This work is licensed under a Creative Commons Attribution-NonCommercial-ShareAlike License. Your use of this material constitutes acceptance of that license and the conditions of use of materials on this site.
Air Toxicants

Michael A. Trush, PhD
Johns Hopkins University
OZONE: A Criteria Air Pollutant
Section A : Sources and Pulmonary Responses to Air Toxicants
Air Pollutants

- Heat from the sun reacts with chemicals from the air (nitrogen oxides, particulate matter) to form photo-chemical smog
- Layer of warm air traps smog
- Population exposed to high levels of
  - Particulate matter
  - Sulfuric Acid and sulfate and nitrate salts
  - Carbon monoxide
  - Lead
A Smog Wave Is Born
How Baltimore experienced six straight days of unhealthful smog

- Winds from the west bring pollutants from as far away as Pittsburgh and the Ohio Valley. A high pressure ridge settles just to the west, keeping clouds from forming.
- Hydrocarbons and nitrogen oxides from cars, smokestacks, and other sources mix solar-heated air with pollutants from the west. The sun’s rays stimulate a chemical reaction, forming ozone.
- The easterly breeze blowing off the Chesapeake Bay acts as a “wall,” holding pollutants alongside the Interstate 95 corridor, allowing smog to build up.

Source: University of Maryland; Maryland Dept of the Environment
Generation of Ozone via the Photolytic Cycle
Photolytic Cycle

ENGINE → NO₂ → NO + O → O₂

UV Radiation from sunlight

O₂ → O₃ → O₂

Continued
Photolytic Cycle

$NO_2 \xrightarrow{\text{UV Radiation from sunlight}} NO + O•$

$O_2 + HC \xrightarrow{} HCO_2$

Continued
Photolytic Cycle

ENGINE

$\text{O}_2 + \text{HC}$

$\rightarrow$ $\text{HCO}_2$

$\rightarrow$ $\text{NO}_2$

$\rightarrow$ $\text{NO} + \text{O}^\cdot$

UV Radiation from sunlight

$\text{O}_2$

$\text{O}_3$

$\text{O}_2$
**EPA Color Coding for Ozone**

<table>
<thead>
<tr>
<th>Ozone Concentration (ppm) (8-hour average, unless noted)</th>
<th>Air Quality Index Values</th>
<th>Air Quality Descriptor</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0 to 0.064</td>
<td>0 to 50</td>
<td>Good</td>
</tr>
<tr>
<td>0.065 to 0.084</td>
<td>51 to 100</td>
<td>Moderate</td>
</tr>
<tr>
<td>0.085 to 0.104</td>
<td>101 to 150</td>
<td>Unhealthy for Sensitive Groups</td>
</tr>
<tr>
<td>0.105 to 0.124</td>
<td>151 to 200</td>
<td>Unhealthy</td>
</tr>
<tr>
<td>0.125 (8-hr.) to 0.404 (1-hr.)</td>
<td>201 to 300</td>
<td>Very Unhealthy</td>
</tr>
</tbody>
</table>
Section B

Responses to Ozone Exposure
Common Symptoms of Irritation from Air Pollution

- Headaches
- Dizziness
- Eye irritation
- Nasal discharge
- Coughing
- Airway constriction
- Sore throat
- Shortness of breath
- Lung damage
Pulmonary Responses to Toxicants

- Local irritation, which results in bronchial constriction and edema; secondary infection (bronchitis) frequently compounds the damage
- Damage to the cells of the airway which results in necrosis
Pulmonary Responses to Toxicants

- Fibrosis (excessive deposition of the extracellular matrix) and emphysema (loss of airways)
- Airway constriction through allergic responses (asthma)
- Lung cancer (ozone may be a tumor promoter)
Acute Effects of Ozone Exposure

1-3 H Acute Ozone Exposure

- Exercise performance decline
  - >10% drop in mean FEV₁
- Increased symptoms
  - >10% drop in individual FEV₁

4-8 H Acute Ozone Exposure

- Increased symptoms
  - >10% drop in individual FEV₁
- Lung cellular injury in animals

Background ozone levels:

- 0.01
- 0.02
- 0.04
- 0.08
- 0.10
- 0.12
- 0.20
- 0.25
- 0.50
- 1.0
Section C: Toxicokinetics and Toxicodynamics of Ozone
**Where Ozone Goes in the Body When Inhaled**

Excess Oxygen-18 in Tissues of Animals Exposed to 1.0ppm $^{18}\text{O}_3$

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Animal</th>
<th>Tissue</th>
<th>n</th>
<th>Tissue Oxygen, Percent of Dry Weight</th>
<th>$^{18}\text{O}$ Enrichment μmoles $^{18}\text{O}$/mole Total Oxygen</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Mouse</td>
<td>Lung</td>
<td>5</td>
<td>19.3 ± 0.5</td>
<td>14.8 ± 7.8</td>
</tr>
<tr>
<td>2</td>
<td>Rat</td>
<td>Lung</td>
<td>5</td>
<td>18.6 ± 1.8</td>
<td>6.6 ± 6.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Trachea</td>
<td>4</td>
<td>21.3 ± 1.2</td>
<td>12.4 ± 8.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nasopharynx</td>
<td>4</td>
<td>19.5 ± 2.3</td>
<td>7.3 ± 1.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Blood</td>
<td>4</td>
<td>22.3 ± 0.3</td>
<td>-1.1 ± 0.0</td>
</tr>
<tr>
<td>3</td>
<td>Rabbit</td>
<td>Lung</td>
<td>4</td>
<td>19.9 ± 0.1</td>
<td>15.6 ± 3.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Alveolar Macrophage</td>
<td>2</td>
<td>14.6 ± 0.4</td>
<td>45.2 ± 3.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lung Surfactant Pellet</td>
<td>2</td>
<td>10.1 ± 0.8</td>
<td>55.0 ± 6.5</td>
</tr>
</tbody>
</table>
Schematic Representation
Ozone Reaction with Monoenoic Fatty Acid

R′CH = CHR → R′C═CHR + CR

R′C═CHR → R′CH = CHR + CR

RCOOH + H₂O → RCHO + H₂O₂
Schematic Representation
Ozone Reaction with Polyenoic Fatty Acid

\[ \text{R'CH} = \text{CH-CH}_2\text{-CH} = \text{CHR} \]

\[ \text{O}_3 \quad \text{H}_2\text{O} \quad \rightarrow \]

\[ \text{R'CHO(COOH)} \quad \text{RCHO(COOH)} \quad \text{CHO(COOH)} \quad + \text{H}_2\text{O}_2 \]

\[ \text{CH}_2 \]

\[ \text{CHO(COOH)} \]
Schematic Representation

*Ozone Reaction with Membrane Proteins*
Infectivity Model
Effect of a Three-Hour Exposure to Various Concentrations of $O_3$ on Mice Challenged with Aerosols of *Streptococcus*
Cellular and Biochemical Changes in BAL of Subjects Exposed to O₃ and Air

- Protein
- Albumin
- IgG
- Fibronectin
- Elastase
- U-PA
- Tissue Factor
- Factor VII
- C3a
- PGE2
- LTB4
- Acid Phosphatase
- PMNs
- Elastase
- Acid Phosphatase
- B-Glucuronidase

FOLD INCREASE (OZONE/AIR)
Generation by PMNs

Myeloperoxidase

Lysozyme

H₂O₂ + Cl⁻ → HOCl

O₂ → O₂⁻

O₂⁻ + NADPH oxidase → NADP⁺ + NADPH
Ozone Can Inactivate Alpha Antitrypsin

Similar to Cigarette Smoke

Inhalation of particles and vapors

Alveolus

Type I pneumocyte

Type II pneumocyte

Alveolar macrophages and PMNs phagocytize tar particles
Elastase

Cigarette smoke $\to O_2^- \to H_2O_2$

A1-antitrypsin (elastase inhibitor)

Hereditary A1-antitrypsin deficiency

Decrease

Neutrophil (PMN)

Protease-containing granules

Serine elastase

Uncontrolled proteolysis

Alveolar wall

Elastic tissue

Deconstruction of elastic tissue $\to$ Emphysema
BENZENE: A HAZARDOUS AIR TOXICANT
Section A: What Is Benzene and Who Is Exposed?
Preventive Interventions

Benzene Exposure → Internal Dose → Biologically Effective Dose → Early Biological Effect → Altered Structure/Function → Disease

Susceptibility Factors
Chemical and Physical Properties

Production and Use of Benzene

- Benzene (C₆H₆) has the following chemical and physical properties:
  - Molecular weight: 78.1
  - Description: Clear, colorless, highly flammable liquid
  - Boiling point: 80.1°C
Chemical and Physical Properties

Production and Use of Benzene

- Melting point: 5.5 °C
- Density: $d_4$ 0.8787
- Refractive index: $n_D^{29}$ 1.5016
- Volatility: Vapor pressure of 74.6 mm Hg at 20 °C

Continued
Chemical and Physical Properties

Production and Use of Benzene

- Solubility: Slightly soluble in water (0.8 part by weight in 1,000 parts of water at 20 °C)
- Miscible with acetone, alcohol, carbon disulfide, carbon tetrachloride, chloroform, ether, glacial acetic acid and oils
Benzene Emissions in the Los Angeles Basin

- Autos (82%)
- Industry (14%)
- Personal & Home (3%)
- Cigarettes (1%)
Sources of Benzene Exposure

Smokers

- Smoking: 89%
- ETS: 2%
- Outdoor air: 4%
- Personal/indoor: 3%
- Driving Car: 2%
Sources of Benzene Exposure

Nonsmokers

- Personal/indoor 31%
- Outdoor air 40%
- Driving Car 19%
- ETS 10%
Exposed Populations and Allowed Levels

- The following population groups may be exposed to benzene:
  - Workers engaged in its production
  - Workers in chemical industries using benzene as an intermediate
  - Workers in industries producing material containing benzene
Exposed Populations and Allowed Levels

- Workers utilizing or handling compounds containing benzene: for instance, as a constituent in gasoline, as a solvent in rubber cement, or as an impurity in industrial toluene

- People living in industrialized towns near factories producing or employing benzene, or compounds containing it

Continued
Exposed Populations and Allowed Levels

- The general overall population since benzene is contained in gasoline and can also be found as a contaminant in drinking water.
**Local Air Is a "Soup" of Pollutants**

Carcinogens in the air

This table shows the concentration of three cancer-causing chemicals in the air at four state monitoring locations in Baltimore. The figures show what toxic levels are generally highest in the downtown area, which state officials attribute to car and truck exhaust and gasoline vapors. All figures are in parts per billion.

<table>
<thead>
<tr>
<th>Chemical</th>
<th>State Limit</th>
<th>1 Downtown</th>
<th>2 Curtis Bay</th>
<th>3 Fairfield</th>
<th>4 Holabird</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzene</td>
<td>0.39</td>
<td>1.84</td>
<td>1.73</td>
<td>1.55</td>
<td>1.34</td>
</tr>
<tr>
<td>1,3- Butadiene</td>
<td>0.016</td>
<td>0.32</td>
<td>0.17</td>
<td>0.22</td>
<td>0.21</td>
</tr>
<tr>
<td>Carbon tetrachloride</td>
<td>0.1</td>
<td>0.12</td>
<td>0.14</td>
<td>0.14</td>
<td>0.13</td>
</tr>
</tbody>
</table>

Source: Maryland Dept. of the Environment
Section B: Health Effects of Acute and Chronic Benzene Exposure
<table>
<thead>
<tr>
<th>Benzene Concentration (ppm)</th>
<th>Duration of Exposure</th>
<th>Effects Observed</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>8</td>
<td>None</td>
<td>Gerarde, 1963</td>
</tr>
<tr>
<td>50-150</td>
<td>5</td>
<td>Headache, lassitude, weariness</td>
<td>Gerarde, 1963</td>
</tr>
<tr>
<td>500</td>
<td>1</td>
<td>Symptoms of illness</td>
<td>Gerarde, 1963</td>
</tr>
<tr>
<td>1,500</td>
<td>1</td>
<td>Serious symptoms</td>
<td>Gerarde, 1963</td>
</tr>
<tr>
<td>1,500-3,000</td>
<td>Several hours</td>
<td>Slight symptoms</td>
<td>Bloomfield, 1951</td>
</tr>
<tr>
<td>1,550-3,100</td>
<td>6</td>
<td>No serious effects</td>
<td>Hamilton, 1934</td>
</tr>
<tr>
<td>1,570 - 3130</td>
<td>Several hours</td>
<td>Slight symptoms</td>
<td>Browning, 1937</td>
</tr>
</tbody>
</table>

Various reported adverse effects in humans after short-term exposure to various concentrations of benzene vapor (Paustenbach et al., 1993)
<table>
<thead>
<tr>
<th>Benzene Concentration (ppm)</th>
<th>Duration of Exposure</th>
<th>Effects Observed</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>3,000</td>
<td>0.5</td>
<td>Endurable</td>
<td>Gerarde, 1963</td>
</tr>
<tr>
<td>3,000</td>
<td>0.5-1</td>
<td>Dangerous</td>
<td>Browning, 1937</td>
</tr>
<tr>
<td>3,000-4,700</td>
<td>1</td>
<td>Maximum concentrations that can be inhaled 1 hour without serious disturbance</td>
<td>Bloomfield, 1951; Von, Oettingen, 1940</td>
</tr>
<tr>
<td>3,130-4,700</td>
<td>0.5-1</td>
<td>Maximum concentration for this period of time</td>
<td>Browning, 1937</td>
</tr>
<tr>
<td>4,650</td>
<td>0.5</td>
<td>Listlessness and confusion</td>
<td>Hamilton, 1931</td>
</tr>
<tr>
<td>4,700</td>
<td>0.5</td>
<td>Confusion</td>
<td>Greenburg, 1926</td>
</tr>
</tbody>
</table>

Adverse effects to benzene exposure
<table>
<thead>
<tr>
<th>Benzene Concentration (ppm)</th>
<th>Duration of Exposure</th>
<th>Effects Observed</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>6,190-9,300</td>
<td>Few hours</td>
<td>Definite symptoms of poisoning</td>
<td>Greenburg, 1926</td>
</tr>
<tr>
<td>6,200-9,300</td>
<td>0.5-1</td>
<td>Immediate or subsequent death</td>
<td>Hamilton, 1934</td>
</tr>
<tr>
<td>7,500</td>
<td>0.5-1</td>
<td>Dangerous to life</td>
<td>Bloomfield, 1951; Gerarde, 1963; Von Oettingen, 1940</td>
</tr>
<tr>
<td>6,200-9,300</td>
<td>Few hours</td>
<td>Loss of consciousness</td>
<td>Hamilton, 1931</td>
</tr>
<tr>
<td>19,000-20,000</td>
<td>Short Exposure</td>
<td>Rapidly fatal</td>
<td>Gerarde, 1963; Browning, 1937; Goldwater and Tewksbury, 1941; Von Oettingen, 1940</td>
</tr>
</tbody>
</table>

Adverse effects to benzene exposure
Chronic Health Effects of Benzene Exposure

- Pancytopenia
- Aplastic Anemia
- Myelodysplasia
- Leukemia

Odor threshold: 1 ppm

[? ppm]
Bone Marrow Diseases

- **Pancytopenia**: diagnosed by examining the peripheral blood; significant decreases in circulating blood cell populations due to effects in the bone marrow.

- **Aplastic anemia**: absence of cells in bone marrow; bone marrow replaced by fat.

- **Myelodysplasia**: Pre-leukemia state; there is a spectrum of myelodysplastic syndromes.

- **Leukemia**: blood cell differentiation arrested at an immature state; immature cells continue to proliferate (divide).
Relationship of benzene exposure to various hematological disorders

A. Causality Proven
   1. Pancytopenia: Aplastic Anemia
   2. Acute Myelogenous Leukemia and Variants
      (Including Acute Myelomonocytic Leukemia, Acute Promyelocytic Leukemia, Erythroleukemia)

B. Causality Suspected
   1. Chronic Myelogenous Leukemia
   2. Chronic Lymphocytic Leukemia
   3. Hodgkin’s Disease
   4. Paroxysmal Nocturnal Hemoglobinuria

C. Association Suggested But Unproven
   1. Acute Lymphoblastic Leukemia
   2. Myelofibrosis and Myeloid Metaplasia
   3. Lymphoma: Lymphocytic, Histiocytic
   4. Thrombocythemia
Section C: Bone Marrow Cellular Structure and Hematopoiesis
Hematopoiesis

- Divided into lymphopoiesis and myelopoiesis (myeloid cells)
- Involves the development and differentiation of various blood and immune cell populations originating from a common stem cell.
- The development and differentiation of blood cells is controlled by a spectrum of cytokines and colony stimulating factors derived from the stromal cell population.
Hematopoiesis

Self-renewing stem cell compartment

Committed stem cell compartment

CFU-S-I → CFU-S-II → CFU-GEMM (mix) → CFU-S → CFU-M → BFU-E → CFU-E

Pre-B-lymphocyte → Pre-T-lymphocyte → Lympholycytic stem cell
Lymphopoiesis

Committed stem cell compartment

| Lymphocytic stem cell | Pre-B-lymphocyte | Pre-T-lymphocyte |

Morphologically identifiable precursor cells

| T cells | B cells |

Mature blood cells
Myelopoiesis

BFU-E

Proerythroblast → Neutrophilic erythroblast → Polychromatophilic erythroblast → Orthochromatic erythroblast → Reticulocyte → Erythrocyte

CFU-M

Megakaryoblast → Promegakaryocyte → Megakaryocyte → Metamegakaryocyte/platelets

CFU-GM

Myeloblast → Promyeloblast → Myelocyte → Metamyelocyte → Band granulocyte → Segmented granulocyte

Monoblast → Monocyte/macrophage
Section D: Relationship between Benzene Biotransformation and Toxicity
Possible pathways relating benzene exposure to acute myelogenous leukemia (after Kekule, 1865)
Benzene: Phase I & II Biotransformation

Phenylmercapturic acid

Glutathione transferase

CO$_2$H
SCH$_2$CH
CH$_3$CNH
O

$\text{H}_2\text{O} + \text{H}_2\text{O}$
$\text{-Alanine}$
$\text{-Glycine}$

Indirect hydroxylation

Direct hydroxilation

$[\text{O}]$

Conjugation

Sulfate esters + Glucoronides Eliminated in urine
**Benzene: Phase I & II Biotransformation**

- **Direct-Indirect hydroxylation**
- **Hydratase**
- **Dehydrogenase**
- **Conjugations**
- **Sulfate esters + Glucuronides**
  - Eliminated in urine
- **Reactions with Nucleophilic Macromolecules:**
  - Proteins, DNA, RNA

*Trans, trans muconic acid*
A Tale of Two Organs

Benzene

First pass effect in the liver

Detoxification and excretion (Toxicokinetics)

Primary metabolites

Bone marrow cell toxicities (Toxicodynamics)
Hydroquinone Toxicokinetics

Hydroquinone → Quinone Reductase (QR) → Hydroquinone

Benzquinone → Conjugation → Sulfate Glucuronides

Benzquinone → GSH → BQ-GSH Conjugate

Hydroquinone Macromolecular Interactions
Bone Marrow Macrophages, Fibroblastoid Stromal Cells

Macrophages

Interleukin 1

Fibroblastoid stromal cells

Hydroquinone

Colony stimulating factors

Myelopoiesis

Interleukins

Lymphopoiesis

Phenol

Benzene
HEPATIC CYP2E1  

BONE MARROW GSH AND QR  

SUSCEPTIBILITY TO BENZENE HEMATOXICITY  

HIGH  

LOW  

HIGH  

HIGH  

LOW  

LOW  

LOW
<table>
<thead>
<tr>
<th>Year</th>
<th>Guideline</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>1941</td>
<td>100 ppm (8-hr TWA)</td>
<td>U.S. DOL</td>
</tr>
<tr>
<td>1947</td>
<td>50 ppm (8-hr TWA)</td>
<td>ACGIH</td>
</tr>
<tr>
<td>1948</td>
<td>35 ppm (8-hr TWA)</td>
<td>ACGIH</td>
</tr>
<tr>
<td>1957</td>
<td>25 ppm (8-hr TWA)</td>
<td>ACGIH</td>
</tr>
<tr>
<td>1963</td>
<td>25 ppm (ceiling value)</td>
<td>ACGIH</td>
</tr>
<tr>
<td>1969</td>
<td>10 ppm (8-hr TWA)</td>
<td>ACGIH</td>
</tr>
<tr>
<td>1971</td>
<td>10 ppm (8-hr TWA)</td>
<td>OSHA</td>
</tr>
<tr>
<td>1974</td>
<td>25 ppm (ceiling value)</td>
<td>OSHA</td>
</tr>
<tr>
<td>1987</td>
<td>1 ppm (8-hr TWA)</td>
<td>OSHA</td>
</tr>
<tr>
<td>1989</td>
<td>Proposed 0.1ppm (8-hr TWA)</td>
<td>ACGIH</td>
</tr>
<tr>
<td>1995</td>
<td>Proposed 0.3ppm (8-hr TWA)</td>
<td>ACGIH</td>
</tr>
</tbody>
</table>

Recommended occupational exposure limit for benzene
## Benzene Exposures and Theoretical Risk in the U.S.

<table>
<thead>
<tr>
<th>Activity</th>
<th>Intake ($\mu$g/day)</th>
<th>Cases/Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smoking</td>
<td>1,800</td>
<td>500</td>
</tr>
<tr>
<td>Passive smoking</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Outdoors</td>
<td>120</td>
<td>150</td>
</tr>
<tr>
<td>Driving car</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>Filling gas tank</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>Occupational</td>
<td>10,000</td>
<td>10</td>
</tr>
<tr>
<td>Other personal</td>
<td>150</td>
<td>200</td>
</tr>
<tr>
<td>Drinking water at the current MCL</td>
<td>10</td>
<td>0.01</td>
</tr>
</tbody>
</table>