Ozone Mode of Action

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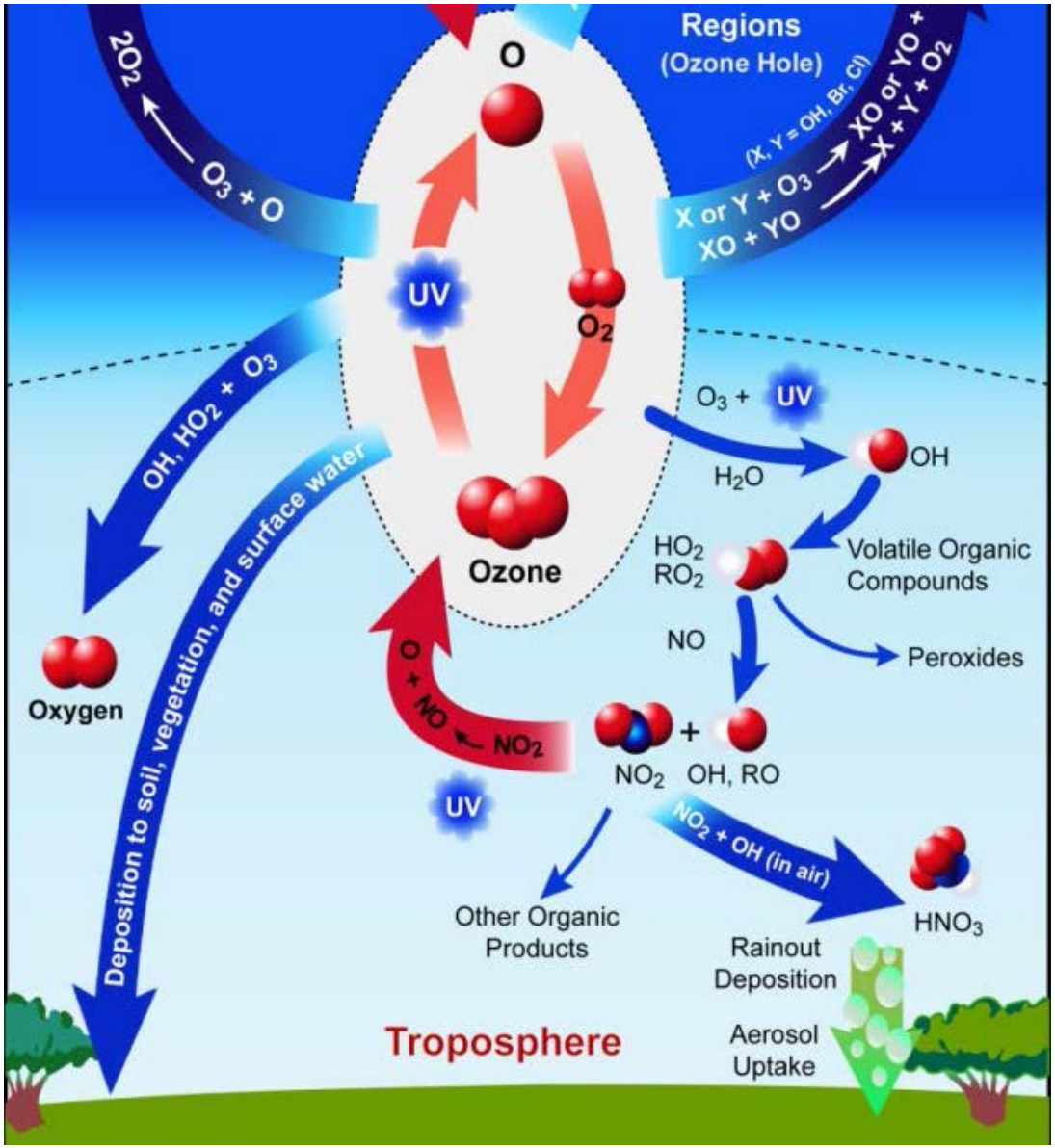
Ozone (O_3)

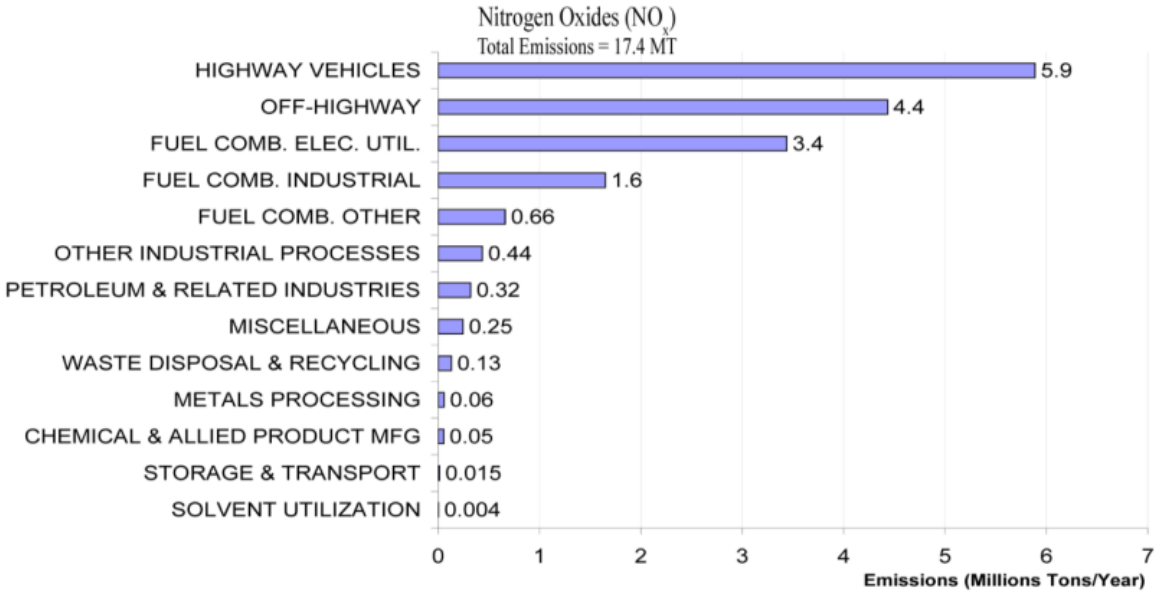
- O_3 is a secondary pollutant, formed when nitrogen oxides (NO_x) and volatile organic compounds (VOCs) react with ultraviolet radiation from sunlight
- O_3 chemistry can be NO_x-limiting or NO_x-saturated (radical dependent) – dictates whether decreases in NO_x will lead to decreases in O_3
- NO_x is responsible for both formation and scavenging of O_3
- O_3 reacts with indoor surfaces and ventilation, scavenging it from indoor air – O_3 is effectively an outdoor pollutant

Ozone Chemistry

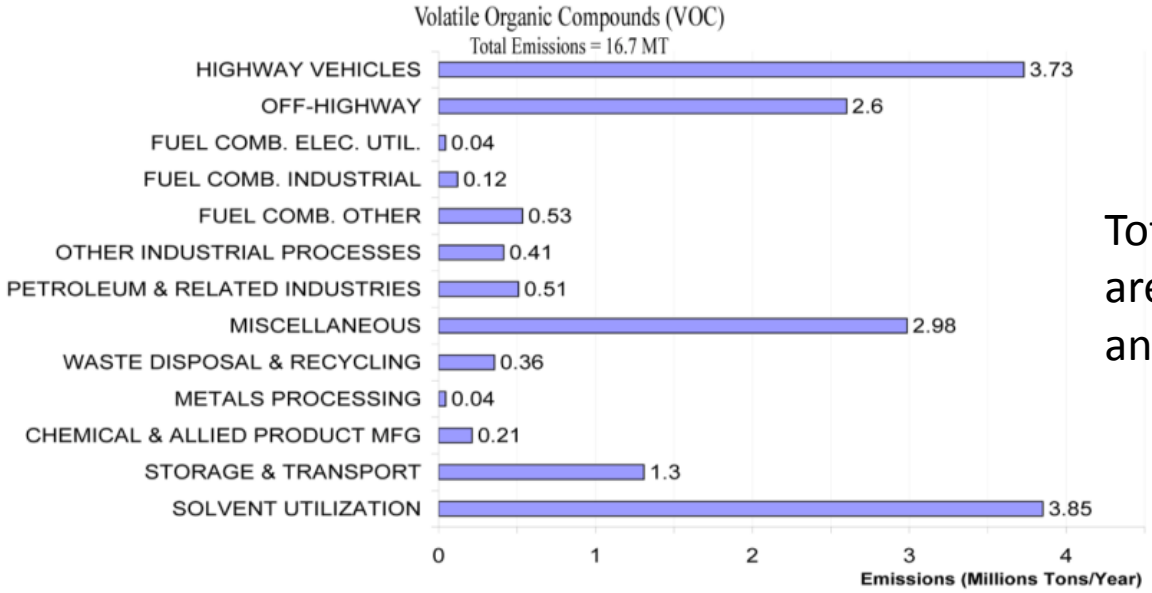


Source: US EPA ISA 2013, Figure 3-1

Ozone Chemistry – Precursor Sources



Source: US EPA ISA 2013, Figure 3-2



Total biogenic VOC emissions are about twice as high as total anthropogenic VOC emissions

Ozone Dosimetry & Uptake

- Ozone is a highly-reactive, poorly water soluble gas at room temperature, and it is a respiratory toxicant

- Ozone dosimetry is typically measured in inhaled dose, which is used as a surrogate for tissue dose (the amount of ozone or secondary reaction products that react with tissues)

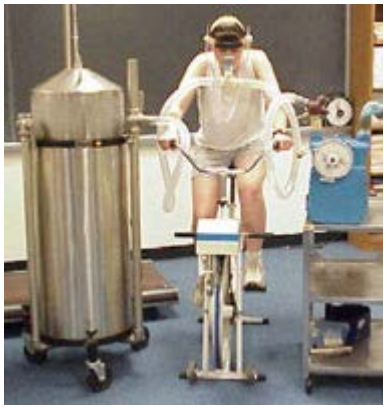
Ozone Dosimetry & Uptake

- Ozone uptake in humans is 80-95% into the entire respiratory tract

- ~50% reacts in the head (nose, mouth, pharynx),~7% in the larynx/trachea and ~43% in the lungs (little in the alveolae).

- With increases in ventilation (ie. exercise) there is an increase in the dose achieved in the lungs and in the alveolae (largely due to a switch from oro-nasal to oral breathing).

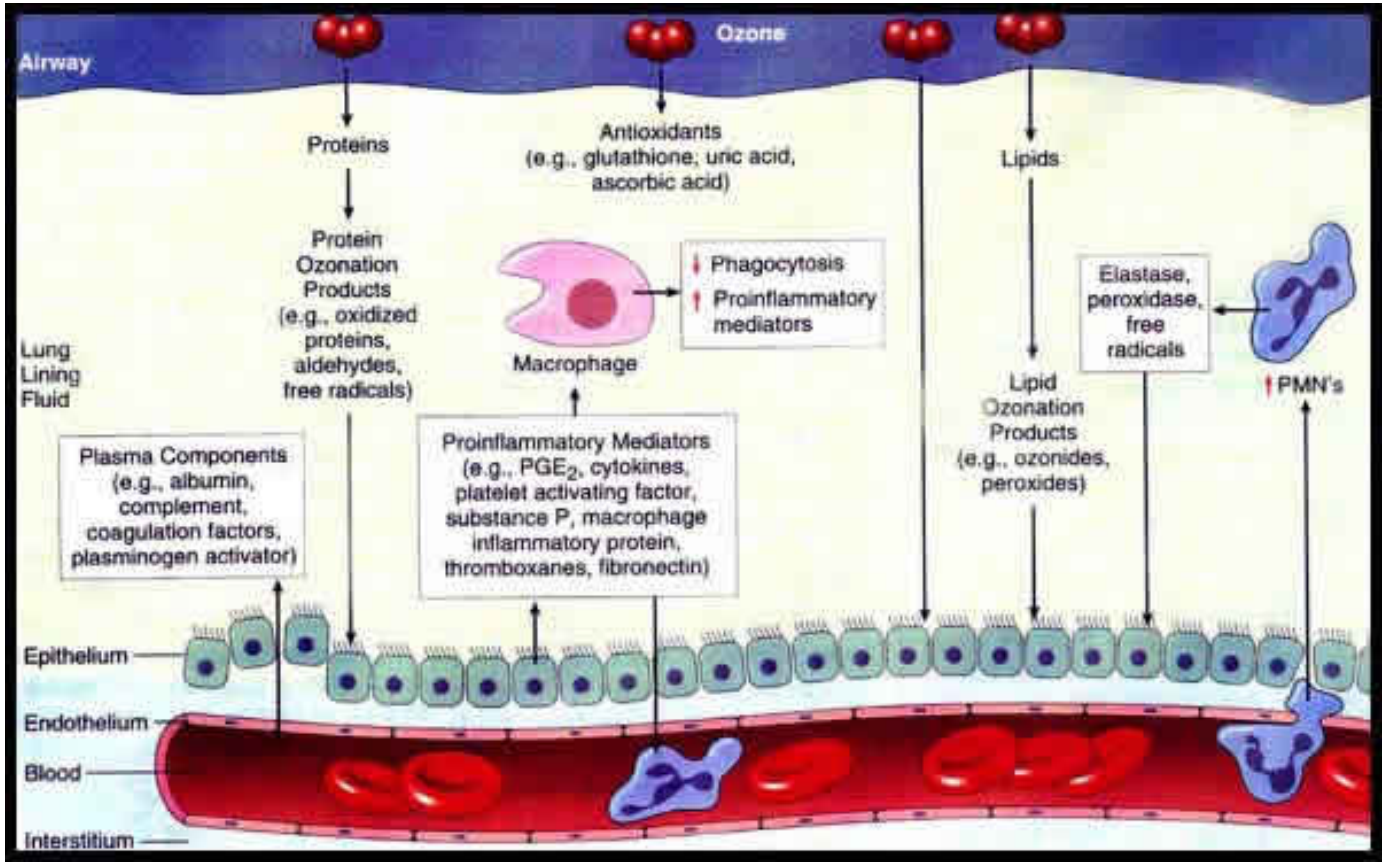
Human Clinical Studies



- These studies measure health effects in volunteers after inhalation of ozone
- They take into account 3 parameters, which make up O_3 ***inhaled dose***:
  - O_3 concentration (in ppm)
  - Time of exposure (in min)
  - Ventilation rate (ie. Exercise level; in L/min)

Ozone Reactions in the Respiratory Tract

- In the respiratory tract ozone diffuses across and reacts with constituents of the epithelial lining fluid (ELF)

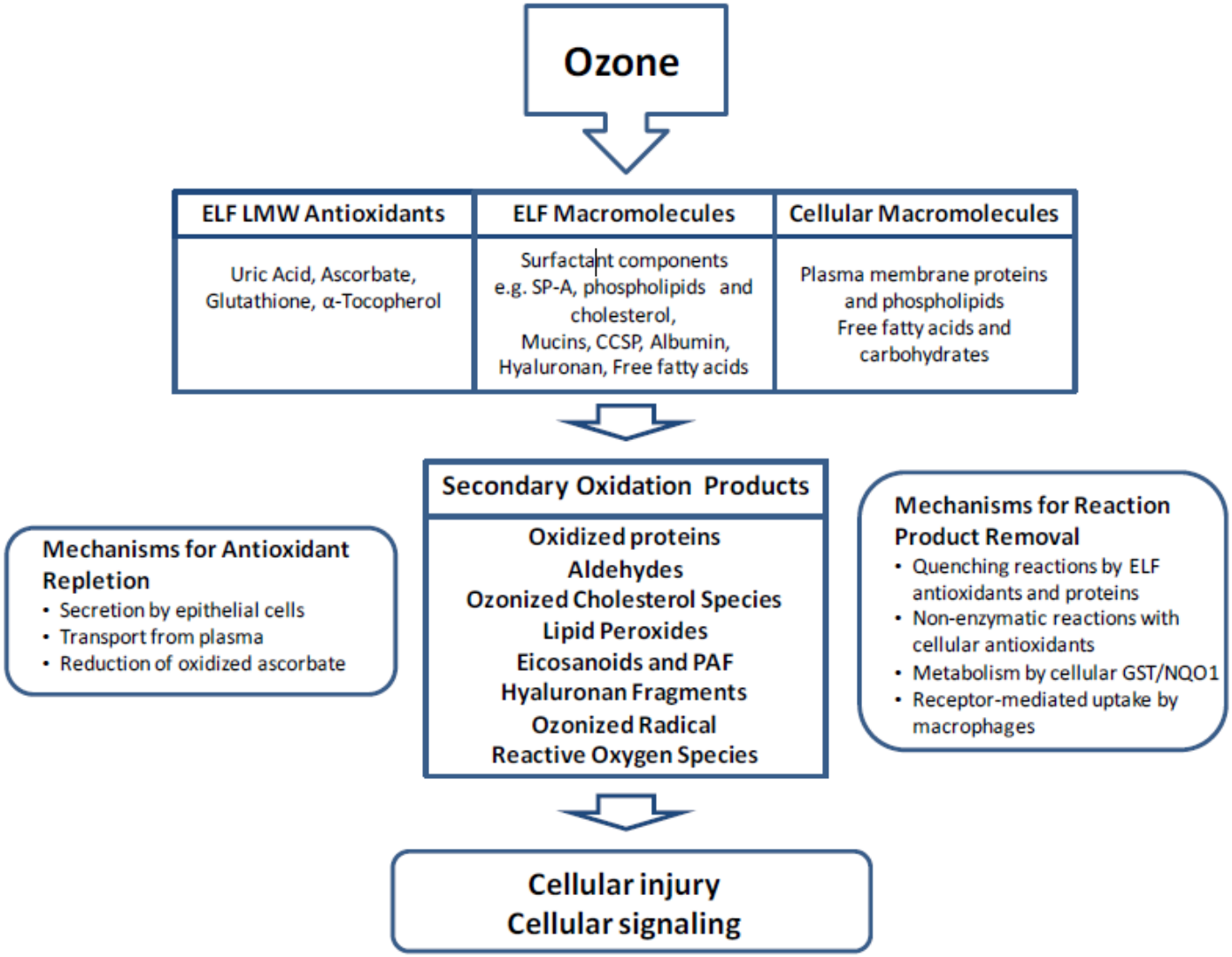


Source: http://www.epa.gov/apti/ozonehealth/population.html

Ozone Reactions in the Respiratory Tract

- The ELF contains antioxidants (primarily ascorbic acid, glutathione, and uric acid) that can react with ozone and prevent it from producing damaging secondary reaction products

- There are several replenishment mechanisms for ELF antioxidants, (so slower dose-rate would mean lower ozone-induced reactions).
  - Eg. Mudway et al (1999) showed that in humans, uric acid in nasal lavage was depleted by ~30% after a 1 hour exposure to 0.2 ppm ozone (with intermittent exercise), but there was no further decrease after the second hour of ozone exposure, suggesting replenishment of uric acid



Note: Contents of this figure not discussed in Section 5.2 will be discussed in Section 5.3. Low molecular weight, LMW; Clara cell secretory protein, CCSP; Surfactant Protein-A, SP-A; Platelet activating factor, PAF. Ozone will react with components of the ELF to produce reaction products that may lead to cellular injury and cell signaling as discussed in Section 5.3.

Figure 5-7 Details of the O_3 interaction with the airway ELF to form secondary oxidation products.

Source: US EPA ISA 2013

Ozone Mode of Action

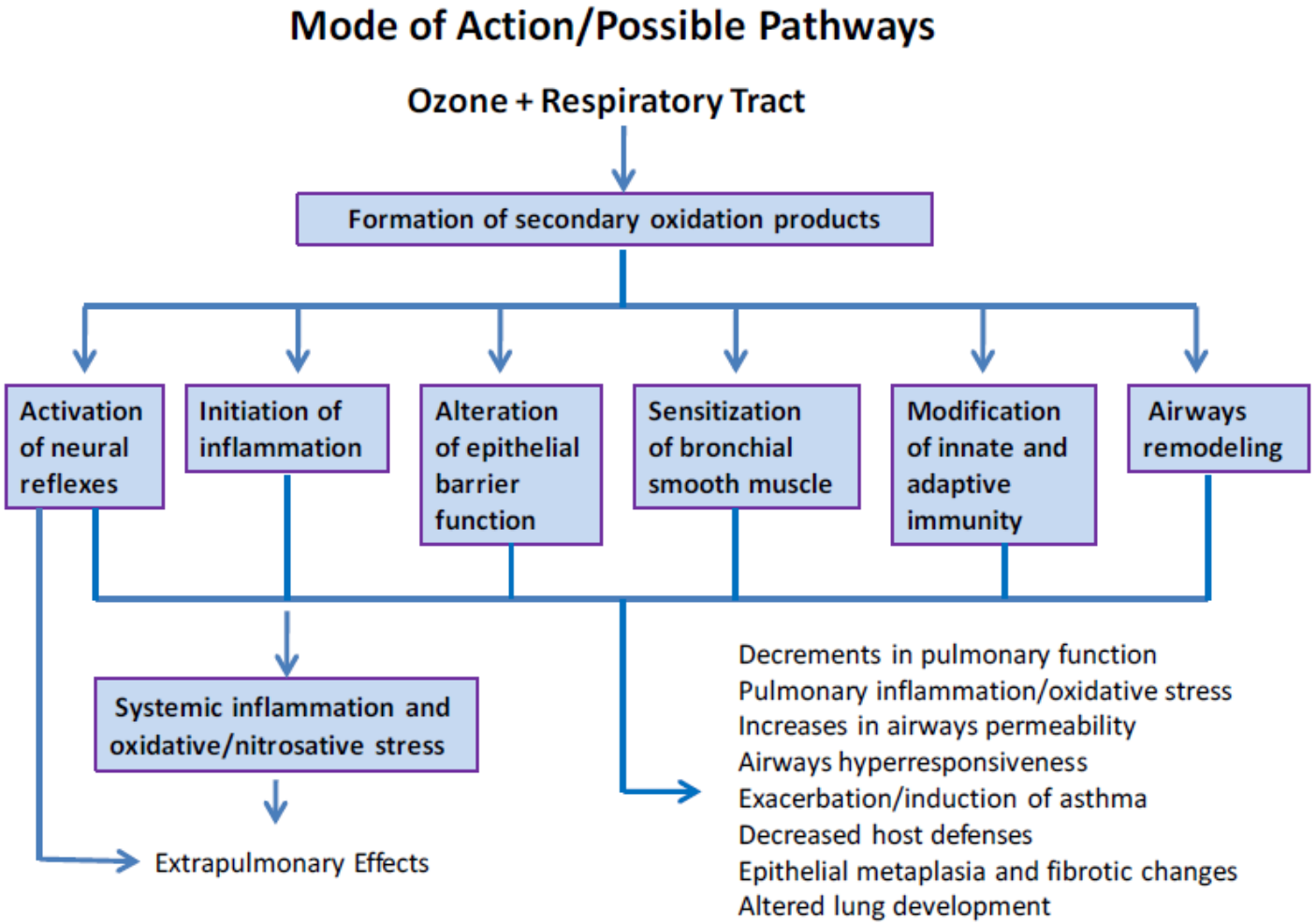


Figure 5-8 **The modes of action/possible pathways underlying the health effects resulting from inhalation exposure to O_3.**

Source: US EPA ISA 2013

Neural Reflexes & Spirometric Responses

- Ozone or ozone secondary products activate the vagal afferent pathway
  - Likely through bronchial C-fibers, activated by binding of lipid reaction products of ozone to TRPA1 receptors
- The vagal afferent nervous response causes spirometric responses
  - Measured by forced expiratory volume in 1 second (FEV_1), forced vital capacity (FVC), etc

Ozone-induced Inflammation

- Damage to cells from ozone/ozone secondary reaction products can initiate inflammatory responses
- Measured in several ways:
  - Influx of inflammatory cells into the lumen of the respiratory tract (primarily neutrophils, but lymphocytes and macrophages have also been observed)
  - Measurement of inflammatory cytokines in lavage fluid (eg. interleukins, eicosanoids, etc)
- Inflammatory biomarkers typically do not correlate with spirometric responses in ozone-exposed individuals

Inflammation Dose-Response

- Mudway & Kelly (2004) did dose-response modeling of neutrophil infiltration into bronchoalveolar lavage fluid – two timepoints: 0-6 hours and 18-24 hours after ozone exposure

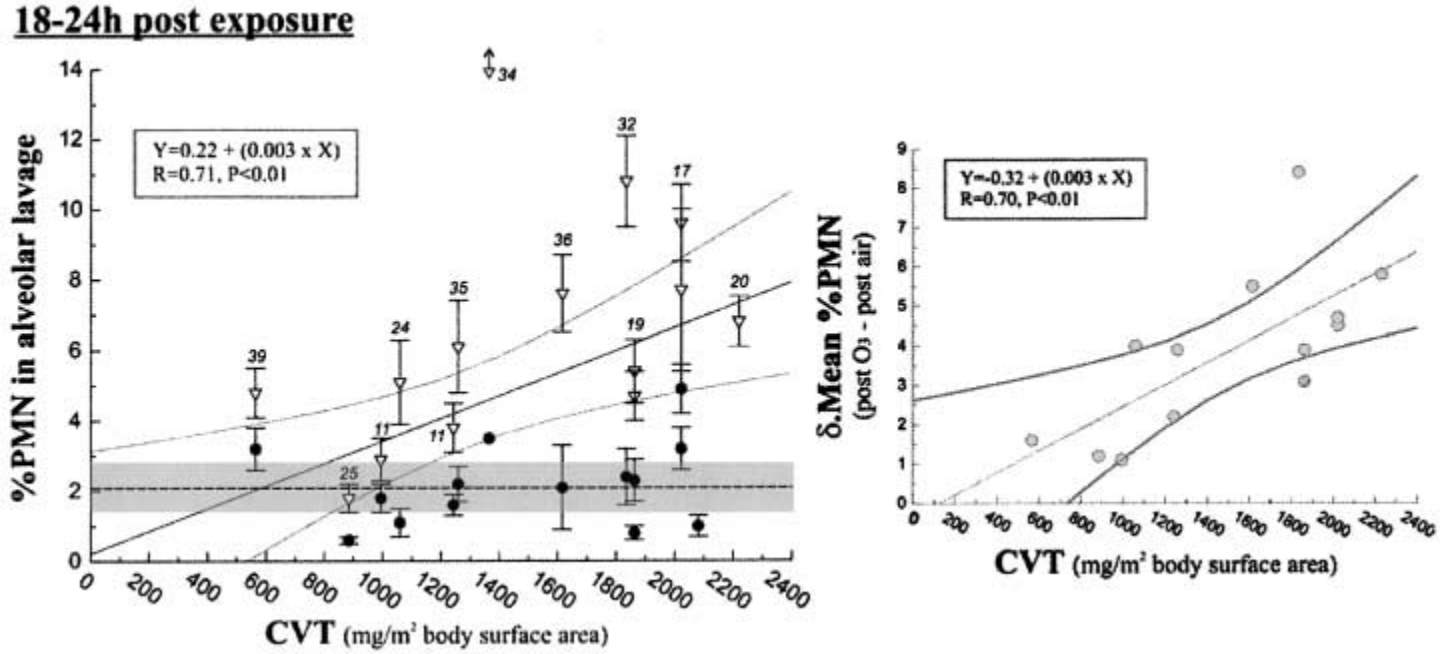


Figure 2, Mudway & Kelly 2004

Inflammation Dose-Response

- Mudway & Kelly (2004) derived two threshold doses of ozone:
  - 645 (408 – 883) µg/m^2 for neutrophil influx at 0-6 hours
  - 810 (491 – 1130) µg/m^2 for neutrophil influx at 18-24 hours

Altered Epithelial Barrier Function

- Damage of the respiratory epithelium caused by ozone or its reaction products can lead to a loss of the barrier function of the epithelium
  - Measured by EM, increased flux of small molecules into the ELF, or by increased protein content in lavage fluids

- Alterations of the respiratory epithelial barrier are typically not correlated with either spirometry or airway hyper-responsiveness

Altered Epithelial Barrier Function

- Mudway & Kelly (2004) investigated dose-response for protein content in lavage fluid
  - Had to exclude several studies to do this analysis, did not derive threshold doses

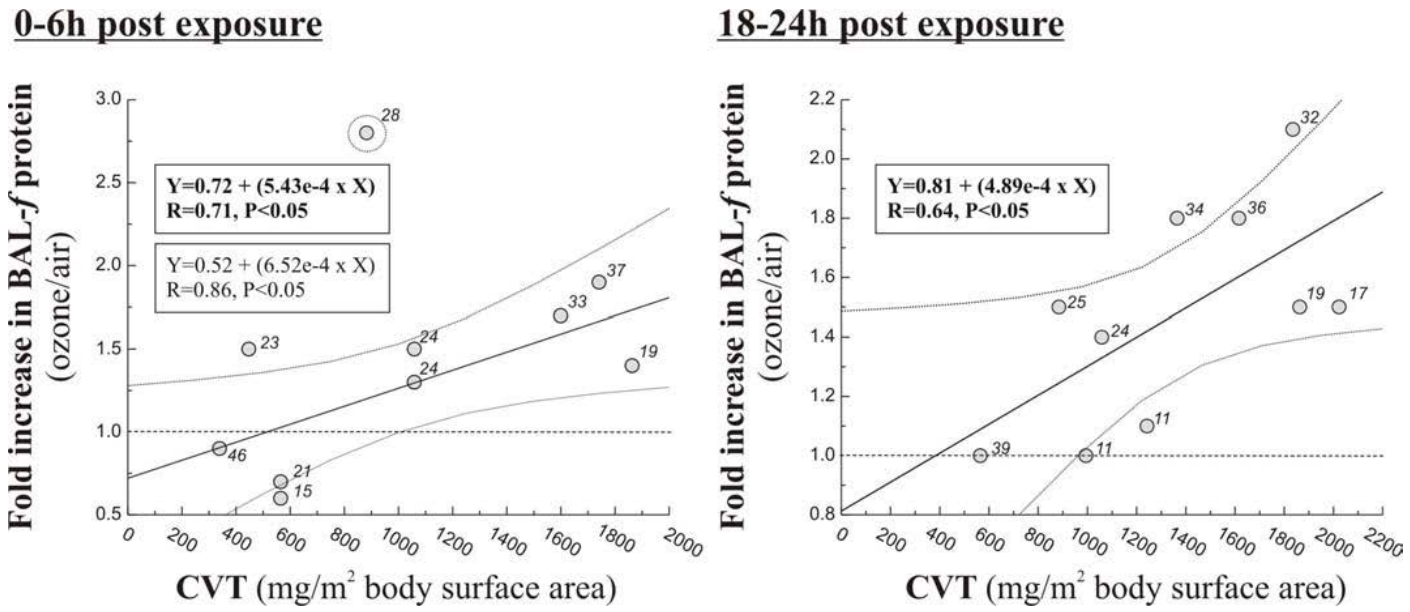


Figure E4, Mudway & Kelly 2004

Bronchial Smooth Muscle Reactivity

- Ozone exposure increases airway hyper-responsiveness to bronchoconstrictive agents such as methacholine and histamine
- Measured as changes in airway resistance (sRaw) or FEV_1 in response to bronchoconstrictive stimuli
- The mechanism of this ozone-mediated response is not clear, and the activation of sensory nerves, inflammation and direct action of ozone secondary reaction products have all been suggested

Uncertainties – Animal studies

- Dose – high dose animal studies may provide MOA that is not relevant to ambient exposures; also need to account for time, concentration and ventilation of exposure

- Species similarities – pathways activated in animals, due to differences in dosimetry, physiology, metabolism, etc, may be different between humans and other animals

Uncertainties – Human studies

- Dose – high dose human studies may provide MOA that is not relevant to ambient exposures, when accounting for time, concentration and ventilation of exposure

- Restricted interventions – the testing that can be done in animals cannot be done in humans, so information that can be obtained from humans is not as extensive

Conclusions

- Ozone is a respiratory toxicant that reacts with antioxidants, proteins, and lipids in the respiratory tract lining fluid
- Ozone (or, more likely, its secondary reaction products) cause several respiratory effects:
  - Nervous system activation → spirometric effects
  - Inflammation
  - Increased epithelial permeability
  - Airway hyper-responsiveness
- Dose-response data and the mechanisms of antioxidant depletion of ozone suggest a threshold of ozone-induced respiratory effects
- Uncertainties in the data include relating experimental doses to ambient doses

References

- US EPA. 2013. "Integrated Science Assessment for Ozone and Related Photochemical Oxidants (Final)." National Center for Environmental Assessment (NCEA). EPA/600/R–10/076F. 1251p., February.
- Mudway, IS; Blomberg, A; Frew, AJ; Holgate, ST; Sandstrom, T; Kelly, FJ. 1999. "Antioxidant consumption and repletion kinetics in nasal lavage fluid following exposure of healthy human volunteers to ozone." *Eur. Respir. J.* 13:1429-1438.
- Mudway, IS; Kelly, FJ. 2004. "An investigation of inhaled ozone dose and the magnitude of airway inflammation in healthy adults: Online data supplement." *Am. J. Respir. Crit. Care Med.* 169:1089-1095.