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 2005) (1,2). 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 vitro 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.

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

TPM cytotoxicity was assessed using BALB/c 3T3 mouse fibroblasts.

Ames bacterial reverse mutation assay

Using TPM five S. typhimurium tester strains TA98, TA100, TA1535, TA1037 and TA102 + metabolic activation (S9) were assessed. For WA exposures, the Ames assay was employed with S. typhimurium tester strains TA98, TA100, TA1535, TA97 and TA102 using a modified methodology (2).

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, promoter protocol.

Results

Figure 2. Schematic drawing of the Vitrocell VC10 smoking robot. (A) controlling software; (B) smoking head; (C) piston; (D) dilution system; (E) exposure module with integrated quartz crystal microbalance (QCM) for dose measurements.

Figure 4. Ames, a representative dataset. (a) Ames TA98 TPM; (b) Ames TA98 WA, presented as a function of deposited mass using QCM technology. Positive responses observed for cigarette smoke in TPM and WA assessments. THP was deemed negative under both exposure scenarios.

Figure 5. A representative dataset. (a) MLA short – S9; (b) Bhas assay. Cigarette smoke deemed positive in both assays under all treatment conditions. THP was deemed negative in both assays under all treatment conditions.

Table 1. Summary of findings

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<th>Treatment condition</th>
<th>NRU TPM</th>
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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 3R4F cigarette smoke was observed in every assay

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

References


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Figure 1. Schematic drawing of THP1.0. (a) Heating device with tobacco consumable inserted; (b) physical construction of the tobacco consumable

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Figure 3. NRU results from cigarette smoke and THP in BALB/c mouse fibroblasts. Cigarette smoke showed a positive cytotoxic response with a full curve and complete cytotoxicity. THP TPM was deemed non-cytotoxicity under test conditions.

Figure 5. A representative dataset. (a) MLA short – S9; (b) Bhas assay. Cigarette smoke deemed positive in both assays under all treatment conditions. THP was deemed negative in both assays under all treatment conditions.

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Related References


Comparative In Vitro Toxicological Evaluation of a Novel Tobacco Heating Product

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Abstract

In vitro studies have been widely used to support the toxicological evaluation of chemicals and complex mixtures including cigarette smoke. More recently the same assays have been employed for the comparative assessment of electronic nicotine delivery systems, such as tobacco heating products (THP) against cigarette smoke.

In this study, traditional in vitro toxicological approaches using total particulate matter (TPM) and advanced aerosol exposure techniques were used to assess cigarette smoke from a Kentucky Reference 3RF cigarette against a commercially available THP. In vitro mutagenicity, cytotoxicity and tumour-promoting activity approaches were employed across TPM and whole aerosol test matrices.

The Ames bacterial mutation assay was employed using Salmonella typhimurium tester strains TA98, TA100, TA1535, TA1537 and TA102 ± metabolic activation (S9). The mouse lymphoma assay (MLA) was assessed ± metabolic activation with short 3hr exposures and longer 24hr-S9 exposures. The Bhas 42 cell transformation assay supplemented traditional approaches and was incorporated as an in vitro alternative for detecting tumour promoters and the neutral red uptake (NRU) cell viability assay provided an acute measure of cytotoxicity. The in vitro micronucleus assay was employed ± S9 with short and long exposures. To complement this in vitro testing strategy, the Ames assay was also employed with S.typhimurium strains TA98, TA100, TA1535, TA97 and TA102 using a scaled down 35mm methodology for the assessment of whole aerosols.

Cigarette smoke from TPM test matrices was deemed positive under almost all test conditions in all assays. For NRU, Ames, MLA, Bhas 42 and IVMN assays, responses were observed at 60µg/mL, 240µg/plate, 60µg/mL, 50µg/mL and 30µg/mL, respectively. In contrast, THP TPM failed to elicit a response in each of the assays up to 240µg/mL. Cigarette smoke was also deemed positive in the Ames assay at doses up to 5µg/cm². THP aerosols were negative at doses exceeded 25µg/cm².

Given a weight of evidence approach, these data demonstrate that THP test matrices are negative at doses equivalent and exceeding those of cigarette smoke where positive responses are observed, suggesting THPs may offer significant reduced risk potential compared to cigarette smoking.

Key words: Tobacco heating products, in vitro, neutral red uptake, Ames, mouse lymphoma, Bhas transformation assay, TPM, whole aerosol