URITIZATION OF A WHOLE SMOKE EXPOSURE SYSTEM FOR THE COMPARISON OF MAINSTREAM CIGARETTE SMOKE, GAS VAPOR PHASE AND TAR MUTAGENICITY

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ABSTRACT

This study was conducted to further optimize smoke exposure procedures, measure the mutagenicity of mainstream cigarette whole smoke (WS) and the contribution the gas vapor phase (GP) and wet and dry particulate matter (WTM) impart to the WS activity, as determined by the Salmonella mutagenicity assay (AMES) test. WS and WTM were prepared from Kentucky Reference 3R4F cigarettes smoked under ISO puff profile (35 mL volume, 2 second puff duration and 1 minute puff inter-arrangement) on a VITROCELL® smoking robot. TA98 and TA100, in the presence (S9+) and absence (S9-) of metabolic activation, were exposed to WS or GVP from three (3) 3R4F cigarettes via the VITROCELL® Dilution / Distribution System with dilution air flow rates set at 1, 2, 4 and 8 L/min, allowing the delivery of four doses of WS or GVP to the Ames exposure modules during each exposure. Per WTM experiments, a Cambridge filter pad was placed in line prior to the puffing syringe in order to remove the smoke particulate fraction, which was subsequently extracted in dimethylsulfoxide (DMSO) for use in WTM exposures. Quantification of several carbonyls verified the delivery of GVP to the bacteria. WTM exposures (S9+) utilized a 30 minute preincubation with DMSO limited to 2.5% v/v final concentration. WS mutagenicity was detected in both strains (S9+&S9-); however, TA100 S9-WS activity was approximately 20% that of TA98 WS activity while TA98 S9 activity was considerably lower at 3% of measured TA98 S9+ WS activity. No GVP mutagenicity was detected in both strains (S9+&S9-). Lack of GVP activity was not due to cytotoxicity since no significant decrease in cell viability was observed over the delivered GVP dose range. WTM activity was detected in both strains (S9+ only) at approximately 67% and 54% of the WS activities measured in TA98 and TA100, respectively. Under the exposure conditions used in this study, the majority of the WS mutagenic activity was found to reside in the particulate fraction, with a smaller portion originating from GVP.

MATERIALS & METHODS

SMOKE PREPARATION & ASSESSMENT

Kentucky Reference 3R4F cigarettes conditioned at least 14 hours in a 60% relative humidity (RH), 21°C environment prior to smoking. Cigarette smoke particulate (PS) was generated using a VITROCELL® smoking robot (Figure 1) following ISO puff profile (35 mL volume, 2 second puff duration and 1 minute puff inter-arrangement). Whole Smoke (WS) and Gas Vapor Phase (GVP) exposures were performed on a VITROCELL® smoking robot with a dedicated system for smoke particulate matter retention. Smoke dosages were delivered using 3, 1, 0.65 or 0.35 L/min smoke delivery rates. GVP and WS were assessed using the standard Ames Assay 6. First, a three strain panel of TA98, TA100 and TA1535 was exposed to WS (S9+/S9-) and WTM (S9+) exposures in both strains resulted in an increase of revertant colonies in a dose dependent manner (Figures 4 & 5). The inability to detect any WTM (S9-) mutagenicity was due to toxicity (cell death), most likely an effect of the preincubation exposure method. Detecting WS (S9-) activity, albeit at low levels, strengthens the argument for utilizing WS exposure methods.

SUMMARY

1. "Real-time" measurement of smoke delivery via deposition has been established with in-line laser photometers. Cigarette volatiles were calculated from a single 3R4F cigarette smoke collection and presented using GraphPad Prism 5.0. /n Analysis of variance, two tailed for comparisons, statistical significance p < 0.05.

2. Activity measured as revertant colonies was calculated from the linear portion of the dose response curve and compared using GraphPad Prism 5.0. /n Analysis of variance, two tailed for comparisons, statistical significance p < 0.05.

REFERENCES
