Introduction

• THS2.2 is a candidate modified risk tobacco product in which the tobacco is heated to a maximum of 350°C, leading to a significant reduction in the formation of harmful and potentially harmful constituents (HPHCs) in the aerosol generated, in comparison to the HPHCs generated during combustion of a conventional cigarette.

• For this evaluation, we have tested the mainstream THS2.2 aerosols from two product variants, regular and menthol, in a battery of regulatory in vitro genotoxicity and cytotoxicity tests, namely the Ames and mouse lymphoma assays (MLA) and the neutral red uptake (NRU) assay.

Materials and Methods

• All studies were performed in full compliance with GLP.

• Neutral Red Uptake Assay: The mouse embryonic fibroblast cell line Balb/c 3T3 (clone A31) was obtained from the European Collection of Authenticated Cell Cultures (Salisbury, UK), and was used to perform the NRU cytotoxicity assay according to INVITOX protocol 3a (INVITOX, 1990), with some modifications. Sodium dodecyl sulfate was used as the positive control. The EC50 was determined with the SAS® Enterprise guide® 4.3 (SAS, Cary, NC, USA).

• Mouse Lymphoma Assay: The L5178Y tk−/− cell line (sub-clone 3.7.2C (IVGT) was obtained from Public Health England (Salisbury, UK) and used for the performance of the microwell version of the MLA according to the OECD TG 490 guideline (OECD, 2015). The cells were treated for 4 hours in the presence (+S9; Moltox, Boone, NC, USA) and absence (-S9) of metabolite activation and 24 h in the absence of metabolite activation (-S9) treatment conditions. 74 mutants were detected following culture in trifluorothymidine (TFT)-containing growth medium (Sigma–Aldrich, St. Louis, MO, USA) for typically 14 days and with the mutation frequencies calculated according to published methods (Clements, 2000).

• Bacterial reversion tests: Mutagenic activity was evaluated using the Salmonella/mammalian microsome tester strains TA98, TA100, TA102, TA1535, and TA1537 and with and without an S9 enzymatic fraction (Moltox, Boone, NC, USA), by following a pre-incubation method (Maron and Ames, 1983) and the OECD 471 test guideline. The S9 metabolising fraction was obtained from A rodor 1254-induced male Sprague-Dawley rat liver (Moltox, NC, USA). His+ revertant colonies were counted using an automatic colony counter (Sorcerer, Perceptive Instruments, Bury Saint Edmunds, UK). The mutagens used were positive controls in the S9- group: 4- nitrophenylenediamine (10 µg/plate) for TA98 and TA100, sodium azide (1.25 µg/plate) for TA1535 and TA1537, and cumene hydroperoxide (2.5 µg/plate) for TA102. For the S9+ group, benzo[a]pyrene (1 µg/plate) was used for TA98, and 2-aminoanthracene (2.5 µg/plate) was used for TA100, TA102, TA1535, and TA1537. DMSO (50 µl/plate) served as the solvent control. All positive control chemicals were obtained from either Sigma-Aldrich (St. Louis, MO, USA) or Moltox (Boone, NC, USA).

• Sample generation: 3RF research cigarettes and THS2.2 tobacco sticks were conditioned for at least 48 at 22 ± 1°C and 60 ± 3% relative humidity (ISO standard 3402) prior to being used for in vitro generation. Following conditioning, aerosols were generated using an RMB80 smoking machine (Burghart, Tabaktechnik GmbH, Wedel, Germany) according to the Health Canada Intense (HCI) smoking regimen (Health Canada, 2000). The generated aerosol and smoke were trapped to analyze the aerosols.

Results: Neutral Red Uptake: Cytotoxicity

Figure 1: The cytotoxicity responses induced by aerosol fractions derived from THS2.2 and 3RF in the NRU assay over three independent tests expressed on a per-ng nicotine basis. A.C. Gas Vapour Phase. B.D. Total Particulate Matter. E. Cytotoxicity of TPM and GVP, expressed as 1/EC50 (milting nicotine).

Results: Mouse Lymphoma Assay

Figure 2: Revertant colonies obtained following exposure to the TPM (1 mg per plate) from THS2.2, THS2.2 M, or 3RF. The concentration of S9 was fixed at 10%. All doses were tested in triplicate and on two independent test occasions. The maximum dose tested of THS2.2 was 10 mg and slim for THS2.2 M. No biologically relevant mutagenicity was detected up to these doses.

Results: Bacterial Reversion Test

Table 1: The mutagenic responses induced by aerosol fractions derived from THS2.2 and 3RF in the 4 h + S9 treatment condition in two independent tests expressed on a per-ng nicotine basis.

Analytical Results

Table 2: Analyte yields from THS2.2, THS2.2 M, and 3RF obtained under HCI machine-smoking conditions and expressed on a per-cigarette/tobacco stick basis.

Summary and Conclusion

The mutagenic and cytotoxic potencies of the mainstream aerosol fractions from THS2.2, when evaluated by the mouse lymphoma, and NRU assays were reduced by at least 85%–95% compared with the mainstream smoke aerosol of 3RF. The Ames assay yielded no biologically relevant mutagenicity.

The low operating temperature of THS2.2 results in significantly lower concentrations of HPHCs in the smoke aerosol of 3RF, compared with the mainstream smoke aerosol of 3RF, the Ames assay yielded no biologically relevant mutagenicity.

While a conclusion underlying the mechanism(s) of these in vitro results cannot be definitively made on the basis of these data, it is reasonable to suggest that the overall reduction in the burden of toxicants present in the THS2.2 aerosol may play a role in the manifestation of the reduced cytotoxic and mutagenic potency in vitro.

References

