

US EPA ARCHIVE DOCUMENT



Ozone

Example Approaches to Understanding Human Health Risks
Associated with Environmental Exposures to Chemicals

Dr. Robert Devlin

U.S. EPA, Office of Research and Development



Using Ozone to Validate a Systems Biology Approach to Toxicity Testing

**Robert Devlin
Environmental Public Health Division
US EPA**

**Advancing the Next Generation (NextGen) of Risk Assessment:
Public Dialog Conference**

**February 15-16, 2011
Washington, DC**

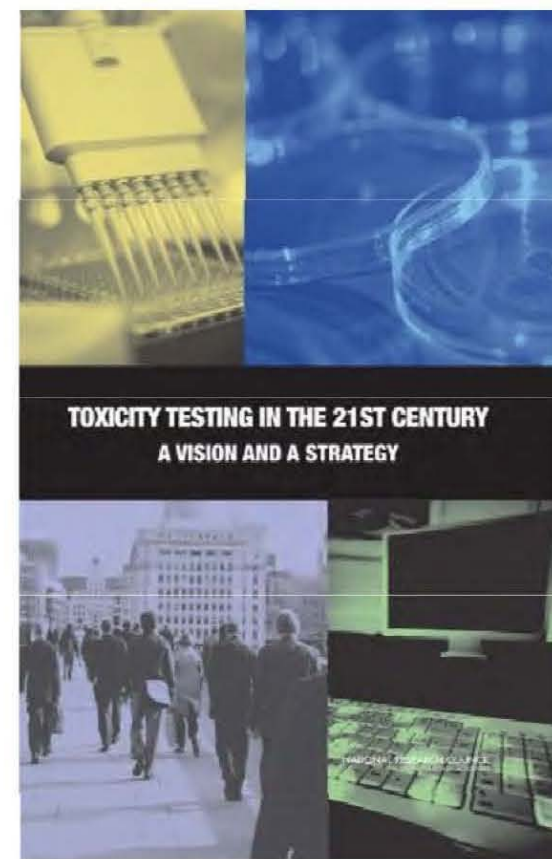
Challenges in Toxicity Testing

- **The need to evaluate thousands of chemicals is a challenge confronting pharmaceutical and chemical industries, as well as regulatory agencies such as the EPA.**
- **The need to consider cumulative effects of mixtures (e.g. synergistic effects) greatly complicates the the problem.**
- **In addition, there is increasing pressure to limit the use of animal testing.**
- **These challenges require a new approach to toxicity testing.**

Toxicity Testing in the 21st Century: A Vision and Strategy

Recognizing that the time has come for more innovative approaches to toxicity testing, in 2004 the US EPA asked the NRC to develop a long range vision and a strategy to advance toxicity testing.

The conclusion of the NRC report is that a conversion to in vitro techniques is the only way to screen the ever increasing number of environmental chemicals that must be regulated.



Key Points from the Report

- Toxicology evaluation of chemicals is poised to take advantage of the on-going revolution in biology and biotechnology
- It is increasingly possible to study the effects of chemicals using cells, cellular components, and tissues – preferably of human origin – rather than whole animals.
- New tests should illuminate changes at the molecular level, helping scientists better predict how chemical exposures do or do not lead to certain health effects and how they affect susceptible populations such as children.
- They should enable rapid screening of chemicals and reduce animal use.

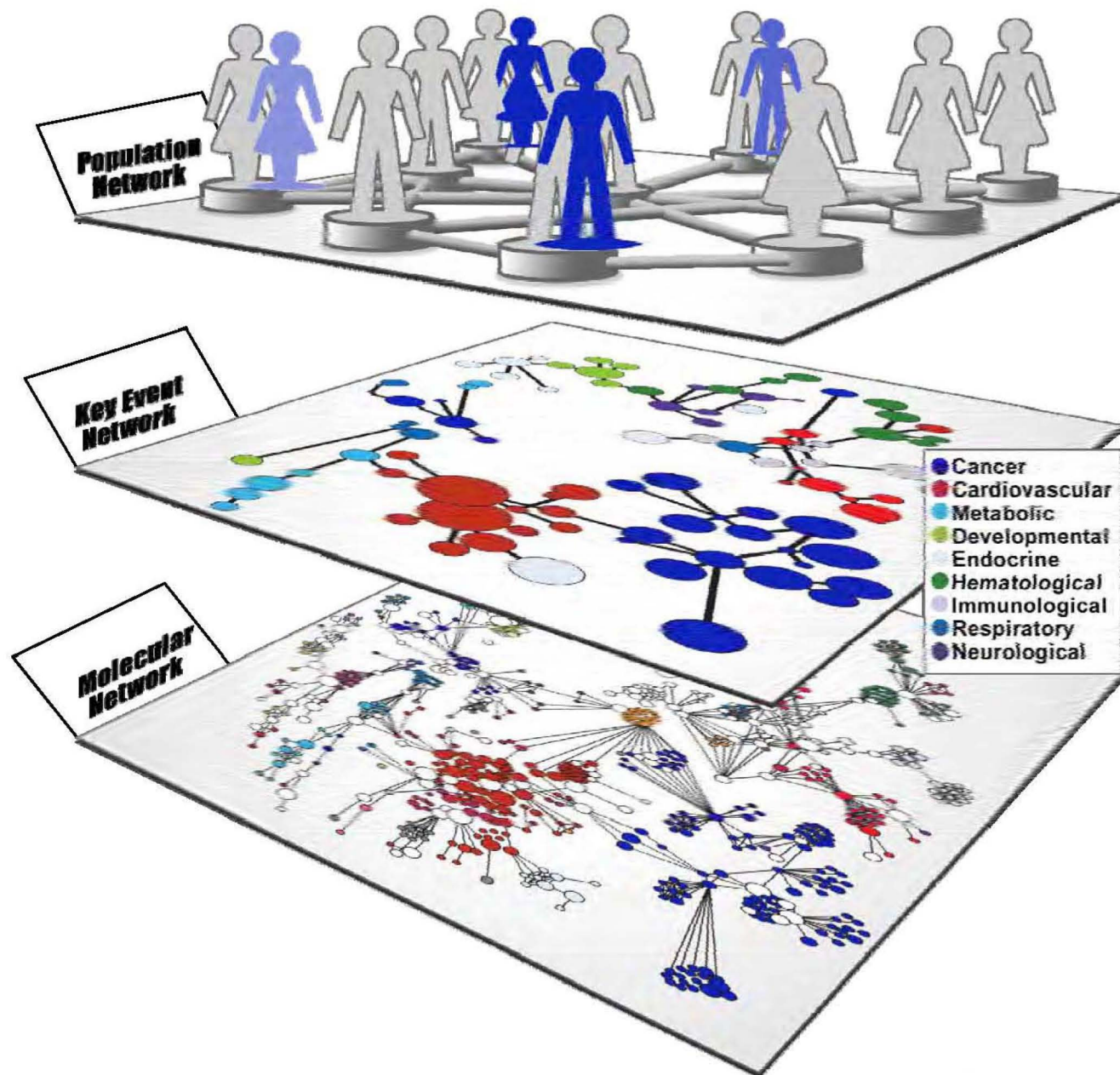
New Technologies Can Transform Existing Approaches

- High throughput techniques developed by the pharmaceutical industry use efficient automated methods to test biologic activities of thousands of chemicals that used to be studied in animals.
- Systems Biology approaches use computational models and laboratory data from **toxicity pathway studies** to describe and understand biologic systems as a whole and how they operate.
- Bioinformatics applies computational techniques to vast amounts of data to understand how cells and cell systems work and link responses to exposure and dose delivered to target organs (e.g. virtual organs).

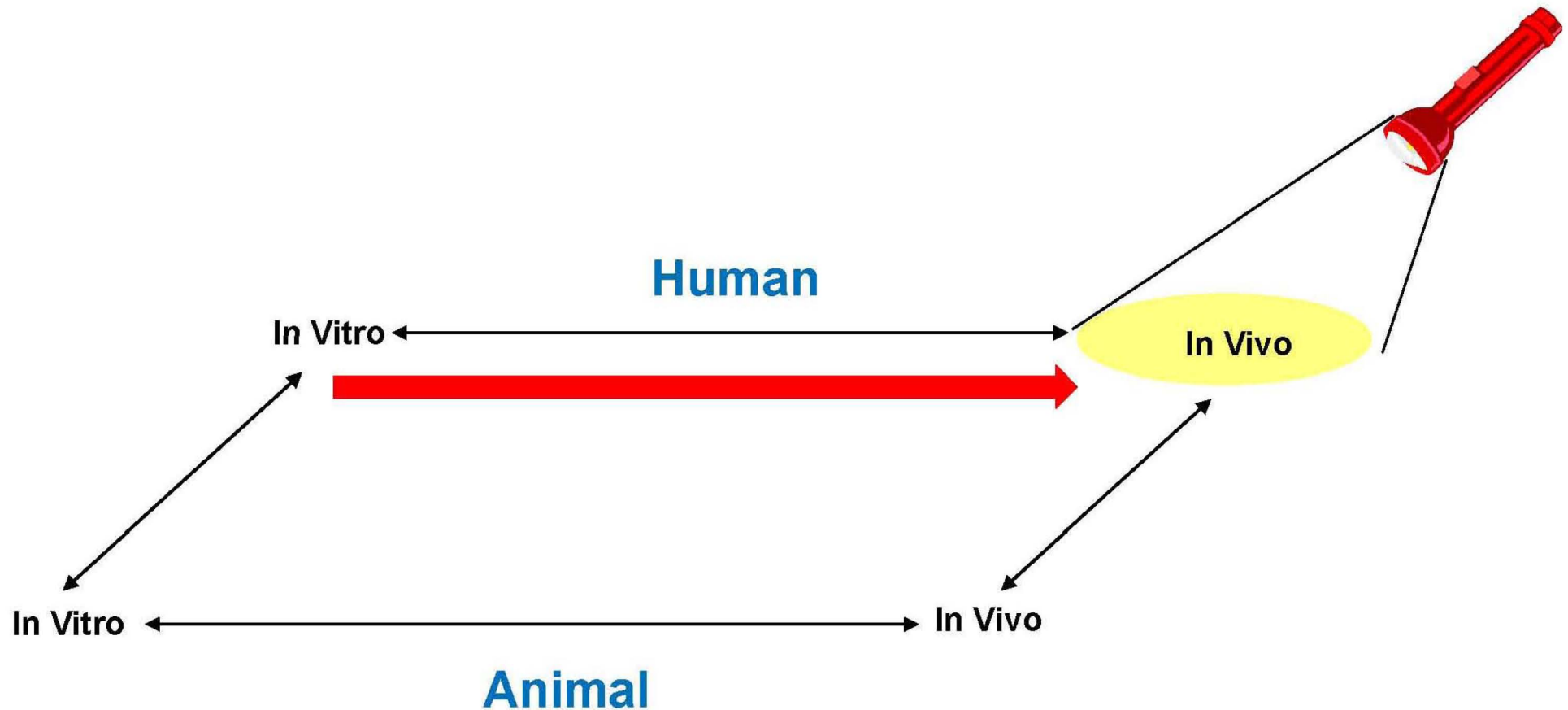
Systems Biology Approach

The report envisions a new toxicity-testing system that relies mainly on understanding **toxicity pathways** – the cellular response pathways that can result in adverse health effects when sufficiently perturbed.

This approach leverages animal and in vitro studies to make predictions about which perturbations to normal biological processes (molecular and key event networks) are sufficient to cause adverse outcomes in humans.



How Well Do in Vitro Assays Predict Human In Vivo Responses?



Goals of the NextGen Ozone Project

- **Characterize toxicity pathways in human lung cells exposed in vitro to ozone**
- **Characterize toxicity pathways (and downstream pathophysiological responses) in human volunteers exposed to ozone**
- **Develop models that can assess how accurately the in vitro pathways predict human responses.**

Why Ozone?

- There are 20 years of controlled human exposure studies that have characterized lung function, inflammatory, and host defense changes in humans exposed to ozone

The current standard rests largely on these studies

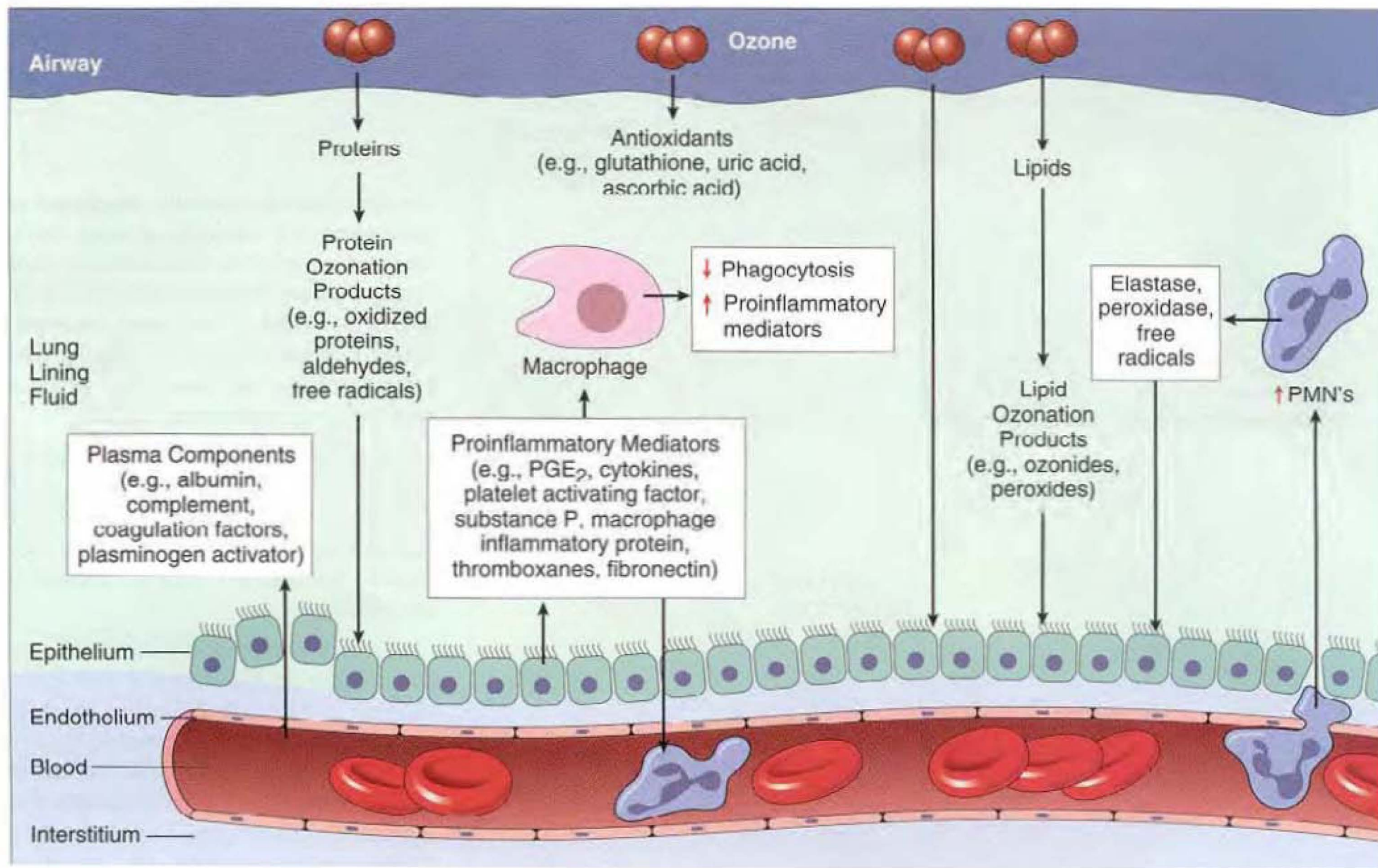
- There are 20 years of animal toxicology studies that have confirmed and extended the human database

Examination of end points and exposure scenarios not possible with human studies

- There are in vitro toxicology studies that have characterized some of the mechanisms by which ozone causes effects in humans

These studies are somewhat dated and did not use the type of technology available today

Effects of Ozone on Respiratory Tract Cells



In Vivo Exposure Studies

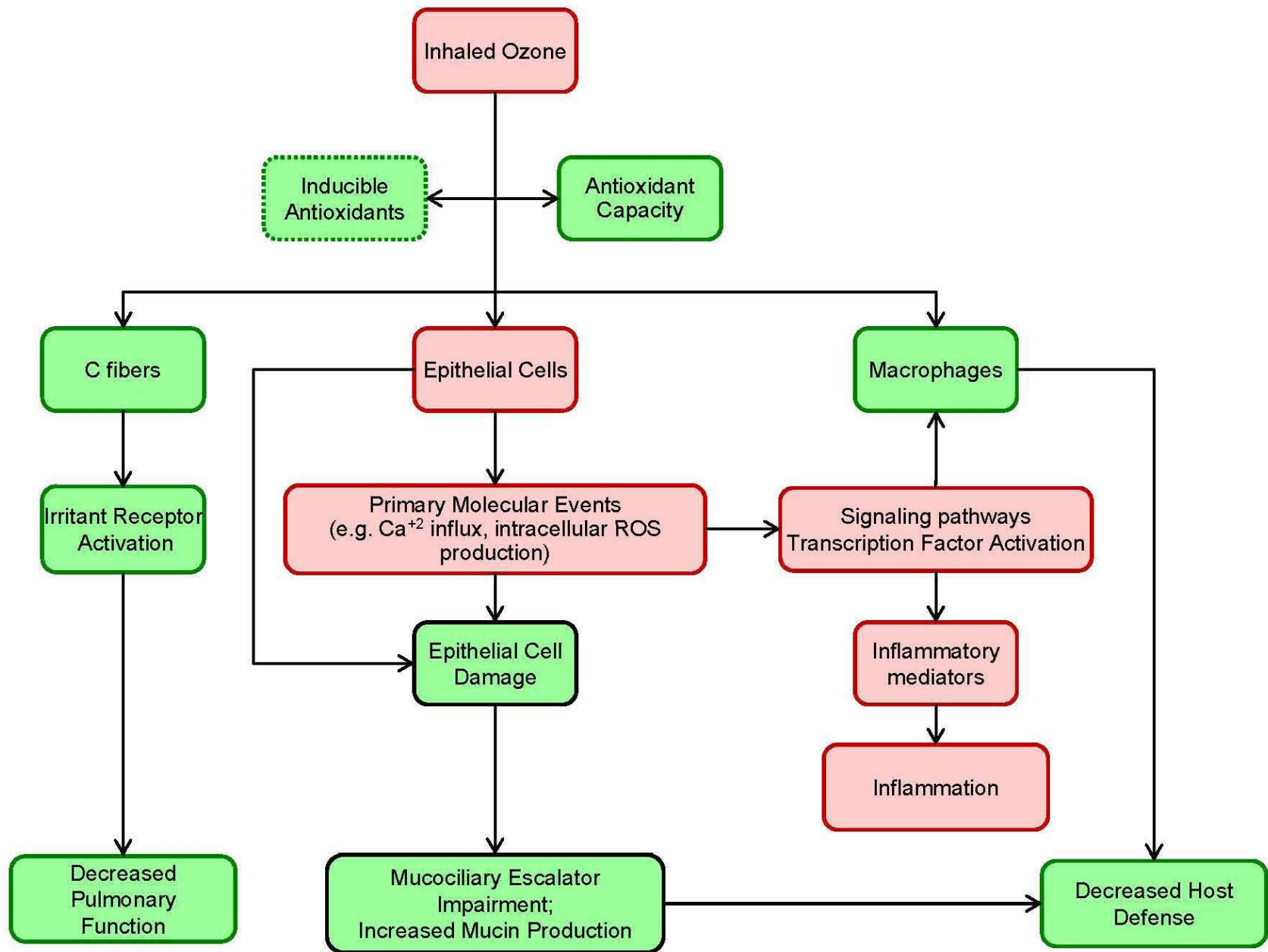
- Each human volunteer is exposed to and air and ozone on two occasions and markers of lung injury and inflammation measured.
- Airway epithelial cells are removed by brush biopsy and micro array technology used to define toxicity pathways induced in those cells by ozone compared with air.
- Quantitative 2D gel electrophoresis coupled with mass spec is used to correlate changes in mRNAs with their protein counterparts and to examine post-transcriptional modifications (e.g. phosphorylation)

In Vitro Exposure Studies

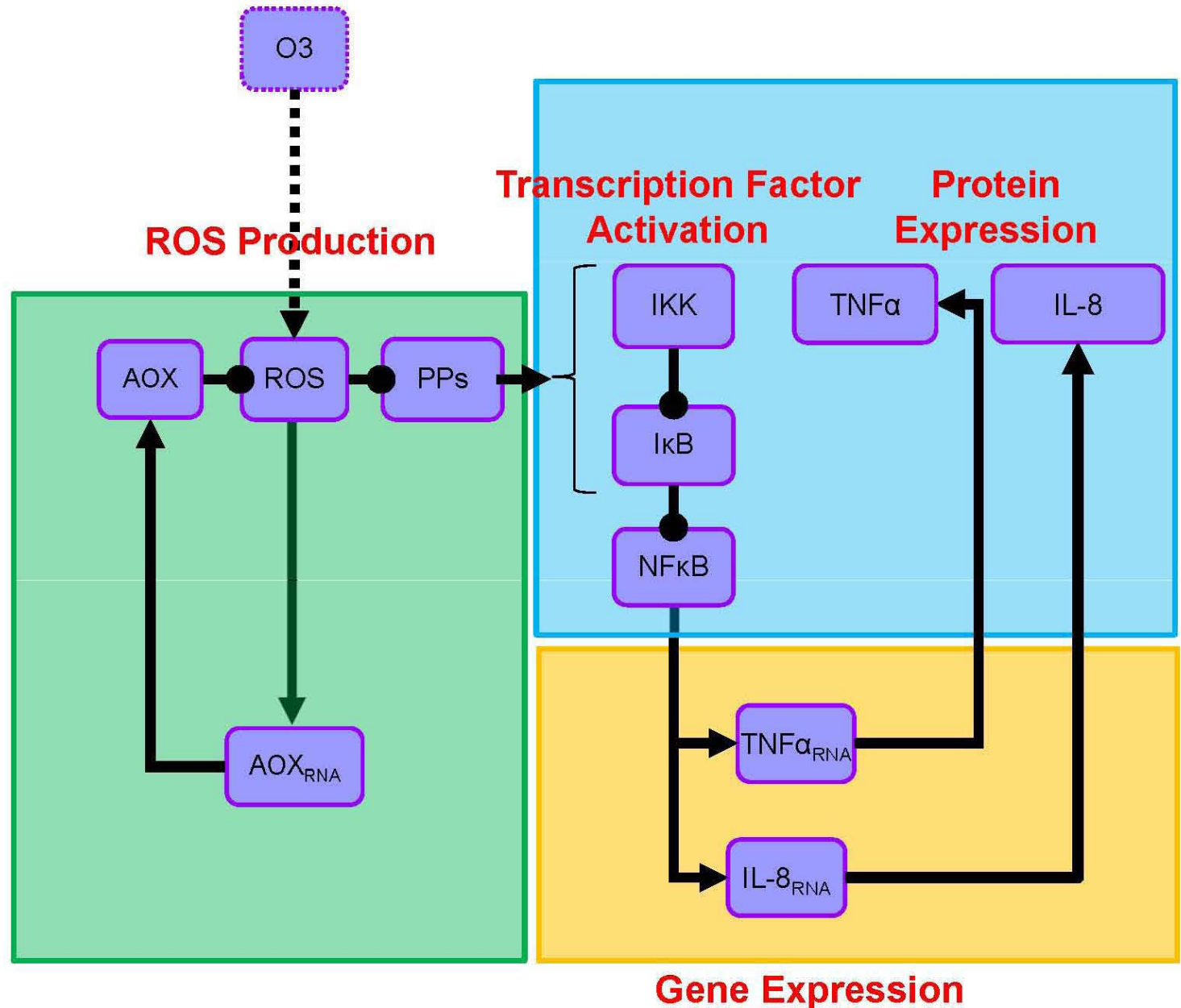
- Airway cells removed following exposure to air are cultured in vitro and exposed to varying concentrations of ozone.
- Micro array technology is used to define toxicity pathways induced in those cells by ozone compared with air.

Proteomics also performed

- Computational modeling used to determine how well toxicity pathways induced in vitro predict those induced in vivo.
- Molecular approaches used to characterize upstream events (e.g. ROS production, MAPK signaling, transcription factor analysis) which will be incorporated into the computational models.



Toxicity Pathway Modeling



Data Incorporation

- **Multiple data sources and types**
- **Different levels of organization**
 - Intracellular (microarray, RT-PCR, 2D gels)
 - Tissue (neutrophils, prostoglandins, LDH, plasma leakage)
 - Organ physiology (e.g. lung function)
- **Integrated into single model**

How Realistic Are In Vitro Experiments?

- Cells removed from their normal three dimensional environment.
little or no cell-cell interaction
- No blood supply with potentially important factors.
- Exposure may not be the same as in vivo.

BUT

Some cellular pathways are activated in the absence of neighboring cells or factors.

EGFR, AH Receptor

Appropriate cell types can be chosen and a realistic dose response curve generated

Normalization of O₃ Dose Delivered to Cultured Cells and Human Target Tissues Following in Vivo Exposure

The use of ¹⁸Ozone

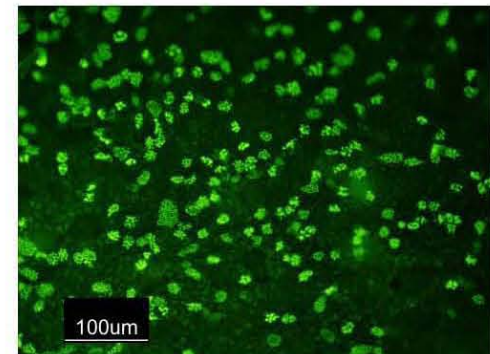
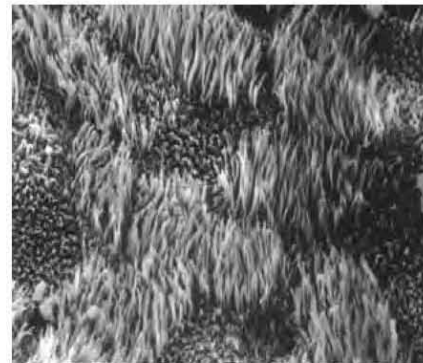
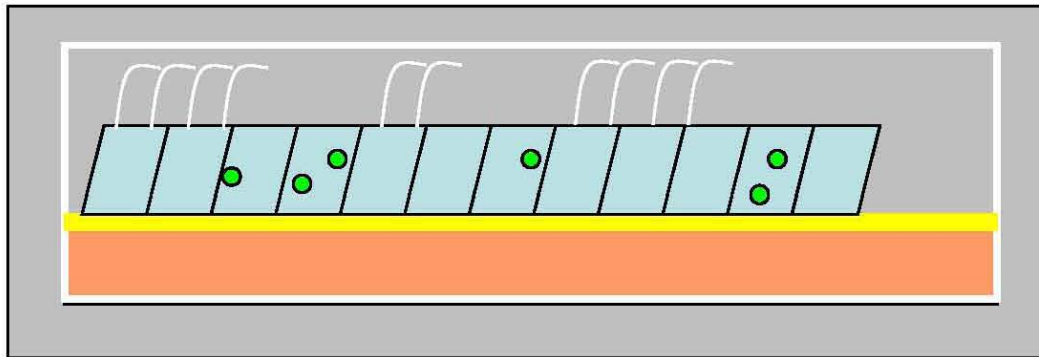
Stable non-radioactive isotopes are safe for human studies.

Ozone reaction products are present on tissues, cells and biomolecules.

Will be used to ensure that cells exposed in vitro receive the same dose of ozone as epithelial cells exposed in vivo.

Human Primary Airway Cells

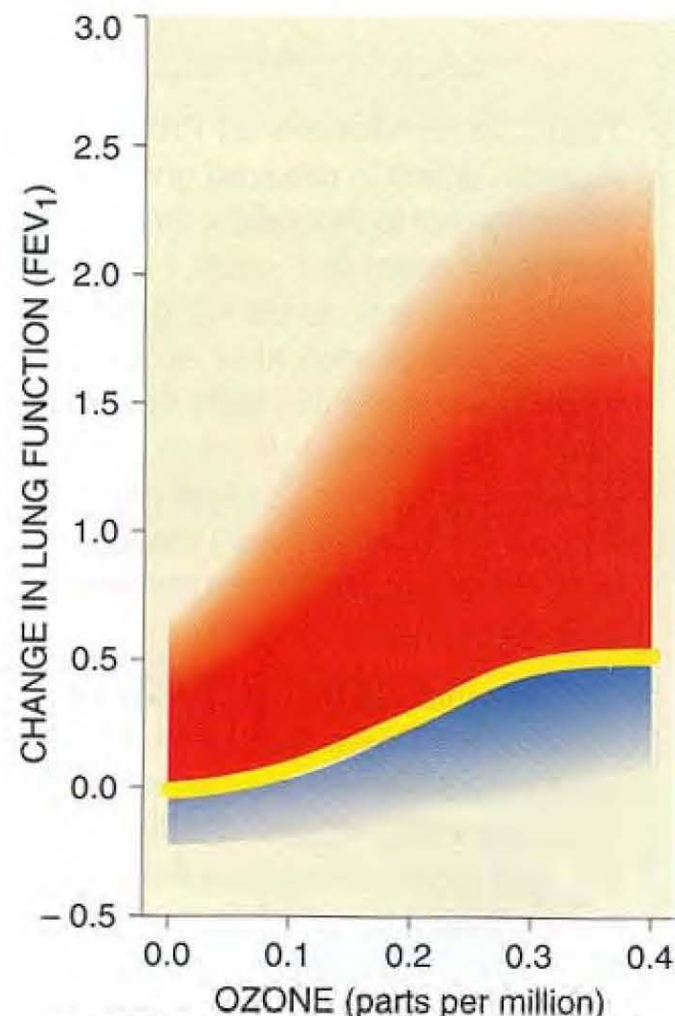
- No animal to human extrapolation needed
- Primary cells respond more “realistically” than immortalized or transformed cells
- Can look at genetic/epigenetic, disease, age and other factors



Genetic Susceptibility

- There are a number of polymorphisms that have been shown to be associated with susceptibility to air pollutants
e.g. GSMT1
- Respiratory tract cells can be obtained from people with specific SNPs and exposed to ozone in vitro to understand the underlying toxicity pathways.

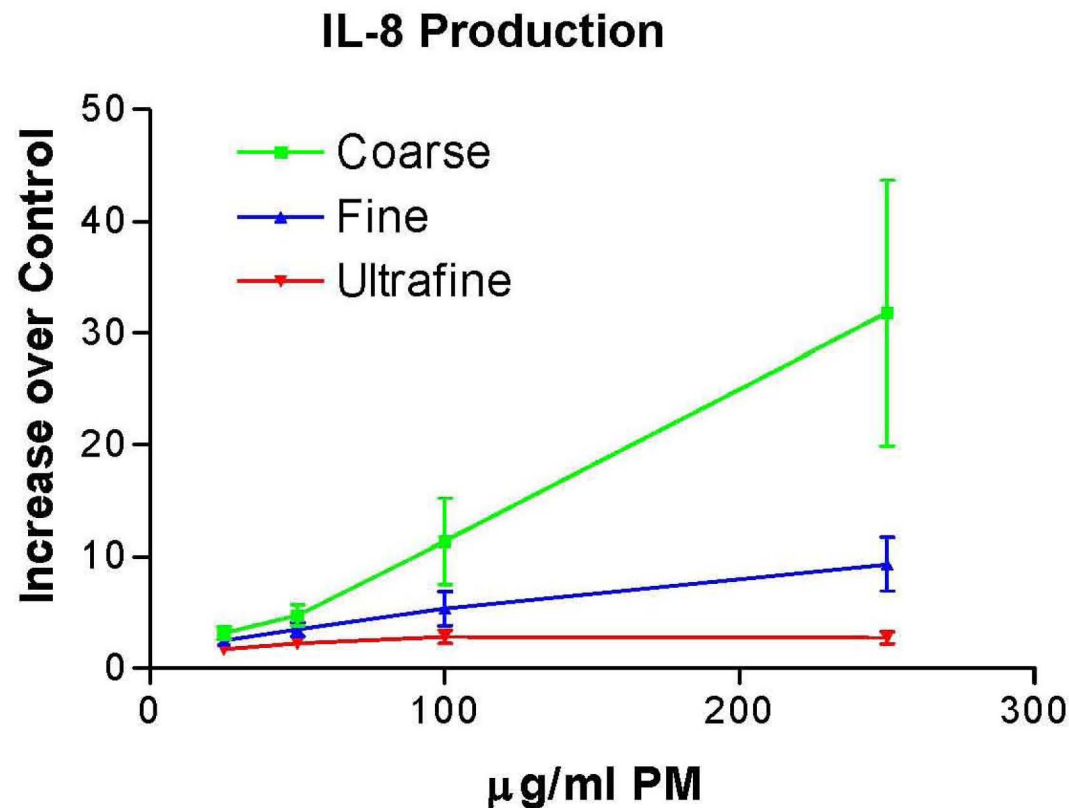
We will be examining both genetic and epigenetic factors might contribute to ozone-induced inflammation.



ADAPTED FROM WILLIAM F. McDONNELL ET AL., AMERICAN REVIEW OF RESPIRATORY DISEASE 147:818-825, APRIL 1993.

Are There Examples of in Vitro Tests Predicting in Vivo Responses?

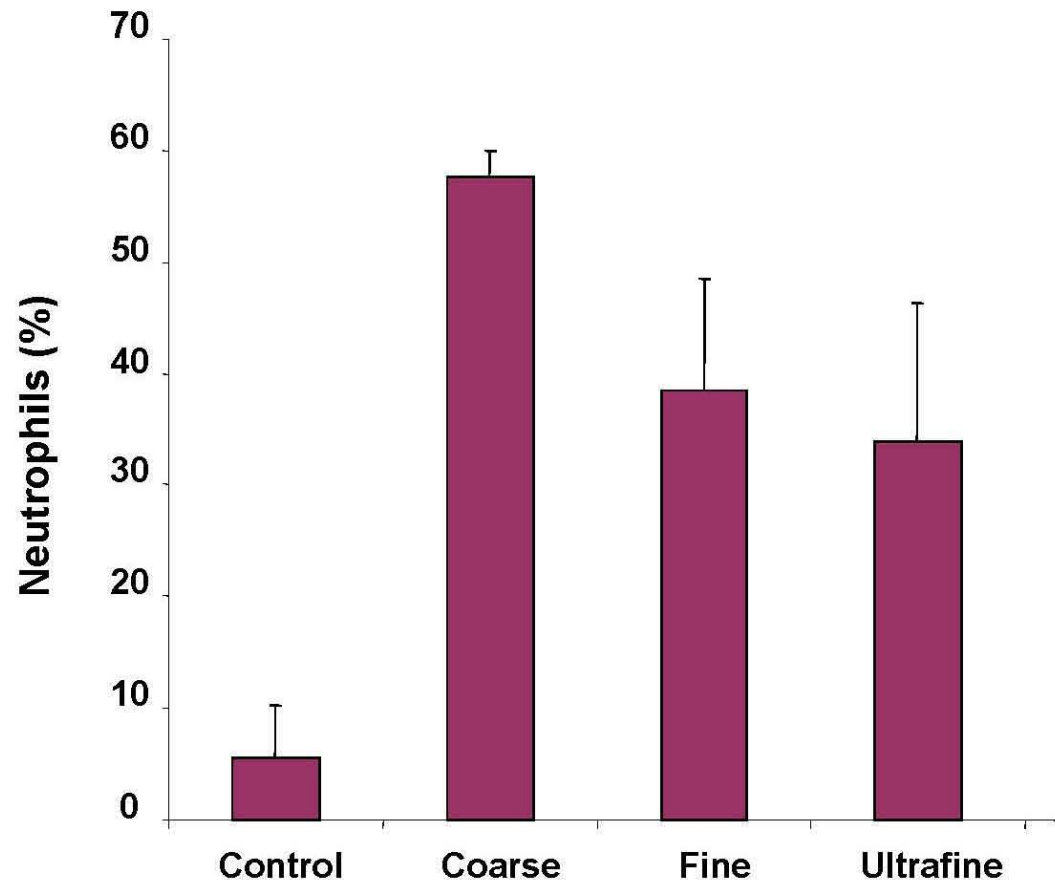
- Cultured human lung cells exposed to different size fractions of PM
- Markers of inflammation measured
- Coarse PM more potent than fine or ultrafine PM



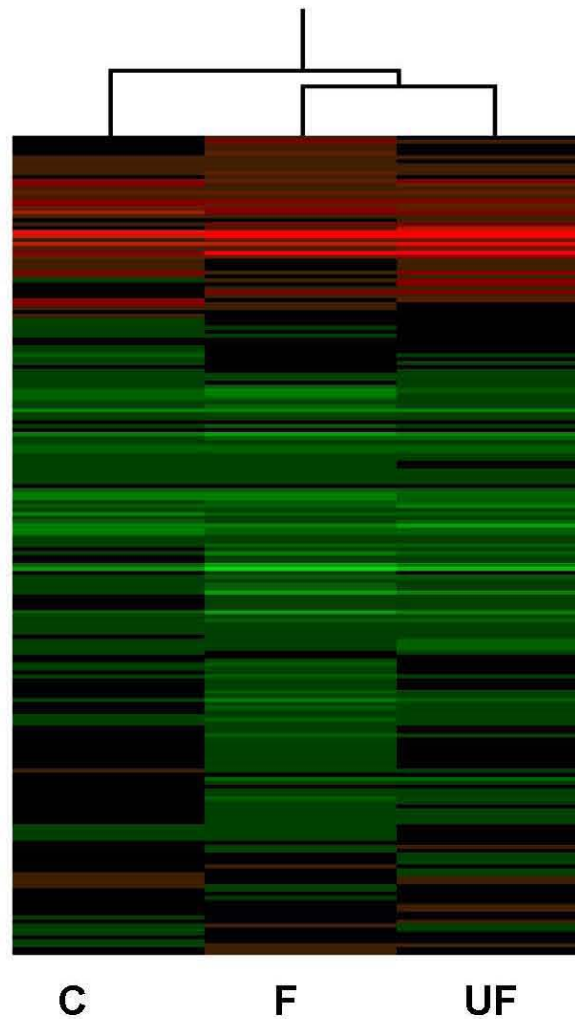
Coarse PM Causes More Inflammation in Mice

Saline and coarse, fine, or ultrafine PM were instilled into mice.

Lavage was performed 24 hrs later and inflammation assessed.



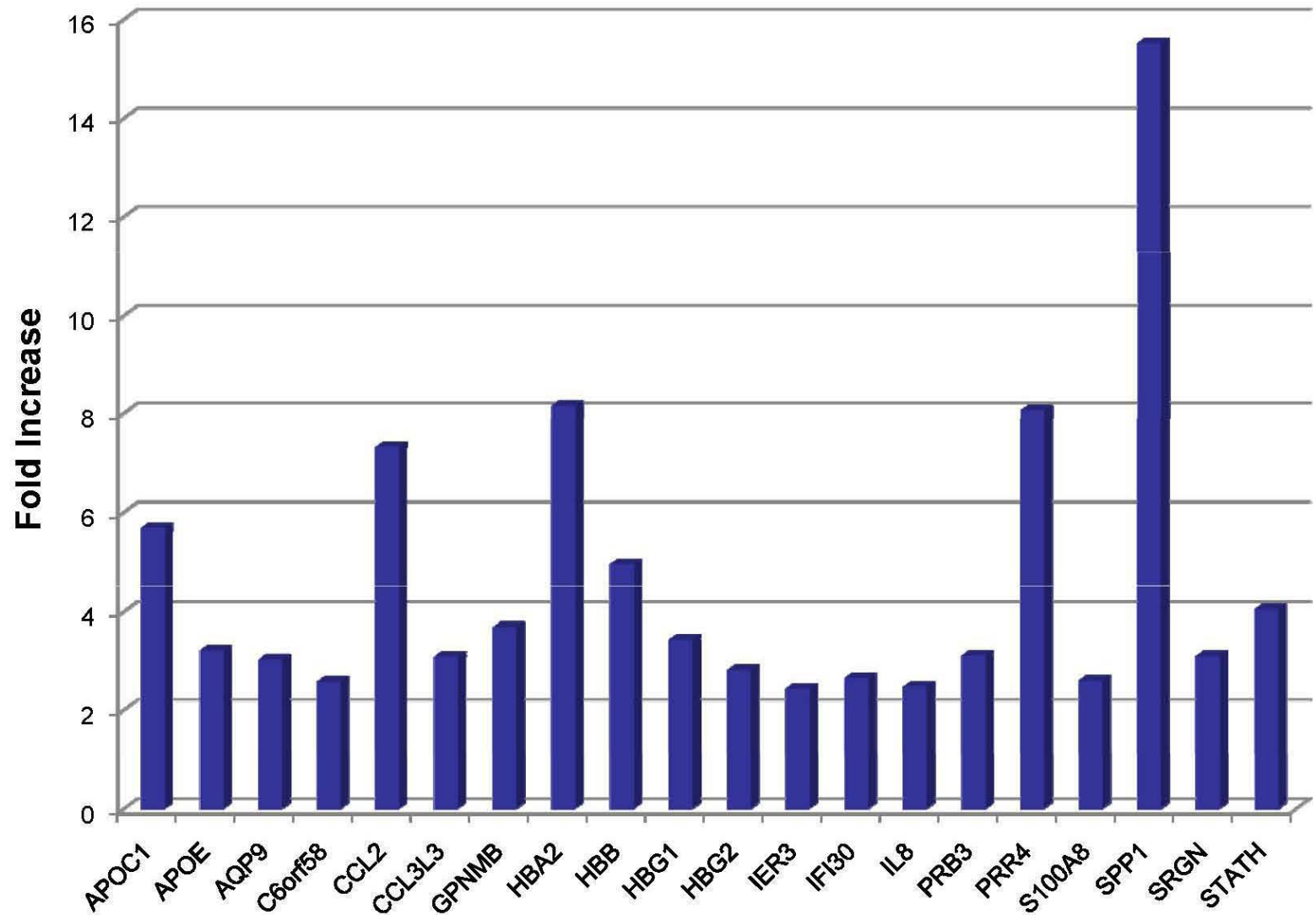
UF, F and C PM Each Elicit the Expression of a Unique Set of Genes



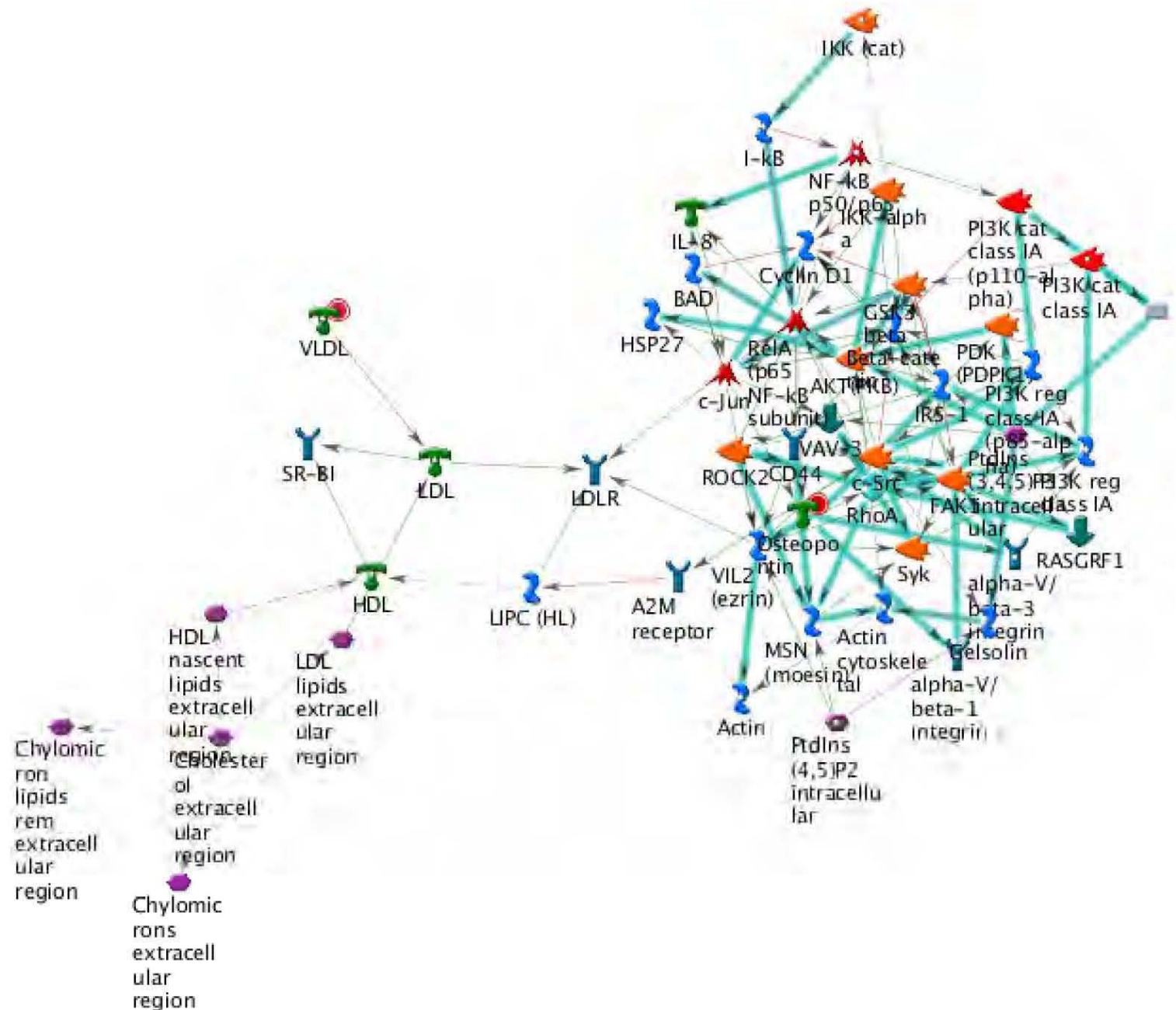
Preliminary In Vivo Data

- **Seven people exposed to ozone and clean air**
30 people needed for study
- **Epithelial cells removed and RNA Isolated**
- **Micro-array analysis performed**

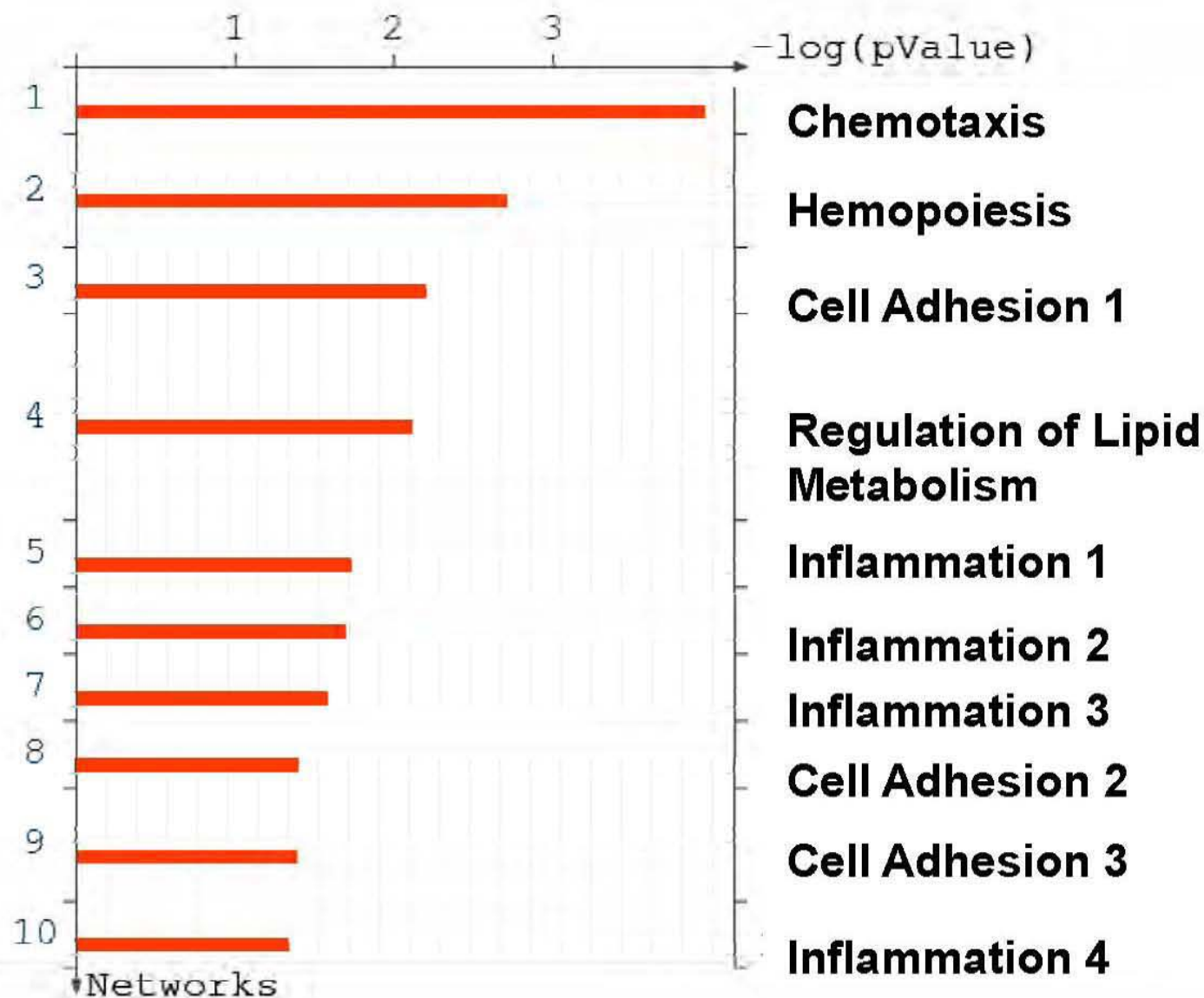
Genes up-Regulated by O3



Top Scored Networks for Filtered Probes



Top Cell Pathways Induced by O₃



Summary

- **There is good reason to believe that in some systems in vitro assays might be used in lieu of animal or human exposure studies.**
- **We are about half way through this study in which we hope to define in a quantitative manner just how accurate the in vitro assays really are using ozone as a model toxicant.**
- **We would then expand this approach beyond ozone to include other toxicants.**

NextGen Ozone Research Team

Kelly Duncan

Lisa Dailey

Lyle Burgoon

Gary Hatch

James Samet

Steve Edwards

David Miller

Rory Conolly

Hisham El-Masri