



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

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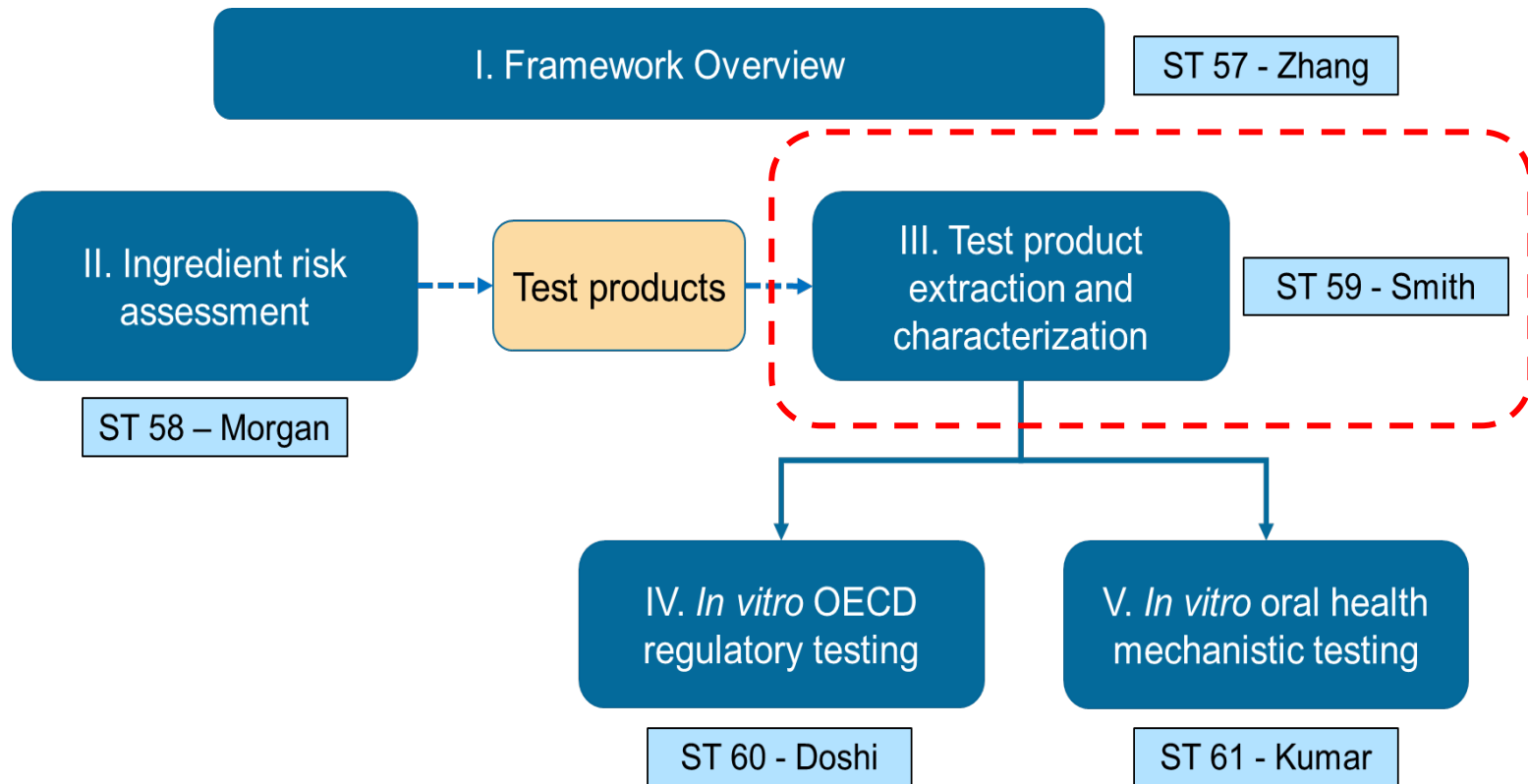
*Center for Research and Technology*

**CORESTA Smoke-Techno (SSPT) Conference - [ST 59]**

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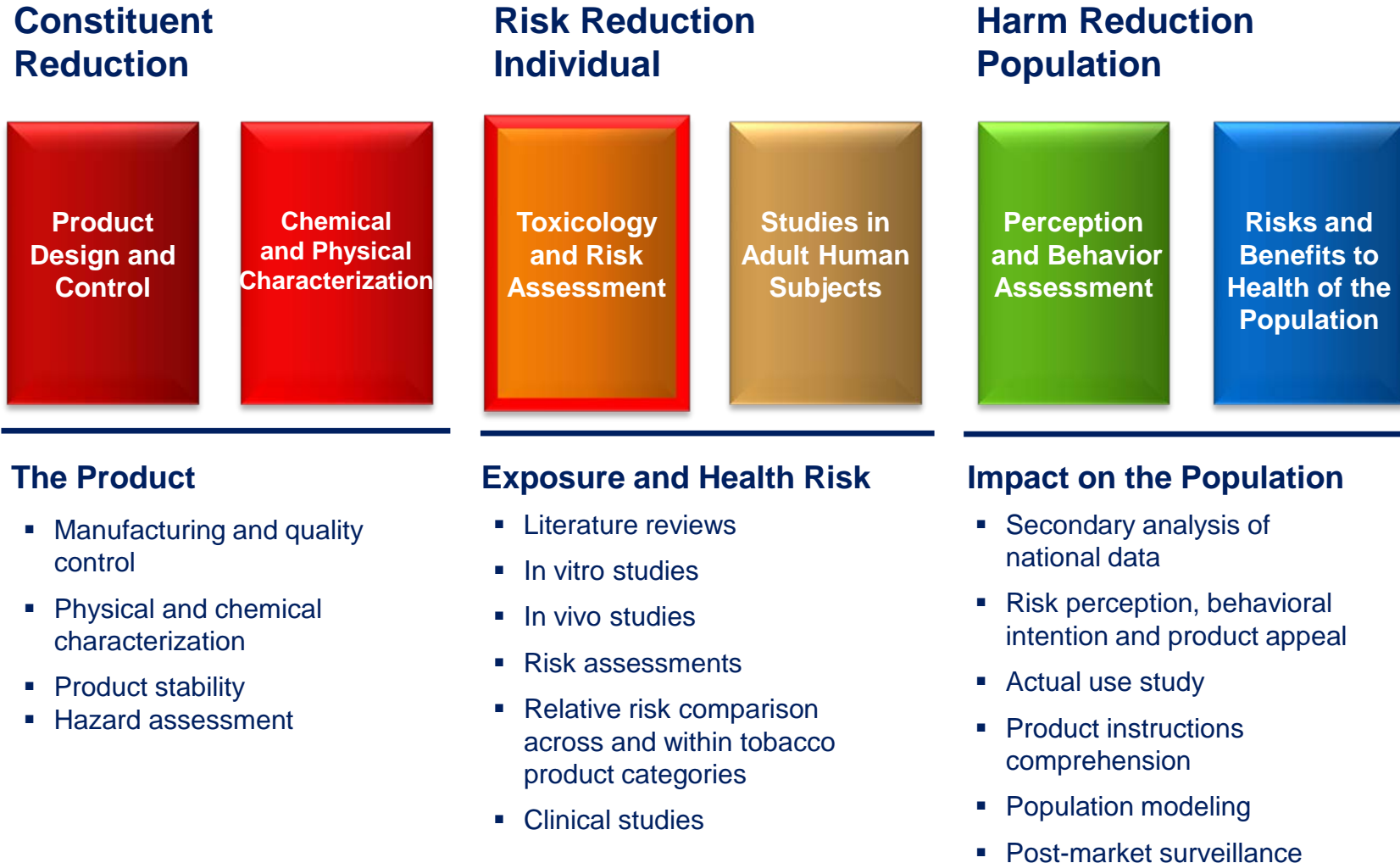
# Framework Overview



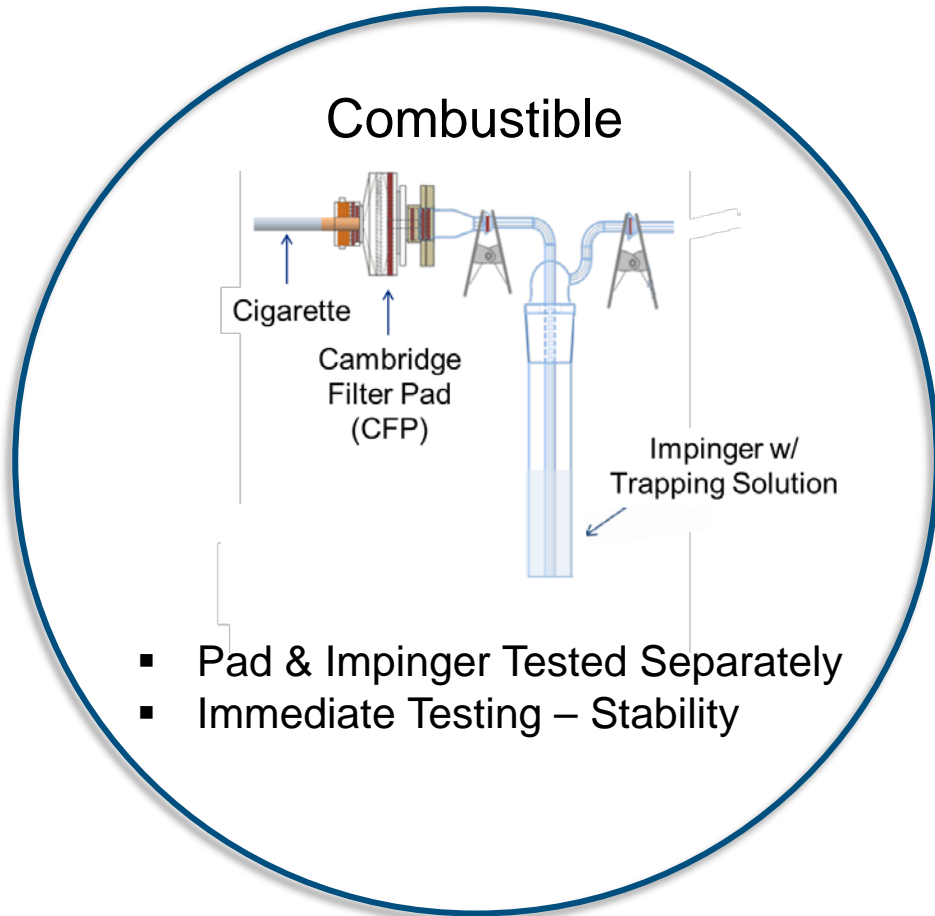
- Introduction
- Extraction
  - Strategy & Approach
  - Method
- Characterization
  - Results
  - Stability
- Summary



# Tobacco Harm Reduction Framework



# Introduction

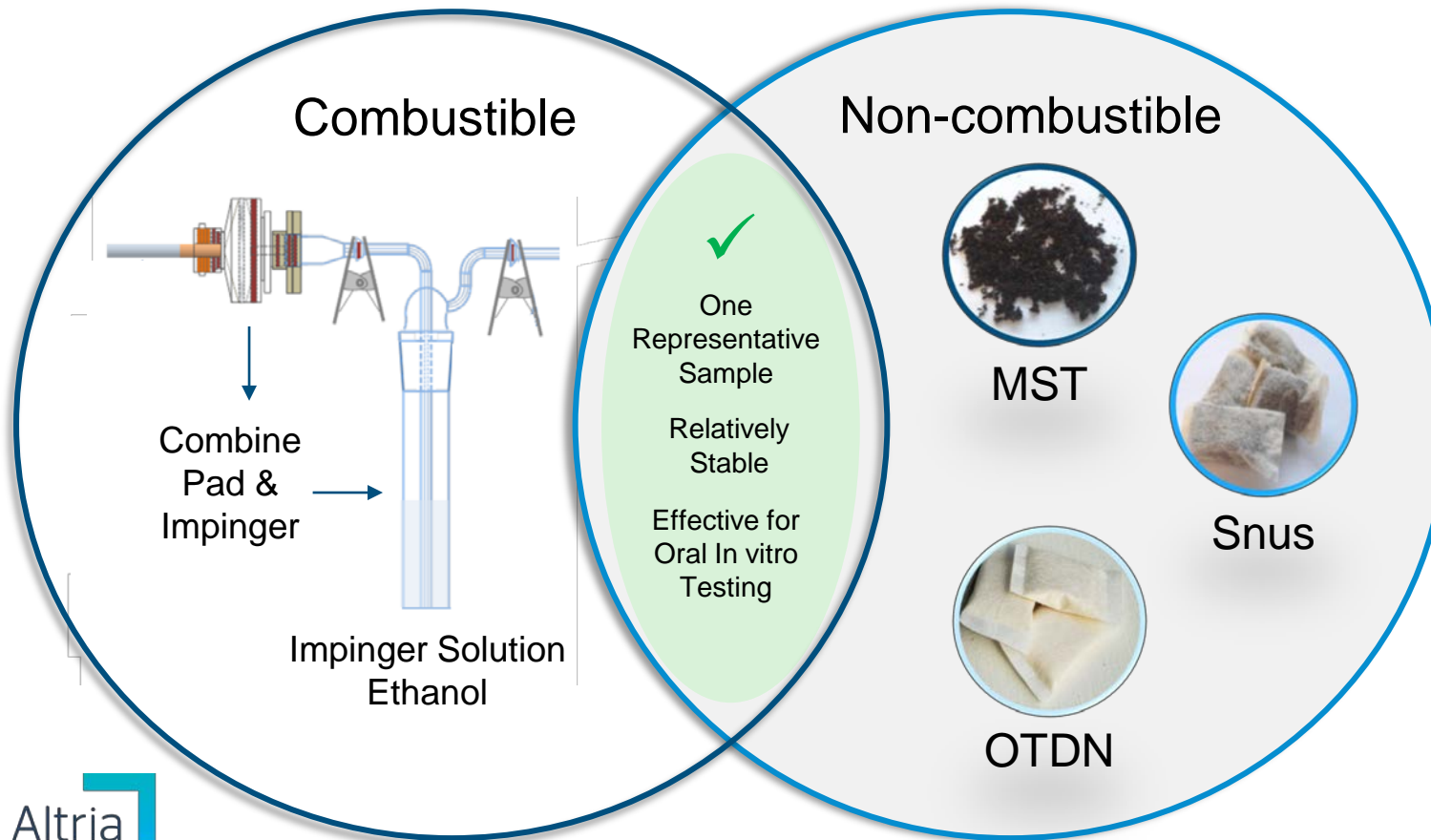


# Extraction Methods Across Tobacco Categories

## Extraction 1

**Solvent:** Ethanol (EtOH)

**Test Products:** 3R4F, CRP 2.1 (MST), CRP 1.1 (Snus), OTDN 6 mg Nicotine (Mint)



## 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

## Combustible

### 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  $\mu\text{m}$  filtration
- Stored at  $-70^{\circ}\text{C}$

## Non-combustible

### <sup>1</sup>Oral Tobacco Product Extract (EtOH/AS)

- 10 % w/v (e.g., 5 g in 50 mL)
- 2 hrs. at  $37^{\circ}\text{C}$  at 250 rpm
- Centrifuged for 10 mins at  $\sim 3000$  g
- 0.2  $\mu\text{m}$  filtration
- Stored at  $-70^{\circ}\text{C}$

1. Bishop, E., et al. "An approach for the extract generation and toxicological assessment of tobacco-free 'modern' oral nicotine pouches." Food and Chemical Toxicology 145 (2020): 111713.

# Extract Characterization - Selected Analytes

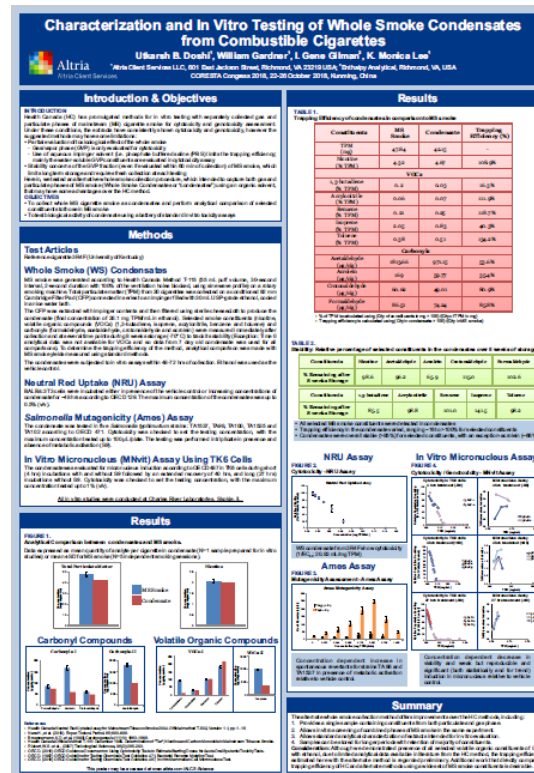
## Key Extraction Analytes

- Nicotine
- Tobacco-specific nitrosamines (TSNAs)

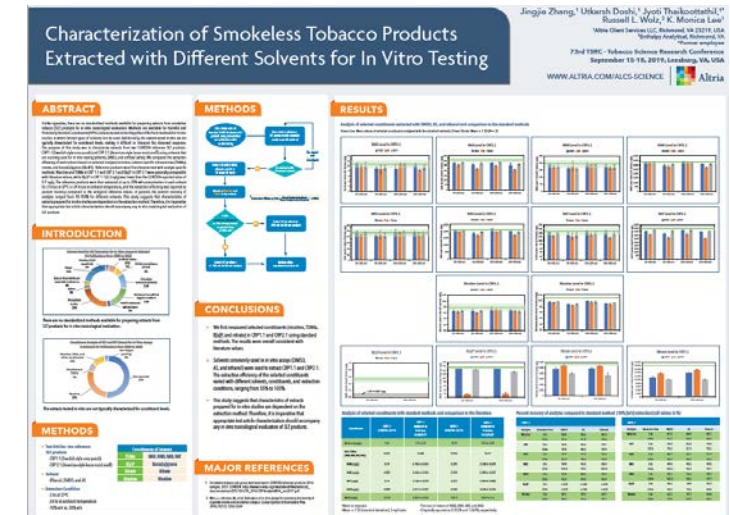
## Additional Analytes

- Carbonyls (EtOH)
- Benzo[a]pyrene (BaP) (EtOH)
- Metals (AS)

### Characterization and In Vitro Testing of Whole Smoke Condensate from Combustible Cigarettes (CORESTA, Doshi et al. 2018)<sup>2</sup>



### Characterization of Smokeless Tobacco Products Extracted with Different Solvents for In Vitro Testing (73<sup>rd</sup> TSRC, Zhang et al. 2019)<sup>3</sup>



# Nicotine analyte recovery above 80% for all product categories

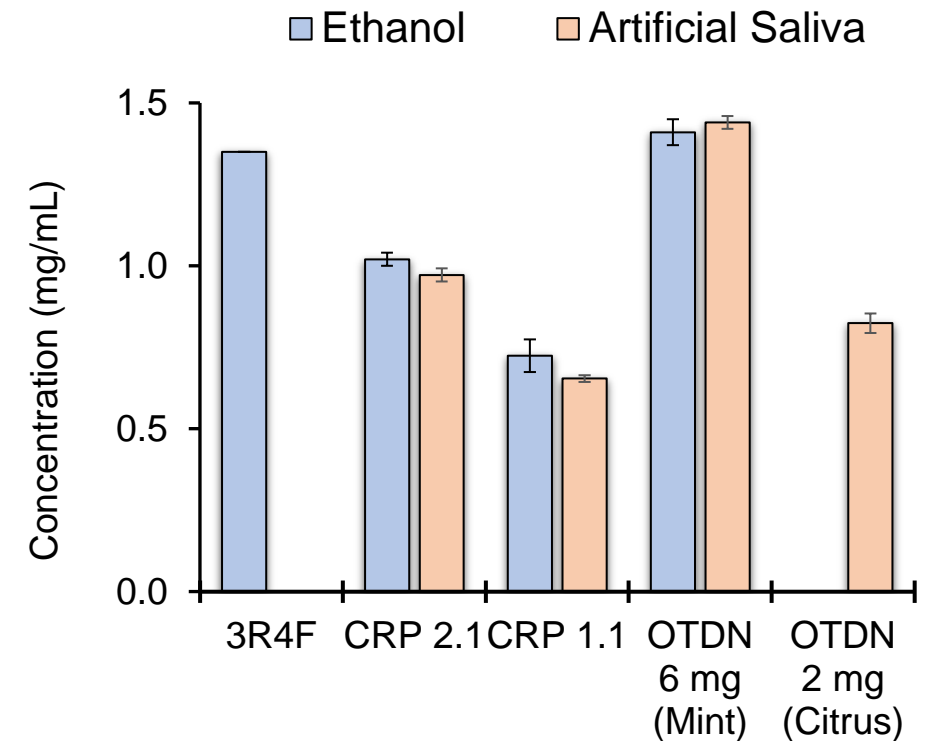
EtOH

Test Product	Measured	Reference <sup>4,5</sup>	Analyte Recovery
3R4F (mg/cig) <sup>4</sup>	1.94 – 2.03	1.99	97% - 102%
CRP 2.1 (mg/g) <sup>5</sup>	9.95 ± 0.41	10.8	92%
CRP 1.1 (mg/g) <sup>5</sup>	7.02 ± 0.38	7.48	94%
OTDN 6 mg Mint* (mg/pouch)	5.64 ± 0.16	6*	94%

AS

Test Product	Measured	Reference <sup>5</sup>	Analyte Recovery
CRP 2.1 (mg/g) <sup>5</sup>	9.72 ± 0.20	10.8	90%
CRP 1.1 (mg/g) <sup>5</sup>	6.54 ± 0.07	7.48	87%
OTDN 6 mg Mint* (mg/pouch)	5.76 ± 0.08	6*	96%
OTDN 2 mg Citrus* (mg/pouch)	1.65 ± 0.06	2*	83%

\*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)

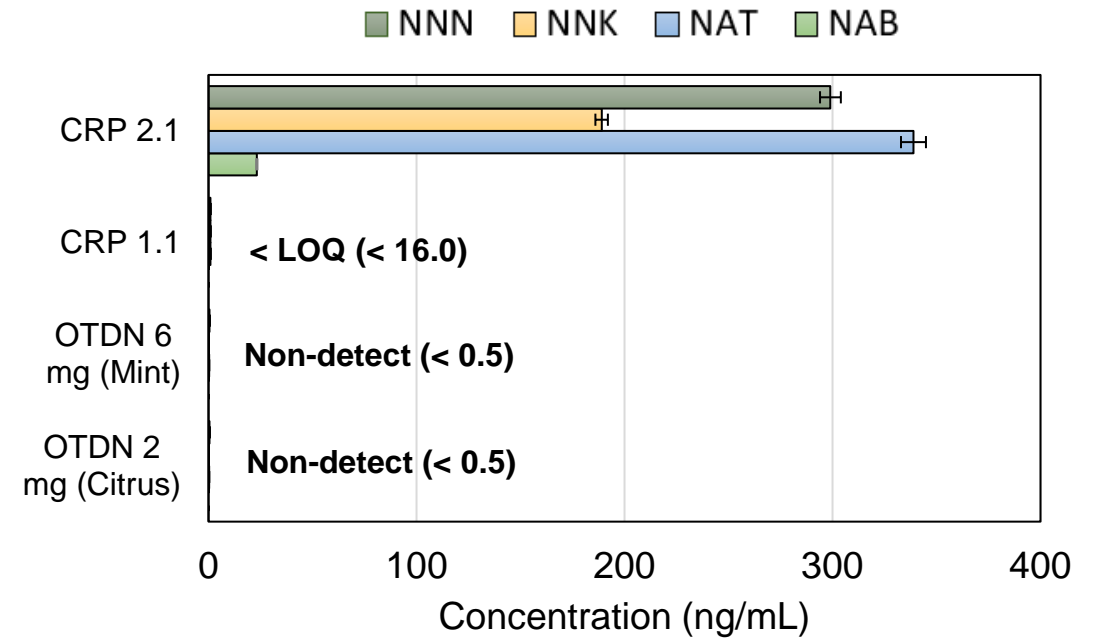
3R4F (Cigarette Smoke)

TSNA	Measured (Ethanol)	Reference <sup>6</sup>	Analyte Recovery
NNN (ng/cig)	302 – 315	297 ± 73	100%
NNK (ng/cig)	273 – 285	252 ± 58	100%
NAT (ng/cig)	286 – 293	279 ± 63	100%
NAB (ng/cig)	35.0 – 35.8	31.2 ± 7.7	100%

CRP 2.1 (MST)

TSNA	Measured (Ethanol)	Reference <sup>7</sup>	Analyte Recovery
NNN (µg/g)	3.25 ± 0.04	3.46	94%
NNK (µg/g)	1.96 ± 0.09	2.09	94%
NAT (µg/g)	3.77 ± 0.11	3.97	95%
NAB (µg/g)	0.234 ± 0.011	0.267	88%

## Extraction 2 (Artificial Saliva)



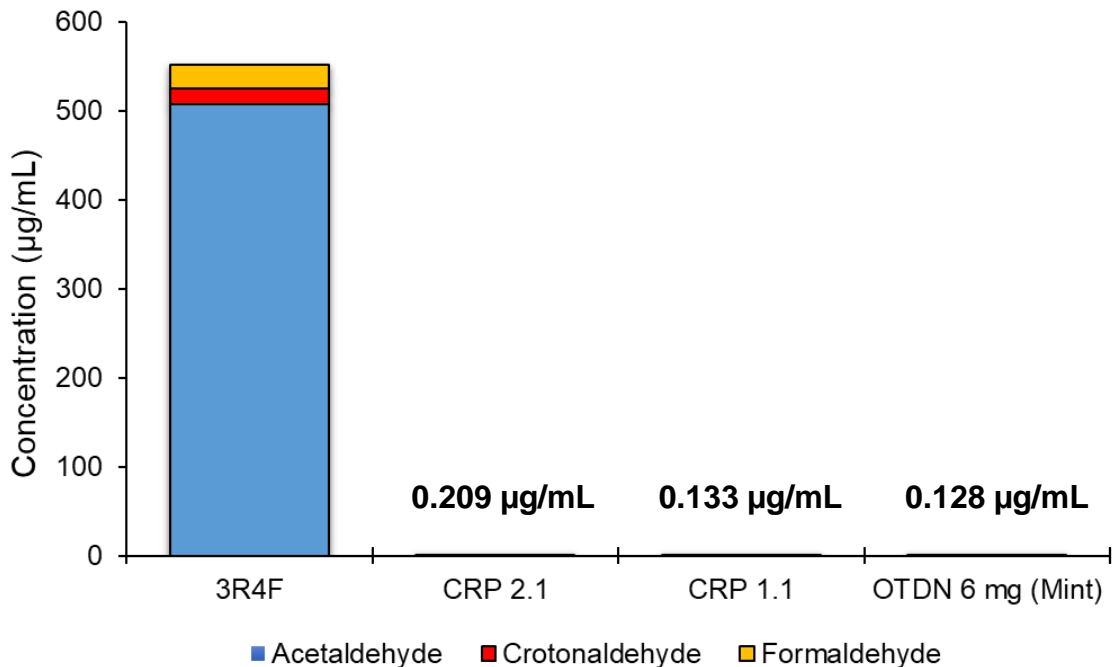
CRP 2.1 (MST)

TSNA	Measured (AS)	Reference <sup>7</sup>	Analyte Recovery
NNN (µg/g)	2.99 ± 0.05	3.46	86%
NNK (µg/g)	1.89 ± 0.03	2.09	90%
NAT (µg/g)	3.39 ± 0.06	3.97	85%
NAB (µg/g)	0.232 ± 0.003	0.267	87%

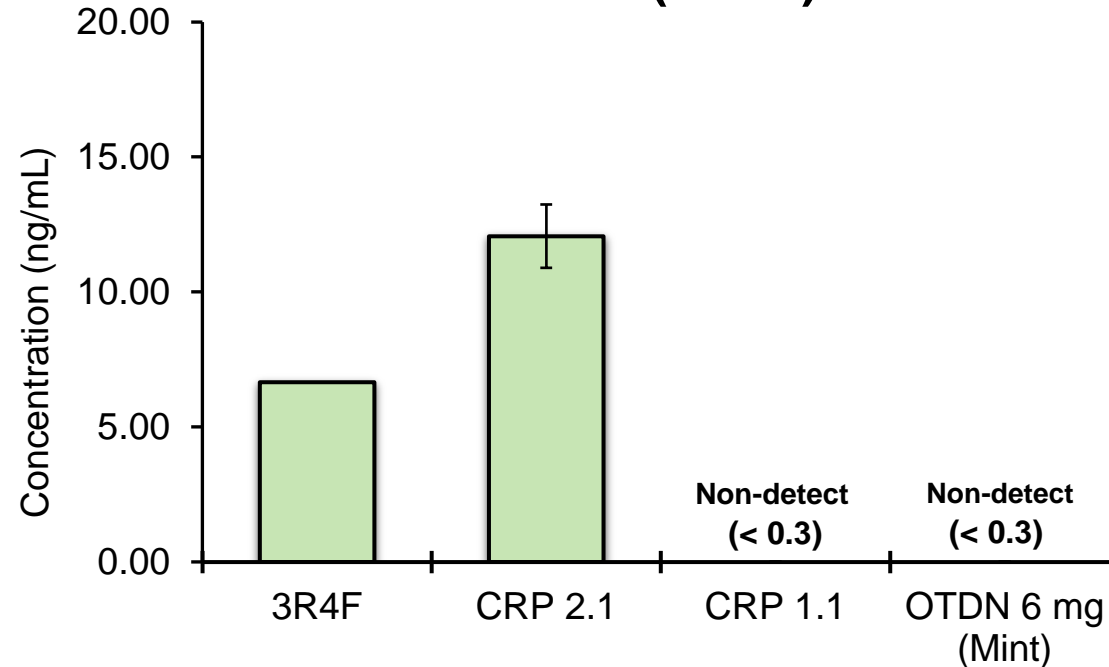


# Additional analytes recoveries consistent with literature and previous work

## Total Carbonyls (EtOH)



## BaP (EtOH)



**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)

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

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





# 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

1. Bishop, E., et al. "An approach for the extract generation and toxicological assessment of tobacco-free 'modern' oral nicotine pouches." Food and Chemical Toxicology 145 (2020): 111713.
2. Doshi, U., et al. "Characterization and In Vitro Testing of Whole Smoke Condensate from Combustible Cigarettes." CORESTA Congress, Kunming, China (2018).
3. Zhang, J., et al. "Characterization of smokeless tobacco products extracted with different solvents for in vitro testing." 73<sup>rd</sup> TSRC, Leesburg, VA (2019).
4. Jaccard, G., et al. "Mainstream smoke constituents and in vitro toxicity comparative analysis of 3R4F and 1R6F reference cigarettes." Toxicology reports 6 (2019): 222-231.
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](#)
7. [CORESTA Ref. TTPA-220-CTR – CORESTA Reference Products - 2019 Analysis | CORESTA](#)





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