated DI water bath
Electric heater
Diffusion dryer
Pump
The VACES does not appreciably effect the Aerosol H₂O₂, because most of the H₂O₂ is generated by the Particles

Comparison of H₂O₂ associated with particles with (dark red) and without (yellow) the VACES in line.

Hydrogen Peroxide is Taken Up by the Water Bath

H₂O₂ is also elevated in other condensed water (liquid, ice) that collects in various places in the instrument.

Jung, Arellanes, Zhao, Paulson, Anastasio and Wexler, submitted to AS&T
The Effect of the VACES can be to Concentrate OR Enrich soluble gasses, depending on its Operating Conditions

The enrichment step concentrates soluble gasses; the water bath etc depletes them.
Conclusions: VACES & soluble gasses

• Soluble gasses are taken up in the water bath and other condensed phases

• Soluble gasses are concentrated by the particle enrichment step.

• Since the two processes act in opposite directions, the gas phase concentrations of soluble gasses are generally only moderately perturbed. Presumably due to the same combination of processes, nitric acid is fairly depleted by the VACES, and ammonia moderately enriched.
Acknowledgements

Research Group: Chuautemoc Arellanes, Ying Wang, Shishan Hu, Leila Lackey, Daniel Curtis, Hwajin Kim, Albert Chung & Brian Barkey

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