THE BIOLOGICAL ASSESSMENT OF A NOVEL TREATMENT, DESIGNED TO REDUCE THE LEVELS OF CERTAIN TOBACCO LEAF CONSTITUENTS

Jane Collard & Clive Meredith.
British American Tobacco, Group Research and Development, Southampton, SO15 8TL, UK.
Correspondence: jane_collard@bat.com

INTRODUCTION
The US Institute of Medicine has encouraged the pursuit and development of potential reduced-exposure products (PREPs), tobacco products that substantially reduce exposure to one or more tobacco toxicants and can reasonably be expected to reduce the risk of one or more specific diseases or other adverse health effects (1).

BACKGROUND
As proteins and amino acids have been predicted to be a number of potentially toxic constituents of tobacco smoke, such as 2-amino-phenanthrene, 4-amino-phenyl and mutagenic heterocyclic amines (2), a novel tobacco treatment process has been developed (3) which not only reduces the levels of proteins in cut tobacco (Table 1) but also leads to decreases in mainstream smoke yields of both nitrogen-containing and phenolic toxicants (Figure 1).

The novel process involves the sequential extraction of the tobacco with water and an aqueous protease enzyme solution, followed by addition of adsorbents and then reapplication of the soluble materials to the extracted tobacco (3).

OBJECTIVES
The studies described here were designed to evaluate the biological effect of this tobacco treatment in a battery of in vitro studies, and a 90-day sub-chronic non-oral rodent inhalation study and so determine whether significant changes in tobacco leaf and mainstream smoke chemistry was associated with reduced toxicity when compared to the effects of a non-treated tobacco control product.

EXPERIMENTAL CIGARETTES
A series of cigarettes were manufactured in the British American Tobacco QMRC Centre in the UK. The cigarettes were based on a conventional Virginia style tobacco blend containing both tobacco leaf lamina and stam, although no casings or flavourings were added to the products. The same tobacco leaf lamina was used for all samples, although some of it had been subjected to the novel process prior to blending (3).

Furthermore, different filters were incorporated in the control and experimental products. These were a conventional cellulose acetate filter for the control product, and a cavity filter (see Figure 2) containing either activated charcoal, or a mixture of activated charcoal and DanemCR® (an amine functionalised porous carbon) (3).

All papers and adhesives used in the cigarette manufacture were standard materials used in commercial products.

Specific details of the control and test cigarettes are given in Table 2.

TABLE 1: TOBACCO CHEMISTRY (DRY WEIGHT BASIS)

<table>
<thead>
<tr>
<th>Tobacco Constituent</th>
<th>Untreated Blend</th>
<th>Treated Blend</th>
<th>% Difference Treated vs. Untreated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrate (mg/gram)</td>
<td>2.5</td>
<td>2.3</td>
<td>-2</td>
</tr>
<tr>
<td>Reducing Sugars (mg/gram)</td>
<td>10.3</td>
<td>12.6</td>
<td>+16</td>
</tr>
<tr>
<td>Protein (mg/gram)</td>
<td>1.5</td>
<td>1.3</td>
<td>-14</td>
</tr>
<tr>
<td>Total Nitrogen (mg/gram)</td>
<td>1.09</td>
<td>0.45</td>
<td>-59</td>
</tr>
</tbody>
</table>

**FIGURE 1: PERCENTAGE DIFFERENCE IN MAINSTREAM TOXICANT/TAR RATIOS DUE TO TOBACCO TREATMENT**

**FIGURE 2: CONSTRUCTION OF A CAVITY FILTER**

**TABLE 2: EXPERIMENTAL CIGARETTE DESIGN**

<table>
<thead>
<tr>
<th>Cigarette Code</th>
<th>Blend</th>
<th>Filter</th>
</tr>
</thead>
<tbody>
<tr>
<td>W860</td>
<td>30% Untreated Lamina + 20% Stem</td>
<td>Cellulose-Acetate</td>
</tr>
<tr>
<td>W861</td>
<td>30% Untreated Lamina + 20% Stem</td>
<td>Cavity with 60mg DanemCR® + 20mg Charcoal</td>
</tr>
<tr>
<td>W862</td>
<td>30% Untreated Lamina + 20% Stem</td>
<td>Cavity with 60mg DanemCR® + 30mg Charcoal</td>
</tr>
<tr>
<td>W863</td>
<td>30% Untreated Lamina + 20% Stem</td>
<td>Cavity with 60mg Charcoal</td>
</tr>
<tr>
<td>W864</td>
<td>40% Untreated Lamina + 40% Treated Lamina + 20% Stem</td>
<td>Cavity with 60mg Charcoal</td>
</tr>
</tbody>
</table>

**IN VITRO STUDIES**

**Experiments**

Cigarette smoke particulate matter (PM) was tested in the following assays:

- **Neutral Red uptake cytotoxicity assay**

Cytotoxicity (IC₅₀) was evaluated in V79 male Chinese Hamster lung cells.

- **Salmonella mutagenicity assay** (Ames test)

Mutations were determined in Salmonella typhimurium tester strains TA98, TA100, TA102, TA1535 and TA1537 in the presence and absence of S9.

- **Mouse lymphoma assay mammalian cell mutation assay**

Mutations were detected at the thymidine kinase (tk) locus of mouse lymphoma L5178Y cells, in the presence and absence of S9.

- **In vitro micronucleous test**

V79 male Chinese Hamster lung cells.

**TABLE 3: SALMONELLA ASSAY RESULTS FOR TA98 WITH S9**

**Conclusion**

The results of this test battery demonstrated that whilst the use of a tobacco containing reduced levels of proteins and polyphenols did reduce the genotoxicity associated with tobacco smoke constituents (including heterocyclic amines), it did not ameliorate any of the other biological endpoints (either in vitro or in vivo) investigated in these studies, when compared to an untreated tobacco control product.

Furthermore, it is postulated that the limited changes observed in the rodent inhalation toxicity were due to the presence of filters containing charcoal.

**REFERENCES**