Genotoxicity Evaluation of Tobacco and Nicotine Delivery Products
Part 1: Mouse Lymphoma Assay

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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. In this study a variety of test matrices from different tobacco and nicotine delivery products was assessed against a Kentucky reference (3R4F) cigarette. The mouse lymphoma assay (MLA) is underrun by OECD guideline 490 and ICH S2(R1) guidance and is a recognized in vitro genotoxicity test battery assay. The aim of this study was to assess the suitability of the MLA with a variety of tobacco and nicotine product test matrices. Testing was conducted in general accordance to OECD Guideline 490 and ICH S2(R1) test guidance. The same samples were also assessed using the in vitro micronucleus assay; results are reported separately (Part 2).

Full Text
Introduction
Next generation tobacco and nicotine products (NGPs), which are comprised of heating product (THP) using the mouse lymphoma assay (MLA) at a final concentration of 1% v/v (100-fold dilution), giving maximum concentrations of 500 µg/mL for THP and ENDS TPM and e-liquid, and 200 µg/mL for 3R4F Kentucky Reference. Cultures treated with DMSO only were used as vehicle controls.

Cell Culture & Treatments:
• Mouse lymphoma (L5178Y) cells (originating from Dr. Donald Clive), which are heterozygous at the thymidine kinase locus (tk-), were used.

Materials and Methods
Sample Preparation:
TPM and e-liquid

Experiment 1

Table 1: Summary of Findings

<table>
<thead>
<tr>
<th>Treatment</th>
<th>PLA</th>
<th>THP</th>
<th>ENDS</th>
<th>TPM</th>
<th>ENDS TPM</th>
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<td>Negative</td>
<td>Negative</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>PLA ENDS</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>THP TPM</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>THP ENDS</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
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<td>Negative</td>
</tr>
<tr>
<td>ENDS TPM</td>
<td>Negative</td>
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</table>

Conclusions
A clear and reproducible concentration-related response for the induction of MF by cigarette smoke was observed under all three test conditions and across two independent experiments.

3R4F TPM positive responses were observed at concentrations exceeding 60 µg/mL (3 hr -S9), 120 µg/mL (3 hr +S9) and 25 µg/mL (24 hr -S9), all within the 10-20% tolerances for RTG (relative total growth).

Conversely, at concentrations far exceeding those of cigarette smoke TPM, 3R4F TPM and ENDS TPM were negative. Both assays showed comparable findings. The data are presented in Tables 1-4 and summarized in Table 5.

FIGURE 1: Induced mutant frequencies (IMF; number of mutants/106 viable cells) under three treatment conditions, data expressed as a function of TPM or e-liquid concentration (µg/mL). (A) 3 hr -S9; (B) 3 hr +S9; (C) 24 hr -S9. Type 1 errors from Experiment 1. The two experiments showed comparable findings. The data are presented in Tables 1-4.

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References