Genotoxicity Assessment of Electronic Nicotine Delivery Systems (ENDS) and Combustible Cigarette Aerosols/Smoke in the Ames and In Vitro Micronucleus Assays

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Abstract

In vitro toxicological assessment of combustible and next generation tobacco products, including Electronic Nicotine Delivery Systems (ENDS), have utilized standard genotoxicity methods. To determine the genotoxic potential of aerosols generated from five ENDS (Vuse Alto®) and a combustible cigarette (CC), the bacterial reverse mutation (Ames) and *in vitro* micronucleus (IVMN) assays were conducted using pad collected material [total particulate matter, TPM (CC); aerosol collected material, ACM (ENDS)] and gas vapor phase (GVP) preparations. Aerosols were generated from the ENDS using a nonstandard puffing regimen (80 mL puff volume, 15 sec puff interval, 5 sec puff duration) or the CC under the Health Canada Intense (HCI) smoking regimen (55 mL puff volume, 30 sec puff interval, 2 sec puff duration, 100% vent blocking). TPM/ACM trapped on a Cambridge filter pad and extracted in DMSO, and GVP captured in PBS, were each prepared at concentrations of 10 mg TPM/mL (CC) or 100 mg ACM/mL (ENDS), followed by quantification of nicotine (TPM/ACM) and carbonyl constituents (GVP). Combined samples (1:1) of the TPM/ACM + GVP (Health Canada T-502) to represent a "whole aerosol" were used for Ames and IVMN exposures. Ames preincubation assays were conducted with tester strains TA98, TA100, TA1535, TA1537, and TA102 with and without metabolic activation, per HC T-501 and OECD 471 guidelines. For the IVMN, CHO cells were exposed under the three schedules (3 hr ±S9 and 24 hr -S9) outlined in HC T-503 and OECD 487 (-cytochalasin-B) guidelines.

Materials and Methods

Generation/Preparation of Test Matrices:

Five different flavor variants of a single ENDS product were assessed under GLP conditions at a contract research laboratory using standard nonclinical regulatory toxicology procedures. For each test article, two aerosol fractions (TPM/ACM + GVP) were generated and combined in a 1:1 ratio forming a single test matrix as described below and shown in Figure 1.

The combustible cigarette TPM was generated using an automated rotary smoking machine and collected on 92 mm Cambridge filter pads. Each ENDS test article ACM was generated using a linear smoking machine and collected on 44 mm Cambridge filter pads. For all test articles, mainstream GVP was bubbled into a cooled glass impinger containing phosphate-buffered saline (PBS). DMSO was used to elute the TPM/ACM from the pads to a stock concentration of 100 mg/mL for ENDS and 10 mg/mL for the combustible cigarette. The volume of the trapped GVP was adjusted with PBS to achieve 100 mg ACM equivalent/mL for ENDS and 10 mg TPM equivalent/mL for the combustible cigarette.

Results

Figure 1: TPM+GVP Test Sample Generation

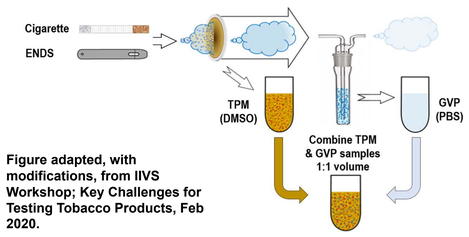


Figure adapted, with modifications, from IIVS Workshop; Key Challenges for Testing Tobacco Products, Feb 2020.

Table 1: Overall Ames Results Summary

Test Article	Overall Ames Result (if positive, strain ±S9)
Vuse Alto Golden Tobacco 5%	Negative
Vuse Alto Golden Tobacco 2.4%	Negative
Vuse Alto Rich Tobacco 5%	Negative
Vuse Alto Menthol 5%	Negative
Vuse Alto Mixed Berry 5%	Negative
Market Combustible	Mutagenic (TA98 +S9, TA 100 ±S9, TA1537 +S9)

Figure 2: Positive Ames Data for Select Strains (+/-S9)

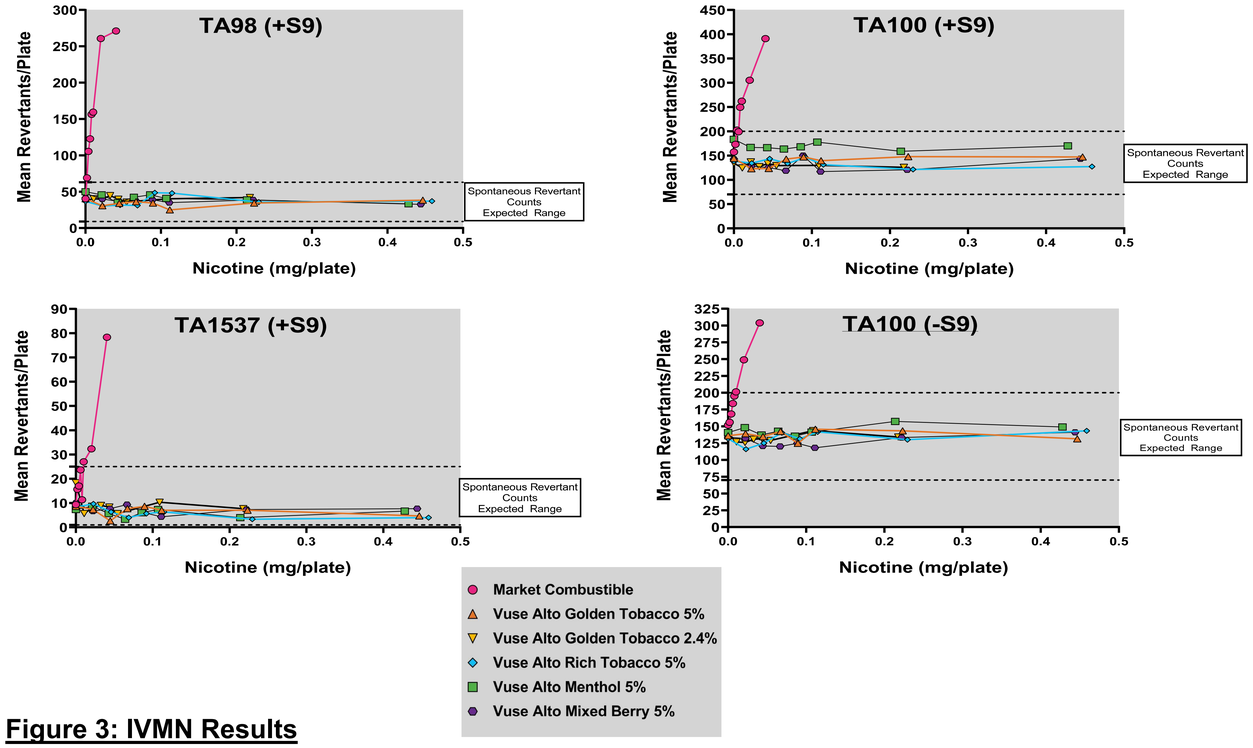
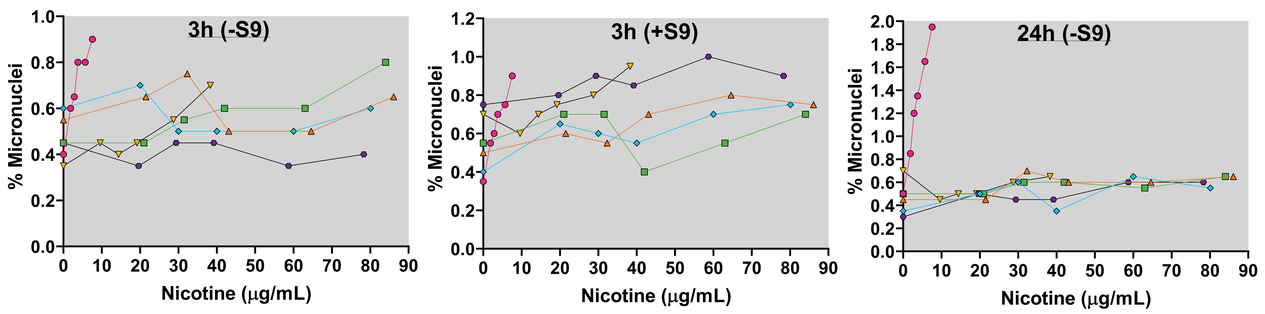


Figure 3: IVMN Results



Materials and Methods (cont.)

Combined samples (1:1) of the TPM/ACM + GVP were used for Ames and IVMN exposures. The GVP samples were tested in each of the biological assays (Ames and IVMN) within 1 hour of generation.

In order to avoid potential artifactual positive response, the highest concentration of test article assessed in these assays was limited to one that did not produce excessive toxicity (as indicated by a decrease in background lawn for Ames and %RICC <60% at the highest dose tested for IVMN), precipitation in the culture medium, or marked changes in pH or osmolality.

Ames bacterial reverse mutation assay:

TPM/ACM + GVP test sample exposures were conducted to the principles of OECD 471 and HC T-501, using 5 bacterial (*Salmonella typhimurium)* strains: TA98, TA100, TA1535, TA1537, and TA102; each with and without metabolic activation (5% S9 mix). Overall assay results for each test article are summarized in Table 1. Results for select strains and conditions are reported in terms of the average number of observed revertants (i.e., colony mutations) per plate following exposure to the test sample (see Figure 2).

***In vitro* micronucleus genotoxicity assay:**

In alignment with the principles of OECD 487 and HC T-503, TPM/ACM and GVP test sample genotoxicity was determined as follows. CHO cells were exposed to the test sample in one of three conditions: ± S9 with short 3h exposure and 21h recovery or a longer 24h exposure (-S9). Results for the IVMN assay are presented as (average) % MN observed following exposure to test sample (Figure 3).

Summary & Conclusions

Ames:

- For the ENDS test articles, mutagenicity was not observed in any bacterial strain tested, with or without exogenous metabolic activation, at test concentrations up to the maximum concentration (5000 µg/plate of TPM/ACM + GVP) as defined by OECD
- Mutagenic responses were observed for the TPM + GVP test samples generated from the combustible cigarette in strains TA98 (+S9), TA100 (±S9), & TA1537 (+S9) (see Table 1)

IVMN:

- Genotoxicity in the IVMN assay was not observed for combined ACM + GVP from any ENDS test article variant, even at exposure schedules lasting up to 24 hours
- Although genotoxic responses were also not observed for combined TPM + GVP from the combustible cigarette after 3 hours in the presence or absence of S9, TPM + GVP from the combustible product did result in a dose-dependent significant increase in micronuclei under the 24h exposure schedule (-S9), thus the combustible samples are considered genotoxic

Based on these results the ENDS test articles demonstrated lower mutagenic and genotoxic results compared to the combustible comparator.