Biomonitoring of Human Exposure to Environmental Chemicals

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Exposome

2005 – Chris Wild – a cancer epidemiologist - first coined the term “exposome” to encompass “the totality of human environmental exposures, from conception onwards, complementing the genome”

Exposomics is the study of the exposome and relies, among other tools, on biomonitoring to assess internal exposure and its effects through the measurement of biomarkers.
Different domains constituting the Exposome

External environment

- Tobacco
- Consumer products
- Physical activity
- Diet
- Water
- Climate
- Green spaces
- Urban environment
- Traffic
- Social capital

Specific external environment

General external environment

Life course dimension

Internal environment

Transcriptomics
Proteomics
Metabolomics

Health risk and impact assessment

Health outcome

Tools for the assessment of the Exposome

Valérie Siroux, Lydiane Agier, Rémy Slama. The exposome concept: a challenge and a potential driver for environmental health research, European Respiratory Review Jun 2016, 25 (140) 124-129
Biomonitoring of exposures

Biomonitoring: “...direct measurement of people's exposure to toxic substances by measuring the substances or their metabolites in human specimens, such as blood or urine”.

“...are the most health-relevant assessments of exposure because they indicate the amount of the chemical that actually gets into people”*

*CDC www.cdc.gov/biomonitoring/ 2006
Benefits of Biomonitoring

• Types of chemicals and body burdens
• Track changes in chemical exposures over time
• Identify whether some groups of people (e.g., infants, anglers) are more exposed than others
• Determine demographic factors that affect exposures, to develop policies for mitigating exposures
• Make timely and appropriate public health decisions based on human exposure

Interdisciplinary science that involves chemists, biologists, epidemiologists, clinicians, and public health practitioners
Are we assessing exposures accurately?

External dose (ExD) (Environmental)

Internal dose (InD) (Biomonitoring)

\[ Dose = f'(\text{concentration in blood}) \]

Concentration in tissue = function of dose
Exposure assessment of phthalates

- **Plasticizers** (to soften plastics; primarily PVC). Phthalates make products flexible, durable, and cheap.

- **High Production Volume**

- **Six phthalates are regulated in toys and child care products** (DEHP, DBP, BBP, DINP, DIDP and D-n-OP)

- **Reproductive toxicants**

<table>
<thead>
<tr>
<th>Name</th>
<th>Acronym</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dimethyl phthalate</td>
<td>DMP</td>
</tr>
<tr>
<td>Diethyl phthalate</td>
<td>DEP</td>
</tr>
<tr>
<td>Di-n-butyl phthalate</td>
<td>DBP</td>
</tr>
<tr>
<td>Diisobutyl phthalate</td>
<td>DIBP</td>
</tr>
<tr>
<td>Di-n-hexyl phthalate</td>
<td>DNHP</td>
</tr>
<tr>
<td>Butyl benzyl phthalate</td>
<td>BzBP</td>
</tr>
<tr>
<td>Di(2-ethylhexyl) phthalate</td>
<td>DEHP</td>
</tr>
</tbody>
</table>
Phthalate applications

- Automotives
- Building & construction materials
- Flooring (DINP, DEHP, BBP, DIHP)
- Cosmetics (DBP, DEP, and DMP)
- Medical devices (DEHP)
- Toys and babycare products (temporary ban on the use of DEHP, DBP, BBP, DINP, DIDP and DNOP)

Phthahalate exposure: an example of metabolite analysis

Phthalate monoesters have a biologic half-life of approximately 12 h

Phthalate

<table>
<thead>
<tr>
<th>DMP</th>
<th>mMP</th>
</tr>
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<tbody>
<tr>
<td>DEP</td>
<td>mEP</td>
</tr>
<tr>
<td>DEHP</td>
<td>mEHP/mECHP/mCMHP/mEHHP/mEOHP</td>
</tr>
</tbody>
</table>

Human Urine, Breast Milk, Serum, and Saliva
Multinational survey of phthalate metabolites in urine

- Total concentrations, Asia: 2.6 to 19300 ng/mL (2006-2010)
- Median level in the USA: 240 ng/mL (2007-2008)

Median values are in red
Human exposure dose to phthalates

Urinary Concentration → Exposure Dose

\[ DI = CV \times \frac{M_1}{M_2} \times \frac{1}{f} \]

DI is the total daily intake of phthalates (μg/day);

C is the urinary phthalate metabolite concentration (μg/L);

V is human daily excretion volume of urine (L/day);

\( M_1 \) and \( M_2 \) are the respective molecular weights of parent phthalate and its metabolite (g/mol);

f is the molar fraction of the urinary monoester metabolite excreted in relation to the ingested amount of phthalate.
Estimated daily exposure dose to phthalates *(Median: µg/day)*

<table>
<thead>
<tr>
<th></th>
<th>Reference dose</th>
<th>China</th>
<th>India</th>
<th>Japan</th>
<th>Korea</th>
<th>Malaysia</th>
<th>Kuwait</th>
<th>Vietnam</th>
<th>USAd</th>
</tr>
</thead>
<tbody>
<tr>
<td>DEP</td>
<td>800a</td>
<td>285</td>
<td>1228</td>
<td>181</td>
<td>177</td>
<td>693</td>
<td>3900 (2)b</td>
<td>64.0</td>
<td>244</td>
</tr>
<tr>
<td>DBP</td>
<td>100</td>
<td>580 (2)</td>
<td>178</td>
<td>140</td>
<td>110</td>
<td>124</td>
<td>822 (5)</td>
<td>173</td>
<td>35-63 (37)</td>
</tr>
<tr>
<td>DEHPc</td>
<td>20</td>
<td>182 (2)</td>
<td>339 (7/22)</td>
<td>92.5</td>
<td>102</td>
<td>97.7 (1)</td>
<td>435 (21/46)</td>
<td>112</td>
<td>245-420</td>
</tr>
</tbody>
</table>

- **a** Reference doses of U.S. EPA (µg/kg bw/day);
- **b** Number of samples exceeded the estimated reference doses;
- **c** Average values calculated from urinary mEHHP and mEOHP.
- **d** US values from Christensen et al. 2014, Reg Toxcol Pharmacol 69, 380

**US Adult median exposure to phthalates: 500-600 µg/day**
Sources of phthalate exposure?

A Survey of Phthalates and Parabens in Personal Care Products from the United States and Its Implications for Human Exposure

Ying Guo and Kurunthachalam Kannan

*Environ. Sci. Technol. 2013, 47, 14442–14449

**Perfumes, deodorants, nail polish - 10s - 1000s of μg/g
Adult female exposure: 22 μg/day

Comparative Assessment of Human Exposure to Phthalate Esters from House Dust in China and the United States

Ying Guo† and Kurunthachalam Kannan‡,*,∥

Environ. Sci. Technol. 2011, 45, 3788–3794

†Concentrations in indoor air: 100s – 5000s ng/m³
‡Concentrations in indoor dust: 10s - 100s of μg/g
∥Adult female exposure: 16 μg/day

Phthalate Concentrations and Dietary Exposure from Food Purchased in New York State

Arnold Schecter, Matthew Lorber, Ying Guo, Qian Wu, Se Hun Yun, Kurunthachalam Kannan, Madeline Hommel, Nadia Imran, Linda S. Hynan, Yun Cheng, Justin A. Colacino, and Linda S. Birnbaum

Environmental Health Perspectives • Volume 121 | Number 4 | April 2013

Concentrations in food: few – 100 ng/g
Adult female exposure: 60 μg/day
Exposure doses: Environmental vs Biomonitoring

**Total 550 µg/d >** Known sources 22+16+60 = 98 µg/d

Only one-fifth of the exposure sources is accounted for.
Many unknown phthalate metabolites are present in urine

Secondary metabolites and adducts
Many unknown phthalate metabolites are present in urine

XIC of -MRM: 291.000/121.000: mEOHP

mEOHP

XIC of -MRM: 307.000/159.000: mECPP/mCMHP

mECPP/mCMHP

mEOHP

IS_mEOHP

mECPP

IS_mECPP

mCMHP

IS_mCMHP
Unsuspected sources of phthalate exposure

- Baby teethers
- Medical devices & Pharmaceuticals
  - Pads
  - Panty liners
  - Bactericidal creams and solutions
  - Deodorant sprays
- Plastic products
- Textiles, inner garments
Bisphenol A: BPA

- Used in the synthesis of polycarbonate plastics and epoxy resins (as protective inner can coating)
Chemical alternatives: BPS and BPA in urine from several countries

**BPS**
- USA (31)
- China (89)
- India (38)
- Japan (36)
- Korea (33)
- Kuwait (30)
- Malaysia (29)
- Vietnam (29)
- All (315)

**BPA**
- China (116)
- India (21)
- Japan (36)
- Korea (32)
- Kuwait (32)
- Malaysia (29)
- Vietnam (30)
- All (296)

**GM concentration (ng/mL; µg/g Cre)**
Unconventional sources: BPA and BPS in thermal receipt papers

**BPA**

This study

0 5 10 15 20

BPA concentration (mg/g)

Albany, NY, USA
Other cities, USA
Two cities, Japan
Incheon, Korea
Hanoi, Vietnam
Wilmington, MA, USA (28)
Zurich, Switzerland (29)

(73) (10) (6) (11) (3) (103) (10) (13)

**BPS**

0 5 10 15 20

BPA concentration (mg/g)

Albany, NY, USA
Other cities, USA
Two cities, Japan
Incheon, Korea
Hanoi, Vietnam
All
BPA in Paper Currencies

- **Brazil**: 36.1 µg/g
- **Czech**: 29.2 µg/g

BPA concentrations: 0.001 to 82.7 µg/g

Mean: 3.40 µg/g
Underestimation of exposures: Parabens – unknown metabolic pathways??

Broad spectrum antimicrobial agents in foods, cosmetics and pharmaceuticals
Protocatechuates – major metabolites of parabens in urine

Parabens (MeP+EtP) = 2% ; Alkyl protocatechuates = 3%; 3,4-DHB = 27%; 4-HB = 68% of total paraben concentrations
Urinary paraben concentrations in Asian countries, Greece and the USA

Total Parabens concentration

Urinary concentration (ng/mL)

China, India, Japan, Korea, Kuwait, Saudi Arabia, Vietnam, Greece, U.S.

227
Urinary chlorophenol levels in Asian countries, Greece and the USA
Urinary pesticide levels in Asian Countries, Greece and the USA

OPs, pyrethroids and phenoxy herbicides

Malathion and chlorpyrifos – major pesticides
Question: Does "shared environment" play a significant role in co-exposure patterns of the exposome?

Familial design of the LIFE study with extensive chemical exposure information - to answer this question.

13 classes of 128 chemicals in 501 couple urine and serum

Finding: Only 9% of the chemicals were significantly correlated between couples living in same homes

Fig. Exposome correlation globe showing the relationships of biomarkers between females, males and couples.
Appropriate sample size for biomonitoring in the Exposome

Exposome-wide association study of semen quality: Systematic discovery of endocrine disrupting chemical biomarkers in fertility require large sample sizes

Ming Kei Chunga, Germaine M. Buck Louisb,c, Kurunthachalam Kannanda, Chirag J. Patelb,a,*

**Question**: Exposome is dynamic and not stable (as genome). Need to consider several covariates (confounders) to design an exposome study.

**Objective**: EWAS study of semen quality and measured 128 EDCs belonging to 15 chemical classes in urine/serum of men.

**Conclusion**: EWAS research on male fertility requires a sample size of 1795-3625.

The average size of four major studies used a sample size of 201 men.
Biomonitoring of effect biomarkers

• Biomonitoring can be applied in the detection and quantification of disease markers – urine specimens have been used in the identification of markers of cardiovascular diseases

• Biomonitoring can be used in the assessment of health status of individuals – e.g., measurement of clinical markers including oxidative stress markers

• Oxidative stress (OS) is the leading cause of many diseases: OS arises from free radicals damaging endogenous molecules – DNA, proteins, lipids, uric acid, etc

• Various OS biomarkers (MDA, 8-OHdG, prostaglandins, allantoin) are not equally sensitive to the same stressor!
Oxidative stress

8-iso-Prostaglandin F$_{2\alpha}$ (8-PGF$_{2\alpha}$)
11β-Prostaglandin F$_{2\alpha}$ (11-PGF$_{2\alpha}$)
8-iso-15(R)-Prostaglandin F$_{2\alpha}$ (8,15-PGF$_{2\alpha}$)
15(R)-Prostaglandin F$_{2\alpha}$ (15-PGF$_{2\alpha}$)

PUFA

ROS

Arachidonic acid

Proteins

DNA

diTyrosine (diY)

8-hydroxy-2′-deoxyguanosine (8-OHdG)

Malondialdehyde (MDA)
2. Multiparameter method for the analysis of OSBs in urine

1. Optimization of the separation and detection by HPLC-MS

 dicks

**HPLC**: Optimize the separation to improve selectivity and sensitivity.

**Column**: Eclipse plus C18 3.5µm

**Mobile Phase**: A. MeOH:ACN 70:30 + 0.1% Acetic acid
B. H2O + 0.1% Acetic acid

**Gradient**: Flow: 0.4 ml min\(^{-1}\)

**Injection volume**: 20 µl
Daily variability in oxidative stress levels among 19 healthy individuals over 30 days
Urinary bisphenol A, phthalates, and couple fecundity: the Longitudinal Investigation of Fertility and the Environment (LIFE) Study

Phthalate exposure in males increases time to achieve pregnancy in women

Phthalates affect semen quality parameters; plasma oxidative enzymes

Elevated oxidative stress biomarkers in urine

Infertility / adverse reproductive health
Summary

➢ **Environmental chemical** exposure is an important contributor to a variety of **noninfectious diseases**. Exposomics can provide tools to study that.

➢ **Biomonitoring** studies permit discoveries of **novel chemical exposures** in human specimens.

➢ **Biomonitoring** offers **better ways of quantifying environmental** exposures.

➢ Use of appropriate **sample matrix and sample size** is important for meaningful interpretation of biomonitoring data in exposomics.

➢ **Biomonitoring of effect biomarkers** can delineate adverse outcome pathways in identifying etiology of diseases. [exposure-pathways-molecular effects-disease].
Acknowledgements

NICHD

NIEHS

CDC