In Vitro Cytotoxicity of Commercial JUUL® Product E-Liquids and Aerosol Condensates

Kubilay Demir1, Utkarsh Doshi1, Candace Laxamana1, Jenny Yao1, Ramsey Atallah2, K. Monica Lee1, Guy Lalonde1

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

Abstract
To help understand the health risks associated with the JUUL Electronic Nicotine Delivery System (ENDS) products, four JUUL ENDS products and the reference 3R4F cigarette were tested for cytotoxicity in the invitro medium red uptake (NRU) assay. Each JUUL ENDS product was tested as an e-liquid and as aerosol condensates, collected on a Cambridge filter paper followed by an impinger filled with ethanol at 0°C, using intense and non-intense puffing regimens. Cigarette smoke was collected with a similar apparatus and tested as smoke condensate using the ISO 20778:2018 protocol. The NRU assay was conducted in accordance with OECD TG129 using two cell lines: the murine fibroblast BALB/c 3T3 cell line and the human lung adenocarcinoma A549 cell line. No cytotoxicity was observed with the JUUL ENDS e-liquids, condensates (volatile organic compounds, VOCs), and carbonyls. Analysis of primary constituents, VOCLs and carbonyls over a period of eight weeks after collection indicated that the JUUL products had insignificant cytotoxicity relative to vehicle control; ISO 10993-5:2009 (ISO 2009) up to the highest tested concentration.

Introduction
In “Guidance for Industry: Premarket Tobacco Product Applications for Electronic Nicotine Delivery Systems” (FDA 2019), the Food and Drug Administration requires applicants to provide information regarding studies assessing toxicity and, in Section IV(D), “recommends providing a full assessment of the toxicological and pharmacological profile associated with the new tobacco product.” In an effort to address the toxicological profile, the JUUL Labs Inc. nonclinical toxicology program included in vitro cytotoxicity (Neutral Red Uptake Assay [NRU]) evaluation of product-specific e-liquids and aerosol condensates. Results from these in vitro studies are compared to those from the 3R4F Kentucky reference combustible cigarette.

Methods
In vitro toxicological studies were conducted on three types of samples: 1. The e-liquid from JUULpod ENDS; 2. The condensate of the aerosol produced by the JUUL System; 3. The condensate of mainstream smoke (hereafter referred to as smoke) from the JUUL reference cigarette. The four JUUL ENDS products tested were Virginia Tobacco (5.0%, 3.0%) and Menthol (5.0%, 3.0%). The ENDS e-liquids were obtained by partially disassembling pods and collecting the fluid by centrifugation. The condensate samples were prepared by capturing aerosols or smoke through a filter, followed by ethanol extraction of the filter. The four JUUL ENDS products tested were Virginia Tobacco (5.0%, 3.0%) and Menthol (5.0%, 3.0%). The ENDS e-liquids were obtained by partially disassembling pods and collecting the fluid by centrifugation. The condensate samples were prepared by capturing aerosols or smoke through a filter, followed by ethanol extraction of the filter. The four JUUL ENDS products tested were Virginia Tobacco (5.0%, 3.0%) and Menthol (5.0%, 3.0%). The ENDS e-liquids were obtained by partially disassembling pods and collecting the fluid by centrifugation. The condensate samples were prepared by capturing aerosols or smoke through a filter, followed by ethanol extraction of the filter. The four JUUL ENDS products tested were Virginia Tobacco (5.0%, 3.0%) and Menthol (5.0%, 3.0%). The ENDS e-liquids were obtained by partially disassembling pods and collecting the fluid by centrifugation. The condensate samples were prepared by capturing aerosols or smoke through a filter, followed by ethanol extraction of the filter. The four JUUL ENDS products tested were Virginia Tobacco (5.0%, 3.0%) and Menthol (5.0%, 3.0%). The ENDS e-liquids were obtained by partially disassembling pods and collecting the fluid by centrifugation. The condensate samples were prepared by capturing aerosols or smoke through a filter, followed by ethanol extraction of the filter. The four JUUL ENDS products tested were Virginia Tobacco (5.0%, 3.0%) and Menthol (5.0%, 3.0%). The ENDS e-liquids were obtained by partially disassembling pods and collecting the fluid by centrifugation. The condensate samples were prepared by capturing aerosols or smoke through a filter, followed by ethanol extraction of the filter. The four JUUL ENDS products tested were Virginia Tobacco (5.0%, 3.0%) and Menthol (5.0%, 3.0%). The ENDS e-liquids were obtained by partially disassembling pods and collecting the fluid by centrifugation. The condensate samples were prepared by capturing aerosols or smoke through a filter, followed by ethanol extraction of the filter. The four JUUL ENDS products tested were Virginia Tobacco (5.0%, 3.0%) and Menthol (5.0%, 3.0%). The ENDS e-liquids were obtained by partially disassembling pods and collecting the fluid by centrifugation. The condensate samples were prepared by capturing aerosols or smoke through a filter, followed by ethanol extraction of the filter.

Results
E-liquid
Fig 1 Comparison of Relative Viability Between the JUUL System E-Liquids in the NRU Assay Using BALB/c 3T3 Cells and A549 Cells

Non-Intense Condensate
Fig 2 Comparison of Relative Viability Between the Non-Intense JUUL System Condensates and the Reference 3R4F Smoke Condensate in the NRU Assay Using BALB/c 3T3 Cells and A549 Cells

Intense Condensate
Fig 3 Comparison of Relative Viability Between the Intense JUUL System Condensates and the Reference 3R4F Smoke Condensate in the NRU Assay Using BALB/c 3T3 Cells and A549 Cells

Discussion
None of the JUUL System e-liquids caused cytotoxicity (defined as a viability < 70%) relative to vehicle control. ISO 10993-5:2009 (ISO 2009) up to the highest tested concentration (0.5% v/v) in either the BALB/c 3T3 or A549 cell lines (Figure 1). None of the JUUL System ENDS aerosol condensates demonstrated cytotoxicity up to the highest concentration (77 µg nicotine/mL) tested in either of the cell lines. In contrast, the 3R4F smoke condensate caused a dose-dependent increase in cytotoxicity (a decrease in viability) in both BALB/c 3T3 and A549 cell lines (IC50: 3T3: 3.00 ± 0.14 µg/mL nicotine, A549: 4.98 ±1.57 µg/mL nicotine). The effects from the 3R4F smoke condensate exposure were observed at lower nicotine-normalized concentrations compared to the JUUL System ENDS non-intense and intense condensates (Figures 2 and 3). These results support the conclusion that the JUUL System is less cytotoxic than combustible cigarettes.

References


Juul Labs Science
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