The exposome and exposomics in inhalation toxicology

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Rollins School of Public Health
Emory University
Atlanta, GA
Outline

• The exposome concept
• Current approaches
• Applications to inhalation toxicology
G \times E = P

- Genes x environment = phenotype

- John Locke’s (1690) view that we are born *tabula rasa*- a blank slate

- Without input from our environment we are feeble. Humanity requires the environment.
Nature vs. Nurture

• Truly a false dichotomy
  (see “Nature vs. Nurture: Death of a Dogma” in Neuron, 2010 by Traynor and Singleton)

• Whether you call it nature vs. nurture or genes vs. environment, life occurs at the interface.

• For the sake of human health, we need a richer understanding of nurture
The Exposome: a Wild idea

Complementing the Genome with an “Exposome”: The Outstanding Challenge of Environmental Exposure Measurement in Molecular Epidemiology

Christopher Paul Wild
Molecular Epidemiology Unit, Centre for Epidemiology and Biostatistics, Leeds Institute of Genetics, Health and Therapeutics, Faculty of Medicine and Health, University of Leeds, Leeds, United Kingdom

Defined the “Exposome” as all exposures from conception onwards, including those from lifestyle, diet and the environment.

<table>
<thead>
<tr>
<th>Table 3.1 Omics, Omics, Omics, The Major Omic Technologies and their Usage</th>
</tr>
</thead>
<tbody>
<tr>
<td>sone (Year Coined)</td>
</tr>
<tr>
<td>Genome (1920)</td>
</tr>
<tr>
<td>Proteome (1994)</td>
</tr>
<tr>
<td>Transcriptome (1997)</td>
</tr>
<tr>
<td>Epigenomics (1990s)</td>
</tr>
<tr>
<td>Toxicogenomics’ (1999)</td>
</tr>
<tr>
<td>Exposome (2005)</td>
</tr>
</tbody>
</table>

A search of PubMed for the sone term and its corresponding root word was conducted. The ratio between the sone term and the root term gives a sense of the acceptance and use of the sone term. Genome and proteome are well represented, while toxicogenomics and exposome are not. There is a strong denominator effect here in that there were only 54 citations of the work exposome as of July, 2013.

*Toxicogenomics, like pharmacogenomics, never used the -ome suffix, only the -omic suffix.

Substituting environment (1,101,485) for exposure yields a 20,398-fold differential.
The Nature of Nurture: Refining the Definition of the Exposome

Gary W. Miller and Dean P. Jones

Rollins School of Public Health, School of Medicine, Emory University, Atlanta, Georgia 30322

Exposome: The cumulative measure of environmental influences and associated biological responses throughout the lifespan, including exposures from the environment, diet, behavior, and endogenous processes
The exposome as a foil to the genome

Oxford English Dictionary: Foil (noun):

A person or thing that contrasts with and so emphasizes and enhances the qualities of another.
The Exposome in the EU

The Exposome at Emory

The Exposome a la Chris Wild

The Exposome Rappaport and Smith

NAS Exposome Workshop

Complementing the Genome with an “Exposome”:
The Outstanding Challenge of Environmental Exposure Measurement in Molecular Epidemiology

Christophe Paul RMI
Department of Environmental and Molecular Biology, Risk Science, University of Geneva, Geneva, Switzerland.

Editors' Note

The term “exposome” was coined in 2005 by Richard Lepley and colleagues to describe the totality of environmental exposures and their effects on health. The exposome includes both external and internal exposures, such as dietary intake, physical activity, and genetic factors. The purpose of this review is to highlight the importance of understanding the exposome in order to improve public health outcomes.
HRM Metabolomics

- 2500 encoded
- 1000+ pharmaceutical
- 80,000 synthetic
- 200,000 plant-based
- 100,000+ microbial

Technological advances:

- IX LC-FTICR w/ apLCMS (2000)
- Dual chromatography (5000)
- Dedicated Orbitrap (10,000)
- Dedicated MSAnalyzer (25,000)
- Hybrid apLCMS w/ MSAnalyzer (50,000)
- Qxactive HF (200,000 to 800,000)

Informatic advances:

- The million exposome...
Affordable analysis of thousands of analytes: exogenous, endogenous, biological pathways

- plasticizers or pesticides
- microbial metabolites of medicines
- tyrosine or tryptophan pathways

~$250/sample for over 20,000 well-characterized and reliable features
The Exposome in the EU

The Exposome at Emory

Japan, other U.S. and European institutions

1966

The Exposome a la Chris Wild

NAS Exposome Workshop

The Exposome Rappaport and Smith

Big data bioinformatics

2016
Are we entering the early phase of a paradigm shift?

- The Exposome in the EU
- The Exposome at Emory
- Japan, other U.S. and European institutions
- Big data bioinformatics
- The Exposome a la Chris Wild
- The Exposome Rappaport and Smith
- NAS Exposome Workshop

1966: TS Kuhn’s Structure published

2016: Helix


2026:

2036:

2046:

2056:

2066:
Postscript to 2nd edition-1969
Kuhn on the term “paradigm”

“...it stands for the entire constellation of beliefs, values, techniques, and so on shared by the members of a community.”

“...it denotes one sort of element in that constellation, the concrete puzzle-solutions which, employed as models or examples, can replace explicit rules as a basis for the solution of the remaining puzzles in normal science.”
How do we assess our surroundings?

- Global
- National
- Regional
- State
- County
- City
- Neighborhood
- Home
- Personal space
NASA has selected two proposals for new Earth science investigations that will put new in particulate air pollutants and study the development of tropical cyclones.

Images from NASA and Dr. Yang Liu laboratory
Air Pollution in World: Real-time Air Quality Index Visualisation.
Similar transformative technologies, such as remote sensing and wearables, are expanding our definition of exposure science.

http://nas-sites.org/emergingscience/meetings/personal-exposure-measurement/workshop-agenda/
Meet Speck.
The indoor air quality monitor empowering you to breathe easier.

Air Quality Matters
Protecting you and your family from harmful air

SPARROW™ wearable air monitor with a powerful app

SPARROW is a wearable environmental health and safety monitor that measures Carbon Monoxide (CO), along with temperature, pressure and relative humidity. It can be attached to your smartphone case, clothes, bag, purse, stroller, bike, and even placed in your car.

GET SPARROW

SPARROW also works in conjunction with the OuterBox UNIVERSE Case System, the innovative smartphone protection that allows for interchangeable accessories to slide directly onto the back of the phone case, making it easier to protect yourself from toxic air quality wherever you go.

How Can Speck Help You?
There is intense competition for data scientists. Toxicology/Environmental Health must invest in data science infrastructure and aggressively recruit, train, and retain those individuals who will be solving our problems in the next 50 years.
Exposome-outside in

• The exposome includes our complex surroundings. The source, timing, duration, and physiochemical properties of these exposures are critical.

• At Emory, we have been focusing most of our efforts on the components of the exposome that can be measured inside the body.
Funded in 2013 (ES P30-019776, renewal pending)

Core facilities for:
- data sciences (Waller, Voit, and Kemp)
- targeted exposures (Barr)
- untargeted exposures (Jones)
- patient studies (Ziegler)
- pilot awards (Morgan)
- career development (Tolbert)
- community engagement (Kegler/Pearson)
Environmental chemicals are present at 3-4 orders of magnitude lower concentration than endogenous metabolites Rappaport S. et al., Environ Health Perspect, 2014
High resolution metabolomic methods provide reliable metabolite measurements in human plasma in millimolar and micromolar range…

and reliable environmental and dietary chemical measurements in *nanomolar* and *subnanomolar* range

<table>
<thead>
<tr>
<th>Compound</th>
<th>No of Subjects</th>
</tr>
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<tbody>
<tr>
<td>Betaine (μM)</td>
<td><img src="image1.png" alt="Graph" /></td>
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<tr>
<td>Choline (μM)</td>
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<tr>
<td>Glucose (mM)</td>
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<td>Cotinine (nM)</td>
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<tr>
<td>Caffeine (μM)</td>
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</tr>
<tr>
<td>Hippurate (µM)</td>
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</tr>
<tr>
<td>Styrene (nM)</td>
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<tr>
<td>N8-Acetylcytidine (nM)</td>
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</tr>
<tr>
<td>Oxoproline (μM)</td>
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<tr>
<td>Oleic acid (µM)</td>
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<tr>
<td>1-Methylhistidine (µM)</td>
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<tr>
<td>Triphenylphosphate (nM)</td>
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<td>Triethylphosphate (nM)</td>
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<tr>
<td>Chlorobenzolate (nM)</td>
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<tr>
<td>Chlorophenylacetate (nM)</td>
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<td>Bilirubin (µM)</td>
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<td>Hypoxanthine (µM)</td>
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<td>Urate (µM)</td>
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<tr>
<td>Uridine (µM)</td>
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<tr>
<td>Pirrimicarb (nM)</td>
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<td>Methmyoglobin (nM)</td>
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<td>Tetraethylene glycol (nM)</td>
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<td>Octylphenol (nM)</td>
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<td>Di-butylphthalate (nM)</td>
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<td>Di-sodinylphthalate (nM)</td>
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<td>Dipropylylphthalate (nM)</td>
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<tr>
<td>Di(2-ethylhexyl)adipate (nM)</td>
<td><img src="image27.png" alt="Graph" /></td>
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</tbody>
</table>
Environmental chemicals are present at 3-4 orders of magnitude lower concentration than endogenous metabolites Rappaport S. et al., Environ Health Perspect, 2014

HRM measures metabolites over >7 orders of magnitude Go et al Tox Sci 2015
Analyte collection using microneedle patch

Collection of Analytes from Microneedle Patches

Andrey V. Romanyuk,† Vasiliy N. Zvezdin,‡ Pradnya Samant,† Mark I. Grenader,† Marina Zemlyanova,§ and Mark R. Prausnitz*†
Detected 7,044 total peaks
6,615 in plasma; 6,012 in ISF (peak present in ≥ 1 sample in matrix)

- **Plasma**: 1,032 (15%)
- **Common**: 5,583 (79%)
- **ISF**: 429 (6%)
Strongly-correlated features between plasma and ISF (peak present in ≥ 4 samples in both plasma and ISF)

<table>
<thead>
<tr>
<th>m/z</th>
<th>Retention time</th>
<th>Spearman r</th>
<th>Compound</th>
<th>Adduct</th>
<th>Source</th>
<th>No. positive in plasma</th>
<th>No. positive in ISF</th>
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<tr>
<td>136.0425</td>
<td>87.441</td>
<td>0.98</td>
<td>Homocysteine</td>
<td>M+H</td>
<td>xMSannotator</td>
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<td>141.0715</td>
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<td>0.96</td>
<td>Dipropyl sulfide</td>
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<td>269.1255</td>
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<td>7</td>
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<td>Dimethylglycine</td>
<td>M+H</td>
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<tr>
<td>119.0162</td>
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<td>0.90</td>
<td>Dihydro-4-mercapto-3(2H)-furanone</td>
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<td>Caffeine</td>
<td>M+H</td>
<td>HMDB</td>
<td>6</td>
<td>4</td>
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<tr>
<td>522.2577</td>
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<td>0.89</td>
<td>LysoPE(0:0/20:5(5Z,8Z,11Z,14Z,17Z))</td>
<td>M+Na</td>
<td>HMDB</td>
<td>10</td>
<td>8</td>
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<tr>
<td>182.0811</td>
<td>84.235</td>
<td>0.88</td>
<td>L-Tyrosine</td>
<td>M+H</td>
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<tr>
<td>478.2920</td>
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<td>0.88</td>
<td>LysoPE(0:0/18:2(9Z,12Z))</td>
<td>M+H</td>
<td>HMDB</td>
<td>10</td>
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<tr>
<td>138.0549</td>
<td>120.073</td>
<td>0.87</td>
<td>m-Aminobenzoic acid</td>
<td>M+H</td>
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<td>Diethylene glycol monoethyl ether</td>
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<td>342.2635</td>
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<td>L-Acetylcarnitine</td>
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<td>Decanoylcarnitine</td>
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<td>120.0656</td>
<td>121.825</td>
<td>0.81</td>
<td>L-Threonine</td>
<td>M+H</td>
<td>xMSannotator</td>
<td>10</td>
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</tr>
</tbody>
</table>
High resolution metabolomics provides a **central reference platform** to link exposure data, internal burden and biological response to exposure.

Exposure assessment | Metabolomics | Molecular response
--- | --- | ---
Environmental Chemicals | Core Biological Metabolome | Commercial Products
Microbiome-related Chemicals | Endogenous metabolome | Supplements and Pharmaceuticals
Non-nutritive Chemicals in Diet | Environmental metabolome | Environmental Chemicals
Supplements and Pharmaceuticals | Commercial Products | Environmental Chemicals

Courtesy of Dean Jones
SV2C Genotype | Frequency | Relative PD risk in smokers
--- | --- | ---
Homozygous, major alleles | 23% | (0.4) ↓↓↓↓
Heterozygous | 75% | (.8-1.9) ↑ or ↓
Homozygous, minor alleles | 2% | (3.3) ↑↑↑

GWAS for protective effect of nicotine in human PD and fly PD model both identified SV2C/ortholog

Identification, characterization, and molecular cloning of a novel transporter-like protein localized to the central nervous system

Jay A. Gingrich, Peter H. Andersen, Mario Tiberi, Salah El Mestikawy, Per N. Jorgensen, Robert T. Fremeau Jr. and Marc G. Caron

SV2, a Brain Synaptic Vesicle Protein Homologous to Bacterial Transporters

Sandra M. Bajjalieh, Karen Peterson, Rajesh Shinghal, Richard H. Scheller
Synaptic vesicle glycoprotein 2C (SV2C) modulates dopamine release and is disrupted in Parkinson disease


*Department of Environmental Health, Rollins School of Public Health, Emory University, Atlanta, GA 30322; bCenter for Neurodegenerative Diseases, Emory University School of Medicine, Atlanta, GA 30322; †Transgenics Section, National Institute on Aging, National Institutes of Health, Bethesda, MD 20892; bDepartment of Pharmacology, Emory University School of Medicine, Atlanta, GA 30322; and †Department of Neurology, Emory University School of Medicine, Atlanta, GA 30322

Edited by Reinhard Jahn, Max Planck Institute for Biophysical Chemistry, Göttingen, Germany, and approved January 30, 2017 (received for review October 11, 2016)
A new avenue of research because a single geneticist decided to include a couple of “environmental” factors into the analysis

When it comes to the exposome we do not need to measure everything. We need to provide a valid set of parameters for “E” that can be used in conjunction with existing studies that employ genomics, epigenomics, proteomics, etc. to study diseases and health outcomes.
Examples of exposomics for inhalation toxicology
Lung inflammation after H1NI infection

Chandler et al. AJPRICP, 2016
Figure 1. Conceptual diagram for lung regeneration. After accumulation of lifelong exposures and adaptive responses in disease development, a conditioning phase is needed to enable efficient regeneration of lung function.
Short-Term Exposures and Acute Biological Responses

Goal: Identify pathways of human metabolism that change with respect to traffic air pollution exposure in commuting settings

Hypothesis Driven

In-vehicle: Particulate Matter, Organic Carbons, Metals, Noise

Commuter Exposure

Targeted Biomarkers

Respiratory: Spirometry
Dried Blood Spots: IL-6, IL-8, IL-1β, hs-CRP, TNF-α, sICAM, sVCAM

High Resolution Metabolomics: Plasma profiling of +/- ions

Metabolome

Response

Prior Knowledge ↑ Few Targets Well-Characterized

Discovery 10^4-10^5 Targets Many Unknown

Courtesy of Dr. Chandresh Ladva and Dr. Jeremy Sarnat
Short-Term Exposures and Acute Biological Responses

Commuter Exposure

1. **Targeted Biomarkers**
   - **Respiratory**: Spirometry
   - **Dried Blood Spots**: IL-6, IL-8, IL-1β, hs-CRP, TNF-α, sICAM, sVCAM

2. **High Resolution Metabolomics**: Plasma profiling of +/- ions

**In-vehicle**: Particulate Matter, Organic Carbons, Metals, Noise

**Response**

**Metabolome**
Short-Term Exposures and Acute Biological Responses

Commuter Exposure → Targeted Biomarkers

Targeted Biomarkers → Metabolome

Metabolome → Response

Response

Biological Response:
Metabolic Pathways, Oxidative Stress, Systemic Inflammation
Particulate Metals Perturb Metabolome

• Null associations for many pollutant models

• Particulate Pb, Al, Fe associated with metabolic changes
  • Pb: 150 features differed
  • Components indicative of road dust

• First evidence of pollutant-mediated metabolic response in ACE panel

- Y-axis: Significance of pre-post metabolite intensities
  • \(-\log_{10}(p)\)
- X-axis: Retention time of individual ions (N = 14,000)

- Thick Dashed line: Bonferroni (\(\alpha < 0.05\))
- Colored Points: FDR < 0.05
  • Direction of average pre-post changes in metabolite intensities indicated by red (decrease) and blue (increase)
Human Metabolic Pathway Enrichment

Exposure Associated Metabolic Pathways

- Androgen and estrogen (3/72)
- Arachidonic acid (3/51)
- Cytochrome P450 (3/41)
- Glycerophospholipid (5/51)
- Gly, Ser, Ala and Thr (4/51)
- Leukotriene (3/53)
- Lysine (3/24)
- Methionine and cysteine (5/53)
- Selenoamino acid (3/23)
- Tryptophan (3/67)
- Tyrosine (3/98)
- Ubiquinone Biosynthesis (3/8)
- Vitamin B9 (folate) (3/19)
- Vitamin E (3/35)
- Metals (Pb, Fe, Al)

Biomarker Associated Metabolic Pathways

IL-6
IL-1β
IL-8
Metals (Pb, Fe, Al)

Enrichment Score

0 1 2 3 4
Figure 4. The cumulative lifelong perturbations in metabolic and cellular functions contribute to lung disease. The lungs are exposed to airborne particulates, environmental chemicals, and infectious agents. Within the lungs, the body burden of these environmental agents causes many types of molecular responses. Central panel reprinted by permission from Reference 85; right panel is from wikipedia.org for idiopathic pulmonary fibrosis (https://en.wikipedia.org/wiki/Idiopathic_pulmonary_fibrosis). ATSDR = Agency for Toxic Substances and Disease Registry.

Go and Jones, 2016
Are we entering the early phase of a paradigm shift? YES

The Exposome in the EU

The Exposome at Emory

Japan, other U.S. and European institutions

exponential expansion of data

1966

TS Kuhn’s Structure published

The Exposome a la Chris Wild

The Exposome Rappaport and Smith

Big data bioinformatics

2066

NAS Exposome Workshop
Submit your best inhalation toxicology to ToxSci!

EDITORIAL

Data Sharing in Toxicology: Beyond Show and Tell
Gary W. Miller

EDITORIAL

More than Manuscripts: Reproducibility, Rigor, and Research Productivity in the Big Data Era
Lance A. Waller and Gary W. Miller

EDITORIAL

Making Data Accessible: The Dryad Experience
Gary W. Miller

EDITORIAL

Young Investigators in Toxicology: Is There a Crisis?
Gary W. Miller

EDITORIAL

Preprints in Toxicology
Gary W. Miller