An Evaluation of Cigarette Smoke Aerosols in Vitro Using a Modified Ames Methodology and the Balb/c 3t3 Neutral Red Uptake Cytotoxicity Assay

Michael Hollings, Amber Woodhams, Adam Seymour, Mark Ballantyne and Julie Clements; Covance Laboratories Ltd., Harrogate, UK

Introduction
Cigarette smoke is a complex aerosol consisting of two fractions; the particulate phase constituting 4-9% and the significantly larger vapour phase constituting the remaining >90%. Historically the tobacco industry has tested the particulate phase (TPM) by trapping the particulate matter on glass fibre pads and eluting it in dimethyl sulfoxide, and the vapour phase (GVP) by capturing the remaining vapour phase in a combination of matrices, prior to testing them independently in the in vitro genotoxicity battery. The challenge now for the tobacco industry is developing an air/liquid interface (ALI) exposure systems to allow assessment of whole smoke. Whole smoke exposures take into account the synergistic interactions that may be taking place between the vapour and particulate phases. The negative aspect of TPM is that the complete vapour phase cannot be captured in a single solvent matrix and testing can only be performed in solution. Whole smoke exposures aim to increase the biological relevance of any results and enable better comparison to human exposure conditions. It is therefore important to develop systems that enable exposure of both the particulate and vapour phase of whole smoke at the air/liquid interface.

Whole Smoke Generation
A Vitrocell® VC 10 Smoking Robot (Vitrocell® Systems, Waldkirch, Germany) was used to generate smoke aerosols from 3R4F, 1R5F (University of Kentucky, USA) and CORESTA Monitor 7 (CM7) cigarettes(supplied by Borgwaldt GmbH, Germany) before being directed to the exposure surface. All cigarettes were conditioned to ISO 3402:199 prior to smoking to ISO 3308:2012 (2 second puff, 35mL, 60 second interval with a 5mL vacuum). Where cigarette puff numbers differed, experiments were matched for exposure time. For all experiments quartz crystal microbalances (QCMs) were included for a quantitative measure of deposited mass.

Methods
Presented at TSRC 2016

References

Image supplied by Vitrocell® Systems GmbH

Figure 1. Percentage relative cell survival in a comparison study of 3R4F, 1R5F and CM7. Cells were exposed to diluted smoke and to an ISO regime for 184 min. Inter-experimental variation is observed in cells at an average relative survival >90%. IC50 values have been calculated and expressed in L/min.

Figure 2. Average mean revertants from exposure to diluted mainstream cigarette smoke generated from 3R4F, 1R5F and CM7 cigarettes in two strains of S. typhimurium (TA98 and TA100) at diluting airflows of 12, 4, 1 L/min, with a constant vacuum of 5mL/min in the presence of S-9 metabolic activation. Positive responses >2-fold were observed in TA98 and TA100 in the presence of metabolic activation.

Conclusions
- Concentration related increases >2 fold observed in TA98 and TA100 in the presence of metabolic activation system, indicating all smoke aerosols tested cause both frame-shift mutations and base pair substitutions.
- TA98 is more responsive and therefore more sensitive than TA100 to whole smoke aerosol.
- Increases in deposition correlate with increases in revertant number; CM7 at 1L/min is the exception due to the presence of toxicity at this dose.
- Cytotoxicity was observed in all smoke aerosols, with varying IC50 values obtained for the three products. CM7 is more toxic than 3R4F, and subsequently 3R4F is more toxic than 1R5F.
- Previously published methodologies are suitable in differentiating between combustible products of varying yield.

Image supplied by Vitrocell® Systems GmbH

Figure 3. Whole smoke dose-response. Average mean revertants from exposure to diluted cigarette smoke (Log Deposition ng/cm²) generated from 3R4F, 1R5F and CM7 for TA98 (A) and TA100 (B).

Image supplied by Vitrocell® Systems GmbH

Figure 4. Whole smoke deposition data and mean revertants from exposure to 3R4F, 1R5F and CM7 for TA98 and TA100. Mean revertants data is highlighted in red, with mean deposition (ng/cm²) in black.