Using Biomarkers to Characterize Human Benzene Metabolism

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University of North Carolina, Chapel Hill
University of California, Berkeley

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Benzene

- **Human carcinogen**
  - First linked with bone marrow toxicity in 1896 (Santesson, C. Arch Hyg Berl 31: 337) and with leukemia in 1928 (Delore, P. and Borgomano, C. J Med Lyon 9: 227)
  - Dose-response poorly defined
  - Uncertain risks, particularly at low exposures

- **Mechanism not completely understood**
  - Linked to metabolism

- **Metabolism is complex**
  - Qualitatively similar in all mammals
  - Important quantitative differences among species
  - Can be affected by genetics (SNPs)

- **Dose-related metabolism poorly characterized in humans**
**Benzene Metabolism**

- Benzene
- Benzene Oxide
- Oxepin
- E,E-Muconaldehyde
- E,E-Muconic Acid
- S-phenylmercapturic acid
- Phenol
- Hydroquinone
- E,E-Muconaldehyde
- 1,2,4-trihydroxybenzene
- 1,4-Benzoquinone
- 1,2-Benzoquinone
Benzene Biomarkers Among Chinese Workers

STUDY #1 (NCI, UC-Berkeley, UNC)

44 Exposed subjects and 44 controls in Shanghai, China

- Very high exposure (med. = 31 ppm)
- Focus on hematology and cytogenetics
- Developed and validated biomarkers of exposure (protein adducts, urinary benzene, and urinary metabolites)
- Evidence of saturable metabolism

Rothman et al., PNAS, 1995
Rothman et al., AJIM, 1996
Rothman et al., EHP 1996
Rothman et al., Cancer Res, 1997
Rothman et al., OEM, 1998
Smith et al., Cancer Res, 1998
Smith et al., PNAS, 2000
Yeowell-O Connell et al., Carcinogenesis, 1998
Yeowell-O Connell et al., CEBP, 2001
Waidyanatha et al., Carcinogenesis, 2001
Waidyanatha et al., Analyt Biochem, 2004
Rappaport et al., J Chromatog B, 2002
Benzene Biomarkers Among Chinese Workers

STUDY #2

134 Exposed subjects and 51 controls in Tianjin, China

Lower exposure (med. = 3.1 ppm)

• Focus upon hematology and biomarkers of exposure

Qu et al., AJIM, 2000
Qu et al., AJIM, 2002
Melikian et al., J Chromatog (B), 2002
Rappaport et al., Cancer Res, 2002
Saturation of benzene metabolism beginning at about 1 ppm (much lower than previously suggested)

Greater unit risk at low exposure levels

Motivated EPA to reduce benzene content in gasoline (2007)

CAN THIS FINDING BE VERIFIED?

Benzene Biomarkers Among Chinese Workers

STUDY #3 (NCI, UC-Berkeley, UNC)

250 Exposed subjects and 140 controls in Tianjin, China

- Similar exposures (med. = 3.7 ppm)
- Focus on exposure–biomarker relationships and mechanism

Vermeulen et al., Ann Occup Hyg, 2004
Lan et al., Science, 2004
Lan et al., Zhang et al., Chem-Biol Interact, 2005
Lan et al., Cancer Res, 2005
Vermeulen et al., PNAS, 2005
Chen et al., Carcinogenesis, 2006
Kim et al., Carcinogenesis, 2006
Kim et al., CEBP, 2006
Kim et al., PGEN, in press
Air Samples (n=2783) and Urinary Analytes (n=620)

Air; Unmetabolized benzene in urine

Minor metabolite

Major metabolites (99% of absorbed dose)
Urinary Benzene vs. Exposure

Urinary benzene detected in all exposed AND control subjects – Used to predict benzene exposures in controls

Kim et al., Carcinogenesis, 27:772 (2006)
Metabolites vs. Exposure (Groups of 30 Workers)

Urinary metabolites not useful for monitoring environmental exposures to benzene due to background sources (other than benzene).

Kim et al., Carcinogenesis, 27:772 (2006)
Groups \((n = 30)\) after adjustment for background levels

- Reduced production of major & total metabolites at low exposures \((0.01 - 1 \text{ ppm, never reported previously})\)

*Kim et al., Carcinogenesis, 27:772 (2006)*
Modeling Metabolite Levels (Natural Splines)

Background levels

Kim et al., CEBP, 2006
# Effects of Covariates

**[GLM+NS]**

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Adj.R²</th>
<th>Covariate</th>
<th>Estimate</th>
<th>p-value</th>
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<td>Smoking</td>
<td>0.338</td>
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- **Age:** metabolite production reduced 1-2%/year of life
- **Sex:** females produce more metabolites than males
- **Smoking:** Cigarette smoke contains HQ and CA

No effect of:
- Alcohol
- Toluene co-exposure

*Kim et al., CEBP, 2006*
Dose-Related Metabolism of Benzene

Predicted levels of total metabolites (background adjusted, female subject, 29 y of age)

9-fold reduction in slope
Effects of Metabolism Genes

- Benzene
- Oxepin
- S-phenylmercapturic acid
- Phenol
- Hydroquinone
- 1,2,4-trihydroxybenzene
- 1,4-Benzoquinone
- 1,2-Benzoquinone
- Hydroquinone
- Catechol
- 1,2,4-trihydroxybenzene

CYPs:
- CYP2E1
- Epoxide Hydrolase
- Nonezymatic Rearrangement

Conjugation via GST:
- S-phenylmercapturic acid

Other metabolites:
- E,E-Muconaldehyde
- E,E-Muconic Acid
Effects of Metabolism Genes

- Benzene
  - Oxepin (conjugate via GST)
  - S-phenylmercapturic acid

- CYP2E1
  - Benzene Oxide
  - E,E-Muconaldehyde
  - E,E-Muconic Acid

- GSTs
  - Conjugate

- EPHX
  - Epoxide Hydrolase
  - Benzoquinone
  - Dihydrodiol dehydrogenase

- Phenol
- Benzene dihydrodiol
- Benzene Diol Epoxide

- Hydroquinone
- 1,4-Benzoquinone

- 1,2,4-trihydroxybenzene
Effects of Metabolism Genes

S-phenylmercapturic acid

Benzene Oxide → Oxepin via CYP2E1

Epoxide Hydrolase

Phenol → Benzene dihydrodiol

CYP2E1

Nonezymatic Rearrangement

Hydroquinone → 1,4-Benzoquinone

CYP2E1

CYP

NQ01 & MPO

1,2,4-trihydroxybenzene

1,4-Benzoquinone

1,2-Benzquinone

Benzene Diol Epoxide

E,E-Muconaldehyde → E,E-Muconic Acid

CYP2E1

dihyrodiol dehydrogenase

Catechol
# Magnitudes of Genetic Effects and Gene-Environment Interactions

**SNP EFFECTS ON BENZENE METABOLITES**

Ratio of (Var:Var)/(Wild:Wild)

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<th>Air concentration (ppm)</th>
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<td>NQO1*2</td>
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Several significant effects, most in expected directions

Most effects are small (< 3-fold)

*Kim et al., PGEN, in press*
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*Most effects vary with conc. due to saturable metabolism and gene-environment interactions*
**Magnitudes of Genetic Effects and Gene-Environment Interactions**

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</table>

Evidence of gene-smoking interactions

*Kim et al., PGEN, in press*
Conclusions

Benzene is metabolized more efficiently at air concentrations below 1 ppm (about 9-fold for total metabolites)

- Observed in two independent Chinese studies using two different biomarkers of exposure (protein adducts and urinary metabolites)

Risk assessments based upon linear models may underestimate risks at low exposure levels

Metabolism varies with age and differs between males and females

Metabolizing genes and gene-environment interactions have detectable but small effects on metabolite production
Conclusions

Biomarkers can be used in observational studies to elucidate effects of metabolism and other phenomena of interest

IF exposures are carefully measured in the same subjects
With Thanks To:

- UNC
  - Sungkyoon Kim
  - Suramya Waidyanatha
  - Karen Yeowell-O’Connell
  - Brent Johnson
- NCI
  - Nat Rothman
  - Roel Vermeulen
  - Qing Lan
  - Stephen Chanock
- NYU
  - Qinshan Qu
  - Roy Shore
  - Beverley Cohen
- UC-Berkeley
  - Martyn Smith
  - Luoping Zhang
- IOM (Beijing)
  - Guilan Li
  - Songnian Yin

Work supported by NIEHS and HEI