In Quest of New Fingerprints of Exposure to VOCs from Consumer Products

Dr Juana Maria Delgado-Saborit
Division of Environmental Health and Risk Management
University of Birmingham, UK
INDEX

1. INTRODUCTION
2. HYPOTHESIS AND OBJECTIVES
3. METHODOLOGY
4. PROGRESS UPDATE & PRELIMINARY RESULTS
5. FUTURE STEPS & CONCLUSIONS
6. RESEARCH TEAM
INTRODUCTION
1. INTRODUCTION

Are VOC concentrations indoors relevant to human exposure?
1. INTRODUCTION

CONTRIBUTION OF INDOOR AIR TO PERSONAL EXPOSURES (PE)

INDOOR SOURCE OF VOCs
Consumer Products

TIME SPENT IN MICROENVIRONMENTS

Indoors __________ 80–93%
In Transit –vehicles ___ 1–7%
Outdoors __________ 2–7%

(Harrison et al. 2009, Brunekreef et al. 2005)

MICROENVIRONMENT
CONTRIBUTION TO PE

Indoors _____ 68% - 77%
In Transit _____ 30% - 6%
Outdoors _____ 2% - 3%

(Bruinen de Bruin et al. 2008, Harrison et al. 2009)
1. INTRODUCTION

Can we trace exposures to VOCs in the human body?
1. INTRODUCTION

PERSONAL EXPOSURES VS LUNG DOSE

PERSONAL EXPOSURES
Measured at the breathing area 2 – 5 µg/m³ in Europe

LUNG DOSE
Mass of pollutant inhaled in the lungs 100 (70 – 500) µg/day in Europe

Depends on:
• Concentration in the breathing area (PE)
• Duration of exposure
• Minute ventilation
  • Age
  • Gender
  • Fitness
  • Level of exertion
  • Presence of disease
1. INTRODUCTION

BIOMARKERS OF EXPOSURE

Exogenous substance within the system, the interactive product between xenobiotic compounds and endogenous components ... related to the exposure.
1. INTRODUCTION

BIOMARKERS OF EXPOSURE

HIGH EXPOSURES

LOW EXPOSURES

(Rappaport et al. 2010. Chem-Biol Interact: 189)

(Harrison et al. 2009. HEI)

1. INTRODUCTION

BIOMARKERS OF EXPOSURE

(Nerbert et al 2002; ATSDR 2007)
1. INTRODUCTION

BIOMARKERS OF EFFECT

Indicator of an endogenous component of the biological system, a measure of the functional capacity of the system, or an altered state of the system that is recognized as impairment or disease.

Xenobiotic (e.g. pollutants) metabolism might affect other metabolic processes...

... Metabolomics might help identify new biomarkers...

- Recognise changes in pattern of endogenous metabolites (natural occurring substances) after xenobiotic exposure
- Connects molecular events to those at the macro level
1. INTRODUCTION

- Source Emissions
- Human Exposure
- Health Effects
- Micro-Environment concentrations
- Biomarkers of Exposure
- PE exposure
- Lung Dose
- Biomarkers of Effect

Human Exposure
Biomarkers of Exposure
Biomarkers of Effect
1. INTRODUCTION

EXPOSURE-EFFECTS CONTINUUM

Source Emissions → Transport & Transformation → Human Exposure → Bio-availability → METABOLISM Accumulation Transformation Elimination → Early Expression of Disease → Altered Function → Clinical Disease

Micro-Environment concentrations

PE exposure

Lung Dose

VOC indoors

VOC PE

VOC inhaled

Biomarkers of Exposure

U-BZ

1,4-BQ

EXOGENOUS

Biomarkers of Effect

Metabolic Profiles

ENDOGENOUS

MARKERS
HYPOTHESIS AND OBJECTIVES
2. HYPOTHESIS AND OBJECTIVES

HYPOTHESIS: EXPOSURE-EFFECTS CONTINUUM

- The general population is exposed to different VOC emitted from consumer products and building materials.

- Doses of inhaled VOCs are metabolised producing biomarkers of exposure (exogenous) and effect (endogenous), that can be detected from urine samples.
### OBJECTIVES

- Characterise VOC concentrations in indoor microenvironments relevant to personal exposures (home and workplace).
- Characterise personal exposures to a common range of chemicals used in consumer products and building materials.
- Model inhalation doses and personal exposures using microenvironment concentrations and subject information.
- Characterise biomarkers of exposure to low-level VOC concentrations.
- Discover biomarkers of effect using metabolomics.
METHODOLOGY
3. METHODOLOGY

WP1: Subject Recruitment

WP2: Personal Exposure Sampling

WP2: MicroEnvironment Sampling

WP2: PE & ME data collection

WP3: PE & ME VOC analysis

WP4: Lung Dose

WP5: VOC urine analysis

WP5: Quinone urine analysis

WP5: Metabonomic analysis

WP6: Biomarker of Exposure

WP6: Biomarker of Effect

WP7: Data Analysis

WP8: Dissemination
PROGRESS UPDATE
4. PROGRESS UPDATE

WP1: Subject Recruitment

WP 2: Personal Exposure Sampling

WP2: MicroEnvironment Sampling

WP 2: Personal Exposure Sampling

WP 2: PE & ME data collection

WP5: Urine Sampling

WP 3: PE & ME VOC analysis

WP 4: Lung Dose

WP 5: VOC urine analysis

WP 5: Quinone urine analysis

WP 5: Metabolomic analysis

WP 7: Data Analysis

Biomarker of Exposure

Biomarker of Effect

WP 8: Dissemination
## 4. PROGRESS UPDATE

### SUBJECT RECRUITMENT

<table>
<thead>
<tr>
<th>GROUP 1 (N=15)</th>
<th>Occupationally Exposed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>• Workers from steel foundries, oil storage depots, petrol station attendants, motor mechanics, bus drivers.</td>
</tr>
<tr>
<td></td>
<td>• “Birmingham is the city of all trades”</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>GROUP 2 (N=15)</th>
<th>Medium – VOC exposed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>• Subjects living / working in new or redecorated / refurbished buildings.</td>
</tr>
<tr>
<td></td>
<td>• New Hospital, Muirhead tower</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>GROUP 3 (N=15)</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>• No occupationally exposed subjects, nor living/working in new or redecorated buildings.</td>
</tr>
<tr>
<td></td>
<td>• Big database of subjects from previous studies</td>
</tr>
</tbody>
</table>
4. PROGRESS UPDATE

SAMPLING STRATEGY
- Group 1 & 3. Sampled once
- Group 2. Sampled twice:
  - 1st: when building new/redecorated
  - 2nd: One year later

SAMPLING EQUIPMENT
- 24-hour personal sample
- Samples collected
  - VOC in sorbent tubes
  - PM2.5 in Teflon filters
  - PAH, quinones
  - BC real time - microAethalometer
- Urine collected the day after

• Concurrent Measurement
  - PERSONAL EXPOSURE
  - HOME
  - WORK
  - URINE COLLECTION
4. PROGRESS UPDATE

RECRUITMENT AND SAMPLING

GROUP 1 \((N=15)\)
Occupationally Exposed

GROUP 2 \((N=15)\)
Medium – VOC exposed

GROUP 3 \((N=15)\)
Control

Percentage of Target (%)

G1 Occupational Exposed

G2 New Buildings

G3 Control Group

Sampled  Recruited
4. PROGRESS UPDATE

RECRUITMENT AND SAMPLING

Recruitment Steps

• PETROCHEMICAL INDUSTRY
  • Discussions with a UK petrochemical company
    • Failed
  • Contact UK Petroleum Industry Association
    • Failed
  • High Concentration measurements
    • Subjects living close to Jeddah Oil refinery
    • 15 subjects living close to the refinery
    • 15 subjects living far from the refinery

• OTHER INDUSTRIES
  • Arizona State University – UoB pilot campaign
    • Test Suitability of Gardeners, Mechanics & Decorators/Painters

GROUP 1 \( (N=15) \)
Occupationally Exposed
4. PROGRESS UPDATE

RECRUITMENT AND SAMPLING

Arizona State University / UoB pilot campaign – Occupational Testing

• Goal:
  – To test various occupational exposures

• Equipment:
  – Hybrid Device – ASU design
  – Shoulder bag

• Procedure:
  – Device is given to user to sample their occupational exposure for approximately 15 minutes
  – Device is taken back by the researchers and set down for approximately 45 minutes as the device runs

The device was fixed in a bag so the user could easily carry the device as they worked.
4. PROGRESS UPDATE

RECRUITMENT AND SAMPLING

Arizona State University / UoB pilot campaign – Occupational Testing

• Three different occupational exposures were considered:
  – Gardening
  – Garage/mechanics
  – Painting
4. PROGRESS UPDATE

RECRUITMENT AND SAMPLING

Arizona State University / UoB pilot campaign – Occupational Testing


G1 Target - Mechanics and Gardeners
4. PROGRESS UPDATE

WP1: Subject Recruitment

WP2: Personal Exposure Sampling

WP2: MicroEnvironment Sampling

WP2: PE & ME data collection

WP3: PE & ME VOC analysis

QA/QC

PE concentration

ME concentration

WP 4: Lung Dose

WP 7: Data Analysis

WP 5: Urine Sampling

WP5: VOC urine analysis

WP5: Quinone urine analysis

WP5: Metabolomic analysis

WP 3: PE & ME analysis

WP 1: Subject Collection

WP 2: Personal Exposure Sampling

WP 2: MicroEnvironment Sampling

WP 2: PE & ME data collection

WP 3: PE & ME VOC analysis

QA/QC

PE concentration

ME concentration

WP 4: Lung Dose

WP 7: Data Analysis

WP 5: Urine Sampling

WP5: VOC urine analysis

WP5: Quinone urine analysis

WP5: Metabolomic analysis

Biomarker of Exposure

Biomarker of Effect

WP 8: Dissemination
4. PROGRESS UPDATE

PE AND ME ANALYSIS

Measurement in recently renovated offices

- Offices:
  - Office conversion
  - New walls & door fitted
  - Walls Painted

- Methodology:
  - Sorbent tubes with pump
    - 24-h sampling
    - 14 days sampling
  - Aeroqual TVOC sensor
    - Continuous measurement
    - 14 days sampling

TVOC (mg/m³)

TVOC (mg/m³)
Subject in a urban route 9-13 hr
4. PROGRESS UPDATE

WP1: Subject Recruitment

WP2: Personal Exposure Sampling

WP2: MicroEnvironment Sampling

WP2: PE & ME data collection

WP5: Urine Sampling

WP 7: Data Analysis

WP 8: Dissemination

QA/QC

PE concentration

ME concentration

WP 4: Lung Dose

WP 5: VOC urine analysis

WP5: Quinone urine analysis

WP5: Metabolomic analysis

Biomarker of Exposure

Biomarker of Effect
4. PROGRESS UPDATE

QUINONE URINE ANALYSIS

Quinone Extraction

- Benzoquinone rearranges in hydroquinone under mild conditions

- Several extraction methods tried:
  - liquid-liquid extraction
  - C-18 SPE
  - Non polar divinylbenzene SPE

- Every methodology has been tried in several replicates and under different conditions

- Best results and reproducibility using Non polar divinylbenzene SPE with acid loading
4. PROGRESS UPDATE

QUINONE URINE ANALYSIS
Quinone Extraction SPE

• 0.3ml of urine are diluted in 0.9ml of 1% formic acid
• Internal Standards are spiked
• The SPE columns are preconditioned with Methanol and equilibrated with water
• The sample is loaded on the column and washed with 5% methanol in water
• The elution is performed with 100% methanol

• The results showed no trace of BQ in the elute:
  • Possibility BQ $\rightarrow$ HQ under acidic conditions.
4. PROGRESS UPDATE

QUINONE URINE ANALYSIS

Quinone Extraction

- Hydroquinone Response urine matrix SPE

![Graph showing hydroquinone response ratio vs. natural STD concentration]

- Response ratio (HQ / HQD4)
- HQ Natural STD Concentration (pg/uL)

- Equation: $y = 1E-04x$
- $R^2 = 0.9521$
4. PROGRESS UPDATE

QUINONE URINE ANALYSIS
Extractions from Real Urine

Hydroquinone in urine

<table>
<thead>
<tr>
<th>Concentration (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>2.5</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>1.5</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>0.5</td>
</tr>
<tr>
<td>0</td>
</tr>
</tbody>
</table>

Non Exposed
Non Exposed
Exposed

This Study
Kim, S. et al. (2006) Carcinogenesis
27(4): 772-781
4. PROGRESS UPDATE

QUINONE URINE ANALYSIS

Extractions from Real Urine

- Poor chromatography results
- Peaks broaden very fast after few injections
- Total ion chromatography & library search have identified:
  - Overlap of HQ peak with benzamide peak (derivative from benzoic acid)
  - Methyl benzoate – derivative (methyl esterification) of benzoic acid in presence of methanol and acid.
  - Mequinol – Monomethyl ether of HQ

Toluene

Hippuric Acid

Benzoic Acid
4. PROGRESS UPDATE

QUINONE URINE ANALYSIS

Extractions from Real Urine

\[ \text{HQ} + \text{BQ} \xrightarrow{\text{Methanol, acid}} \text{4-MP} \]

BQ= benzoquinone; HQ= hydroquinone; 4-MP= 4-methoxyphenol (Mequinol)

JAYANT R. INDURKAR (2008)
FUTURE STEPS
5. FUTURE STEPS

SAMPLING

GROUP 1  • RECRUITMENT & SAMPLING

GROUP 2  • 12-MONTHS RESAMPLING

ANALYSIS - ENVIRONMENTAL SAMPLES  • CONTINUE

ANALYSIS – URINARY BIOMARKERS

BQ-HQ ANALYSIS
• Derivatize HQ to separate peak from benzamide
• Assess contribution of Mequinol in the mass balance of BQ-HQ

U-BZ HS-GCMS ANALYSIS

METABOLOMICS ASSESSMENT
• NMR analysis Viant et al (2007) Metabolomics
6. CONCLUSIONS

- Good progress in recruiting and sampling subjects in Groups 2 (recent redecoration) and 3 (control).

- Difficulties in recruiting subjects occupationally exposed (Group 1) – Neighbours of Oil Refinery as alternative.

- Sampling in recently renovated offices have shown increased VOC concentrations indoors.

- Progress in method development of BQ-HQ method, but new difficulties have arisen (e.g. Toluene interference, Mequinol conversion). Suggestions to address these are in place.

- Next work involves:
  - Analysis of unmetabolised VOCs
  - Metabolomics analysis of urine samples.
RESEARCH TEAM

ADVISORY BOARD

Dr. Peyton Jacob III (UCSF)
Researcher specialist in urinary biomarker analysis

Prof Roy Harrison (UoB)
Head of Division Env Health & Risk Manag.
UK NERC Env. Poll & Human Health Theme Leader

Dr. Mark Viant (UoB)
Director of UK NERC Biomolecular Analysis Facilities Metabolomics

RESEARCH TEAM

Dr. Juana Mari Delgado-Saborit
Research Fellow
Research Coordinator

Mr. Massimiliano Mascelloni
PhD student – 2010 LRI Award funded Urine Analysis and Method Development

Mrs. Barbara Macias Hernandez
PhD student – Mexican Government funded Sampling and Environmental Analysis
Thanks for your attention

Thanks to CEFIC for the 2010 LRI Innovative Science Award

http://www.gees.bham.ac.uk/research/projects/airtoxics/index.shtml