Introduction

Tobacco heating products (THPs) represent a subset of the next-generation nicotine and tobacco product (NGP) category, in which tobacco is heated at temperatures of less than 350°C instead of burning (900°C), having the potential to significantly reduce cigarette smoke toxicants. THPs hold great potential for reducing the harm associated with tobacco use, but this needs to be scientifically proven.

As a complement to chemical analysis of emissions, a battery of in vitro toxicity tests can be used as an initial screen to determine the mutagenic and cytotoxic potential of NGPs. International guidelines have been developed that recommend an appropriate battery of in vitro mutagenicity and carcinogenicity assays to ensure consistency of testing procedures and appropriate assay selection as part of a risk assessment process. Several guidelines exist, including those developed by the International Conference on Harmonisation (ICH) 2011, Health Canada [Health Canada 2009] [4]. In summary, these guidelines recommend the use of: i) a bacterial mutagenicity assay (Ames reverse mutation assay), ii) a mammalian cell based assay for cytogenetics/mutation, either in vivo micronucleus, chromosome aberrations or the mouse lymphoma assay (MLA) and finally, a cytotoxicity based assay. Although the Bhas 42 cell transformation assay is not a recognized assay for use in regulatory testing, an OECD guidance document has been issued. It is considered that this assay, adds value in its ability to detect non-genotoxic carcinogens, and significantly supports a weight of evidence based testing strategy.

Furthermore traditional TPM approaches were supplemented with Whole Aerosol (WA) techniques to support a weight-of-evidence approach.

Aim

To characterise the biological impact of the novel THP: THP1.0 (commercially known as glo™) comparing results to a reference 3R4F cigarette in a comparative study design.

Materials and Methods

Products

Whole aerosol (WA)
A Vitrocell VC10 smoking robot (Vitrocell Systems, Germany) was used to generate whole aerosols for the Ames assay, as previously described [2].

Neutral red uptake (NRU) cytotoxicity assay
Cigarette smoke and THP aerosols were tested up to 2400 μg/mL over 72 h plate incorporation and pre-incubation with S9 + S9 over 3 treatment conditions.

Ames bacterial reverse mutation assay
Using TPM five S. typhimurium strains; TA98, TA100, TA1535, TA1537 and TA102 were tested and metabolic activation (S9) was assessed. For WA exposures, the Ames assay was employed with S. typhimurium tester strains TA98, TA100, TA1535, TA97 and TA102 using a modified methodology [5].

Mouse lymphoma assay (MLA)
TPM was assessed with short 3 h exposures (± S9) and longer 24 h – S9 exposures.

In vitro micronucleus assay (IVMN)
TPM was assessed with short 3 h exposures (± S9) and longer 24 h – S9 exposures.

Bhas cell transformation assay
The potential of TPM from the products to induce tumour development was evaluated using the Bhas 42 cell transformation assay, promotor protocol.

Results

Conclusions

• Cytotoxicity, mutagenicity, clastogenicity and tumour promoting activity assays were used to compare cigarette smoke and commercially available tobacco heating product (THP).

• Responses from THPs were directly compared to a 3R4F reference tobacco product at equivalent doses.

• Clear positive activity from THPs was consistently observed in every assay condition.

• Compared to cigarette smoke, THP showed significantly reduced activity in all assays

References