Characterization of Whole Mainstream Smoke/Aerosol Delivery within the Vitrocell® Ames 48 High Throughput Exposure Module Using Different Tobacco Product Types.

Leverette R, Keyser B, Seymour A and Hollings M

1Scientific & Regulatory Affairs, RAI Services Company, Winston-Salem, NC 27101
2Coventage Laboratories Ltd, Otley Road, Harrogate, North Yorkshire HG3 1PY, UK

Abstract
The preferred means of assessing combustible or other aerosol-generating tobacco products in vitro is by direct exposure of cell cultures to freshly generated whole mainstream smoke/aerosols. This approach eliminates the fractionation of smoke/aerosols that occurs when collecting particulate matter on filter pads and collecting it as a vapor phase via liquid traps. Whole smoke/aerosol exposure systems are commercially available and have been utilized to assess combustible and tobacco heating products (THP) as well as electronic nicotine delivery systems (ENDS) in vitro. However, a challenge with such systems is ensuring a sufficient number of doses and sample throughput for in vitro toxicological studies in a timely manner. Vitrocell® has developed a high throughput whole smoke/aerosol exposure module (Ames 48 module) designed to concurrently deliver up to seven different doses (six wells per dose) of smoke/aerosol and a clean air control to 48 wells of bacterial cell cultures. Characterization of smoke/aerosol delivery within this system was conducted in a series of experiments designed to assess smoke/aerosol delivery and biological responses from a Kentucky Reference 3R4F combustible cigarette or a commercially available THP or ENDS. Dilution air flows consisting of 0.5 – 10 L/min for the THP and ENDS were evaluated. Smoke/aerosol deposition was quantified on a mass delivered basis using fluorescence measurements (Ex 355/Em 485) of captured particulate matter and chemical analysis (e.g., glycerol, nicotine) of either DMSO (3R4F) or PBS (THP, ENDS) liquid traps within the module. The mutagenicity (Ames Assay) of whole smoke from the 3R4F cigarette was also assessed with the Ames 48 module using Salmonella strains TA98 and TA100 (±S9). Results demonstrate a dose-dependent deposition of smoke/aerosol constituents (3R4F, THP and ENDS) and a characteristic dose-dependent increase in revertant counts (3R4F). Current test results from the Ames 48 module are comparable to historical 3R4F results generated using the standard Vitrocell® exposure modules.

Materials and Methods
Smoke/Aerosol Dosimetry and Distribution:
- THP and 3R4F cigarettes were conditioned for at least 48 hrs at 22 ± 1°C, 60 ± 3% relative humidity (ISO 3402)
- AMES 48 module wells contained a 35 mm petri dish with 2 or 4 mL of PBS (THP, ENDS) or DMSO (3R4F)
- THP and ENDS dilution air flow rates (L/min): 0 (undiluted) / 0.25 / 0.5 / 1.0 / 2.0 / 3.0 / 4.0
- THP dilution air flow rates (L/min): 0.5 / 1.0 / 2.0 / 3.0 / 4.0 / 5.0 / 6.0 / 8.0
- HCl regimen (3R4F and THP): 55 mL puff volume, 2 sec puff, 30 sec puff interval, 100% vent blocking for 3R4F only
- CRM81 regimen (ENDS): 55 mL puff volume, 3 sec puff, 30 sec puff interval
- Vacuum flow rate to exposure wells was 5 mL/min
- 8 sec puff exhaust time to deliver smoke/aerosol to exposure module

THP Dosimetry: Pad-collected TPM extracted in DMSO at 24 mg/mL and serially diluted to generate a standard curve (Ex 355 / Em 485) (Figure 1).

3R4F Dosimetry: THP delivered dose was determined by quantifying glycerol captured in aerosol-exposed PBS using Free Glycerol Reagent (Sigma # F09100).

Results

Figure 1: Vitrocell® AMES 48 high throughput exposure module set-up for either THP and ENDS (A) or 3R4F combustible cigarette (B). Diagrams indicate placement of photometers (.), dosimetry modules (a) and addition of air for smoke/aerosol dilution (we).

Figure 2: 3R4F Total Particulate Matter (TPM) and Glycerol standard curves.
(A) Pad-collected 3R4F TPM (µg TPM/mL) was serially diluted and fluorescence (Ex 355 / Em 485) was measured over a range of TPM concentrations. A hyperbola model fit was used to establish the standard curve and extrapolate 3R4F TPM deposition within the exposure module (GraphPad Prism 8.0.1).
(B) Known concentrations of glycerol (µg/mL) were quantified using Free Glycerol Reagent with a linear model used to fit the curve and extrapolate THP and ENDS delivery within the exposure module.

Figure 3: THP, AMES 48 (n = 6).
(A) Deposition of 3R4F TPM (µg/tube) determined by extrapolation of the Ex 355 / Em 485 fluorescence of smoke-exposed DMSO and glycerol depression in PBS to their respective standard curves (Figures 2A and 2B). Values are presented as % of the overall mean (± SD) for each dose. Dashed lines (-) are ±15%.

Figure 5: 3R4F whole smoke Ames Assay: AMES 48.
TA98 (A) and TA100 (B) were exposed to 3R4F whole smoke generated under conditions from 4-6 cigarettes. Delivered dose (µg TPM/tube) determined by extrapolation of the Ex 355 / Em 485 fluorescence of smoke-exposed DMSO. (*P < 0.05 vs control)

Summary and Conclusions
- Freshly generated whole smoke from the 3R4F reference cigarette (HCl, TPM) and aerosol from either a commercially available THP (HCl, glycerol) or ENDS (CRM81; glycerol) was delivered consistently within the Vitrocell® Ames 48 High Throughput exposure module (Figure 3).
- Coefficients of variation (CV) for whole smoke/aerosol deposition within each dose (row) were < 20% (3R4F, TPM) and < 15% (THP and ENDS; glycerol).
- Overall, the data presented demonstrate the consistent delivery of whole smoke/aerosol under controlled conditions and a reproducible in vitro biological response ( Ames) with the Vitrocell® Ames 48 High Throughput exposure module. HCl 3R4F whole smoke exposures do require additional range finding experiments for optimization.
- Ames activity of 3R4F whole smoke, generated under similar conditions, was comparable when using either the Vitrocell® Ames 48 High Throughput exposure module or the Vitrocell® Standard exposure modules (Figure 6).
- The Vitrocell® Ames 48 exposure module is a useful tool to increase sample throughput for the in vitro toxicological assessment of freshly generated whole smoke and aerosols from different tobacco product types (combustible, THP and ENDS). The Ames 48 module allows 7 smoke/aerosol doses (with 6 cultures per dose) per exposure versus only 2 (HCl) - 4 (ISO) doses (with 3 cultures per dose) for the standard exposure modules (when using a VC1® smoking machine).

References