

# Neutral Red Uptake (NRU) Cytotoxicity Analysis of Aerosol Generated from a Temperature-Regulated Nicotine Salt Pod System Utilizing Cotton Wicking Material

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## Introduction

The JUUL Nicotine Salt Pod System (NSPS) is temperature-regulated to minimize the generation of combustion related degradation byproducts in the aerosol. The NSPS Pods contain 5 major ingredients: nicotine, benzoic acid, glycerol, propylene glycol, and flavorants. At present, formulations in 18 mg/mL nicotine concentration are available in ex-US markets. Pods utilizing cotton wicking material to conduct e-liquid for aerosolization were evaluated for cytotoxic potential over a range of dosing concentrations relative to a 3R4F Kentucky Reference Cigarette. The Neutral Red Uptake (NRU) *in vitro* assay assessed the relative toxicity in CHO-WBL cell populations following 24-hour exposure to NSPS aerosol and 3R4F smoke using validated methodologies at an accredited ISO-17025 3<sup>rd</sup> party laboratory (Labstat International).

## Materials and Methods

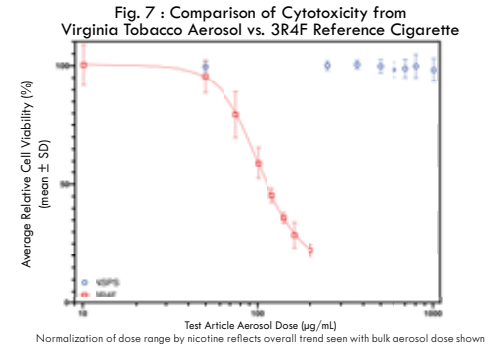
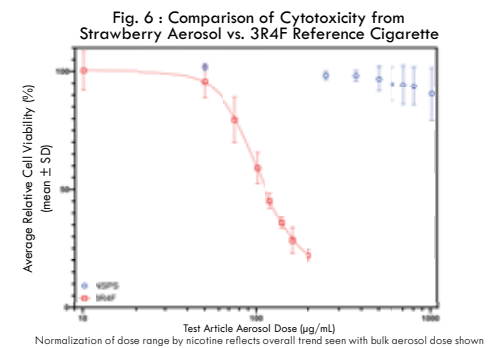
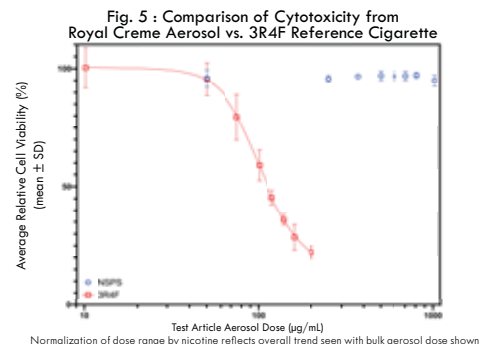
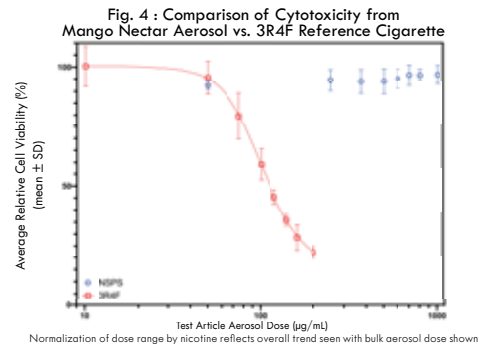
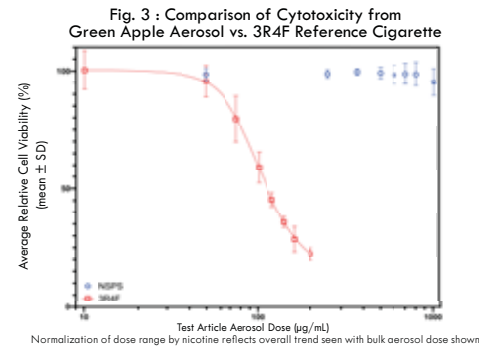
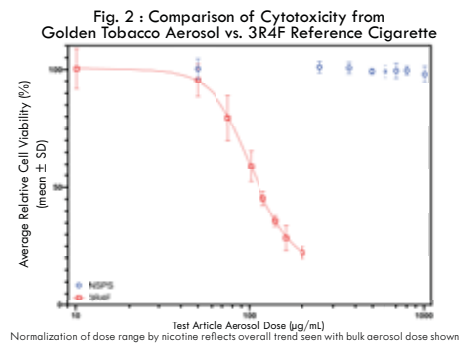
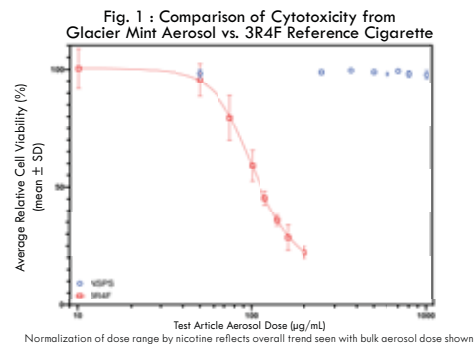
Cytotoxic potential was evaluated using the NRU *in vitro* assay, according to OECD guidelines<sup>1</sup>. CHO-WBL cells were treated with NSPS aerosol condensates, positive control, and vehicle control for 24 hours utilizing a Dimethyl sulfoxide (DMSO) and Calcium Magnesium Free Phosphate Buffer Saline (CMF-PBS) solution extraction methodology<sup>2</sup>. NSPS aerosol was tested for seven different formulations: Glacier Mint, Golden Tobacco, Green Apple, Mango Nectar, Royal Creme, Strawberry, Virginia Tobacco, all at a nicotine concentration of 18 mg/mL.

NSPS with cotton wicking material aerosol collection was prepared under a modified CORESTA recommended method<sup>3</sup>. Collection was performed on a Rotary Smoking Machine using a square wave, 70 mL volume puffed over 3 seconds across 30 second intervals. Generated aerosol was collected on a Cambridge filter pad followed in series by an impinger constituted with CMF-PBS. NSPS aerosol exposure thus consisted of both pad and impinger collections extracted in equal volumes of DMSO and CMF-PBS respectively, to final concentrations of approximately 50 mg/mL. Assay dosing was conducted for combined aerosol collected mass (ACM) and gas vapor phase (GVP) across a concentration range of (0-1000 µg/mL).

Cell viability following NSPS aerosol exposure was subsequently compared against the combined mainstream Total Particulate Matter (TPM) and GVP from 3R4F Kentucky reference cigarettes, following similar sample preparation as NSPS, at a dose range of (0-200 µg/mL) extracted in DMSO. 3R4F cigarette samples were prepared under the Health Canada defined "intense puffing regime" characterized as a 55 mL puff volume over a 2 second draw with a 30 second puff interval. Additionally, the cells were exposed to the positive control, SLS (Sodium Lauryl Sulfate) [CAS No. 151-21-3] at a single dose concentration of 110 µg/mL, a physiological representative dose validated by the accredited 3<sup>rd</sup> party lab (Labstat).

## Results

Study results are presented as the relative percent cell viability and standard deviation (±SD) of the combined ACM and GVP of each test article relative to its respective vehicle control. At the doses tested, EC<sub>50</sub> for the NSPS aerosol and carrier control aerosol could not be calculated because cell viability was greater than 50% at all concentrations tested. Additionally, TPM from the 3R4F reference cigarette demonstrated expected toxicity with a calculated EC<sub>50</sub> of 100.4 µg/mL with an r<sup>2</sup> of 0.97.



## Conclusion

Under the experimental conditions and based on the criteria for Evaluation of Cytotoxic Response (ISO 10993-5), NSPS aerosol condensates generated from the 18mg/mL NSPS test articles were found to be non-cytotoxic.

## References

1. OECD, "Guidance Document on Using Cytotoxicity Tests to Estimate Starting Doses For Acute Oral Systemic Toxicity Tests" (TG 129), July 2010.
2. Labstat International, "Determination of Cytotoxic Response of Mainstream Aerosol Collected Matter and Mainstream Gas Vapor Phase of Electronic Cigarettes and E-Liquids by Neutral Red Uptake Assay", Dec 2018.
3. CORESTA, "Routine Analytical Machine For E-Cigarette Aerosol Generation And Collection - Definitions And Standard Conditions" (Method No. 81), June 2015.
4. M. Misra, G. Gilman, P. Desai, "Neutral Red Uptake (NRU) Cytotoxicity Analysis of Aerosol Generated From a Temperature-Regulated Nicotine-Salt Based Ends Product", Dec. 2018.