A 12-Month Stability Study on JUUL2 Virginia Tobacco and Crisp Menthol Flavored Aerosols using Targeted Analytical Methods.

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Introduction

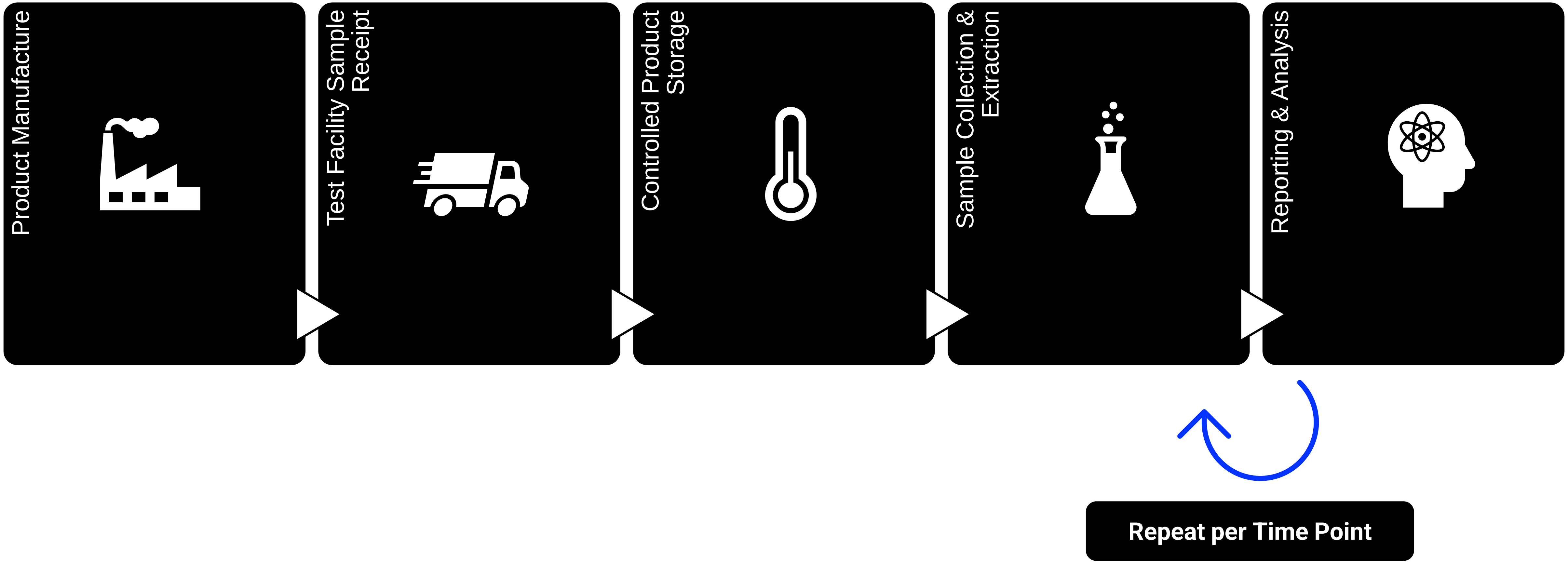
Stability studies provide evidence on how the quality of a substance or product may vary and/or establish re-test period for the product or a shelf life and recommended storage conditions[1]. Similar to a drug substance or drug product, Electronic Nicotine Delivery System (ENDS) products may vary with time under the influence of a variety of environmental factors such as temperature, humidity, and light.

[1] Guidance for Industry Q1A(R2) Stability Testing of New Drug Substances and Products. ICH. November 2003, Revision 2.

Objectives

1. Discuss the parameters of an ENDS stability study using JUUL2 Virginia Tobacco and Crisp Menthol Flavored JUULpods.
2. Present data for select compounds through 12 months and monitor changes in chemistry and stability.
3. Review additional considerations regarding study design, data collection, and interpretation.

Targeted Analysis of ENDS



Study Design

- Design based upon principles of ICH Q1A(R2) for new drug products.
- One production lot of Virginia Tobacco (18 mg/mL) and Crisp Menthol (18 mg/mL) pods utilized with JUUL2 Device.

Storage Condition

Time Point (Months)	Environmental Condition	Duration
Long Term (LT) or Ambient	T0: 22 °C ± 2 °C / 60 % RH ± 5 % Relative Humidity [RH] T3-T12: 25 °C ± 2 °C / 60 % RH ± 5 %	12

Testing Schedule

Time Points	T0	T3	T6	T9	T12
Product Age	1 Month	4 Month	7 Month	10 Month	13 Month
Storage Conditions	LT	LT	LT	LT	LT

*Due to shipping logistics from manufacturing to testing lab, testing started at 1 month of product age.

Study Design (Cont.)

- Targeted compounds were based on the United States Food and Drug Administration Premarket Tobacco Application Guidance for Industry (USFDA; June 2019) [2] and European Tobacco Products Directive (EUTPD; April 2014) guidance[3] using validated methodologies.
- JUUL2 device and pod containing tested formulations were puffed under CORESTA recommended method No. 81 non-intense condition (55 mL, 3 second puff duration, 30 second puff interval) [4] and JUUL product-specific intense puffing condition (110 mL, 6 second puff duration, 30 second puff interval).
- Aerosol constituent levels were based on the number of puffs collected, and puff counts were determined by total device mass loss (also referred to “End of Pod” or “Whole Pod Yield”).
- 5 replicates were analyzed per method.

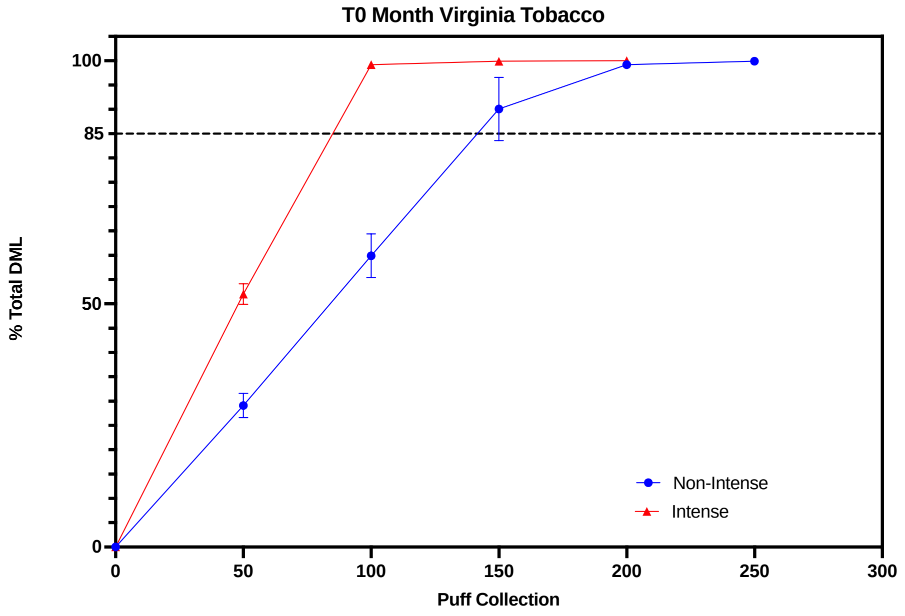
Method	Analytes
Primary Constituents	Propylene Glycol, Glycerol, Nicotine, Menthol, Diethylene Glycol, Ethylene Glycol, Water
Carbonyls	Diacetyl, 2,3-Pentanedione, Formaldehyde, Acetaldehyde, Acrolein, Crotonaldehyde, Butyraldehyde, Furfural
Glycidol	Glycidol
Aromatic Flavourants	Benzyl Acetate, Ethyl Acetate, Ethyl Acetoacetate, Isoamyl Acetate, Isobutyl Acetate, Methyl Acetate, N-butanol
Toxic Trace Metals	Chromium, Iron, Nickel, Copper, Zinc, Arsenic, Cadmium, Tin, Silver, Lead, Beryllium, Cobalt, Selenium, Gold
Volatile Organic Compounds	Acrylonitrile, Benzene, Toluene, Propylene Oxide, 1,3-Butadiene, Isoprene
Nicotine Degradants	β-Nicotyrine, Anabasine, Anatabine, Cotinine, Myosmine, Nicotine-N-Oxide, Nornicotine
Tobacco Specific Nitrosamines	NNK, NNN
Organic Acids	Propionic Acid, Benzoic Acid
pH	pH

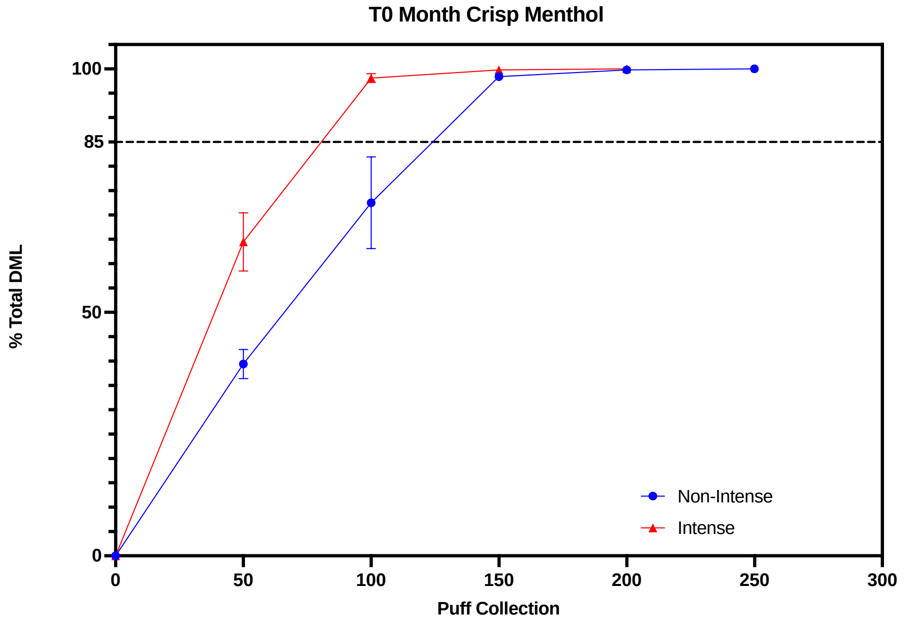
[2] US Dept HHS, 2019, Premarket Tobacco Product Applications for Electronic Nicotine Delivery Systems (ENDS)
[3] Directive 2014/40/EU of the European Parliament and of the Council.
[4] CORESTA Recommended Method No. 81-Routine Analytical Machine for E-Cigarette Aerosol Generation and Collection-Definitions and Standard Conditions, June 2015.

Collection Approach: End-Of-Pod Life Assessments

- To account for emission consistency (i.e., constituents that may change over the life of the pod)[5], end-of-pod life assessments were conducted at each time point across non-intense and intense puffing regimes to determine the average number of puffs required to reach 85 % of total device mass loss for JUUL2 device and Virginia Tobacco and Crisp Menthol JUULpods.

Average # of Puffs Per Regime (T0-T12)				
Regime	Virginia Tobacco		Crisp Menthol	
	Mean	SD	Mean	SD
Non-Intense	134	13	118	5
Intense	85	2	84	3





[5] Selected Harmful and Potentially Harmful Constituents Levels in Commercial e-Cigarettes Maxim Belushkin, Donatien Tafin Djoko, Marco Esposito, Alexandra Korneliou, Cyril Jeannet, Massimo Lazzerini, and Guy Jaccard, Chemical Research in Toxicology 2020 33 (2), 657-668. DOI: 10.1021/acs.chemrestox.9b00470

Results

Measured constituents present at levels above the limit of quantification (T0-T12)

Origin	Constituents			
	Virginia Tobacco (Non-Intense)	Virginia Tobacco (Intense)	Crisp Menthol (Non-Intense)	Crisp Menthol (Intense)
Primary Constituents	Benzoic Acid Glycerol Nicotine Propylene Glycol Water	Benzoic Acid Glycerol Nicotine Propylene Glycol Water	Benzoic Acid Glycerol Nicotine Propylene Glycol Water Menthol	Benzoic Acid Glycerol Nicotine Propylene Glycol Water Menthol
Thermal Degradants	Acetaldehyde Acrolein Formaldehyde Glycidol	Acetaldehyde Acrolein Formaldehyde` Glycidol	Acetaldehyde Formaldehyde Glycidol	Acetaldehyde Acrolein Formaldehyde Glycidol
Nicotine Degradants	β-Nicotyrine Cotinine Myosmine Nicotine-N-Oxide Nornicotine	β-Nicotyrine Cotinine Myosmine Nornicotine	β-Nicotyrine Myosmine Nicotine-N-Oxide Nornicotine	β-Nicotyrine Myosmine Nicotine-N-Oxide Nornicotine

*1,3-Butadiene, 1-Butanol, Acrylonitrile, Benzene, Ethyl Acetate, Ethyl Acetoacetate, Gold, Isoamyl Acetate, Isobutyl Acetate, Isoprene, NNN, NNK, Propylene Oxide, and Toluene were not present at levels above limit of quantification at T0, therefore, were removed for subsequent time points (T3-T12)

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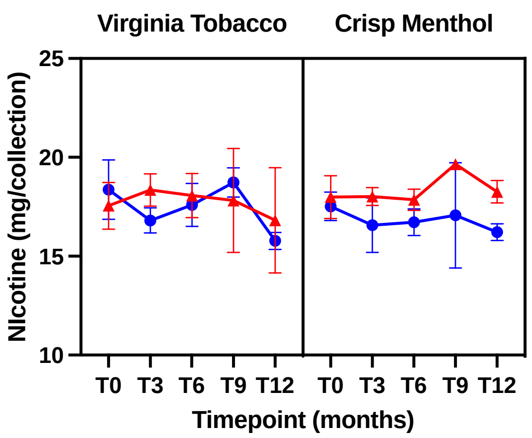
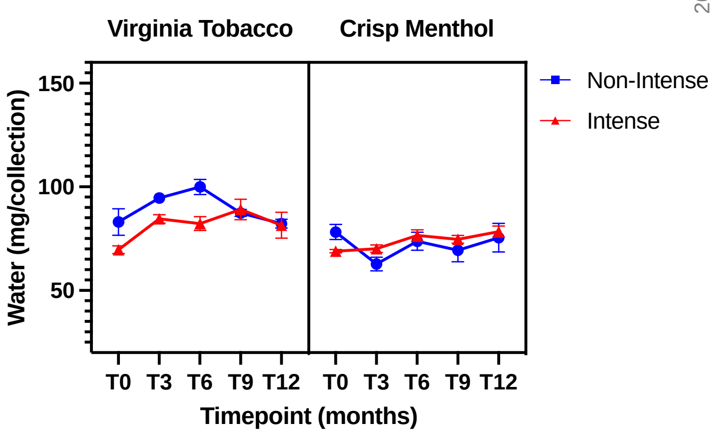
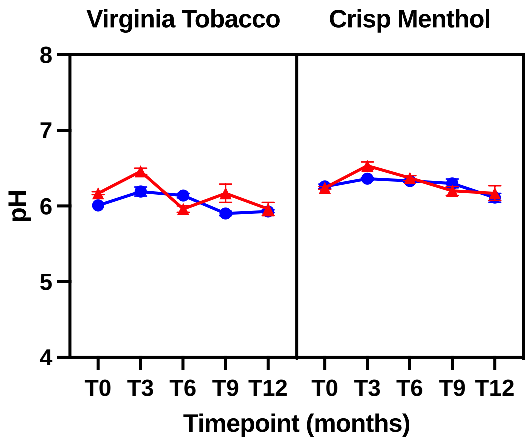
Primary Constituents and pH

Parameter/ Constituent	% Difference (T0 vs T12) Virginia Tobacco		% Difference (T0 vs T12) Crisp Menthol	
	Non-Intense	Intense	Non-Intense	Intense
Benzoic Acid*	NA	NA	NA	NA
Glycerol	-2.0 %	5.5 %	-4.1 %	7.9 %
Menthol	NA	NA	-9.2 %	-1.9 %
Nicotine	-14.1 %	-4.2 %	-7.4 %	1.5 %
pH	-1.3 %	-3.4 %	-2.4 %	-1.1 %
Propylene Glycol	-10.9 %	-1.1 %	-2.4 %	9.4 %
Water	-0.94 %	17.0 %	-3.5 %	13.7 %

*Benzoic Acid % Difference not available due to measurement discrepancy at T0





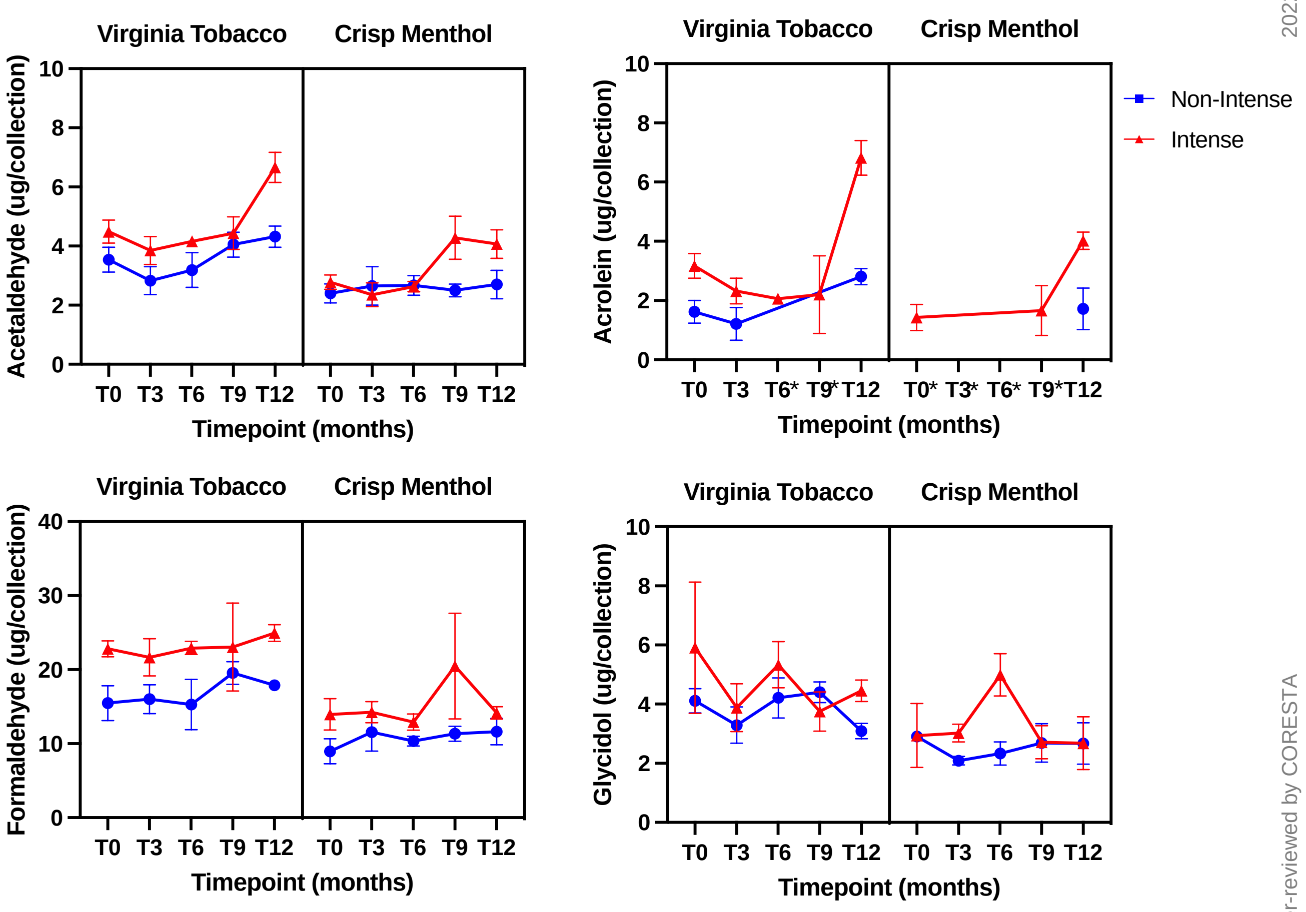


Thermal Degradants

Parameter/ Constituent	% Difference (T0 vs T12)			
	Virginia Tobacco		Crisp Menthol	
	Non-Intense	Intense	Non-Intense	Intense
Acetaldehyde	22.0 % (≥ 99.9 %**)	48.3 % (≥ 99.9 %**)	12.5 % (≥ 99.9 %**)	46.4 % (≥ 99.9 %**)
Acrolein	73.5 % (≥ 99.8 %**)	115.1% (≥ 99.6 %**)	NA (≥ 99.9 %**)	181.1 % (≥ 99.8 %**)
Formaldehyde	15.5 % (≥ 97.0 %**)	9.4 % (≥ 96.6 %**)	29.8 % (≥ 98.0 %**)	1.3 % (≥ 97.6 %**)
Glycidol	-24.8 % (NA***)	-24.7 % (NA***)	-7.9 % (NA***)	-8.8 % (NA***)

**Comparison of nicotine normalized (mg/mg) thermal degradant levels in Virginia Tobacco and Crisp Menthol formulations (18 mg/mL) aerosol levels to 3R4F reference cigarette[6]

*** No 3R4F Glycidol literature comparator data available, comparisons to glycidol not applicable (NA)

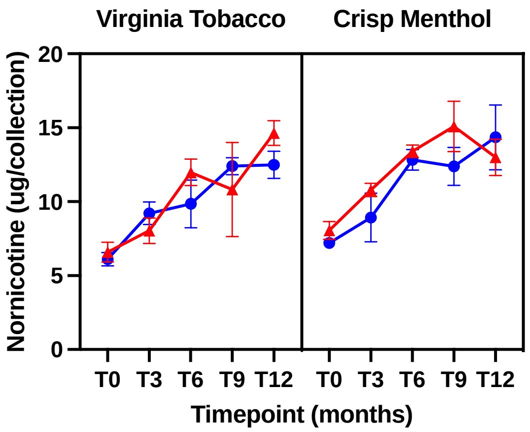


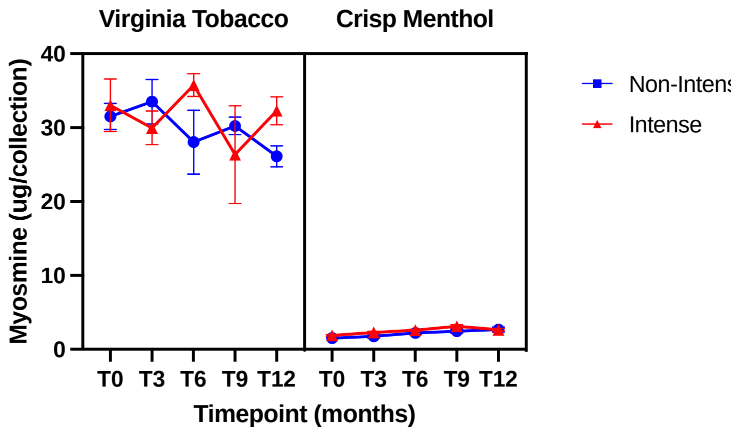
*Acrolein below limits of quantitation for select time points

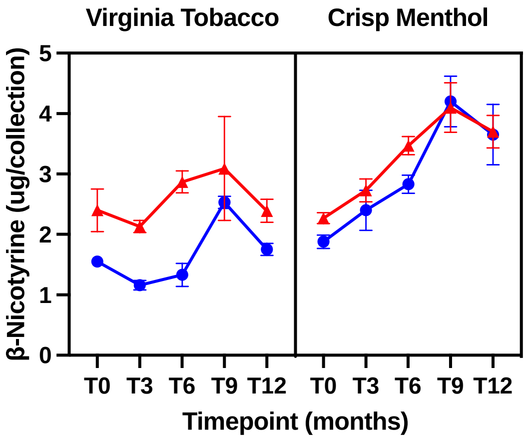
[6]Jaccard G, Djoko D, Korneliou A, Stabbert R, Belushkin M, Esposito M. Mainstream smoke constituents and in vitro toxicity comparative analysis of 3R4F and 1R6F reference cigarettes. Toxicology Reports. 2019;6:222-231. smoke under non-intense and intense puffing/smoking conditions

Nicotine Degradants

Parameter/ Constituent	% Difference (T0 vs T12)			
	Virginia Tobacco		Crisp Menthol	
	Non-Intense	Intense	Non-Intense	Intense
β-Nicotyrine	12.9 %	-0.42 %	94.2 %	63.0 %
Cotinine	3.5 %	20.6 %	NA	NA
Myosmine	-17.2 %	-2.3 %	75.8 %	42.3 %
Nicotine-N-Oxide	NA	NA	10.4 %	-5.3 %
Nornicotine	104.8 %	122.2 %	99.0 %	61.3 %







Additional Considerations

Study Design

- *A Priori* acceptance criteria
- Packaging configurations
- Climactic zones
- Regulatory Requirements

Data Collection

- Scientific rationale and evidence may support reduced measurement frequency for select constituents[7].
- Room air blanks should be incorporated for low level analytes[8].

Interpretation

- Data analysis procedures must be consistent when accounting for mixed results (i.e. below LOD, between LOD and LOQ, greater than LOQ) [7].
- Higher measurement error present when evaluating concentrations close to detection limits.

[7] Wagner KA, Flora JW, Melvin MS, Avery KC, Ballentine RM, Brown AP, McKinney WJ. An evaluation of electronic cigarette formulations and aerosols for harmful and potentially harmful constituents (HPHCs) typically derived from combustion. Regul Toxicol Pharmacol. 2018 Jun;95:153-160. doi: 10.1016/j.yrtph.2018.03.012. Epub 2018 Mar 20. PMID: 29567331.
[8] Plunkett et al. "MarkTen® E-Vapor Stability Study Design and Preliminary Data" 2017 TSRC Poster

Summary

- Nicotine, pH, water and other primary constituents remained consistent, exhibiting no more than a 20% change from T0 levels.
- Thermal Degradants increased, except Glycidol which slightly decreased.
- Relative to measured nicotine concentration, Nicotine Degradants remained below United States Pharmacopeia (USP) percent impurity limit [9].

[9] United States Pharmacopeia and the National Formulary. USP 41; The United States Pharmacopeial Convention Inc.: Rockville, MD, USA, 2018.

Conclusion

- Results support at least a 12-month shelf life for JUUL2 Virginia Tobacco (18 mg/mL) and Crisp Menthol (18 mg/mL) JUULpods.

- Despite increases in thermal degradants (Acetaldehyde, Acrolein, and Formaldehyde) over the 12-month stability study, on average, a ≥ 99 % reduction in yields relative to 3R4F cigarette smoke[10] was found across non-intense and intense puffing regimen.

[10] Chen, X.; Bailey, P.C.; Yang, C.; Hiraki, B.; Oldham, M.J.; Gillman, I.G. Targeted Characterization of the Chemical Composition of JUUL Systems Aerosol and Comparison with 3R4F Reference Cigarettes and IQOS Heat Sticks. Separations 2021, 8, 168. https://doi.org/10.3390/separations8100168

Thank you and Questions