Toxicant and Carcinogen Metabolite Biomarkers: Applications in Studies of Tobacco Use and Wok Cooking

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Overall Goal

Elucidate mechanisms of tobacco-induced cancer and apply this knowledge to cancer prevention.
Approaches

- Identify carcinogens and toxicants in tobacco products and the human environment.
- Determine pathways of carcinogen and toxicant metabolism and DNA damage.
- Using mass spectrometry, develop biomarkers to quantify carcinogen and toxicant uptake in humans.
  - Metabolites in urine or blood
  - DNA or protein addition products
- Use these biomarkers to identify groups and individuals susceptible to cancer.
# Environmental Carcinogen and Toxicant Urinary Biomarkers

## Carcinogen or Toxicant
In environment and tobacco smoke
- Polycyclic aromatic hydrocarbons (PAH)
- Volatiles such as acrolein, crotonaldehyde, ethylene oxide, propylene oxide, butadiene, benzene

Tobacco-specific
- Tobacco-specific nitrosamines
- Nicotine

## Urinary Metabolite Biomarker
Found in all human urine samples
- 1-hydroxypyrene (1-HOP) or phenanthrene tetraol (PheT)
- Mercapturic acids: 2- and 3-HPMA, HMPMA, HEMA, MHBMA, SPMA

Found only in tobacco users or people exposed to tobacco smoke
- NNAL, NNN
- Cotinine (also from NRT)
# Environmental Carcinogen and Toxicant Urinary Biomarkers

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  - Tobacco-specific nitrosamines
  - Nicotine

## Main Effects

- **Carcinogens:** respiratory tract and other tissues
- **Acrolein and crotonaldehyde** - highly toxic irritants; ethylene oxide, propylene oxide, butadiene, benzene - established carcinogens

- **NNK, NNN** – powerful carcinogens to lung, oral cavity, pancreas, esophagus
- **Nicotine** - addictive
A Panel of Urinary Tobacco Carcinogen and Toxicant Biomarkers

<table>
<thead>
<tr>
<th>Urinary biomarkers</th>
<th>Source</th>
<th>Smokers</th>
<th>Non-smokers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total NNAL</td>
<td>NNK</td>
<td>1.1 - 2.9</td>
<td>Not Detected</td>
</tr>
<tr>
<td>Total NNN</td>
<td>NNN</td>
<td>0.049 - 0.24</td>
<td>Not Detected</td>
</tr>
<tr>
<td>Phenanthrene tetraol or 1-HOP</td>
<td>PAH</td>
<td>0.50 - 1.45</td>
<td>0.18 - 0.50</td>
</tr>
<tr>
<td>MHBMA</td>
<td>1,3-Butadiene</td>
<td>15.5 - 322</td>
<td>0.65 - 7.5</td>
</tr>
<tr>
<td>SPMA</td>
<td>Benzene</td>
<td>3.2 - 32.1</td>
<td>0.17 - 3.14</td>
</tr>
<tr>
<td>3-HPMA</td>
<td>Acrolein</td>
<td>5,869 - 11,190</td>
<td>1,131 - 1,847</td>
</tr>
<tr>
<td>HMPMA</td>
<td>Crotonaldehyde</td>
<td>9,825 - 26,000</td>
<td>242 - 3,200</td>
</tr>
<tr>
<td>HEMA</td>
<td>Ethylene oxide</td>
<td>19.1 - 102</td>
<td>6.51 - 38.8</td>
</tr>
<tr>
<td>Cd</td>
<td>Cadmium</td>
<td>2.3 - 12.8</td>
<td>1.34 - 8.04</td>
</tr>
<tr>
<td>8-epi-PGF$_{2\alpha}$</td>
<td>Oxidative damage</td>
<td>1.48 - 2.80</td>
<td>0.62 - 1.13</td>
</tr>
<tr>
<td>Nicotine equivalents</td>
<td>Nicotine</td>
<td>70.4-154 µmol/24 h</td>
<td>Not Detected</td>
</tr>
</tbody>
</table>

Persistence of Biomarkers Study

- 17 smokers provided baseline 24h urine samples.
- Eight days later, they quit smoking and received nicotine replacement therapy.
- They provided 24h urine samples on days 3, 7, 14, 21, 28, 42, and 56 after quitting.
- Urine samples were analyzed by LC-MS/MS for panel biomarkers.

S.G. Carmella, M. Chen, S. Han, A. Briggs, J. Jensen, D. K. Hatsukami, and S. S. Hecht
Mean Urinary 3-HPMA (Acrolein) Reduction on Smoking Cessation, N=17
Mean Urinary HMPMA (Crotonaldehyde) Reduction on Smoking Cessation

N=17
Mean Urinary SPMA (Benzene) Reduction on Smoking Cessation, N=17

% REDUCTION FROM BASELINE SMOKING

DAYS POST CELSSATION
Mean Urinary HEMA (Ethylene Oxide) Reduction on Smoking Cessation

N=17
Mean Urinary MHBMA (Butadiene) Reduction on Smoking Cessation, N=17
Mean Urinary Total NNAL (NNK) Reduction on Smoking Cessation, N=17
Mean Urinary 1-HOP (PAH) Reduction on Smoking Cessation, N=15
Conclusions of the Persistence of Biomarkers Study

- All biomarkers decreased significantly upon smoking cessation (P<0.001)
  - Most rapid for mercapturic acids; 80% decrease
  - Gradual for NNAL
  - 1-HOP, 50% decrease and more variable

- Consistent with levels of the parent compounds in tobacco smoke and differences in biomarker levels between smokers and non-smokers

- Demonstrates that these urinary metabolites are biomarkers of exposure
Biomarkers of Cancer Risk?

Addressed with respect to the following biomarkers:

– Total NNAL (NNK) and total NNN (NNN)
– PheT (PAH)
– Total cotinine (nicotine)

– MHBMA (butadiene)
– HEMA (ethylene oxide)
– SPMA (benzene)
– 3-HPMA (acrolein)
– HMPMA (crotonaldehyde)
Shanghai Cohort Study

• Enrolled 18,244 men aged 45-64 in 1986-89
• In-person interview for smoking and other lifestyle factors
• Baseline blood and urine samples collected
• Incident lung cancer cases identified through follow-up interviews and record linkage
• 475 lung cancer cases and 475 matched controls, all smokers, in this study
• Evaluated total NNAL, PheT, total cotinine, and mercapturic acids with respect to lung cancer
# Biomarker – Lung Cancer Relationships, Shanghai Cohort Study

Adjusted for Smoking Duration and Intensity and the Other Biomarkers

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Adjusted OR</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total NNAL</td>
<td>1.93</td>
<td>1.28-2.90</td>
<td>0.001</td>
</tr>
<tr>
<td>PheT</td>
<td>2.34</td>
<td>1.33-4.11</td>
<td>0.023</td>
</tr>
<tr>
<td>Total Cotinine (S. Murphy lab)</td>
<td>3.52</td>
<td>2.30-5.41</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Urinary Mercapturic Acids and Lung Cancer Risk in the Shanghai Study (N = 735)
Adjusted for Smoking Duration and Intensity AND Total Cotinine

• After adjustment for cotinine, none of the mercapturic acids was significantly related to lung cancer.

• Interpretation: Total cotinine is an effective surrogate for the volatiles because they are weakly or non-carcinogenic to the lung.

• In contrast, NNK and PAH, represented by total NNAL and PheT, are powerful pulmonary carcinogens.

Urinary Total NNN and Esophageal Cancer Risk in the Shanghai Study (N = 77 cases and 223 matched controls)
Adjusted for Smoking Duration and Intensity, Total Cotinine, and Total NNAL

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Adjusted OR</th>
<th>95% C.I.</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total NNN</td>
<td>17.0</td>
<td>3.99 – 72.8</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Total NNAL and total cotinine were not significantly associated with esophageal cancer risk

Summary of the Tobacco Smoke Studies

• A panel of quantitative biomarkers is available to assess exposure to toxicants and carcinogens in tobacco products.
• All toxicant and carcinogen levels are significantly elevated in cigarette smokers.
• Tobacco-specific biomarkers can identify exposures of non-smokers to cigarette smoke or other tobacco products.
• Certain biomarkers in this panel – cotinine, NNAL, NNN, and PheT - are related to risk of lung or esophageal cancer, based on prospective epidemiologic studies.
Background

- Lung cancer, particularly adenocarcinoma, is common among non-smoking women in Singapore and some other parts of Asia.
- Not due to smoking or secondhand smoke exposure
- Different from the use of solid fuels in poorly ventilated areas, as in some parts of China
- Multiple epidemiologic studies find an association between high temperature wok cooking and lung cancer.
- IARC conclusion: emissions from high temperature frying are “probably carcinogenic to humans” (Group 2A).

Biomarker Studies in Chinese Women Who Regularly Cook at Home Using a Wok

Preliminary Study

• Compared urinary biomarker levels in 54 non-smoking women of Chinese ethnicity from Singapore who did not smoke or drink, with 50 women randomly chosen from the non-drinking, non-smoking participants in the Singapore Chinese Health Study.

• Found elevated levels of mercapturic acids of acrolein, crotonaldehyde, and benzene. No elevation of mercapturic acid of butadiene or PAH biomarkers 1-HOP and PheT

• Increases in mercapturic acid levels not nearly as great as in smokers

_Cancer Epidemiol, Biomarkers, and Prev._ **19**: 1185-1192 (2010)
Biomarker Studies in Chinese Women Who Regularly Cook at Home Using a Wok

Enrolled 328 non-smoking, non-drinking women from Singapore, ages 45-74, who did various levels of wok cooking:
   - less than once per week
   - 2 – 6 times per week
   - 7 or more times per week

Subjects filled out a questionnaire and provided a spot urine sample.

Urine was analyzed for 3-HPMA, HMPMA, and SPMA from acrolein, crotonaldehyde, and benzene.
Significant Relationships between Frequency of Wok Cooking and Acrolein and Crotonaldehyde Biomarkers

<table>
<thead>
<tr>
<th>Cooking related variables</th>
<th>N (%)</th>
<th>Geometric means (95%CI) of mercapturic acids (pmol/mg creatinine)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>SPMA 3-HPMA HMPMA</td>
</tr>
<tr>
<td>Cooking frequency, times/week</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 1</td>
<td>90 (27.4)</td>
<td>0.52 (0.40-0.66)</td>
</tr>
<tr>
<td>2-6</td>
<td>143 (43.6)</td>
<td>0.46 (0.40-0.54)</td>
</tr>
<tr>
<td>7+</td>
<td>95 (29.0)</td>
<td>0.52 (0.44-0.64)</td>
</tr>
<tr>
<td>P for trend</td>
<td>0.652</td>
<td>0.018</td>
</tr>
</tbody>
</table>
Summary of Wok Studies

- Consistent elevation of biomarkers of acrolein and crotonaldehyde exposure in non-smoking women who do regular wok cooking.
- Consistent with emissions of these toxicants from heated cooking oil.
- Although neither is a strong carcinogen, both are powerful and intense respiratory irritants for which there is evidence for involvement in lung cancer etiology:
  - Acrolein causes DNA damage in the p53 gene similar to that seen in lung cancer in smokers.
  - Acrolein and crotonaldehyde cause irritation, inflammation, cell proliferation, squamous metaplasia, interference with pulmonary function, immunosuppression, and other effects upon inhalation.
Overall Summary

• A panel of carcinogen and toxicant biomarkers is now available:
  – Quantified by high throughput mass spectrometric methods with high accuracy and precision
  – Can be applied in large studies (MEC, $N = 2200$)
  – Can specifically distinguish tobacco product exposures from those due to other factors
  – Can identify toxicant and carcinogen exposures from cooking
• Application of biomarkers in field studies can provide reliable and objective exposure data.
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Bob Carlson, editorial support
Metabolism of 1,3-Butadiene to Mercapturic Acids


a. GSH, GSTs; b. $\gamma$-glutamyltranspeptidase; c. cysteinyglycine dipeptidase; d. cysteine S-conjugate N-acetyltransferase
Metabolism of 1,3-Butadiene to Mercapturic Acids


1,3-butadiene → P450s → [O] → a-d → HO-S-\(\text{NHAc}\)COOH + AcNH-S-S-OH

\(\text{MFHMA}\)

1,3-butadiene → HO-S-\(\text{NHAc}\)

ADH → HO-CO

a-d → HO-S-\(\text{NHAc}\)

CR → \(\text{DHBMA}\)

a. GSH, GSTs; b. \(\gamma\)-glutamyltranspeptidase; c. cysteinylglycine dipeptidase; d. cysteine S-conjugate N-acetyltransferase
Mean Urinary DHBMA Reduction Upon Smoking Cessation, N=17
Structures of Urinary Biomarkers

Structures of Urinary Biomarkers

\[
\text{MHBMA (from 1,3-butadiene)}
\]

\[
\text{DHBMA (from 1,3-butadiene)}
\]

\[
\text{HPMA (from acrolein)}
\]

\[
\text{HBMA (from crotonaldehyde)}
\]

\[
\text{HEMA (from ethylene oxide)}
\]

\[
\text{SPMA (from benzene)}
\]

Metabolism of Acrolein, Crotonaldehyde, Benzene, and Ethylene Oxide to Mercapturic Acids

- Acrolein:  \( \text{acrolein} \) \( \rightarrow \) \( \text{AcNH} - \text{S} \rightarrow \text{AcNH} \)
- Crotonaldehyde:  \( \text{H}_3\text{C} - \text{acrolein} \) \( \rightarrow \) \( \text{AcNH} - \text{S} \rightarrow \text{AcNH} \)
- Benzene:  \( \text{P450s} \) \( \rightarrow \) \( \text{AcNH} - \text{S} \rightarrow \text{AcNH} \)
- Ethylene oxide:  \( \text{ethylene oxide} \) \( \rightarrow \) \( \text{AcNH} - \text{S} \rightarrow \text{AcNH} \)

- HPMA
- HBMA
- SPMA
- HEMA

a. GSH, GSTs; b. \( \gamma \)-glutamyltranspeptidase; c. cysteinylglycine dipeptidase; d. cysteine S-conjugate N-acetyltransferase