Development and Characterization of an Alternative Rotary-like ENDS Collection Method for in vitro Toxicology Testing

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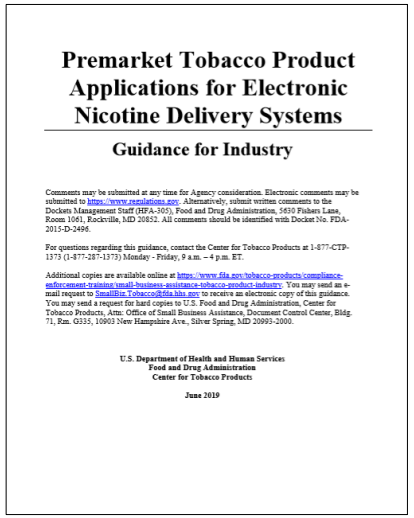
Background- OECD *In Vitro* Toxicology Testing of E-vapor

- FDA PMTA ENDS Final Guidance recommends evaluating products for appropriate hazard identification and the use of OECD.
- Limited by OECD guidelines for the maximum allowable solvent levels (ie. Neutral Red Uptake (NRU) assay is limited to 0.5 % of vehicle (for organic solvents).
- Also limited by the final concentration of the generated aerosol condensate.
- Standard linear smoking machine collection (Ethanol) yields 60-70 mg/ml, with maximum NRU testing dose of 300 µg/mL.
- Testing of higher doses requires exceeding OECD guideline solvent limits or generating more concentrated condensates.



Premarket Tobacco Product Applications for Electronic Nicotine Delivery Systems

Guidance for Industry

Comments may be submitted at any time for Agency consideration. Electronic comments may be submitted to https://www.regulations.gov. Alternatively, submit written comments to the Dockets Management Staff (HFA-305), Food and Drug Administration, 5630 Fishers Lane, Room 1061, Rockville, MD 20852. All comments should be identified with Docket No. FDA-2015-D-2496.

For questions regarding this guidance, contact the Center for Tobacco Products at 1-877-CTP-1373 (1-877-287-1373) Monday - Friday, 9 a.m. – 4 p.m. ET.

Additional copies are available online at https://www.fda.gov/tobacco-products/compliance-enforcement-training/small-business-assistance-tobacco-product-industry. You may send an e-mail request to SmallBiz.Tobacco@fda.hhs.gov to receive an electronic copy of this guidance. You may send a request for hard copies to U.S. Food and Drug Administration, Center for Tobacco Products, Attn: Office of Small Business Assistance, Document Control Center, Bldg. 71, Rm. G335, 10903 New Hampshire Ave., Silver Spring, MD 20993-2000.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Tobacco Products

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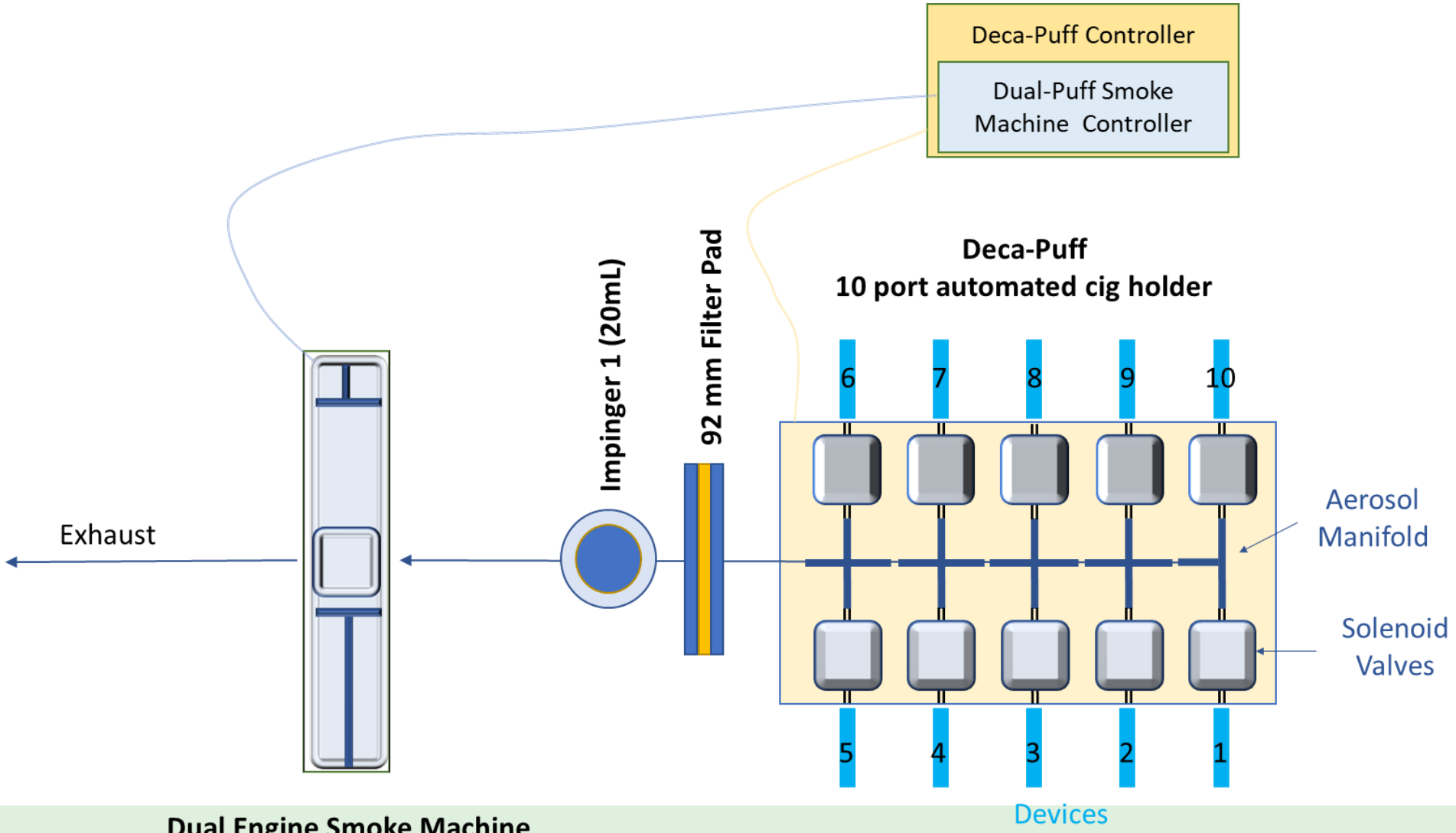
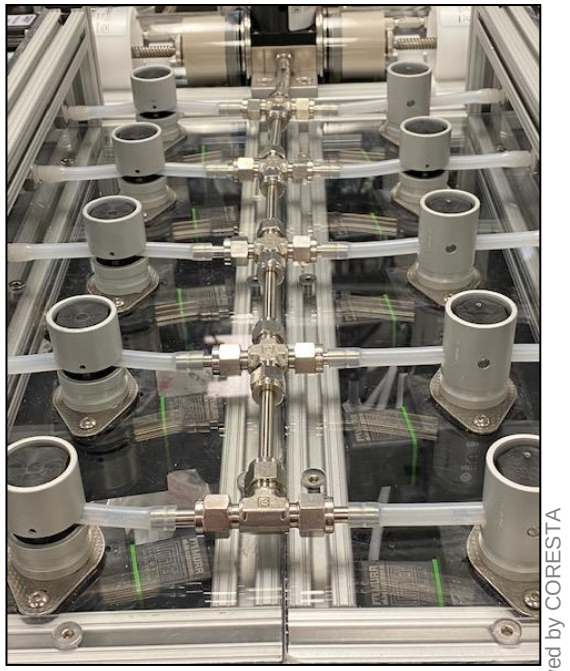
Objective: Develop Rotary Like ENDS Vaping Machine



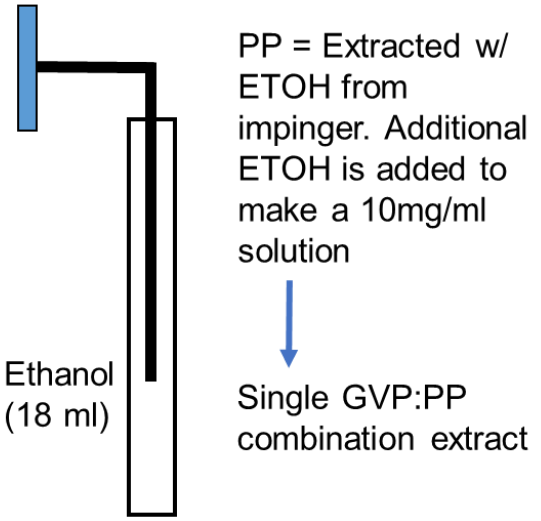
Methods- Deca-puff Aerosol Collections

- Dual engine smoke machine and aerosol captured onto a 92 mm pad (~200 mg/ml).
- The impinger contains ethanol (20 mL) which is also used to extract the CFP, generating a GVP:PP extract.
- Commercially available e-liquids (Tobacco and Menthol flavors) and devices with an output of ~5-6 mg/puff were used.
- Puffing conducted under non-intense (55/3/30) and intense (110/6/30) regimes.
- Nicotine and carbonyl levels measured from 10 pods, vaped for 100 puffs each (1000 puffs total) for a target ACM > 4300 mg.
- Condensate concentration was normalized to 200 mg/mL.



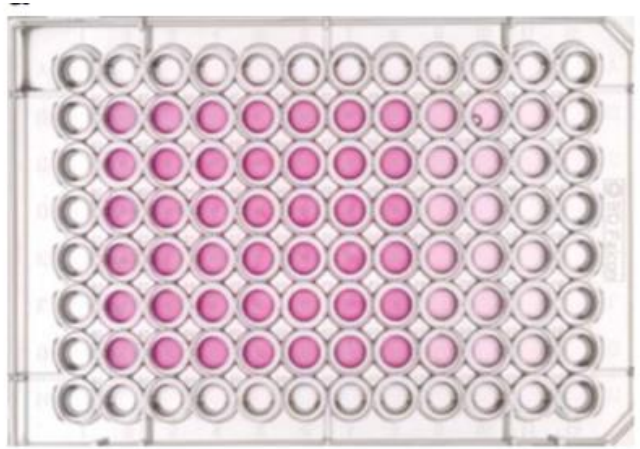
Methods- Standard Linear Aerosol Collections

- Collection of ~1200 mg on a 44 mm pad and aerosol condensates generated.
- Commercially available e-liquids (Tobacco and Menthol flavors) and devices with an output of ~5-6 mg/puff were used.
- Puffing conducted under non-intense (55/3/30) and intense (110/6/30) regimes.
- Nicotine and carbonyl levels measured from a single pod, vaped for 200 puffs until 1200-1300 mg of ACM collected.
- Condensate concentration was normalized to 60 mg/mL.

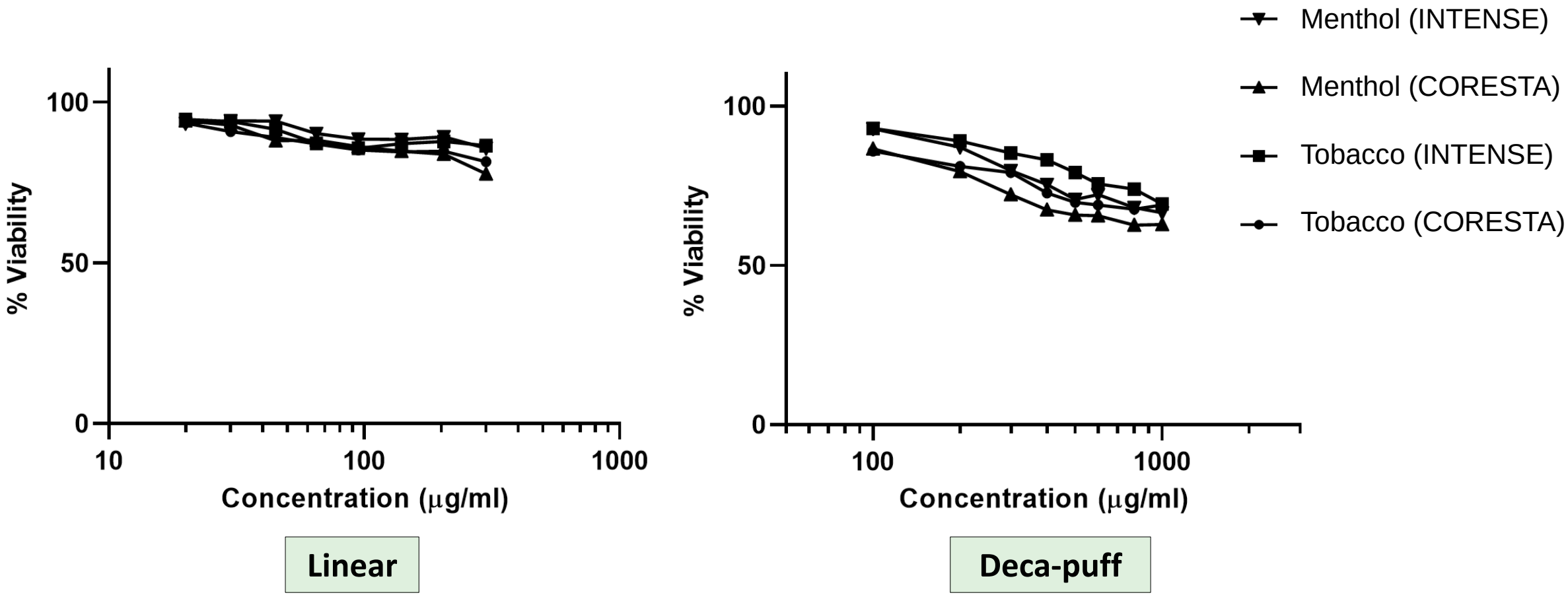


Methods- Neutral Red Uptake Assay

- Balb/c 3T3 (~3,000) or A549 (~10,000) cells were incubated in the presence of:
  - vehicle control (USP Ethanol).
  - positive control (sodium lauryl sulfate).
  - collected aerosol condensates.
- 48-hour exposure according to OECD 129
  - Ethanol limit in the dosing medium was kept at 0.5 % for all tests.



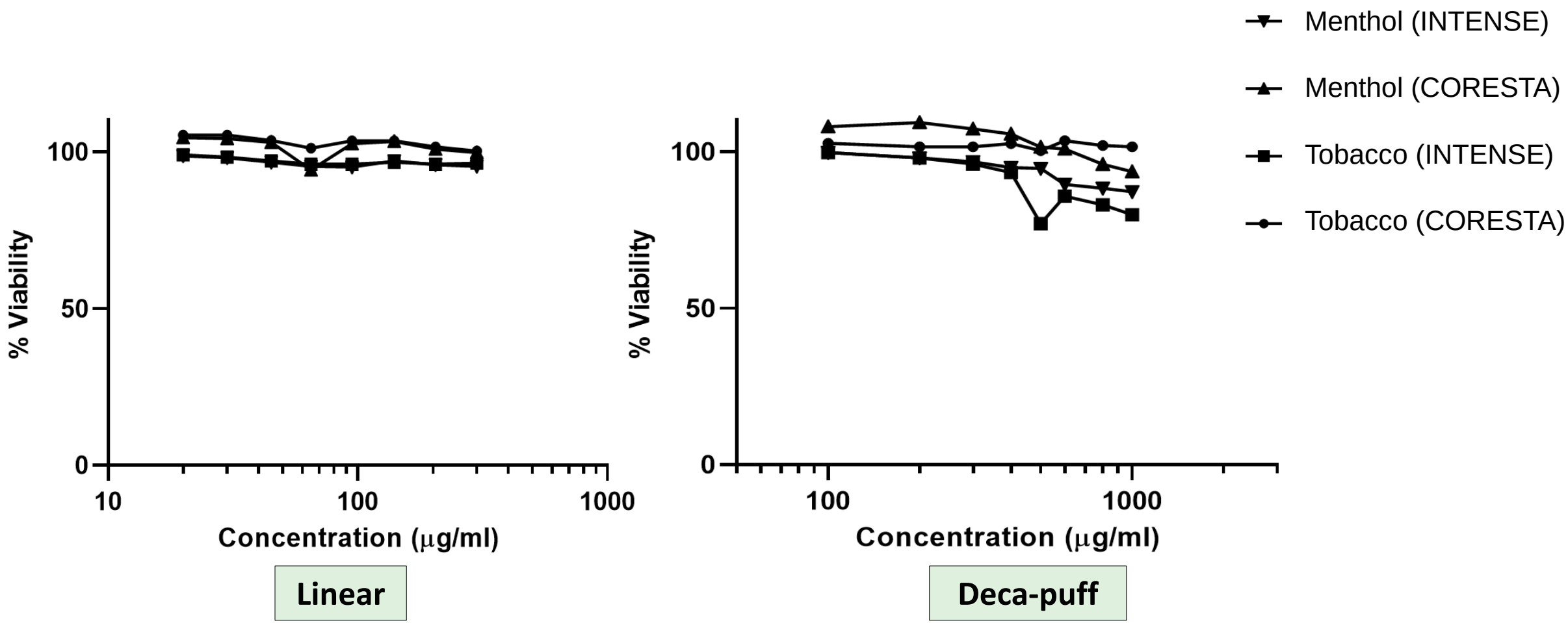
Results- Linear smoking vs. Decapuff in Balb/c 3T3



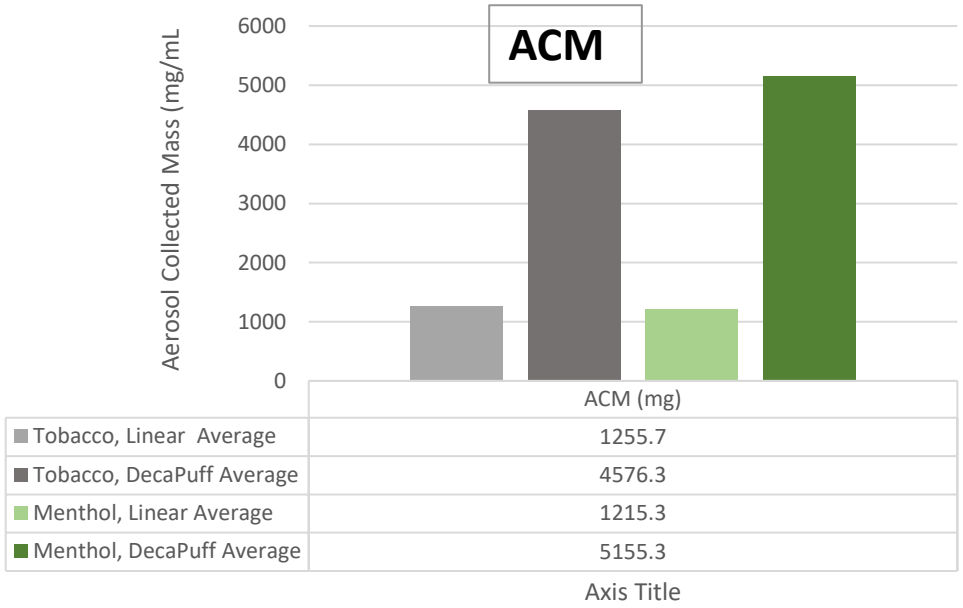
Linear

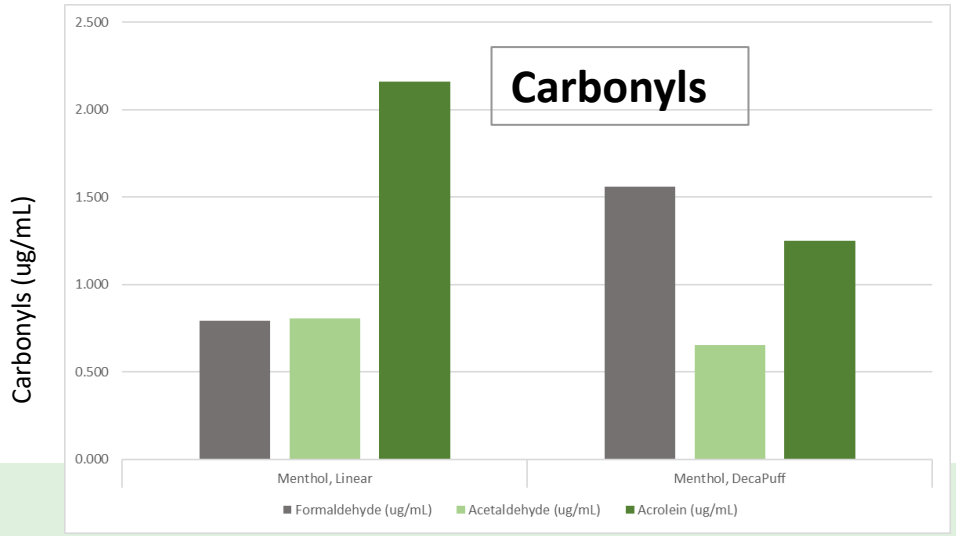
Deca-puff

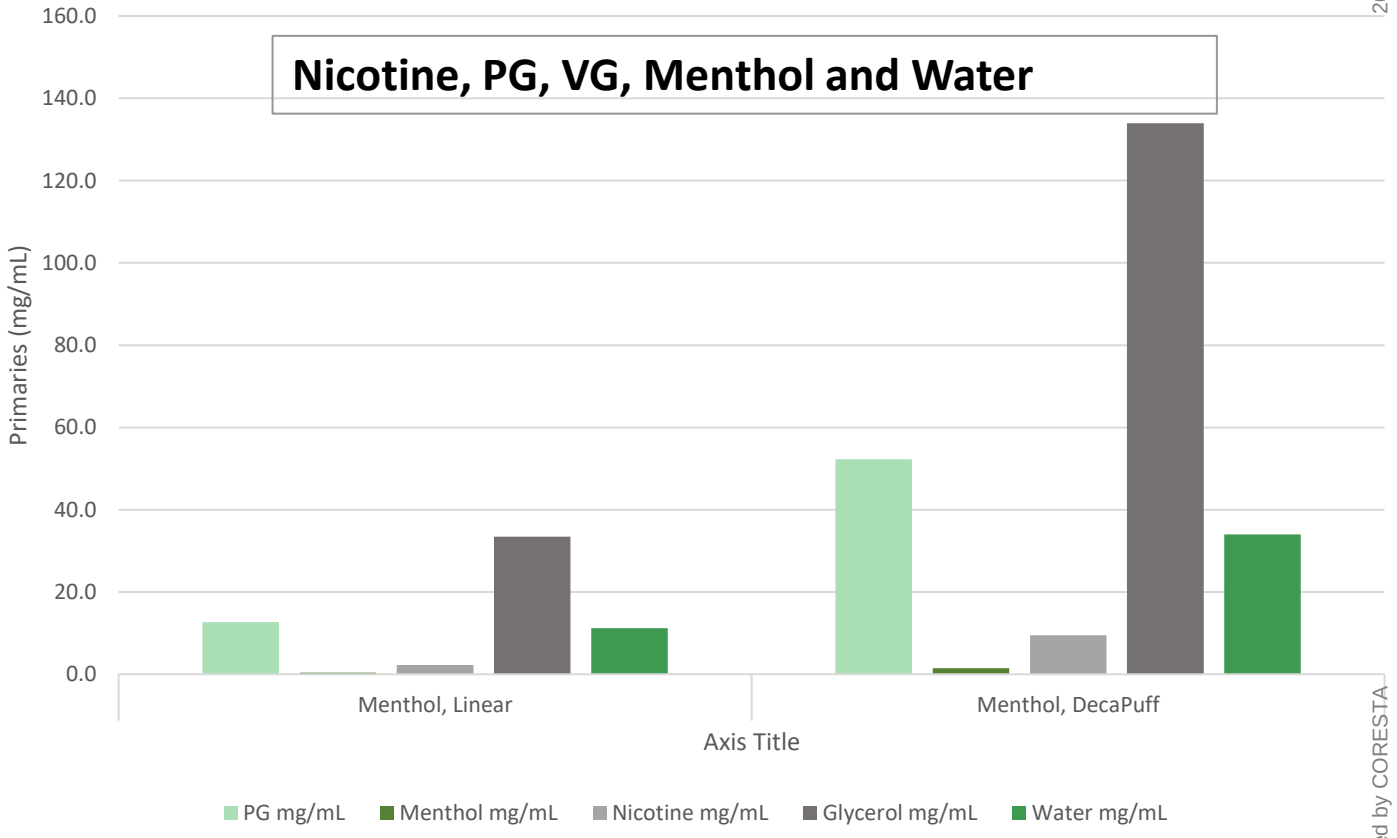
Results- Linear vs. Decapuff in A549 cells



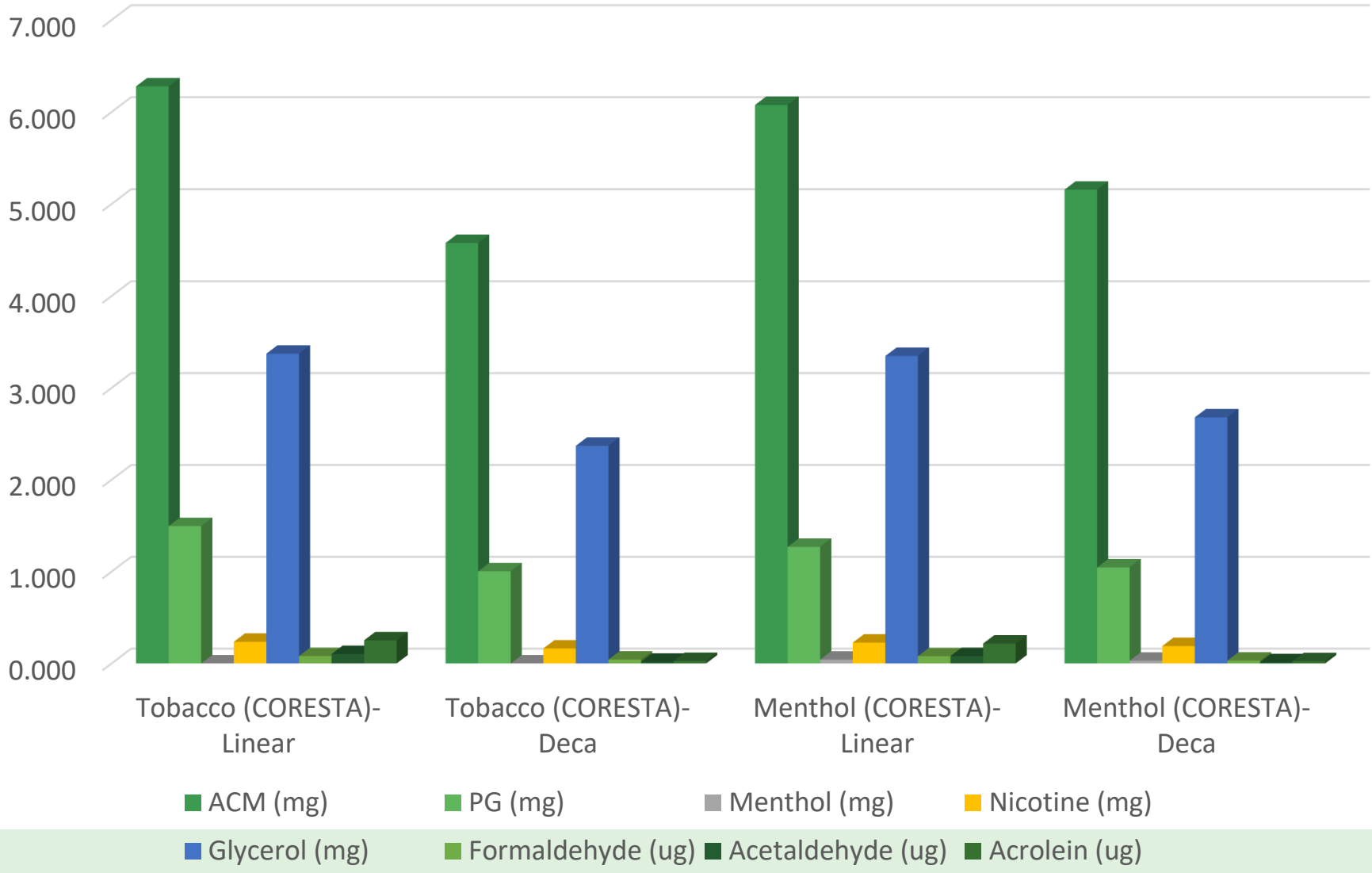
Chemical Analysis of Condensates (per mL) - CORESTA







Chemical Analysis of Condensates (per puff)



Conclusions

- Aerosol collection using the Deca-puff method allowed for collection times that were faster (50 min versus 500 min) and resulted in more concentrated condensates when compared to the standard linear smoking machine collection (~60 vs 200 mg/mL).
- Nicotine, PG, VG, and Menthol total amounts collected were comparable between the two collection methods when normalized on a puff basis.
- However, the selected carbonyls measured were either similar or lower in the Deca-puff collection compared to the linear collection. This may be due to the number of puffs per pod taken (200 for linear vs 100 for deca-puff).
- Differences in cell line specific cytotoxicity was observed in the NRU assay.
- This method is a viable alternative for more concentrated condensate collections.

Q&A Session

Email your questions

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