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

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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 gas 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 airflows consisting of 0.5 – 10 L/min for 3R4F and 0 (undiluted) - 4 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 Delivery and Distribution:

- THP and 3R4F cigarettes were conditioned for at least 48 hrs at 22 ± 1°C, 60 ± 3% relative humidity (ISO 3402)[1]
- 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
- 3R4F dilution air flow rates (L/min): 0.5 / 1.0 / 2.0 / 4.0 / 5.0 / 6.0 / 8.0
- HCI 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

3R4F Dosimetry: Pad-collected TPM extracted in DMSO at 24 mg/mL and serially diluted to generate a standard curve (Ex 355 / Em 485)[2] (Figure 2A) used to extrapolate TPM delivery (smoke-exposed DMSO).

THP and ENDS Dosimetry: THP delivered dose was determined by quantifying glycerol captured in aerosol-exposed PBS using Free Glycerol Reagent (Sigma # FG0100).

3R4F Whole Smoke AMES Assay[3]:

- TA98 and TA100 (±S9)
- Whole smoke from 4-6 (HCI) or 8 (ISO) 3R4F cigarettes was diluted (L/min) as above

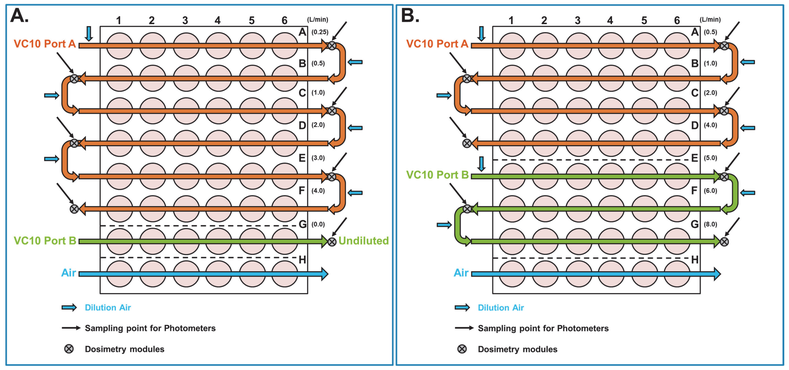

Figure 1: Vitrocell® AMES 48 high throughput exposure module set-up for either THP and ENDS (A) or 3R4F combustible cigarette (B). Diagrams indicate placement of photometers (⟶), dosimetry modules (⊗) and addition of air for smoke/aerosol dilution (⇒).

References

1. ISO 3402 (1999). Tobacco and tobacco products - Atmosphere for conditioning and testing (4th edition).
2. Aufderheide, M. and Gressmann, H. (2007). A modified Ames assay reveals the mutagenicity of native cigarette mainstream smoke and its gas vapour phase. Exp Toxicol Pathol, 58, 383 – 392.
3. Fowler et al. (2018). Development, qualification, validation and application of the Ames test using a VITROCELL® VC10® smoke exposure system. Toxicology Reports, 5, 542–551.

Results

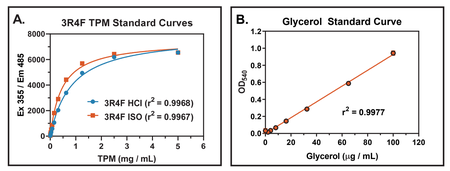

Figure 2: 3R4F Total Particulate Matter (TPM) and Glycerol standard curves.
(A) Pad-collected 3R4F TPM (in DMSO) was serially diluted and fluorescence (Ex 355 / Em 485) was measured over a range of TPM concentrations. A hyperbola model fit was used to establish the standard curve and extrapolate 3R4F 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.

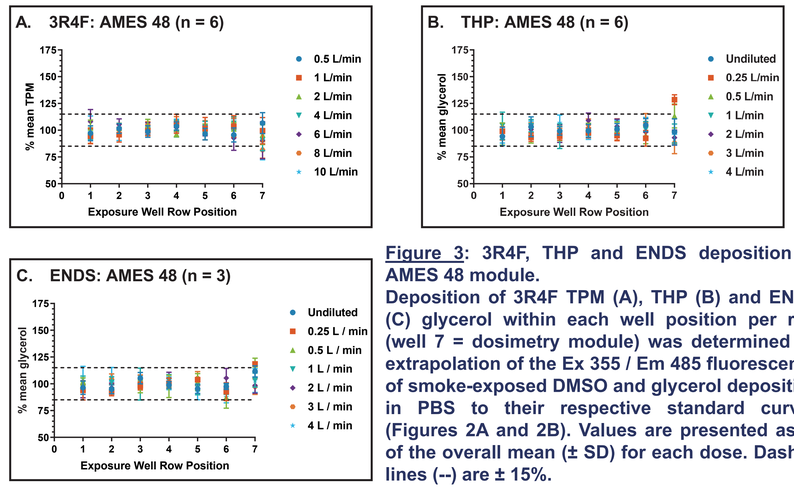

Figure 3: 3R4F, THP and ENDS deposition in AMES 48 module.
Deposition of 3R4F TPM (A), THP (B) and ENDS (C) glycerol within each well position per row (well 7 = dosimetry module) was determined by extrapolation of the Ex 355 / Em 485 fluorescence of smoke-exposed DMSO and glycerol deposition in PBS to their respective standard curves (Figures 2A and 2B). Values are presented as % of the overall mean (± SD) for each dose. Dashed lines (--) are ± 15%.

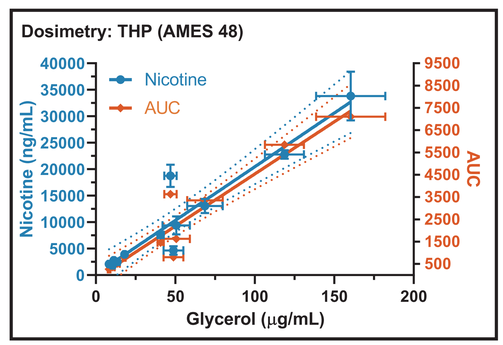

Figure 4: Dosimetry THP AMES 48.
Aerosol delivery monitored in real-time using laser photometer (AUC). Deposition of aerosol determined by quantifying glycerol and nicotine from the PBS solvent trap. Graph shows the linear relationship between the dosimetry measures. Similar results were observed for 3R4F whole smoke. ENDS data still in process.

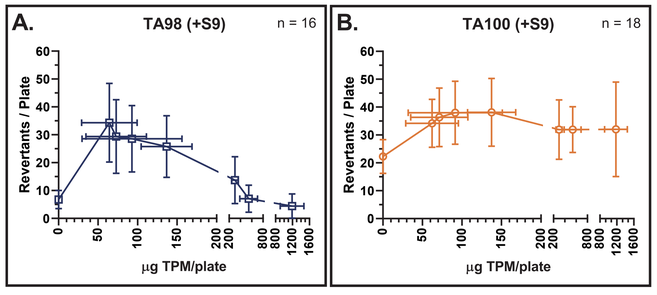

Figure 5: 3R4F whole smoke Ames Assay: AMES 48
TA98 (A) and TA100 (B) were exposed to 3R4F whole smoke generated under HCI conditions from 4-6 cigarettes. Delivered dose (µg TPM/plate) determined by extrapolation of the Ex 355 / Em 485 fluorescence of smoke-exposed DMSO. (-S9 data not shown)

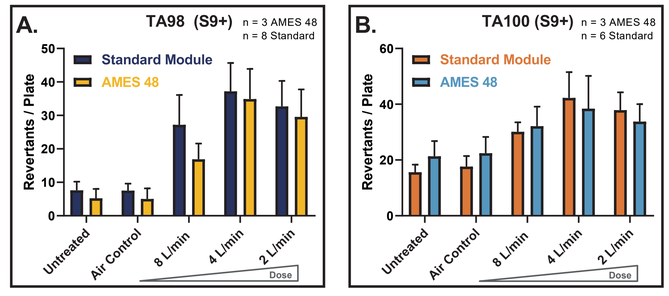

Figure 6: 3R4F whole smoke Ames Assay: Standard vs. AMES 48 Modules.
TA98 (A) and TA100 (B) were exposed to 3R4F whole smoke generated under ISO conditions from 8 cigarettes. Standard module data taken from historical data. Revertant counts compared at equivalent dilution air flows.

Summary and Conclusions

- Freshly generated whole smoke from the 3R4F reference cigarette (HCI; TPM) and aerosol from either a commercially available THP (HCI; glycerol) or ENDS (CRM81; glycerol) were 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. HCI 3R4F whole smoke exposures do require additional range finding experiments for optimization.
- Ames activity of 3R4F whole smoke, when 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 (HCI) - 4 (ISO) doses (with 3 cultures per dose) for the standard exposure modules (when using a VC10® smoking machine).