A Conceptual Model for Assessing Criteria Air Pollutants in a Multipollutant Context:
A modified adverse outcome pathway approach

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Alternative Approaches for Acute Inhalation Toxicity to Address Global Regulatory and Non-regulatory Data Requirements
PETA International Science Consortium (PISC), Ltd.
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Adverse Outcome Pathways (AOPs) and the National Ambient Air Quality Standards (NAAQS)

- NAAQS
- Multipollutant context
- Need for a conceptual model
- Possible frameworks
- AOP approach illustrated with case reports
Figure I  Schematic of the key steps in review of the National Ambient Air Quality Standards.

• **NAAQS are promulgated for individual criteria pollutants:**
  - particulate matter (PM)
  - ozone (O₃)
  - oxides of nitrogen (NOₓ)
  - sulfur oxides (SOₓ)
  - carbon monoxide (CO)
  - lead (Pb)

• **There are two sets of standards:**
  - Primary – based on health effects
  - Secondary – based on welfare effects

• **One of the criteria pollutants (PM) is a mixture**

• **Currently, NOₓ and SOₓ are being reviewed together for the secondary NAAQS**

Recommendation:
Address multiple pollutants in the NAAQS review and standard setting process

“Although the committee does not believe that the science has evolved to a sufficient extent to permit the development of multipollutant NAAQS, it would be scientifically prudent to begin to review and develop NAAQS for related pollutants in parallel and simultaneously”

NOTE: There are Currently no Plans to Attempt the Development of Multipollutant Primary NAAQS
Practical Advancement of Multipollutant Scientific and Risk Assessment Approaches for Ambient Air Pollution

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OBJECTIVES: The U.S. Environmental Protection Agency is working toward gaining a better understanding of the human health impacts of exposure to complex air pollutant mixtures and the key features that drive the toxicity of these mixtures, which can then be used for future scientific and risk assessments.

DATA SOURCES: A public workshop was held in Chapel Hill, North Carolina, 22–24 February 2011, to discuss scientific issues and data gaps related to adopting multipollutant science and risk assessment approaches, with a particular focus on the criteria air pollutants. Expert panelists in the fields of epidemiology, toxicology, and atmospheric and exposure sciences led open discussions to encourage workshop participants to think broadly about available and emerging scientific evidence related to multipollutant approaches to evaluating the health effects of air pollution.

SYNTHESIS: Although there is clearly a need for novel research and analytical approaches to better characterize the health effects of multipollutant exposures, much progress can be made by using existing scientific information and statistical methods to evaluate the effects of single pollutants in a multipollutant context. This work will have a direct impact on the development of a multipollutant science assessment and a conceptual framework for conducting multipollutant risk assessments.

CONCLUSIONS: Transitioning to a multipollutant paradigm can be aided through the adoption of a framework for multipollutant science and risk assessment that encompasses well-studied and ubiquitous air pollutants. Successfully advancing methods for conducting these assessments will require collaborative and parallel efforts between the scientific and environmental regulatory and policy communities.

Groupings of Pollutants and their Effects

By fundamental biological reactivity
• Oxidative injury
• Affinity for neural receptors
• Recognition by immune cells
• Covalent binding to DNA or proteins
  (Mauderly, et al., Inhalation Toxicology 22(S1):1, 2010)

By surrogate marker
• Endothelial function
• Endothelial progenitor cells
• Blood pressure
• ANS measures
• Systemic inflammation
• Insulin resistance
  (S. Rajagopalan, AAAR March 2010)
Characterization of Common Modes of Action and Toxicity Pathways
(Lead: Barbara Buckley)

Do multiple criteria pollutants act through similar pathways to induce health effects?

• Develop framework
• Fit existing information for single pollutants into framework
• Provide case studies illustrating converging effects/converging pathways for multiple pollutants
MOA Paradigm

- Sequence of precursor steps necessary for end result
- Measureable key events or markers of key events
- Toxicokinetic/Toxicodynamics ???
O₃ MOA: Respiratory Effects

Formation of secondary oxidation products

Activation of neural reflexes
- Spirometric changes (↓FEV₁,FVC)
- Mild bronchoconstriction (↑sRaw)

Increased bronchial reactivity (AHR)
- △Epithelial permeability
- Airways neutrophilia

Inflammation and injury
- ^Epithelial permeability
- Airways neutrophilia

Immune system modulation
- Allergic priming & sensitization
- Decreased pathogen clearance

Exacerbation/induction of asthma and allergic responses

Decrements in Pulmonary Function

Repair or remodeling

Impaired host defense/RT infections
Multipollutant MOAs: Respiratory Effects

Formulation of secondary oxidation products in ELF

- SO₂ ↔ PM ↔ O₃

- Activation of neural reflexes
  - ↓ Inspiratory capacity
  - Decrement in pulmonary function

- Increased bronchial reactivity (AHR)
  - Mild/Moderate bronchoconstriction

- Inflammation and injury
  - ↑ Epithelial permeability
  - Airways neutrophilia
  - Immune system modulation
  - Decreased pathogen clearance
  - Impaired host defense/respiratory tract infections

- Exacerbation/induction of asthma and allergic responses
  - Allergic priming & sensitization
Multipollutant MOA: Cardiovascular (CV) Effects

EC: Endothelial cell; RT: Respiratory tract
Common Adverse Outcome Paradigm

Developed by NRC to guide U.S. EPA in conducting a cumulative risk assessment of a class of chemicals (i.e., phthalate esters) which share a common health outcome rather than a common mechanism/mode of action

It broadened the focus to include contributions to the common health outcome resulting from stressors other than phthalates
Common Adverse Outcome Paradigm

Phthalates

Antiandrogenic compounds, other risk factors

Disturbed androgen action

Altered male reproductive outcomes

NRC 2008 Phthalates and Cumulative Risk Assessment - The Task Ahead
Common Adverse Outcome: Air Pollutants

Progression of Atherosclerosis

PM
O₃
Motor Vehicle Exhaust

Altered Cardiovascular Outcomes
Common Adverse Outcome: Air Pollutants

- PM
- Motor Vehicle Exhaust
- Cigarette Smoke
- CO

Altered Vasomotor Function

Altered Cardiovascular Outcomes
Common Adverse Outcome: Air Pollutants

PM  NOX  Motor Vehicle Exhaust  Cigarette Smoke  CO  O3

↑Lipid peroxidation
↓Flow mediated dilation
Systemic inflammation
↑MMP, ET-1
↑Adhesion molecules
↑↓ Coagulation factors

Vascular Toxicity

Altered Cardiovascular Outcomes

MMP: matrix metalloproteinase; ET-1: endothelin-1
Common Adverse Outcome: Air Pollutants

PM  NO$_x$  Motor Vehicle Exhaust  Cigarette Smoke  CO  O$_3$

$\downarrow$ eNOS activity
$\downarrow$ eNOS protein
$\uparrow$ O$_2^-$ generation
$\downarrow$
$\downarrow$ NO bioavailability
$\downarrow$
$\downarrow$ Flow mediated dilation

Vascular Toxicity

Altered Cardiovascular Outcomes

O$_2^-$: superoxide; NO: nitric oxide; eNOS: endothelial nitric oxide synthase
The U.S. Environmental Protection Agency’s Strategic Plan for Evaluating the Toxicity of Chemicals
Figure 1. Toxicity Pathways. Toxicity pathways describe the processes by which perturbations of normal biological processes due to exposure to a stressor (e.g., chemical) produce changes sufficient to lead to cell injury and subsequent events (modified from NRC, 2007).
In Vivo Exposures/In Vitro Assays

Key role of circulating factors

Channell et al., Tox Sci 127: 179, 2012

IL-8: Interleukin-8; sLOX: soluble lectin-like receptor for oxidized low density lipoprotein
Ankley, et al., Env Tox Chem 29: 730, 2010
Ankley, et al., Env Tox Chem 29: 730, 2010
“The AOP framework also illustrates how effects caused by mixtures of chemicals that act via the same molecular initiating event...or affect pathways that converge at common intermediate steps ...can be aggregated for risk characterization.”

“AOPs do not, however, address the question of what dose of chemical will cause sufficient perturbation to drive the pathway to the adverse outcome.”

Ankley, et al., Env Tox Chem 29: 730, 2010
Review

Conceptual model for assessing criteria air pollutants in a multipollutant context: A modified adverse outcome pathway approach

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\textbf{ABSTRACT}

Air pollution consists of a complex mixture of particulate and gaseous components. Individual criteria and other hazardous air pollutants have been linked to adverse respiratory and cardiovascular health outcomes. However, assessing risk of air pollutant mixtures is difficult since components are present in different combinations and concentrations in ambient air. Recent mechanistic studies have limited utility because of the inability to link measured changes to adverse outcomes that are relevant to risk assessment. New approaches are needed to address this challenge. The purpose of this manuscript is to describe a conceptual model, based on the adverse outcome pathway approach, which connects initiating events at the cellular and molecular level to population-wide impacts. This may facilitate hazard assessment of air pollution mixtures. In the case reports presented here, airway hyper-responsiveness and endothelial dysfunction are measurable endpoints that serve to integrate the effects of individual criteria air pollutants found in inhaled mixtures. This approach incorporates information from experimental and observational studies into a sequential series of higher order effects.

The proposed model has the potential to facilitate multipollutant risk assessment by providing a framework that can be used to converge the effects of air pollutants in light of common underlying mechanisms. This approach may provide a ready-to-use tool to facilitate evaluation of health effects resulting from exposure to air pollution mixtures.
Background

- Air pollution is a complex mixture of particulate and gaseous components.
- Conventional epidemiologic and toxicological approaches cannot evaluate all of the possible unique multipollutant mixtures.
- A large number of toxicological studies have been conducted for the purpose of elucidating underlying changes in genes, biomarkers, proteins, etc. in response to air pollutants.
- The utility of toxicological findings is limited because of the inability to link such changes to an adverse outcome that is relevant to risk assessment.
- This type of mechanistic data may be most informative for risk assessment when translated into measurable changes including organ responses, clinical consequences, and impacts to the population at large.
Goals

• Develop a conceptual model for air pollution mixtures that links initiating events at the cellular and molecular level to population-wide impacts

• Identify measurable endpoints which serve to integrate the effects of individual criteria air pollutants found in inhaled mixtures
  o Airway hyperresponsiveness - a key feature of asthma (Case Report 1)
  o Endothelial dysfunction - a risk factor for cardiovascular (CV) disease (Case Report 2)
  o Physiological changes at the organ level which can be measured in the clinic/laboratory

• Incorporate information from experimental and observational studies into a sequence of steps occurring over multiple levels of biological organization
Case Report 1:
Irritant gases, airway responsiveness, and respiratory morbidity

Irritant gases = O$_3$, NO$_2$, and SO$_2$

Airway responsiveness reflects the sensitivity of airway smooth muscle to natural or pharmacological stimuli.

Epidemiologic Studies

• **Short–term exposures and associations with:**
  • Respiratory symptoms
  • Asthma medication use
  • Respiratory-related emergency department (ED) visits
  • Hospital admissions (HA) including those for asthma

• **Long-term exposures and associations with:**
  • Respiratory symptoms
  • Bronchitis
  • Asthma
  • New onset asthma

• **Potential co-pollutant confounding for both short- and long-term studies but more evidence for independent effects in short-term studies**
Case Report 1

Controlled Human Exposure Studies

• Formation of secondary oxidation products in the lung lining fluid

• ↑ immune responses in healthy individuals
  • Neutrophil influx in airways
  • Th2 polarization (repeated exposures)
  • Altered cell surface molecules on monocytes that are characteristic of innate immunity and antigen presentation

• ↑ immune responses in allergic asthmatics
  • Eosinophils, ECP in lung lining fluid
  • Pro-allergic cytokines
  • Activation of the TLR4 pathway

• Physiologic changes in airway smooth muscle (healthy and asthmatics)
  • ↑ airway resistance due to acetylcholine release by airway nerves and to inflammatory mediators

• ↑ Inherent reactivity of airway smooth muscle (healthy and asthmatics)
  • Airway hyperresponsiveness following a direct or allergen challenge

Th2: T helper cell 2; ECP: eosinophil cationic protein; TLR4: Toll receptor 4
Case Report 1

Toxicological Studies

• Formation of secondary oxidation products in the lung lining fluid
• ↑ immune responses
  • Allergic sensitization in naïve animals
    • Activation of the TLR4 pathway
    • Dendritic cell maturation
    • Polarization to Th2 and Th17 phenotype
  • Enhanced allergic responses in allergen-sensitized animals
• Physiologic changes in airway smooth muscle
  • ↑airway resistance due to neural reflexes involving vagus nerve
• ↑Inherent reactivity of airway smooth muscle
  • Hyperreactivity of vagal nerves due to inflammatory mediators
  • Stimulation of local axon reflexes with release of tachykinins
  • Mast cell degranulation
  • Airway remodeling
  • Disruption of the epithelial-mesenchymal unit during lung development

Th17: T helper cell 17
Airway Hyperresponsiveness

- This simplified AOP illustrates a sequential series of higher order effects linking exposure to NO₂, O₃, and SO₂ to an adverse outcome with relevance to risk assessment.
- Airway hyperresponsiveness is a measurable endpoint which can serve to integrate the upstream effects of O₃, NO₂ and/or SO₂ in the respiratory tract.

HA: hospital admissions; ED: emergency department
Case Report 2
PM and $O_3$, endothelial dysfunction, and CV disease

Endothelial dysfunction is defined as impaired blood vessel response to specific vasodilators. It can occur in conduit arteries and microvascular resistance vessels.

Epidemiologic Studies

• **Short-term exposures to PM and associations with:**
  • CV morbidity/mortality
  • Myocardial infarction (MI)
  • Endothelial dysfunction

• **Long-term exposures to PM and associations with:**
  • CV morbidity/mortality
  • Atherosclerosis
  • Endothelial dysfunction

• **Short-term exposures to $O_3$ and associations with:**
  • Clinical CV events related to coronary artery disease, MI, atherosclerosis
Case Report 2

Controlled Human Exposure Studies
• **PM**: Endothelial dysfunction in healthy subjects and subjects with CV disease
• **O₃**: No endothelial dysfunction in healthy subjects

Toxicological Studies
• **PM and O₃**: Endothelial dysfunction

How does PM or O₃ inhalation lead to systemic effects?
• Evidence suggests that pulmonary inflammation/oxidative stress mediates systemic inflammation/oxidative stress

What is the mechanism underlying endothelial dysfunction?
• It is likely due to decreased nitric oxide bioavailability which can occur via several mechanisms
This simplified AOP illustrates a sequential series of higher order effects linking exposure to PM and O₃ to an adverse outcome with relevance to risk assessment.

Cellular responses refer to responses in all vascular cell types.

Endothelial-dependent vasodilation is a measurable indicator of endothelial dysfunction which can serve to integrate the upstream effects of PM and O₃ in the vasculature.

NO: nitric oxide; eNOS: endothelial nitric oxide synthase; BH₄: tetrahydrobiopterin
Relevancy

The proposed model has the potential to facilitate multipollutant risk assessment by:

• Providing a framework that can be used to converge the effects of air pollutants based on common underlying mechanisms
• Identifying data gaps
• Enabling prioritization of targeted research in the most efficient and cost-effective manner possible
• Allowing the incorporation of biomarker data that is predictive of clinically significant outcomes
Limitations

• Population effects may be mediated by alternative mechanisms than the ones identified

• Model does not account for adaptation and repair

• Model does not address dose-response considerations

• Toxicokinetic and toxicodynamics data have not been incorporated
“Dosimetry links exposure and response”

Annie Jarabek

What a Difference the Dose Makes
Webinar Series
April 28, 2016
Advancing AOP and MOA

- Need to define different dose metrics in order to apply key events of adverse outcome pathways (AOP) and mode of action (MOA) in risk assessment
  - Screening dosimetry insufficient for quantitative response analysis
  - Portal-of-entry descriptions
  - Broad context re: both endpoints and chemical classes

- Support transparency, causal linkage and interoperability along continuum: exposure to dose-response analysis

Source: US EPA Human Health Risk Assessment (HHRA) FY16-19 Strategic Research Action Plan
https://www.epa.gov/research/strategic-research-action-plans-2016-2019
Impact

• This approach facilitates the evaluation of health effects resulting from exposure to air pollution mixtures.

• Evidence from epidemiologic, controlled human exposure and toxicological studies of single criteria pollutants can be utilized to develop AOPs for mixtures of these pollutants.

• AOPs may be simplified, as illustrated here, or more detailed, including multiple effects occurring in multiple compartments at each level of biological organization.

• They may be used to indicate the certainty of mechanistic linkages between steps and to portray potential biomarkers of exposure or effect.

• AOPs may allow the incorporation of toxicodynamic, toxicokinetic data into a conceptual model.

• This may lead to the quantitation of exposure-response relationships for multipollutant mixtures.
Future Direction

The outputs from this project are helping to advance EPA’s science assessments, moving us one step closer to explicit consideration of multipollutant evidence in reviews of the NAAQS.
Questions?