In Vitro Mutagenicity Evaluation of Commercial JUUL Product E-Liquids and Aerosol Condensates

Jenny Yao[1], Kubilay Demir[1], Utkarsh Doshi[2], Candace Laxamana[1], K. Monica Lee[2], Guy Lalonde[1]

[1]JUUL Labs Inc., San Francisco, CA, USA, [2]Altria Client Services LLC, Richmond, VA, USA

Introduction

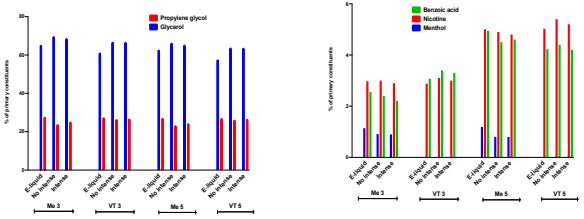
In it's "Guidance for Industry: Premarket Tobacco Product Applications for Electronic Nicotine Delivery Systems" (FDA 2019), the Food and Drug Administration recommends applicants to provide information regarding studies assessing toxicology: in Section IV(H)(2)(A), "a full assessment of the toxicological and pharmacological profile associated with the new tobacco products In this study, four JUUL ENDS products were evaluated in in vitro Ames mutagenicity using product-specific e-liquids and aerosol condensates in accordance with OECD TG471. Results from the ENDS condensate in vitro studies were compared to those from the 3R4F Kentucky reference cigarette condensate.

Methods

- **Test articles**: JUUL ENDS: Virginia Tobacco 3% nicotine (VT3), Menthol 3% nicotine (ME3), Virginia Tobacco 5% nicotine (VT5), Menthol 5% nicotine (ME5) and 3R4F reference cigarette (University of Kentucky).
- **E-liquid collection:** The JUUL e-liquid samples were obtained by partially disassembling pods and collecting the fluid by centrifugation.
- **JUUL ENDS Condensate Collection:** Two types of condensates were prepared for each of the four JUUL products tested: 1. a "non-intense" puffing regimen based on ISO 20768 (55 mL puff volume over 3 seconds with a 30 second interval between puffs), and 2. an "intense" puffing regimen (defined by the longest puff duration possible (6 seconds) given the design of the JUUL Device), 110 mL puff volume over 6 seconds with a 30 second interval between puffs. Condensate was generated by collecting aerosol on a non-conditioned Cambridge Filter pad (CFP, 55mm glass fiber filters, Cerulean (USA) followed in series by an impinger containing 20 ml of USP ethanol chilled in an ice bath (~0°C). The ethanol from the impinger was used to extract the pad to produce the condensate solution. Devices were puffed using linear puffing machines to 100 puffs/device for the non intense regimen (i.e., equivalent 300 puffs/port), and 50 puffs/device for the intense regimen (i.e., equivalent to 150 puffs/port). The final condensate concentration was ~60 mg/mL of aerosol collected mass (ACM). The e-liquids and condensates were analyzed for nicotine, menthol, propylene glycol (PG), glycerol (VG) and benzoic acid immediately after collection and, in the case of condensates, at several time points up to 8 weeks of storage at ≤ -70°C.
- **3R4F Condensate Collection:** 3R4F reference cigarettes were conditioned prior to testing. Mainstream cigarette smoke was generated using a rotary smoking machine and as per ISO 20778-2018 intense smoking regimen. A total of six smoke collections were performed and pooled for analysis, with one collection representing 20 cigarettes (2 smoking runs of 10 cigarettes/run). Smoke was passed through a conditioned 92mm Cambridge Filter Pad (CFP) connected in series to an impinger filled with 30 mL USP grade ethanol chilled in an ice bath (~0°C). The CFP was extracted with impinger contents to produce the condensate (concentration of ~ 25 mg TPM/mL in ethanol). The condensate was analyzed for the compounds listed in Table 2 immediately after collection and at several time points up to 8 weeks of storage at ≤ -70°C.
- **Ames Assay:** The mutagenicity of e-liquids and condensates was evaluated in *Salmonella typhimurium* strains TA98, TA100, TA102, TA1535, and TA1537 with and without an enzymatic metabolizing fraction (S9) using the pre-incubation procedure, as per the OECD TG471 and under GLP guideline. E-liquid and condensate samples were tested to a concentration up to 100µl/plate. All experiments were performed in triplicate. Ethanol and DMSO were used as vehicle controls for condensates and e-liquids, respectively.

Analytical Results: Juul ENDS

Figure 1. Concentrations of primary constituents in e-liquid compared to those in non-intense and intense condensates



Concentrations of primary constituents in condensates expressed as percentage of ACM are similar to those in e-liquid.

Table 1. Percentage (mean ± sd) of primary aerosol constituents in JUUL ENDS aerosol condensates following 8 weeks of storage (at ≤ -70°C) relative to Time 0.

% Compound in Condensate Remaining @ 8 Weeks Relative to Time 0					
Propylene Glycol	Non Intense	100.8 ± 0.3	98.1 ± 0.6	103.8 ± 0.4	102.5 ± 0.8
	Intense	101.5 ± 0.5	95.9 ± 0.6	103.1 ± 0.6	102.8 ± 0.6
Glycerol	Non Intense	100.5 ± 0.4	101.1 ± 0.5	98.6 ± 0.5	98.6 ± 0.9
	Intense	100.2 ± 0.5	101.7 ± 0.6	98.4 ± 0.7	99.3 ± 0.7
Nicotine	Non Intense	103.2 ± 0.1	98.2 ± 0.1	103.3 ± 0.1	108.1 ± 0.2
	Intense	103.3 ± 0.1	98.1 ± 0.1	103.4 ± 0.1	106.2 ± 0.1
Menthol	Non Intense			100 ± 0.0	100 ± 0.0
	Intense			100 ± 0.0	100 ± 0.0
Benzoic acid	Non Intense	82.4 ± 0.4	97.7 ± 0.4	95.8 ± 0.2	88 ± 0.3
	Intense	82 ± 0.3	102.3 ± 0.5	104.5 ± 0.2	87 ± 0.3

No major changes in the concentrations of primary ingredients in JUUL ENDS condensates were observed for the duration of biological testing (up to 8 wks: (>82%)

Analytical Results: 3R4F

Table 2. Selected analyte concentrations in 3R4F smoke condensate after 8 weeks of storage at ≤ -70°C

Concentrations of Selected Analytes in 3R4F Condensate Measured at Time 0 and % Remaining @ 8 Weeks		
Compound (units)	Concentration	% Remaining at 8 weeks relative to Time 0
	Mean (SD)	%
Nicotine (mg/cig)	1.29 (0.08)	100.8 (0.02)
1,3-Butadiene (ug/cig)	11.1 (0.64)	82.4 (0.64)
Acetonitrile (ug/cig)	24.3 (0.7)	104.9 (0.7)
Benzene (ug/cig)	98.2 (2.2)	98.8 (2.2)
Isoprene (ug/cig)	473.3 (2.2)	84.8 (19.5)
Toluene (ug/cig)	183.7 (8.26)	108.5 (8.3)
Acetaldehyde (ug/cig)	942.8 (19.3)	93.8 (19.3)
Acrolein (ug/cig)	48.5 (27.2)	83.7 (27.2)
Crotonaldehyde (ug/cig)	26.4 (2.9)	114.1 (3.0)
Formaldehyde (ug/cig)	45.5 (3.3)	93.8 (19.3)

No major changes in the concentrations of tested compounds in 3R4F condensates were observed for the duration of biological testing (up to 8 wks: (>~84%))

AMES Test Results

Mutagenicity of 3R4F Condensate and of JUUL ENDS Non-Intense & Intense Condensates

	Presence S9	Absence S9
3R4F	Positive (TA1537, TA98, TA100)	Negative
VT3	Negative	Negative
VT5	Negative	Negative
ME3	Negative	Negative
ME5	Negative	Negative

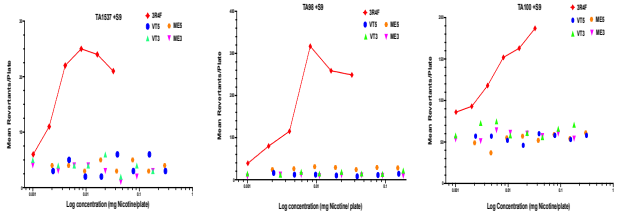


Figure 2 : Comparison of JUUL ENDs Non-Intense and 3R4F condensate in the Ames test . Dose dependent increases of revertants were observed for 3R4F condensate in strains TA98, TA100 and TA1535 in presence of metabolic activation (S9) relative to vehicle control.

All JUUL ENDS e-liquids and condensates (both intense and non-intense) were found not mutagenic at the concentrations tested (all data not shown).

Summary and Conclusion

E-liquids & condensates collected from JUUL ENDS and 3R4F condensates were characterized for selected constituents. The concentrations of these constituents were found not to change substantially over the duration of biological testing. The 3R4F smoke condensate treated with S9 metabolic activation mixture was found mutagenic in strains TA98, TA100 and TA1537 at concentrations as low as 0.01mg Nicotine/plate. In contrast, the e-liquid and the condensates from all JUUL ENDS were negative in all strain tested, up to the highest nicotine concentrations; 0.3 mg Nicotine /plate. In summary, the four JUUL END S e-liquids and aerosol condensates were not found mutagenic under the tested conditions.

References

D M Maron, BN Ames 1983 Revised methods for the Salmonella mutagenicity test. Mutat Res, 113(3-4):173-215

Utkarsh Doshi, K. Monica Lee et al. Society of Toxicology 57th Annual Meeting, March 11 - 15, 2018, San Antonio, TX, USA

Health Canada Official Method T-115: December 1999. Determination of "Tar", Nicotine and Carbon Monoxide in Mainstream Tobacco Smoke

Organization for Economic Co-operation and Development, 1997. Test No. 471: Bacterial Reverse Mutation Test. OECD Publishing.