A comparison of human nicotine dose estimates from filter analysis with nicotine metabolites analysis
Kelley St.Charles a, George Krautter a,b & Derek Mariner c
a) Formerly Brown & Williamson Tobacco, b) R.J. Reynolds Tobacco, c) British American Tobacco

Objective
Compare and correlate three methods of nicotine estimation
- Filter Analysis Method
- Saliva Cotinine Method
- Urinary Nicotine + 5 Metabolites
- Cotinine + glucuronide
- 3-Hydroxycotinine + glucuronide

Experimental Design
- 5 day Clinical Study at Covance Clinical Research in Madison, WI to assure compliance
- 74 subjects smoking their own brand
- Allowed to smoke ad-lib in a ventilated smoking area
- ~ 10 subjects/week within same tar band
- Wide range of brands to assure robustness of correlations

Sample Collection
- 24-hour urine (Days 1-5) stored @ -70°C
- Saliva at 18:30 (Days 1-5) stored @ -20°C
- All cigarettes smoked (Days 1-5)
- Subject returned smoked cigarette to clinician
- Filter removed and stored in jar
- Clinician issued another of the same brand
- Jars shipped overnight to lab each day
- 1 cm of mouth end of filter cut day of receipt
- Stored @ -20°C in glass jar

Correlation Results

<table>
<thead>
<tr>
<th>Source of Urinary Metabolites</th>
<th>Cotinine &amp; 3-Hydroxycotinine</th>
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</thead>
<tbody>
<tr>
<td><strong>Individual</strong></td>
<td><strong>5 Day Average</strong></td>
</tr>
<tr>
<td>Urine vs. Filter</td>
<td>Y = 0.67 x + 4.3 mg/day</td>
</tr>
<tr>
<td>Urine vs. Saliva (R&lt;0.64 in ref 4)</td>
<td>Y = 0.44 x + 5.4 mg/day</td>
</tr>
<tr>
<td>Filter vs. Saliva</td>
<td>Y = 0.042 x + 5.9 mg/day</td>
</tr>
<tr>
<td>Creatine Normalized Urine vs. Filter</td>
<td>Y = 0.044 x + 6.9 mg/day</td>
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</tbody>
</table>

Summary
- Each measure correlates significantly with the other two (intercept & slope  p < 0.01)
- Strongest correlation between 24 hr. urinary metabolites and filter method
  - Highest R2 and lowest standard error
  - Significant improvement with 5 day average
- Normalization of urinary metabolites to creatinine degrades correlation
- Urinary nicotine and metabolites are an amalgam of intake over 2 days
- Approximately 70% of the cigarette yield appears as urinary nicotine, cotinine, 3-hydroxy cotinine + respective glucuronides

References

Abstract
Human nicotine intake during smoking has been estimated by either analyzing the metabolites of nicotine in body fluids or by analyzing filters from smoked cigarettes. However, no comparison of the filter analysis method with body fluid analysis methods has been published. Consequently, an inpatient study was conducted with 75 smokers of 1-17mg FTC tar products smoking their own brands. The subjects stayed in a clinic for 5 days and were allowed to smoke in a smoking room whenever they wished. Each smoked cigarette had to be returned to a clinician before another was issued. The filters were analyzed to estimate the daily mouth intake of nicotine. 24-hour urine samples were collected and analyzed for cotinine, 3-OH cotinine and their respective glucuronides conjugates. Saliva samples were collected at 18.30 each day for cotinine analyses. On the fourth day, additional saliva samples were collected at 8.30 and 13.30 to assess any diurnal variations in saliva cotinine levels. Each method correlated significantly (p<0.01) with the other two, but the best correlation was between nicotine mouth intake and urinary nicotine and metabolites. Averaging the results over 5 days improved the mouth intake / urinary cotinine correlation even further but had little effect on the saliva cotinine correlations. Multiple regression analysis implies that urinary output is an amalgam of the nicotine input from multiple days.