Perchlorate and Tobacco Updates from CDC’s National Biomonitoring Program

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Perchlorate

- Component of propellant for rockets and missiles
- Explosives, fireworks, road flares, dyes, leather tanning, matches
- Can form naturally in atmosphere
Perchlorate in the Environment

• Manmade leaching from industrial sites
  – Perchlorate freely soluble in water
  – Ground water contamination plumes
  – Lower Colorado River

• Natural atmospheric formation/deposition
  – Trace levels (ng/L in precipitation)
  – Can accumulate in arid regions
  – Chilean fertilizer was ~0.15% perchlorate, applied to crops

• Formation in sodium hypochlorite and in some water distribution systems
Perchlorate Contamination in Colorado River (2002)

- In 1997, 450 kg/day entered Lake Mead
- Measurable entire 300 miles to Mexico
- Used by Las Vegas, Los Angeles, San Diego, Phoenix; water supply for approx. 25 million people
- Treatment reduced ClO₄ to 1 ppb in 2012
Major Agricultural Districts Grow Food Crops Using Lower Colorado River Water

Potential sources for human exposure

- Direct consumption of contaminated water
- Crops grown with contaminated water, fertilizer or soil
  - Food crops
  - Forage crops
Perchlorate Inhibits of Iodide Uptake at Sodium-Iodide Symporter (NIS)

NIS function

- Active transport of I⁻ and ClO₄⁻ across cell membrane using sodium ion gradient

NIS Inhibitors: ClO₄⁻, SCN⁻, NO₃⁻

ClO₄⁻ is transported by NIS 30-fold more avidly than iodide

NIS and Pendrin transport ClO₄⁻ across cell membranes

Tran et al. (2008); Dohan et al. (2008); Attanasio et al. (2011)
Perchlorate Biomonitoring Update

- Sources of perchlorate exposure: synthetic vs. natural
- Trends in perchlorate exposure for the U.S. population (urinary perchlorate from NHANES)
Study to Characterize Perchlorate Exposure From Synthetic vs. Natural Sources

- Perchlorate formed synthetically is subtly different from perchlorate formed naturally
- Analysis of chlorine isotopes $^{35}\text{Cl}, ^{36}\text{Cl}, \text{and} ^{37}\text{Cl}$ requires >0.1 mg perchlorate (~30 L of typical U.S. resident urine)
- Study design:
  - Isolate perchlorate from urine collected from residents of Atlanta (U.S.) and Talca (Chile)
  - Compare urinary perchlorate chlorine isotope pattern with isotope patterns from reference perchlorate (synthetic and naturally-formed)
Identification of Perchlorate Exposure Sources

1. Extract Perchlorate from complex aqueous matrix and recrystallize as TPA ClO₄⁻
2. Stable Cl isotope Analysis ($\delta^{37}$Cl) by Secondary Ion Mass Spectrometry (SIMS)
3. $^{36}$Cl Abundance Analysis by Accelerator Mass Spectrometry (AMS)
4. Comparative Isotopic Pattern: $^{36}$Cl/Cl vs $\delta^{37}$Cl (‰)

References:
Distinguishing Among Perchlorate Exposure Sources

Western USA
Atlanta, USA

Atacama
Taltal, Chile
Synthetic

$^{36}\text{Cl}/(10^{15})$
$\delta^{37}\text{Cl} (\%/o)$
Conclusions

• Chlorine isotopes patterns differ among perchlorate formed by various natural and synthetic processes

• Perchlorate isolated from urine collected from Atlanta residents resembles naturally-formed perchlorate that is found in the Western U.S.

• Perchlorate isolated from urine collected from Talca (Chile) residents resembles naturally-formed perchlorate that is found in Northern Chile

• Next step is to compare NHANES urine pool with perchlorate worker urine pool
Perchlorate Trend Analysis 2001-2012

- Have urinary perchlorate concentrations decreased since 2001 as states limit drinking water perchlorate?
  - No Federal MCL
  - No regulation of food perchlorate levels
  - Reduced perchlorate in the Lower Colorado River

- Urinary perchlorate measured in NHANES across 12 years using IC-MS/MS method

- Population weighted results stratified by age
Perchlorate exposure in the U.S. has not changed significantly over the 12 year period studied.

Higher urinary perchlorate levels in children compared to other age groups likely results from consuming more perchlorate-rich foods per kg of body weight.
Biomarkers of Exposure to Tobacco and Smoke
Biomarkers of Exposure to Tobacco and Smoke

- Who has been exposed to smoke?
- How intensely are people using which tobacco products?
- How much has each person been exposed in comparison with toxicological benchmarks?
- What are the exposure consequences of different tobacco product use?
- Do interventions reduce exposure?
- Are study participants forthcoming about their tobacco use?
Current Tobacco-Related Biomarker Methods at CDC

- Cotinine in serum (2)
- Nicotine metabolites and tobacco alkaloids in urine (11)
- TSNAs in urine (4)
- VOCs in blood (54)
- Aldehyde adducts in serum (15)
- Aldehyde/VOC metabolites in urine (29)
- Aromatic amines in urine (13)
- Heterocyclic amines in urine (9)
- Volatile nitrosamines in urine (7)
- PAHs in urine (11)
- Toxic anions in urine (4)
- Toxic metals in urine and blood (10)
Cotinine is the leading biomarker of tobacco exposure

**Comparison of biochemical markers of tobacco smoke consumption**

<table>
<thead>
<tr>
<th>Marker</th>
<th>Specificity</th>
<th>Sensitivity</th>
<th>Limitations</th>
<th>Cost</th>
<th>Advantages</th>
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<tbody>
<tr>
<td>Carbon Monoxide</td>
<td>Good</td>
<td>Good short term</td>
<td>Time of sampling, Variable absorption and elimination</td>
<td>Inexpensive</td>
<td>Direct toxin, Noninvasive (expired CO)</td>
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<tr>
<td>Thiocyanate</td>
<td>Good</td>
<td>Good long term</td>
<td>Dietary interference, Qualitative measure</td>
<td>Moderate</td>
<td>Noninvasive (saliva)</td>
</tr>
<tr>
<td>Nicotine</td>
<td>Excellent</td>
<td>Excellent short term</td>
<td>Time of sampling, Variable elimination rate</td>
<td>Expensive</td>
<td>Direct measurement of reinforcer</td>
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<tr>
<td>Cotinine</td>
<td>Excellent</td>
<td>Excellent intermediate term</td>
<td>Variable (?) elimination rate</td>
<td>Moderate</td>
<td>Measurement of nicotine consumption</td>
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*Reprinted from Benowitz 1983*
<table>
<thead>
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<th>Year</th>
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<th>2013 -</th>
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<tr>
<td>Analyte</td>
<td>Cotinine</td>
<td>Cotinine</td>
<td>Cotinine and Trans-3’-Hydroxycotinine</td>
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<tr>
<td>Pre-screening</td>
<td>ELISA</td>
<td>ELISA</td>
<td>None</td>
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<tr>
<td>Samples/Day</td>
<td>100</td>
<td>100</td>
<td>384</td>
<td></td>
</tr>
<tr>
<td>Time to prep 100 samples</td>
<td>12 hours</td>
<td>5 hours</td>
<td>2 hours</td>
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<tr>
<td>Sample Volume</td>
<td>500 uL</td>
<td>500 uL</td>
<td>200 uL</td>
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<tr>
<td>LC</td>
<td>Hewlett-Packard 1090L</td>
<td>Shimadzu 10A Agilent Injector</td>
<td>Shimadzu 20A</td>
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<tr>
<td>Mass Spec</td>
<td>API 3</td>
<td>API 4000</td>
<td>API 6500</td>
<td></td>
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<tr>
<td>Cost/Sample</td>
<td>&gt;$200</td>
<td>$115</td>
<td>$60</td>
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</table>
Harmonization of Methods

- Common accuracy basis
- NIST reference materials
  - CRMs
  - SRMs
- PhenX, other open exchange of methodological info
- Round robin sample exchanges

Nicotine Biomarkers Crucial for Assessing Tobacco Exposure

- Serum Cotinine: gold standard/historical data
- Urine Nicotine Metabolites
  - Measures nicotine and 7 metabolites (mass balance, ~95% of nicotine in urine)
  - Functional assessment of nicotine metabolism, with implications for addiction and exposure
  - Measure minor alkaloids to monitor compliance to nicotine replacement therapy (NRT)
  - Need to adjust for dilution caused by variable hydration
Measuring Nicotine Equivalents

Hukkanen et al. 2005
Importance of combustion biomarkers for differentiating nicotine sources

- Acrylonitrile metabolite CYMA
- PAHs
- Aromatic amines
- Heterocyclic aromatic amines
- 2,5-Dimethylfuran
- Other VOCs, including aldehydes
Harmful VOCs in mainstream cigarette smoke

• 4 of the 5 top carcinogens in tobacco smoke are VOCs
  – 1,3-Butadiene
  – Acrylonitrile
  – Acetaldehyde
  – Benzene

• Acrolein, Acetaldehyde, and Acrylonitrile are among the most potent respiratory irritators
  ((Fowles & Dybing, Tobacco Control 2003)

• “…cigarette smoking is a primary source of benzene, toluene and styrene and an important source of ethylbenzene and xylene exposure for the U.S. population…”
  ((Chambers, et. al. Environ. Int. 2011))
Challenges in measuring HPHCVOCs in smoke and human matrices

Aldehydes:
- Acetaldehyde
- Acrolein
- Butanal
- Crotonaldehyde

Monoaromatics:
- Benzene
- Toluene
- Ethylbenzene
- m/p-Xylene
- o-Xylene
- Styrene
- 3-Ethyltoluene
- Pyridine
- Nitrobenzene

Ketones:
- Methyl vinyl ketone
- 2,3-Butanedione
- 2-Butanone
- 2-Pentanone
- 3-Pentanone

Nitro:
- Nitromethane
- 2-Nitropropane

Aldehydes:
- Acetaldehyde
- Acrolein
- Butanal
- Crotonaldehyde

Ketones:
- Methyl vinyl ketone
- 2,3-Butanedione
- 2-Butanone
- 2-Pentanone
- 3-Pentanone

Unsaturated hydrocarbons:
- Vinyl chloride
- 1,3-Butadiene
- Furan
- 2,5-Dimethylfuran
- Vinyl acetate
- Acrylonitrile

Broad boiling point range
- Vinyl chloride -13.4 °C
- Nitrobenzene 210.9 °C

Broad range of polarities (Henry’s Constant)
- Vinyl Chloride \( \frac{1}{k_H} = K_{H_2O/air} = 0.51 \)
- Nitrobenzene \( \frac{1}{k_H} = K_{H_2O/air} = 741 \)

Reactive and non-reactive compounds
- Some compounds can polymerize if not stored properly

Contamination of solvents and labware
Monoaromatic VOCs are highly correlated in blood collected from smokers

VOCs Are Highly Correlated in Cigarette Smoke
Pattern of monoaromatic VOCs is similar in cigarette smoke and smoker’s blood

Tobacco Smoke Exposure in the U.S. Population

National Health and Nutrition Examination Survey (NHANES)
## Summary of Tobacco-Related Biomarkers in NHANES 2011 - 2016

<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>serum cotinine</td>
<td>all 3+</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>urine nicotine metabolites and minor alkaloids</td>
<td>1/3 subset 6+; all adult smokers</td>
<td>x</td>
<td>x</td>
<td>x</td>
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<tr>
<td>serum aldehydes</td>
<td>1/3 subset 12+; all adult smokers</td>
<td>x</td>
<td>x</td>
<td>x</td>
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<tr>
<td>urine aromatic amines</td>
<td>1/3 subset 6+; all adult smokers</td>
<td>x</td>
<td>x</td>
<td>x</td>
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<tr>
<td>urine NNAL/TSNAs</td>
<td>all 3+</td>
<td>x</td>
<td>x</td>
<td>x</td>
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<tr>
<td>urine NNAL/TSNAs</td>
<td>1/3 subset 6+; all adult smokers</td>
<td></td>
<td></td>
<td>x</td>
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<tr>
<td>urine heterocyclic amines</td>
<td>1/3 subset 6+; all adult smokers</td>
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<td></td>
<td>x</td>
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<tr>
<td>urine volatile nitrosamines</td>
<td>1/3 subset 6+; all adult smokers</td>
<td></td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>blood VOCs</td>
<td>1/3 subset 6+; all adult smokers</td>
<td>x</td>
<td>x</td>
<td>x</td>
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<tr>
<td>urine VOC metabolites</td>
<td>1/3 subset 6+; all adult smokers</td>
<td>x</td>
<td>x</td>
<td>x</td>
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<tr>
<td>urine thiocyanate</td>
<td>1/3 subset 6+; all adult smokers</td>
<td>x</td>
<td>x</td>
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<tr>
<td>urine metals</td>
<td>1/3 subset 6+; all adult smokers</td>
<td>x</td>
<td>x</td>
<td>x</td>
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<tr>
<td>urine arsenic</td>
<td>1/3 subset 6+; all adult smokers</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>urine hydroxyPAHs</td>
<td>1/3 subset 6+; all adult smokers</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
</tbody>
</table>

*Environmental subsets are ages 3+ after 2012*
Tobacco-Related Exposure Patterns in NHANES 1999 – 2012

• Download all publicly available NHANES data
  – N ranges from ~6,700 – 56,000 individuals

• Categorize use based on smoking questionnaire (SMQ680 and SMQ690)
  – Never user
  – exclusive smokeless tobacco
  – exclusive combusted tobacco

• Plot histogram of log10-transformed data
Serum cotinine higher in tobacco users

Tobacco Use Last 5 Days: SMQ680 and SMQ690 A-F
Log10 Cotinine, serum [ng/ml]

- None
- Smokeless Tobacco (exclusive)
- Combustion Tobacco (exclusive)

Un-weighted statistics of log10-transformed data
NHANES 1999-2012
Urine NNAL is higher in tobacco users.

Tobacco Use Last 5 Days: SMQ680 and SMQ690 A-F
Log10 NNAL, urine [ng/g Cr]

- None
- Smokeless Tobacco (exclusive)
- Combustion Tobacco (exclusive)

Un-weighted statistics of log10-transformed data
NHANES 2007-2012
Urine arsenic levels unrelated to tobacco use

Tobacco Use Last 5 Days: SMQ680 and SMQ690 A-F
Log10 Arsenic acid, urine [ng/g Cr]

Un-weighted statistics of log10-transformed data
NHANES 2003-2012 (subsample)
Urine CYMA higher in tobacco smokers

Tobacco Use Last 5 Days: SMQ680 and SMQ690 A-F
Log10 [Acrylonitrile] CYMA, urine [ng/g Cr]

None
Smokeless Tobacco (exclusive)
Combustion Tobacco (exclusive)

Un-weighted statistics of log10-transformed data
NHANES 2005-2006 (subsample) and 2011-2012 (smoking subsample)
Blood 2,5-dimethylfuran higher in tobacco smokers

Tobacco Use Last 5 Days: SMQ680 and SMQ690 A-F
Log10 2,5-Dimethylfuran, blood [ng/ml]

Un-weighted statistics of log10-transformed data
NHANES 2003-2006 (subsample)
CDC National Exposure Report includes smoking categorization

![CDC National Exposure Report Cover](http://www.x.gov/biomonitoring/pdf/FourthReport_UpdatedTables_Feb2015.pdf)

### Urinary N-Acetyl-S-(2-cyanoethyl)-L-cysteine

<table>
<thead>
<tr>
<th>Cigarette Smokers</th>
<th>Geometric mean (95% confidence interval)</th>
<th>50th</th>
<th>75th</th>
<th>90th</th>
<th>95th</th>
<th>Sample size</th>
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</thead>
<tbody>
<tr>
<td>Total</td>
<td>122 (105-141)</td>
<td>140</td>
<td>277</td>
<td>504</td>
<td>717</td>
<td>889</td>
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<tr>
<td>Age group</td>
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<td></td>
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<tr>
<td>20-49 years</td>
<td>114 (99-143)</td>
<td>132</td>
<td>265</td>
<td>515</td>
<td>717</td>
<td>532</td>
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<tr>
<td>50 years and older</td>
<td>137 (118-159)</td>
<td>146</td>
<td>306</td>
<td>481</td>
<td>705</td>
<td>357</td>
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<tr>
<td>Gender</td>
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<td></td>
</tr>
<tr>
<td>Males</td>
<td>123 (108-153)</td>
<td>156</td>
<td>306</td>
<td>552</td>
<td>780</td>
<td>536</td>
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<tr>
<td>Females</td>
<td>121 (104-140)</td>
<td>128</td>
<td>240</td>
<td>403</td>
<td>652</td>
<td>353</td>
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<tr>
<td>Nonsmokers</td>
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<tr>
<td>Total</td>
<td>1.50 (1.35-1.67)</td>
<td>1.33</td>
<td>2.35</td>
<td>4.81</td>
<td>12.1</td>
<td>1308</td>
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<td>Age group</td>
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<tr>
<td>20-49 years</td>
<td>1.67 (1.38-2.02)</td>
<td>1.45</td>
<td>2.49</td>
<td>5.84</td>
<td>13.4</td>
<td>655</td>
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<td>50 years and older</td>
<td>1.34 (1.18-1.53)</td>
<td>1.23</td>
<td>2.19</td>
<td>3.85</td>
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<td>653</td>
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<td>Gender</td>
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<tr>
<td>Males</td>
<td>1.60 (1.65-2.05)</td>
<td>1.44</td>
<td>2.56</td>
<td>5.05</td>
<td>14.7</td>
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<td>Females</td>
<td>1.24 (1.06-1.45)</td>
<td>1.19</td>
<td>1.90</td>
<td>3.55</td>
<td>6.84</td>
<td>683</td>
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</table>
Marijuana smoke contains similar chemicals as tobacco smoke (Moir et al. 2008)

Adjusted geometric means of urinary concentrations of PAH and VOC metabolites (creatinine corrected) among nonusers (NU), marijuana users (MU) and cigarette users (CU). Error bars indicate 95% confidence intervals. Wei et al (2016).
Acknowledgements

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• The dedicated staff of the Tobacco and Volatiles Branch
• Funding from CDC and FDA Center for Tobacco Products
Analytical measurement of biomarkers of exposure to harmful and addictive chemicals is crucial for characterizing and countering the epidemic of disease caused by tobacco use.

For more information, please contact

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