The Role of Air Pollution Particles as a Potent Adjuvant that Causes Allergic Disease and Asthma

David Diaz-Sanchez
UCLA Department of Medicine; Division of Clinical Immunology & Allergy
Is there a link between air pollution and airway disease?
What components are involved?
What are the mechanisms involved?

What confers susceptibility?

What can we do about it?
PREVALENCE OF ALLERGIC RHINITIS SINCE THE INDUSTRIAL REVOLUTION

GENETIC PREVALENCE LIMIT FOR ALLERGIC RHINITIS

- Bostock 1 case 1819
- Bostock 28 cases 1828
- Lloyd Common 1907

Prevalence of allergic rhinitis since the Industrial Revolution.
EXPERIMENTAL RESEARCHERS

OF THE

CAUSES AND NATURE

OF

CATARRHUS AESTIVUS

(HAY-FEVER OR HAY-ASTHMA)

BY

CHARLES H. BLACKLEY, M.R.C.S. ENG

LONDON

DAWSON’S OF PALL MALL

1873
and I have shown that large numbers of the people have been transferred from the country to the workshops and mills of the towns, and have thus been placed in circumstances where the predisposition to hay-fever would be most rapidly developed in those who rise to a place amongst the educated class. And lately, I have shown that the production of the exciting cause has of late years been largely increased.

Taking all these circumstances into account it is a highly probable that hay-fever was at one time altogether unknown, and it is tolerably certain that it has not only been much more frequent of late, but that, as population increases and as civilization and education advance, the disorder will become more common than it is at the present time.
The Effect of Air Pollution on Lung Development from 10 to 18 Years of Age

W. James Gauderman, Ph.D., Edward Avol, M.S., Frank Gilliland, M.D., Ph.D., Hita Vora, M.S., Duncan Thomas, Ph.D., Kiros Berhane, Ph.D., Rob McConnell, M.D., Nino Kuenzli, M.D., Fred Lurmann, M.S., Edward Rappaport, M.S., Helene Margolis, Ph.D., David Bates, M.D., and John Peters, M.D.

1759 kids
1993-2001
12 communities
FEV1, FVC, MMER
Allergen Avoidance as a Treatment for Perennial Rhinitis and Asthma.

Components of Air Pollution

A. Primary-secondary pollutants
   (i) Primary: pollutants emitted directly into the atmosphere (eg, SO₂, some NOₓ species, CO, PM)
   (ii) Secondary: pollutants that form in the air as a result of chemical reactions with other pollutants and gases (eg, ozone, NOₓ, and some particulates)

B. Indoor-outdoor pollutants
   (i) Indoor pollutants
      (a) Sources: cooking and combustion, particle resuspension, building materials, air conditioning, consumer products, smoking, heating, biologic agents
      (b) Products: Combustion products (eg, tobacco and wood smoke), CO, CO₂, SVOC (eg, aldehydes, alcohols, alkanes, and ketones), microbial agents and organic dusts, radon, manmade vitreous fibers
   (ii) Outdoor pollutants
      (a) Sources: industrial, commercial, mobile, urban, regional, agricultural, natural
      (b) Products: SO₂, ozone, NOₓ, CO, PM, SVOC

C. Gaseous-particulate pollutants
   (i) Gaseous: SO₂, NOₓ, ozone, CO, SVOC (eg, PAH, dioxins, benzene, aldehydes, 1,3-butadiene)
   (ii) Particulate: coarse PM (2.5-10 μm; regulatory standard = PM₁₀), fine PM (0.1-2.5 μm; regulatory standard = PM₂.₅); ultrafine PM (<0.1 μm; not regulated)

NOₓ, Nitrogen oxides; SVOC, specific volatile organic compounds.
Nasal DEP  Inhaled Diesel  Inhaled ETS
Diesel- a model combustion particulate pollutant

- Respirable median dia 0.2 microns
- Carbon core surrounded by chemicals
  - PAH
  - Metals
- Major source of particulates especially in Europe and Japan

Figure 2. Highway Sources of Pollution

Particulate Matter (Soot) (15% of Fuel-Burning Sources)

- Diesel Trucks and Buses 58%
- Passenger Cars 23%
- SUVs and Light Trucks 16%
- Gasoline Trucks 3%
Diesel Exhaust Particles Affect Four Phases of Allergic Airway Disease

Immediate phase response
- Increased mediator release and symptoms

• Short term response
  - Release of chemokines, cytokines and increased cellular inflammation

• Intermediate term response
  - Enhanced IgE antibody response to allergens

• Long term response
  - Primary allergic sensitization
Nasal lavage as a tool in assessing acute inflammation in response to inhaled pollutants.

Koren HS, Hatch GE, Graham DE.

Clinical Research Branch, U.S. Environmental Protection Agency

Toxicology 1990 Jan-Feb;60(1-2):15-25
Nasal Lavage/challenge Model Of Allergic Inflammation
(Fishing In The Nose)

- Day O, Nasal Lavage
  - Baseline levels

- Nasal challenge with:
  - Allergen
  - DEP (300 µg)

- Measure response in min, hrs, days
Diesel Particles Enhances Allergy Symptoms and Histamine release

Diaz-Sanchez D, Penichet-Garcia M, Saxon A. *J Allergy Clin Immunol* 2000
Diesel Particles Reduce Allergen Threshold

Only 1/5th of the normal amount of allergen is needed to get allergic symptoms when diesel particles are present.

Diaz-Sanchez D, Penichet-Garcia M, Saxon A. J Allergy Clin Immunol 2000
DEP enhances the inflammatory response

Chemokine levels (pg/mL)

- IL-8
- TNF-alpha
- GM-CSF

Saline 30 ug 100 ug 300 ug

DEP challenge dose
DEP enhances allergen-IgE responses

Sensitization

• Cause an allergy to a substance which was previously “harmless”

• Can Pollution make subjects allergic to a “neo-allergen”?

• Keyhole Limpet Haemocyanin (KLH)
Sensitization

• KLH alone --> NO IgE
  IgG, IgA

• KLH + DEP --> 60-90% subjects sensitized
  KLH-IgE apparent at Day 32
  (3rd exposure)
  allergic symptoms upon rechallenge

(Diaz-Sanchez et al., J. Allergy Clin Immunol 1999; 104:1183-8)
Challenge with DEP can define high and low responder populations
Augmentation of allergen-IgE production by DEP is reproducible and intrinsic.

$r=0.83, p<0.01$

Effect of diesel exhaust on healthy human lower airways

- Sandström and co-workers
- Increased number of inflammatory cells in the airways
- Increase in histamine levels
- Increased levels of inflammatory mediators and molecules
- Decreased macrophage function
Diesel exhaust enhances airway responsiveness in asthmatic subjects

- 14 nonsmoking, atopic asthmatics
- 300 µg/m³ DE or air for 1 h

- Increase in:
  - hyperresponsiveness to methacholine
  - airway resistance
  - sputum levels IL-6

- Nordenhall et al., Eur Respir J 2001 17:909-15
Diesel Exposure

BUILDING WALL

INSIDE

OUTSIDE

INTERCHANGEABLE INSULATED NOZZLE

TRUCK EXHAUST PIPE

INSULATED REDUCER AND COUPLING

INSULATED RAW EXHAUST PIPE

TR COUPLING

DILUTION TUNNEL

SAMPLE PORT

SAMPLE PORT

BUTTERFLY VALVE OR OTHER ADJUSTABLE RESTRICTION

FILTER BOX FOR INLET DILUTION AIR

FLEX DUCT

200 CFM

ROOTS BLOWER

CHAMBER
A double-blind randomized cross-over controlled exposure to filtered air, diesel exhaust (100 ug/m$^3$) and nitrogen dioxide for 2 hours.

**Chamber**
350-ft$^3$ plexiglass chamber, 74-81°F; relative humidity 35-50%, air exchange rate of 17 changes/hour

**Subjects**
18-45 years of age
Ten mild asthmatic subjects with skin test sensitivity to at least one allergen
Four non-allergic, non-asthmatic subjects.

**Endpoints**
*Lung function*
FEV1, Airway hyperresponsiveness, Airway Resistance

*Immune function*
Cell influx, allergic antibody, cytokine, chemokine and mediator production in sputum

*Symptoms*
Continual health questionnaire, EKG monitoring, pulse oximetry
Comparison of mass distributions between different diesel exposures
### The PM mass loadings for individual human exposures ($\mu g/m^3$)

<table>
<thead>
<tr>
<th>Filter ID</th>
<th>SMPS Set Point (ug m$^{-3}$)</th>
<th>OC (ug m$^{-3}$)</th>
<th>EC (ug m$^{-3}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0003</td>
<td>no data</td>
<td>77.4</td>
<td>32.9</td>
</tr>
<tr>
<td>0004</td>
<td>no data</td>
<td>66.4</td>
<td>30.6</td>
</tr>
<tr>
<td>0009</td>
<td>96.2</td>
<td>51.3</td>
<td>32.7</td>
</tr>
<tr>
<td>0010</td>
<td>96.2</td>
<td>49.4</td>
<td>28.8</td>
</tr>
<tr>
<td>0005</td>
<td>107.6</td>
<td>69.4</td>
<td>30.2</td>
</tr>
<tr>
<td>0006</td>
<td>107.6</td>
<td>67.2</td>
<td>34.0</td>
</tr>
<tr>
<td>0008</td>
<td>112.1</td>
<td>61.4</td>
<td>36.2</td>
</tr>
<tr>
<td>0011</td>
<td>112.1</td>
<td>68.1</td>
<td>31.1</td>
</tr>
<tr>
<td>0012</td>
<td>110.2</td>
<td>67.7</td>
<td>34.3</td>
</tr>
<tr>
<td>0013</td>
<td>110.2</td>
<td>65.6</td>
<td>32.5</td>
</tr>
<tr>
<td>0014</td>
<td>104.9</td>
<td>53.6</td>
<td>33.6</td>
</tr>
<tr>
<td>0015</td>
<td>104.9</td>
<td>56.3</td>
<td>35.6</td>
</tr>
<tr>
<td>40910</td>
<td>no data</td>
<td>57.9</td>
<td>31.3</td>
</tr>
<tr>
<td>xx</td>
<td>no data</td>
<td>59.1</td>
<td>30.8</td>
</tr>
<tr>
<td>0032</td>
<td>104.2</td>
<td>65.1</td>
<td>29.9</td>
</tr>
<tr>
<td>0033</td>
<td>104.2</td>
<td>62.6</td>
<td>30.2</td>
</tr>
<tr>
<td>0157</td>
<td>103.8</td>
<td>no data</td>
<td>no data</td>
</tr>
<tr>
<td>0158</td>
<td>103.8</td>
<td>no data</td>
<td>no data</td>
</tr>
<tr>
<td>0155</td>
<td>96.8</td>
<td>64.4</td>
<td>24.0</td>
</tr>
<tr>
<td>0156</td>
<td>96.8</td>
<td>62.0</td>
<td>27.6</td>
</tr>
<tr>
<td>0137</td>
<td>101.5</td>
<td>61.9</td>
<td>31.5</td>
</tr>
<tr>
<td>0159</td>
<td>101.5</td>
<td>61.4</td>
<td>32.1</td>
</tr>
</tbody>
</table>

Note SMPS set point. Values are within 6% of target (100 $\mu g/m^3$ diesel particles)
<table>
<thead>
<tr>
<th></th>
<th>Healthy</th>
<th>Asthmatic</th>
<th>All</th>
</tr>
</thead>
<tbody>
<tr>
<td>Filtered Air</td>
<td>+38 (71)</td>
<td>-24 (302)</td>
<td>-2 (239)</td>
</tr>
<tr>
<td>NO₂</td>
<td>-20 (43)</td>
<td>+59 (136)</td>
<td>+30 (115)</td>
</tr>
<tr>
<td>Diesel Exhaust</td>
<td>+30 (107)</td>
<td>+17 (186)</td>
<td>+22 (155)</td>
</tr>
</tbody>
</table>

**Bronchial reactivity to methacholine**

Rank 1 indicated highest bronchial reactivity.

Filtered Air  2.2
NO₂  2.2
Diesel Exhaust  1.7

p>0.05
The rank order of key endpoints of airway inflammation following diesel exhaust exposure

P = 4.87 x 10^{-7}
ETS exposure protocol

-2h  NL
Oh   2hr Clean air/ETS exposure
2h   NL
   Allergen challenge
24h  NL
96 h NL
Generation of ETS

FTC guidelines

RM G1
Borgwaldt
Smoking machine

1R4F
reference cigarettes
9.2 mg Tar
0.8 mg nicotine
ETS exposure

- Five cigarettes
- Two hour period
- Carbon monoxide < 5 ppm
- PM level ≈ 400 µg/m³
Secondhand smoke exposure exacerbates IgE responses

Diaz-Sanchez et al., *J Allergy Clin Immunol*, 2006
ETS enhances allergen-induced histamine release

Diaz-Sanchez et al., *J Allergy Clin Immunol*, 2006
In mouse model ETS

• Induces sensitization in low responder strain
  – OVA-IgE formation
  – Eosinophilia
  – Th2 cytokines in BAL

• Enhances secondary allergic response in high responder strain

Whitekus & Diaz-Sanchez, *J. Immunol*, 2004
DEP and/or ETS Affect Four Phases of Allergic Airway Disease

- **Immediate phase response**
  - Increased mediator release and symptoms

- **Short term response**
  - Release of chemokines, cytokines and increased cellular inflammation

- **Intermediate term response**
  - Enhanced IgE antibody response to allergens

- **Long term response**
  - Primary allergic sensitization
Individuals Respond the Same to DEP and ETS

**IgE increase (fold) following DEP challenge**

**IgE increase (fold) following ETS challenge**

\[ R^2 = 0.4553 \]
What makes some people sensitive to the pro-inflammatory/pro-allergenic effects of ETS/DEP and others not?
Macrophages and APCs interact with the airway epithelium to initiate an immune response. Macrophages secrete IL-10, which inhibits Th0 cells. APCs present antigens to Th0 cells, which differentiate into Th1 and Th2 cells. Th1 cells secrete IL-12, which promotes Th1 cell differentiation. Th2 cells secrete IL-4 and IL-13, which promote B cell differentiation into plasma cells and mast cell activation. Plasma cells secrete IgE, which binds to specific IgE receptors on mast cells. Mast cells release proinflammatory cytokines such as IL-8 and Rantes, GM-CSF, and others, which contribute to the inflammatory response.
Inhaled DEP-induces Oxidative Stress in Murine Lungs

BALB/c mice exposed to saline, OVA, or OVA plus DEP by aerosolized inhalation daily for 10 days

Assays for carbonyl protein content (A) and lipid hydroperoxides (B) in the lung homogenates

Metabolic Pathway for Detoxification of DEP chemicals

Phase II enzymes can metabolize reactive oxygen species (ROS) by conjugating them into small hydrophilic moieties, thereby rendering them soluble and excretable.
ETS or DEP effects on allergic inflammation mediated by oxidative stress are modulated by Phase II enzymes.

Quinones, oxy-PAH $\rightarrow$ redox cycling $\rightarrow$ Phase II enzymes

Phase II enzymes (e.g. NQO1, GSTM1)
Quinones $\rightarrow$ hydroquinones
Is the enhancement of allergic/immune responses caused by DEP dependent on genotype?

Allergic subjects

Cross-over study

Challenge with:
  allergen/saline
  allergen/DEP
Member of the Glutathione S-transferases family

Involved in oxy-PAH detoxification

Identified in lung and nasal tissue

Homozygous GSTM1*0/GSTM1*0 (i.e. null genotype) completely lack activity

Null Frequency is 20-50%
Effect of glutathione-S-transferase M1 and P1 genotypes on xenobiotic enhancement of allergic responses: randomised, placebo-controlled crossover study

Frank D Gilliland, Yu-Fen Li, Andrew Saxon, David Diaz-Sanchez

<table>
<thead>
<tr>
<th></th>
<th>GSTM1 Null (n=14)</th>
<th>GSTM1 Present (n=5)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>IgE</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clean air and allergen</td>
<td>6.9 (2.6–24.3)</td>
<td>8.9 (4.3–18.8)</td>
<td>0.40</td>
</tr>
<tr>
<td>DEP and allergen</td>
<td>106.6 (8.8–534.8)</td>
<td>49.8 (14.2–79.4)</td>
<td>0.15</td>
</tr>
<tr>
<td>Difference</td>
<td>102.5 (1.0–510.5)</td>
<td>45.5 (–1.5–60.6)</td>
<td>0.03</td>
</tr>
<tr>
<td><strong>Histamine</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clean air and allergen</td>
<td>2.9 (1.3–5.9)</td>
<td>2.8 (1.9–6.7)</td>
<td>0.96</td>
</tr>
<tr>
<td>DEP and allergen</td>
<td>16.9 (2.9–27.6)</td>
<td>9.8 (3.1–19.0)</td>
<td>0.08</td>
</tr>
<tr>
<td>Difference</td>
<td>14.0 (–0.2–24.7)</td>
<td>7.4 (1.2–12.3)</td>
<td>0.02</td>
</tr>
</tbody>
</table>

NQO1 over-expression decreases IL-8 production in DX-stimulated BEAS-2B cells

Ritz et al., Am J Physiol Lung Cell Mol Physiol. 20
Sulforaphane
Sulforaphane inhibits DEP increased cytokine expression by NHBECs

Ritz et al., Am J Physiol Lung Cell Mol Physiol. 20
GSTP1 Gene Expression and DEP Challenge Dose
GSTP1 Gene Expression and Total Cell Count after DEP challenge

![Graph showing the relationship between GSTP1 fold increase and total cell count. The graph includes data points and a line of best fit with an R² value of 0.1972.](image-url)
Dietary Sulforaphane - brocco shakes!

Step-up dose-ranging randomised placebo-controlled study

Oral dosing begins at 200 $\mu$mol = 25 grams (1/2 cup) BroccoSprouts®/daily for 4 days

Follow-up visits:

- 400 $\mu$mol (50 grams)
- 600 $\mu$mol (75 grams)
- 800 $\mu$mol (100 grams)
- 1000 $\mu$mol (125 grams) daily
Dietary Sulforaphane increases phase II expression in nasal cells.
Individuals Respond the Same to DEP and ETS

\[ R^2 = 0.4553 \]
AJRCCM Articles in Press. Published on October 5, 2006 as doi:10.1164/rccm.200509-1424OC

Gluathione-S-Transferase M1 and P1 Prevent Aggravation of Allergic Responses by Secondhand Smoke

Frank D. Gilliland, M.D., Ph.D.1, Yu-Fen Li, Ph.D.1,2, Henry Gong, Jr., M.D.1,

David Diaz-Sanchez, Ph.D.1
Glutathione S transferase deficiency and passive smoking increase childhood asthma

M Kabesch, C Hoefler, D Carr, W Leupold, S K Weiland, E von Mutius


Background: It has been suggested that the genetically determined deficiency of glutathione S transferase (GST) enzymes involved in the detoxification of environmental tobacco smoke (ETS) components may contribute to the development of asthma.

Methods: A large population of German schoolchildren (n=3054) was genotyped for deficiencies of the GST isoforms M1 and T1. The association between GSTM1 and GSTT1 genotypes and asthma as well as atopy was investigated with respect to current and in utero ETS exposure.

Results: In children lacking the GSTM1 allele who were exposed to current ETS the risk for current asthma (OR 5.5, 95% CI 1.6 to 18.6) and asthma symptoms such as wheeze ever (OR 2.8, 95% CI 1.3 to 6.0), current wheezing (OR 4.7, 95% CI 1.8 to 12.6) and shortness of breath (OR 8.9, 95% CI 2.1 to 38.4) was higher than in GSTM1 positive individuals without ETS exposure. Hints of an interaction between ETS exposure and GSTM1 deficiency were identified. In utero smoke exposure in GSTT1 deficient children was associated with significant decrements in lung function compared with GSTT1 positive children not exposed to ETS.

Conclusions: GSTM1 and GSTT1 deficiency may increase the adverse health effects of in utero and current smoke exposure.
Glutathione S-transferase P1 gene polymorphism and air pollution as interactive risk factors for childhood asthma


Nox
SO2
Genetic polymorphism of GSTM1 and antioxidant supplementation influence lung function in relation to ozone exposure in asthmatic children in Mexico City


Background: We recently reported that antioxidant supplementation with vitamins C and E mitigated ozone related decline in forced expiratory flow (FEF25–75) in 158 asthmatic children in an area with high ozone exposure in Mexico City.

Methods: A study was undertaken to determine whether deletion of glutathione S-transferase M1 (GSTM1 null genotype), a gene involved in response to oxidative stress, influences ozone related decline in FEF25–75 and the benefit of antioxidant supplementation.

Results: GSTM1 null children receiving placebo had significant ozone related decrements in FEF25–75 (percentage change per 50 ppb of ozone 2.9 (95% CI −5.2 to −0.6), p=0.01); GSTM1 positive children did not. Conversely, the effect of antioxidants was stronger in children with the GSTM1 null genotype.

Conclusions: Asthmatic children with a genetic deficiency of GSTM1 may be more susceptible to the deleterious effects of ozone on the small airways and might derive greater benefit from antioxidant supplementation.
Gene x Gene x Environment

<table>
<thead>
<tr>
<th>GSTM1</th>
<th>GSTP1</th>
<th>n</th>
<th>IgE difference</th>
<th>Histamine difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present</td>
<td>I/I</td>
<td>2</td>
<td>26.1 (6.7–45.5)</td>
<td>7.73 (3.13–12.32)</td>
</tr>
<tr>
<td>Present</td>
<td>I/V</td>
<td>3</td>
<td>48.9 (−1.5–60.6)</td>
<td>7.44 (1.22–7.48)</td>
</tr>
<tr>
<td>Null</td>
<td>I/I</td>
<td>11*</td>
<td>137.0 (29.9–510.5)</td>
<td>14.33 (8.14–24.67)</td>
</tr>
<tr>
<td>Null</td>
<td>I/V</td>
<td>3</td>
<td>9.1 (1.0–46.2)</td>
<td>2.98 (−0.22–19.59)</td>
</tr>
</tbody>
</table>

Values are median (range). *p=0.0034 for IgE and p=0.0073 for histamine calculated by the Wilcoxon test comparing GSTM1 null/GSTP1 I/I with the other three genotype groups combined.

Table 4: IgE (U/mL) and histamine (nmol/L) differences by joint GSTM1 and GSTP1 genotype

Joint effects of genotypes on median nasal allergic endpoints when exposed to allergen plus clean air or allergen plus second hand smoke (SHS) exposure.

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Second hand smoke exposure response</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IgE (U/mL)</td>
<td>IL-4 (U/mL)</td>
<td>IFN-γ (ng/L)</td>
<td>Histamine (nM/L)</td>
<td></td>
</tr>
<tr>
<td><strong>GSTM1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>GSTP1 Ile105Val</strong></td>
<td>n Median (min~max)</td>
<td>Median (min~max)</td>
<td>Median (min~max)</td>
<td>Median (min~max)</td>
<td></td>
</tr>
<tr>
<td>+ Ile/Ile</td>
<td>2</td>
<td>59.8 (24.6~95.0)</td>
<td>2.7 (0.0~5.4)</td>
<td>-0.5 (-0.9~0.1)</td>
<td>9.3 (8.4~10.2)</td>
</tr>
<tr>
<td>+ Ile/Val</td>
<td>3</td>
<td>46.7 (8.9~74.7)</td>
<td>3.2 (0.0~3.3)</td>
<td>-0.2 (-1.0~0.2)</td>
<td>3.2 (-0.9~10.1)</td>
</tr>
<tr>
<td>- Ile/Ile</td>
<td>11</td>
<td>184.4 (47.6~725.5) *</td>
<td>2.9 (0.4~13.1)</td>
<td>-0.2 (-0.8~0.1)</td>
<td>10.3 (2.3~20.6) **</td>
</tr>
<tr>
<td>- Ile/Val</td>
<td>3</td>
<td>55.2 (11.3~423.6)</td>
<td>11.3 (-0.4~12.2)</td>
<td>-0.9 (-1.5~0.1)</td>
<td>6.0 (-0.9~6.3)</td>
</tr>
</tbody>
</table>

Gililland et al., Am J Respir Crit Care Med, 2006
## Cell number changes following DEP challenge

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Change from saline</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median (min~max)</td>
</tr>
<tr>
<td><strong>GSTM1</strong></td>
<td></td>
</tr>
<tr>
<td><strong>GSTP1 Ile-105Val</strong></td>
<td>n</td>
</tr>
<tr>
<td>+ Ile/Ile</td>
<td>8</td>
</tr>
<tr>
<td>+ Ile/Val</td>
<td>12</td>
</tr>
<tr>
<td>- Ile/Ile</td>
<td>31</td>
</tr>
<tr>
<td>- Ile/Val</td>
<td>9</td>
</tr>
<tr>
<td><strong>GSTM1 TNFα G-308A</strong></td>
<td>n</td>
</tr>
<tr>
<td>+ GG</td>
<td>13</td>
</tr>
<tr>
<td>+ GA</td>
<td>5</td>
</tr>
<tr>
<td>- GG</td>
<td>36</td>
</tr>
<tr>
<td>- GA</td>
<td>6</td>
</tr>
<tr>
<td><strong>GSTM1 NQO1 Pro-187Ser</strong></td>
<td>n</td>
</tr>
<tr>
<td>+ Pro/Pro</td>
<td>6</td>
</tr>
<tr>
<td>+ Pro/Ser or Ser/Ser</td>
<td>10</td>
</tr>
<tr>
<td>- Pro/Pro</td>
<td>22</td>
</tr>
<tr>
<td>- Pro/Ser or Ser/Ser</td>
<td>22</td>
</tr>
</tbody>
</table>
**GSTM1 and GSTP1 and respiratory health in asthmatic children exposed to ozone**

Isabelle Romieu\(^1\), Matiana Ramirez-Aguilar\(^1\), Juan José Sienra-Monge\(^2\), Hortensia Moreno-Macías\(^1\), Blanca Estela del Rio-Navarro\(^2\), Gloria David \(^3\), Jacqui Marzec\(^3\), Mauricio Hemández-Avila\(^1\), Stephanie London\(^3\),

**ERJ 2006**

Table 4. Effect of ozone (20 ppb) on the risk of reporting difficulty breathing on a given day according to combined GSTM1 and GSTP1 genotypes among 151 asthmatic children in Mexico City.

<table>
<thead>
<tr>
<th></th>
<th>GSTM1 null and</th>
<th>GSTM1 positive and</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GSTP1 Val/Val</td>
<td>GSTP1 Ile/Ile and Ile/Val</td>
</tr>
<tr>
<td></td>
<td>( n=22 )</td>
<td>( n=61 )</td>
</tr>
<tr>
<td>Ozone 1-day lag</td>
<td>1.08 (1.00-1.17)</td>
<td>1.00 (0.94-1.05)(^+)</td>
</tr>
<tr>
<td>Ozone 2-day average</td>
<td>1.12 (1.02-1.23)</td>
<td>1.00 (0.94-1.05)(^+)</td>
</tr>
<tr>
<td>Ozone-6-day average</td>
<td>1.22 (1.07-1.40)</td>
<td>1.04 (0.96-1.12)(^+)</td>
</tr>
</tbody>
</table>

\(^+\) Odds ratio and 95% CI calculated using GEE for logistic regression separately for each genotype cross-classification adjusting for previous day temperature and chronological time

\(^+\) \( p \leq 0.05 \). \( p \)-value obtained comparing ORs within ozone exposure categories between combined genotype groups (GSTM1 null and GSTP1 Val/Val versus GSTM1 positive and GSTP1 Ile/Ile, Ile/Val).
Greetings from Los Angeles

Inhale Warm Medical Smoke
It goes where many forms of internal medication cannot reach.

Blosser’s Cigarettes

What Can We Do About It?
Nasal Glucocorticoids are NOT effective at blocking the effects of DEP challenge

Fifteen ragweed- rhinitic, nonsmoking volunteers, age 18 to 50 years old

Treatment with fluticasone propionate at recommended dose—two sprays (50 ug each) per nostril once daily

7 days prior to & including the day of challenge

Challenge with 300 ug DEP

No effect on DEP-induced nasal IgE, IgE-secreting cells and Th2 cytokines

Diaz-Sanchez et al., Clin
### TABLE III. Interventions reported to decrease the effect of pollutants*

<table>
<thead>
<tr>
<th>Intervention</th>
<th>Ozone</th>
<th>DEP</th>
<th>LPS</th>
<th>PM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decreased exposure to motor vehicle exhaust</td>
<td>Decreased ED hospital use by asthmatic subjects</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Decreased ED hospital use by asthmatic subjects</td>
</tr>
<tr>
<td>Decreased point source emissions</td>
<td>Unclear</td>
<td>Unclear</td>
<td>Unclear</td>
<td>Decreased ED hospital use by asthmatic subjects, decreased death rates</td>
</tr>
<tr>
<td>Inhaled corticosteroids</td>
<td>Decreased response to ozone by allergic asthmatic subjects, no protection in healthy volunteers</td>
<td>Ineffective in one study</td>
<td>Decreased response to LPS by allergic asthmatic subjects, mild protection in healthy volunteers</td>
<td>Unknown</td>
</tr>
<tr>
<td>Vitamins C and E Non-steroidal anti-inflammatory agents</td>
<td>Reports of protection from asthma exacerbation Protection from immediate decrease in lung function caused by ozone in both asthmatic and healthy volunteers, not a clear effect on allergic inflammation</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

*Many items listed as unknown are currently under study, and existing current studies are inconclusive.

*DEP, Diesel exhaust particle.*
The avoidable health effects of air pollution in three Latin American cities: Santiago, São Paulo, and Mexico City

Michelle L. Bell\textsuperscript{a,\,*}, Devra L. Davis\textsuperscript{b}, Nelson Gouveia\textsuperscript{c}, Víctor H. Borja-Aburto\textsuperscript{d}, Luis A. Cifuentes\textsuperscript{e}
Comparative study of two systems:

Actual vs. Control

Control: Existing technologies
Transport, Residencial and Industrial
Reduction of 10% in PM10 y O3 in 20 years
## Benefit of a control strategy in Mexico City 2000 to 2020

<table>
<thead>
<tr>
<th>Health endpoint</th>
<th>Mexico City</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mortality</strong></td>
<td></td>
</tr>
<tr>
<td>Adult</td>
<td>33,084</td>
</tr>
<tr>
<td>Infant (&lt;1 year)</td>
<td>2648</td>
</tr>
<tr>
<td><strong>Medical visits</strong></td>
<td></td>
</tr>
<tr>
<td>Children’s medical visits (3 to 15 years)</td>
<td>113,623</td>
</tr>
<tr>
<td>Hospital admissions (cardiovascular)</td>
<td>2919</td>
</tr>
<tr>
<td>Hospital admissions (respiratory)</td>
<td>46,275</td>
</tr>
<tr>
<td>Children’s hospital admissions (&lt;13 years for PM$_{10}$, &lt;5 years for O$_3$)</td>
<td>4836</td>
</tr>
<tr>
<td>Emergency room visits (respiratory)</td>
<td>537,826</td>
</tr>
<tr>
<td><strong>Bronchitis and asthma</strong></td>
<td></td>
</tr>
<tr>
<td>Asthma attacks</td>
<td>2,988,077</td>
</tr>
<tr>
<td>Acute bronchitis</td>
<td>78,528</td>
</tr>
<tr>
<td>Chronic bronchitis</td>
<td>28,371</td>
</tr>
<tr>
<td><strong>Activity effects</strong></td>
<td></td>
</tr>
<tr>
<td>Restricted activity days (18 to 65 years)</td>
<td>12,722,033</td>
</tr>
<tr>
<td>Work loss days</td>
<td>4,412,424</td>
</tr>
</tbody>
</table>
Table 2  Summary of pooled percentage difference (95% confidence intervals) for effect of parental smoking on lung function

<table>
<thead>
<tr>
<th></th>
<th>No. of studies</th>
<th>% difference (95% CI) fixed effect</th>
<th>% difference (95% CI) random effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>FVC</td>
<td>19</td>
<td>-0.2 (-0.4 to +0.1)</td>
<td>-0.4 (-0.8 to +0.0)</td>
</tr>
<tr>
<td>FEV₁</td>
<td>21</td>
<td>-0.9 (-1.2 to -0.7)</td>
<td>-1.4 (-1.9 to -1.0)</td>
</tr>
<tr>
<td>MEF</td>
<td>19</td>
<td>-4.8 (-5.4 to -4.3)</td>
<td>-5.0 (-6.6 to -3.3)</td>
</tr>
<tr>
<td>EEF</td>
<td>9</td>
<td>-4.3 (-5.3 to -3.3)</td>
<td>-4.3 (-5.5 to -3.1)</td>
</tr>
</tbody>
</table>

FVC = forced vital capacity; FEV₁ = forced expiratory volume in one second; MEF = mid expiratory flow rate; EEF = end expiratory flow rate.

Cook and Strachan *Thorax* 1999;54:357–366

“ETS exposure clearly confers an increased risk of acute lower respiratory disease in young children”

California EPA review
### TABLE 4. ADJUSTED* ODDS RATIOS AND 95% CI FOR THE JOINT EFFECTS OF IN UTERO EXPOSURE TO MATERNAL SMOKING AND GSTM1 GENOTYPE ON ASTHMA AND WHEEZE, ODDS RATIO AND 95% CONFIDENCE INTERVAL

<table>
<thead>
<tr>
<th>Outcomes</th>
<th>No in utero</th>
<th></th>
<th>In utero</th>
<th></th>
<th>In utero</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GSTM1 (+)</td>
<td>OR (95% CI)</td>
<td>GSTM1 (+)</td>
<td>OR (95% CI)</td>
<td>GSTM1 (+)</td>
</tr>
<tr>
<td>Asthma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ever asthma</td>
<td>Reference group</td>
<td>1.0 (0.8, 1.2)</td>
<td>0.9 (0.6, 1.4)</td>
<td>1.4 (0.9, 2.1)</td>
<td></td>
</tr>
<tr>
<td>Active asthma</td>
<td>Reference group</td>
<td>0.8 (0.6, 1.1)</td>
<td>0.8 (0.5, 1.3)</td>
<td>1.7 (1.1, 2.8)</td>
<td></td>
</tr>
<tr>
<td>Medication for asthma</td>
<td>Reference group</td>
<td>0.9 (0.7, 1.2)</td>
<td>0.7 (0.4, 1.2)</td>
<td>1.8 (1.1, 2.8)</td>
<td></td>
</tr>
<tr>
<td>Early onset asthma</td>
<td>Reference group</td>
<td>0.9 (0.7, 1.2)</td>
<td>0.9 (0.7, 1.4)</td>
<td>1.6 (1.0, 2.5)</td>
<td></td>
</tr>
<tr>
<td>Persistent asthma</td>
<td>Reference group</td>
<td>1.0 (0.8, 1.2)</td>
<td>0.9 (0.6, 1.4)</td>
<td>1.6 (1.1, 2.4)</td>
<td></td>
</tr>
<tr>
<td>Wheezing</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ever wheezing</td>
<td>Reference group</td>
<td>1.0 (0.8, 1.2)</td>
<td>1.3 (1.0, 1.8)</td>
<td>1.8 (1.3, 2.5)</td>
<td></td>
</tr>
<tr>
<td>Wheeze with cold</td>
<td>Reference group</td>
<td>1.0 (0.8, 1.2)</td>
<td>1.1 (0.8, 1.7)</td>
<td>1.8 (1.2, 2.7)</td>
<td></td>
</tr>
<tr>
<td>Wheeze without cold</td>
<td>Reference group</td>
<td>1.0 (0.8, 1.3)</td>
<td>1.1 (0.7, 1.8)</td>
<td>2.3 (1.4, 3.5)</td>
<td></td>
</tr>
<tr>
<td>Persistent wheeze</td>
<td>Reference group</td>
<td>0.8 (0.6, 1.2)</td>
<td>1.6 (0.9, 2.8)</td>
<td>2.2 (1.3, 4.0)</td>
<td></td>
</tr>
<tr>
<td>Attacks of wheezing</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shortness of breath</td>
<td>Reference group</td>
<td>1.0 (0.7, 1.3)</td>
<td>1.4 (0.9, 2.3)</td>
<td>2.3 (1.4, 3.8)</td>
<td></td>
</tr>
<tr>
<td>Awakened at night</td>
<td>Reference group</td>
<td>0.9 (0.7, 1.3)</td>
<td>1.1 (0.6, 1.9)</td>
<td>1.8 (1.0, 3.1)</td>
<td></td>
</tr>
<tr>
<td>Wheeze with exercise</td>
<td>Reference group</td>
<td>0.9 (0.7, 1.2)</td>
<td>1.0 (0.6, 1.6)</td>
<td>2.1 (1.3, 3.3)</td>
<td></td>
</tr>
<tr>
<td>Treatments for wheezing</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medication for wheeze</td>
<td>Reference group</td>
<td>1.0 (0.7, 1.2)</td>
<td>1.0 (0.6, 1.5)</td>
<td>2.2 (1.4, 3.4)</td>
<td></td>
</tr>
<tr>
<td>Emergency room for wheeze</td>
<td>Reference group</td>
<td>0.9 (0.5, 1.5)</td>
<td>1.0 (0.4, 2.4)</td>
<td>3.7 (1.9, 7.3)</td>
<td></td>
</tr>
</tbody>
</table>

*Adjusted for age, race, sex, birth order, parity, history of asthma in the child, and smoking status.
How many smokers keep smoking when pregnant?

75%

Large population-based survey from 33 states
8803 pregnant women
Prevalence of smoking among pregnant women = 16.3% in 1987
11.8% in 1996
Quitting rate = 26.3% in 1987
25.2% in 1996

Early Asthma Risk Factor Study [EARS]

691 pregnant women 1975 and 1986
Prevalence of smoking among pregnant women
First trimester 19%
second trimester 13%
third trimester 12%
cessation rate 15%
Children’s Health Study Southern California.

338 children with asthma diagnosed in first 5 years of life,

570 control subjects were countermatched on in utero exposure to maternal smoking within grade, sex, and community of residence.

Gilliland et al., Chest. 2005;127:1232-1241.)
“Grandma's Behavior While Pregnant Affects Her Grandkids' Health”

Table 4—Multivariable Analysis of the Joint Associations of Maternal and Child’s In Utero Exposure to Maternal Smoking With Child’s Asthma Risk, OR, and 95% CI*

<table>
<thead>
<tr>
<th>In Utero Exposure to Maternal Smoking</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR</td>
</tr>
<tr>
<td></td>
<td>No.†</td>
</tr>
<tr>
<td>Unexposed</td>
<td>118/151</td>
</tr>
<tr>
<td>Exposed</td>
<td>27/58</td>
</tr>
<tr>
<td>Exposed Unexposed</td>
<td>165/34</td>
</tr>
<tr>
<td>Exposed Exposed</td>
<td>102/36</td>
</tr>
</tbody>
</table>

*Models are adjusted for race/ethnicity, gestational age, and SHS exposure.
†Number of countermatched control subjects/case patients.
Testing the Grandmother effect

Pregnant mice receive either 1 h ETS or saline daily for duration of pregnancy

Fathers receive either 1 h ETS or saline for 10 consecutive days before mating

Offspring bred

F2 at 6 weeks receive:

  1% OVA for 20 min for 10 days

OVA i.t and methacholine challenge 48h later
Exposure of maternal Grandmother to ETS increases airway responsiveness.
Are Children at Increased Risk?
Children vs. Adults

Children spend more time outdoors
They are more active outside
Their total inhaled volume/mass is greater
They breathe through their mouth more
More difficult to avoid smoke
Health effects can last a life time

Their immune system is different
### Allergic Endpoints Are Greater After ETS/OA Exposure in Younger Mice

<table>
<thead>
<tr>
<th></th>
<th>Day</th>
<th>Young mice (2-3 weeks)</th>
<th>Mature mice (8-12 weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum OA-IgE (U/mL)</td>
<td>12</td>
<td>1032</td>
<td>528</td>
</tr>
<tr>
<td>Serum OA-IgG1 (ng/mL)</td>
<td>12</td>
<td>216</td>
<td>48</td>
</tr>
<tr>
<td>IL-5 (pg/mL)</td>
<td>31</td>
<td>104</td>
<td>69</td>
</tr>
<tr>
<td>IL-2 (pg/mL)</td>
<td>31</td>
<td>142</td>
<td>73</td>
</tr>
<tr>
<td>IFN-γ (pg/mL)</td>
<td>31</td>
<td>N.D.</td>
<td>5</td>
</tr>
<tr>
<td>GM-CSF (pg/mL)</td>
<td>31</td>
<td>439</td>
<td>198</td>
</tr>
<tr>
<td>Total cells in BAL (% increase over OA alone)</td>
<td>31</td>
<td>492%</td>
<td>226%</td>
</tr>
</tbody>
</table>
Effects of DEP challenge on Adults vs. Children

**DESIGN**
A single-blind randomized exposure design
4 different DEP doses – 0 (control), 30, 100, 300 µg
nasal lavage immediately before and 24 hours after DEP challenge
four week wash-out period between each DEP exposure

**Study Population**
Twenty adults (25-55 years of age)
Fifteen children (10-15 years of age)
Matched for sex/ethnicity
Children are more susceptible to the pro-inflammatory effects of particles.
Children have reduced antioxidant defense to pollutants

![Graph showing GSTP1 increase over baseline (fold) for DEP nasal challenge doses of 30 ug, 100 ug, and 300 ug for both Adults and Children.](image)
¡Kids are not mini-adults!