Dosimetry Methods for Tobacco Smoke Aerosol *In Vitro* Exposures

#1841

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Introduction

Recent technological developments in the testing of tobacco products have seen the widespread introduction of equipment that is capable of exposing cultured cells or media to the whole smoke aerosol, adding an additional capability to the well established regimes for testing tobacco smoke Total Particulate Matter *in vitro* for genotoxicity and cytotoxicity.

The Salmonella reverse mutation assay[1] (Ames test) has been used to demonstrate both mutagenicity and toxicity of main stream cigarette smoke.[2] Using a Vitrocell® smoking robot, dilution and exposure system, the dose of smoke bacteria are exposed to is controlled by cigarette type, speed of diluting air flowing through the system, volume of smoke passed over the cells and number of cigarettes smoked. As such, accurate dosimetry is not possible without further measurements.

Carbon monoxide (CO) and smoke particulate concentrations were selected for measuring gas vapour phase (GVP) and particulate phase exposure respectively. Dosimetry methods have been developed to measure these constituents, within whole smoke and cigarette GVP diluted with air flowing at speeds ≥2 L/min.

Measuring constituents of diluted smoke or GVP used to expose air-liquid-interface cultures, allows better understanding and a greater comparative ability of mutagenicity and toxicity.

Methods

Diluted smoke from three 3R4F reference cigarettes was generated using a Vitrocell® VC 10 smoking machine and dilution system and passed over surface cultures of *S. typhimurium* strains YG1042 or TA100 in the presence of S9 (for metabolic activation).

Smoke particulate concentration was measured by light scattering, in a flow-through photometer, attached in-line to the exposure module (Figure 1).

CO was measured spectrophotometrically using a Signal 7000fm machine. Two CO methods were developed, real-time measurement from the dilution system exhaust and at-line measurement via a gas bag filled passively from the final sample port of the dilution bar (Figure 1).

For GVP exposures, a Cambridge filter pad was placed between the smoking head and piston to remove all particulate matter.

Results

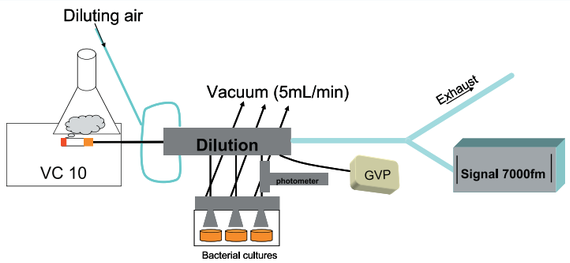

Figure 1. Schematic diagram of equipment set up.
Smoke generated by a VC 10 smoking machine enters a Vitrocell® dilution bar and is diluted by air flowing at a defined speed. Diluted smoke is pulled from the dilution bar and through a Vitrocell® exposure module at 5 mL/min.

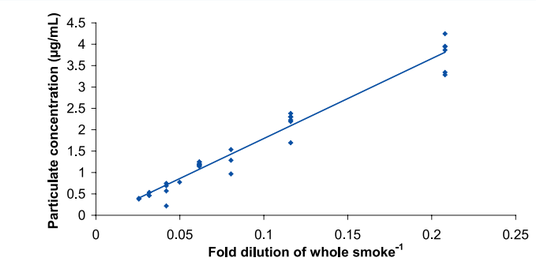

Figure 2. Smoke particulate concentration vs. smoke dilution.
Diluting air flow rates were set between 1 and 10 L/min. to produce 3R4F smoke dilutions of 4.8-39.1 fold. A positive correlation (R^2=0.960, P<0.001) was observed when comparing the calculated smoke dilution with the measured particulate concentration across the entire dose range tested. Particulate concentrations ranged from 0.4-4.2 µg/mL.

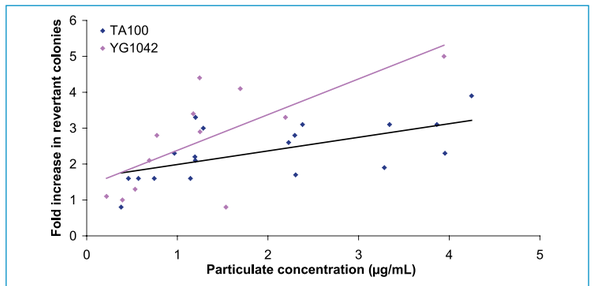

Figure 3. Ames response vs. smoke particulate concentration.
Both TA100 (R^2=0.375, p<0.01) and YG1042 (R^2=0.497, p<0.01) strains of *S. typhimurium* demonstrated a dose related increase in revertant colonies following exposure to smoke generated from 3 3R4F cigarettes under the ISO smoking regime.

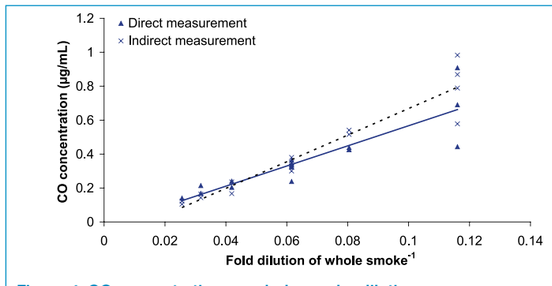

Figure 4. CO concentration vs. whole smoke dilution.
Both direct (R^2=0.788, p<0.001) and indirect methods (R^2=0.912, p<0.001) revealed a positive correlation between the calculated smoke dilution range with the measured CO concentration. At high smoke concentrations (<2 L/min. diluting air flow, data not shown) interexperimental variability in CO concentration increases requiring further optimization.

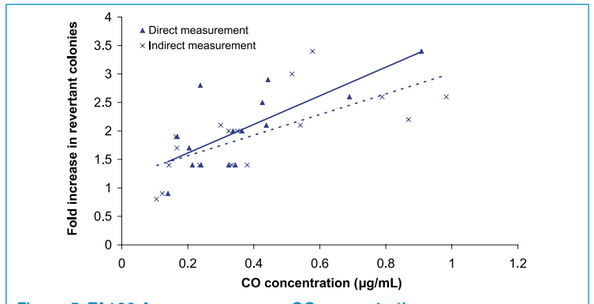

Figure 5. TA100 Ames response vs. CO concentration.
A dose related correlation was observed between CO concentration measured directly (R^2=0.550, p<0.001) and indirectly (R^2=0.486, p<0.01) and revertant colony numbers following TA100 exposure to diluted whole cigarette smoke.

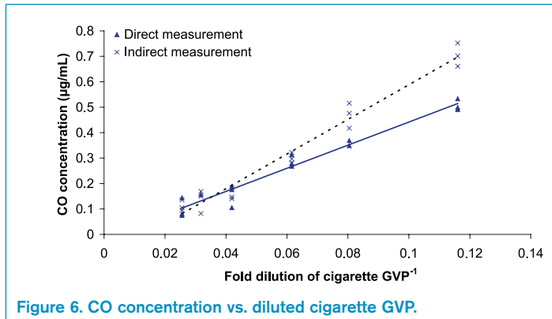

Figure 6. CO concentration vs. diluted cigarette GVP.
Both direct (R^2=0.962, p<0.001) and indirect (R^2=0.969, p<0.001) methods revealed a positive correlation between the calculated GVP dilution range with the measured CO concentration. Lower CO concentrations were measured for GVP exposures compared with whole smoke of an equal dilution.

Conclusion

- We have developed a photometric method to measure the smoke particulate concentration entering *in vitro* exposure modules.
- Good correlation was observed between calculated smoke dilution and smoke particulate concentration over the dilution range tested (1-10 L/min. diluting air flow) (Figure 2).
- The YG1042 strain of *S. typhimurium* had a greater Ames response than TA100, inducing more revertant colonies following exposure to the same concentration of smoke particulates (Figure 3). This may be attributed to the increased nitroreductase and acetyltransferase activity of YG1042.[3]
- Inter-experimental variation occurs through both biological variation and the complexity of the variables influencing the smoke dilution (Figure 3).
- We have developed methods that allow the CO concentration in diluted smoke to be measured, both real-time and at-line. Good correlation was observed between calculated smoke/GVP dilution and CO concentration over the dilution range tested (2-10 L/min. diluting air flow) (Figures 4 and 6).
- A dose dependent correlation was observed between CO levels in diluted whole smoke and Ames response in TA100 (Figure 5).
- Puff profiles obtained from the photometer and real-time CO measurements (data not shown) also provide a valuable QC check for the system.

Future Work

- Further optimization is required to reproducibly quantify CO for smoke diluted with air flowing at <2 L/min.
- Further dosimetry investigation will be performed to include other assay end points and the measurement of single constituents from cigarette smoke.

References

1. Maron, DM. and Ames, BM. 1983. Mutat Res. 113 (3-4):173-215.
2. Aufderheide, M and Gressmann, H. 2007. Exp Toxicol Pathol. 58:383-392.
3. Hagiwara, Y., et al., 1993. Mutat Res. 291(3):171-80.

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