A modified Ames methodology for the assessment of mainstream cigarette smoke genotoxicity using an aerosol-based exposure system

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Introduction

The development of whole smoke exposure systems has been driven by the fact that traditional smoke exposure techniques are based on the particulate phase of tobacco smoke and not the complete whole smoke aerosol. To overcome these challenges, whole smoke exposure systems have been developed which expose cell cultures to diluted tobacco smoke and capture the full interactions of both smoke phases1,3. Furthermore, standard methodologies, governed by regulatory guidelines are not necessarily compatible with complex aerosols, such as cigarette smoke.

Aim

To develop a modified version of the Ames reverse mutation assay suitable for whole smoke exposure. For this study, five strains were selected and exposed to diluted 3R4F mainstream cigarette smoke using the Vitrocell® VC 10 Smoking Robot. Quartz crystal microbalances (QCM)2 gave further confidence in the exposure system and enabled biological responses to be presented as a function of real-time obtained deposited mass.

Materials and Methods

Cigarette Smoke Generation

A Vitrocell® VC 10 Smoking Robot (Serial Number VC10/090610) was used to expose bacteria to mainstream cigarette smoke generated from 3R4F reference cigarettes (Fig 1). Cigarettes were conditioned according to ISO 3402:2000 and smoked according to ISO 30308:2000, with an 8 second exhaust. Mainstream cigarette smoke was passed into a constant flow of diluting air set at varying flow rates (1-12 L/min) to achieve different dosages. The diluted smoke was drawn through the modules using a constant vacuum of 5.0 mL/min for all experiments.

Ames Assay

Four strains of S. typhimurium (TA98, TA100, YG1024 and YG1042) and one strain of E. coli (WP2 uvrA pKM101) were exposed to diluted mainstream smoke in the presence or absence of S9. Approximately 2x10⁶ cells were plated onto 35mm Vogel-Bonner agar plates using spread plate methodology such that bacteria were exposed at an air-agar interface. Plates were exposed to a total of 3 cigarettes smoked over 24 minutes. Concurrent negative (air and untreated) and positive controls were included with each exposure. Following exposure, plates were incubated at 37°C for 3 days before revertant colony numbers were counted using an automated scoring system.

Measurement of Particulate Dose

A QCM (Fig 2) was placed in the fourth position of the exposure module for all whole smoke exposures in order to quantify the dose delivered by measuring deposition of particulate mass. At the end of the whole smoke exposure period, the final deposited mass reading on each QCM was recorded once a plateau in the deposition curve was observed2.

Conclusions

• Concentration-related increases in revertant numbers were observed in S. typhimurium strains TA98, TA100, YG1024 and YG1042 up to maximum mean fold increases of 5.6, 1.7, 24.8 and 5.3-fold, respectively, following 24 minute exposure to diluted 3R4F mainstream cigarette smoke in the presence of S-9.

• No response to whole smoke was observed in E. coli WP2 uvrA pKM101 in the absence or presence of S-9.

• Measurement of real-time deposited particulate mass using QCMs in ali of whole smoke exposure demonstrated that the increases in revertant numbers observed in the four Salmonella strains in the presence of S-9, correlated with increasing particulate deposition.

• Our results indicate, that using a 5.0 mL/min vacuum, the GVP fraction alone does not induce mutation. However, alternative vacuum rates have yet to be assessed.

• In the absence of a metabolic activation system, whole smoke failed to induce mutation, indicating that direct acting smoke constituents cannot be detected, under these conditions.

Future Directions

• We intend to develop this modified assay alongside additional strains to create a multi-strain-testing approach.

• This work will be further supplemented by assessing strains in order to identify the optimal strains for testing cigarette smoke.

• We would like to complement QCM measurements with a measure of the vapour phase dose – a technique is required for this as none currently exists.

References

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Poster Number #STPOST15

Presented at:-
CORESTA
SSPT Meeting 2013
Sept 29th - 03 Oct
Seville, Spain.