Nonclinical Toxicity Assessment of Oral Tobacco-Derived Nicotine Products: III. Extraction and Test Material Characterization

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CORESTA Smoke-Techno (SSPT) Conference - [ST 59]
October 27, 2021
Framework Overview

I. Framework Overview

II. Ingredient risk assessment
- ST 58 – Morgan

III. Test product extraction and characterization
- ST 59 - Smith

IV. In vitro OECD regulatory testing
- ST 60 - Doshi

V. In vitro oral health mechanistic testing
- ST 61 - Kumar

- Introduction
- Extraction
  - Strategy & Approach
  - Method
- Characterization
  - Results
  - Stability
- Summary
Tobacco Harm Reduction Framework

Constituent Reduction

- Product Design and Control
- Chemical and Physical Characterization

Risk Reduction Individual

- Toxicology and Risk Assessment
- Studies in Adult Human Subjects

Harm Reduction Population

- Perception and Behavior Assessment
- Risks and Benefits to Health of the Population

The Product

- Manufacturing and quality control
- Physical and chemical characterization
- Product stability
- Hazard assessment

Exposure and Health Risk

- Literature reviews
- In vitro studies
- In vivo studies
- Risk assessments
- Relative risk comparison across and within tobacco product categories
- Clinical studies

Impact on the Population

- Secondary analysis of national data
- Risk perception, behavioral intention and product appeal
- Actual use study
- Product instructions comprehension
- Population modeling
- Post-market surveillance
Extraction Methods Across Tobacco Categories

**Extraction 1**
- **Solvent:** Ethanol (EtOH)
- **Test Products:** 3R4F, CRP 2.1 (MST), CRP 1.1 (Snus), OTDN 6 mg Nicotine (Mint)

- One Representative Sample
- Relatively Stable
- Effective for Oral In vitro Testing

**Extraction 2**
- **Solvent:** Artificial Saliva (AS)
- **Non-combustible Only**
- **Test Products:** CRP 2.1 (MST), CRP 1.1 (Snus), OTDN 6 mg (Mint), OTDN 2 mg (Citrus)

- 10+ Previous Studies (ST)
Extraction Methods

**Cigarette Smoke Extract (EtOH)**
- ISO 20778 (Health Canada Intense) (55 mL, 30 sec., 2 sec., 100% blocked, rotary)
- TPM of 20 cigarettes collected on 92 mm CFP with 30 mL EtOH impinger (ice bath)
- CFP extracted in EtOH impinger solution for 20 mins. at 200 rpm
- Centrifuged for 10 mins at 1000 g
- 0.2 µm filtration
- Stored at -70°C

**1Oral Tobacco Product Extract (EtOH/AS)**
- 10 % w/v (e.g., 5 g in 50 mL)
- 2 hrs. at 37°C at 250 rpm
- Centrifuged for 10 mins at ~3000 g
- 0.2 µm filtration
- Stored at -70°C

**Combustible**

**Non-combustible**

Key Extraction Analytes

- Nicotine
- Tobacco-specific nitrosamines (TSNAs)

Additional Analytes

- Carbonyls (EtOH)
- Benzo[a]pyrene (BaP) (EtOH)
- Metals (AS)
Nicotine analyte recovery above 80% for all product categories

<table>
<thead>
<tr>
<th>Test Product</th>
<th>Measured</th>
<th>Reference</th>
<th>Analyte Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>3R4F (mg/cig)</td>
<td>1.94 – 2.03</td>
<td>1.99</td>
<td>97% - 102%</td>
</tr>
<tr>
<td>CRP 2.1 (mg/g)</td>
<td>9.95 ± 0.41</td>
<td>10.8</td>
<td>92%</td>
</tr>
<tr>
<td>CRP 1.1 (mg/g)</td>
<td>7.02 ± 0.38</td>
<td>7.48</td>
<td>94%</td>
</tr>
<tr>
<td>OTDN 6 mg Mint* (mg/pouch)</td>
<td>5.64 ± 0.16</td>
<td>6*</td>
<td>94%</td>
</tr>
</tbody>
</table>

*Reference value based on product label

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<table>
<thead>
<tr>
<th>Test Product</th>
<th>Measured</th>
<th>Reference</th>
<th>Analyte Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP 2.1 (mg/g)</td>
<td>9.72 ± 0.20</td>
<td>10.8</td>
<td>90%</td>
</tr>
<tr>
<td>CRP 1.1 (mg/g)</td>
<td>6.54 ± 0.07</td>
<td>7.48</td>
<td>87%</td>
</tr>
<tr>
<td>OTDN 6 mg Mint* (mg/pouch)</td>
<td>5.76 ± 0.08</td>
<td>6*</td>
<td>96%</td>
</tr>
<tr>
<td>OTDN 2 mg Citrus* (mg/pouch)</td>
<td>1.65 ± 0.06</td>
<td>2*</td>
<td>83%</td>
</tr>
</tbody>
</table>

EtOH

AS

Concentration (mg/mL)

- Ethanol
- Artificial Saliva

*Reference value based on product label
TSNAs analyte recovery above 80% for 3R4F (Smoke) and CRP 2.1 (MST) reference products and non-detect in OTDN Products

### Extraction 1 (Ethanol)

<table>
<thead>
<tr>
<th>TSNA</th>
<th>Measured (Ethanol)</th>
<th>Reference</th>
<th>Analyte Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>NNN (ng/cig)</td>
<td>302 – 315</td>
<td>297 ± 73</td>
<td>100%</td>
</tr>
<tr>
<td>NNK (ng/cig)</td>
<td>273 – 285</td>
<td>252 ± 58</td>
<td>100%</td>
</tr>
<tr>
<td>NAT (ng/cig)</td>
<td>286 – 293</td>
<td>279 ± 63</td>
<td>100%</td>
</tr>
<tr>
<td>NAB (ng/cig)</td>
<td>35.0 – 35.8</td>
<td>31.2 ± 7.7</td>
<td>100%</td>
</tr>
</tbody>
</table>

### CRP 2.1 (MST)

#### TSNA Measured (Ethanol) Reference Analyte Recovery

<table>
<thead>
<tr>
<th>TSNA</th>
<th>Measured (Ethanol)</th>
<th>Reference</th>
<th>Analyte Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>NNN (µg/g)</td>
<td>3.25 ± 0.04</td>
<td>3.46</td>
<td>94%</td>
</tr>
<tr>
<td>NNK (µg/g)</td>
<td>1.96 ± 0.09</td>
<td>2.09</td>
<td>94%</td>
</tr>
<tr>
<td>NAT (µg/g)</td>
<td>3.77 ± 0.11</td>
<td>3.97</td>
<td>95%</td>
</tr>
<tr>
<td>NAB (µg/g)</td>
<td>0.234 ± 0.011</td>
<td>0.267</td>
<td>88%</td>
</tr>
</tbody>
</table>

### Extraction 2 (Artificial Saliva)

#### CRP 2.1

- Concentration (ng/mL)
- < LOQ (< 16.0)
- Non-detect (< 0.5)

#### Non-detect (< 0.5)

<table>
<thead>
<tr>
<th>TSNA</th>
<th>Measured (AS)</th>
<th>Reference</th>
<th>Analyte Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>NNN (µg/g)</td>
<td>2.99 ± 0.05</td>
<td>3.46</td>
<td>86%</td>
</tr>
<tr>
<td>NNK (µg/g)</td>
<td>1.89 ± 0.03</td>
<td>2.09</td>
<td>90%</td>
</tr>
<tr>
<td>NAT (µg/g)</td>
<td>3.39 ± 0.06</td>
<td>3.97</td>
<td>85%</td>
</tr>
<tr>
<td>NAB (µg/g)</td>
<td>0.232 ± 0.003</td>
<td>0.267</td>
<td>87%</td>
</tr>
</tbody>
</table>
Additional analytes recoveries consistent with literature and previous work

**Total Carbonyls (EtOH)**

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3R4F</td>
<td>0.209</td>
</tr>
<tr>
<td>CRP 2.1</td>
<td>0.133</td>
</tr>
<tr>
<td>CRP 1.1</td>
<td>0.128</td>
</tr>
<tr>
<td>OTDN 6 mg (Mint)</td>
<td>0.128</td>
</tr>
</tbody>
</table>

**BaP (EtOH)**

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3R4F</td>
<td>Non-detect (&lt; 0.3)</td>
</tr>
<tr>
<td>CRP 2.1</td>
<td>Non-detect (&lt; 0.3)</td>
</tr>
<tr>
<td>CRP 1.1</td>
<td>Non-detect (&lt; 0.3)</td>
</tr>
<tr>
<td>OTDN 6 mg (Mint)</td>
<td>Non-detect (&lt; 0.3)</td>
</tr>
</tbody>
</table>

**Metals (AS)** – Cadmium analyte recoveries were < 25% for CRP 2.1 (105 ng/g) and CRP 1.1 (57.6 ng/g). Nickel analyte recoveries were near LOQ (500 ng/g) for CRP 1.1 and CRP 2.1. No quantifiable levels of arsenic, chromium or lead were observed in any non-combustible product extracts.
Nicotine levels in all extracts, irrespective of solvent, were stable for a minimum of 8 weeks (stored at -70°C)

Storage Condition of Extracted Test Material (Post Filtration) – Freezer (-70°C)

<table>
<thead>
<tr>
<th>Extraction Solvent</th>
<th>Test Product</th>
<th>Initial Measured</th>
<th>8 week Timepoint</th>
<th>% Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol</td>
<td>3R4F (mg/mL)</td>
<td>1.29 ± 0.08</td>
<td>1.29 ± 0.05</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td>CRP 2.1 (mg/mL)</td>
<td>0.954 ± 0.012</td>
<td>0.930 ± 0.001</td>
<td>-2.5%</td>
</tr>
<tr>
<td></td>
<td>CRP 1.1 (mg/mL)</td>
<td>0.653 ± 0.010</td>
<td>0.668 ± 0.002</td>
<td>2.3%</td>
</tr>
<tr>
<td>Artificial Saliva</td>
<td>OTDN 6 mg Mint (mg/mL)</td>
<td>1.47 ± 0.02</td>
<td>1.35 ± 0.02</td>
<td>-8.2%</td>
</tr>
<tr>
<td></td>
<td>OTDN 2 mg Citrus (mg/mL)</td>
<td>0.818 ± 0.029</td>
<td>0.831 ± 0.008</td>
<td>1.6%</td>
</tr>
</tbody>
</table>

% Difference = \( \frac{(\text{Timepoint Conc.} - \text{Initial Conc.})}{\text{Initial Conc.}} \times 100 \)
Summary

- The results showed that ethanol and artificial saliva were efficient at the extraction of nicotine and TSNAs (>80% of CORESTA reference values) for combustible and non-combustible products.

- The nicotine extraction of OTDN was efficient (>80% of product label) in both solvents, with little to no detectable levels of additional HPHCs in either solvent.

- The nicotine levels in all extracts, irrespective of solvent, were stable for a minimum of 8 weeks (stored at -70°C)

- The results support the use of both ethanol and artificial saliva in preparing test materials from various tobacco product categories needed for in vitro toxicological assessment
References


5. CORESTA Ref. TTPA-193-1-CTR – 2019 Collaborative Study for the Determination of Nicotine in Tobacco and Tobacco Products

6. CORESTA 2011 Collaborative Study - Determination of Tobacco Specific Nitrosamines in Cigarette Mainstream Smoke


Note, reference values used throughout the presentation can be found in the documents section on the CORESTA website if links above are unavailable. https://www.coresta.org/
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Questions? Email us at altriascience@altria.com