EVIDENCE RELATED TO THE HEALTH RISK OF IQOS USE

EVALUATION OF NONCLINICAL STUDIES

Presented by
Mayo J. Wright, Ph.D.
Toxicologist
Office of Science
Center for Tobacco Products
U.S. Food and Drug Administration

Disclaimer: This is not a formal dissemination of information by FDA and does not represent Agency position or policy.
The information in these materials is not a formal dissemination of information by FDA and does not represent agency position or policy. The information is being provided to TPSAC to aid in its evaluation of the issues and questions referred to the committee.

This presentation contains information prepared by the FDA for the members of the TPSAC. The presentation describes assessments and/or conclusions and recommendations written by individual FDA reviewers. Such conclusions and recommendations do not necessarily represent the final position of the individual reviewers, nor do they necessarily represent the final position of the Review Division or Office. This presentation may not include all issues relevant to FDA’s decision on the applications and instead is intended to focus on issues identified by FDA for discussion by TPSAC. The FDA will not make its determination on the issues at hand until input from TPSAC and from the public comments has been considered and all FDA reviews have been finalized. FDA’s determination may be affected by issues not discussed at the TPSAC meeting.
• Chemicals found in HeatStick aerosols and reference cigarette smoke, including harmful and potentially harmful constituents
• In vitro studies
  − Cytotoxicity and mutagenicity studies
  − Organotypic studies
• In vivo studies
  − 90-day rat inhalation studies
  − 8-month cessation and switching study with ApoE -/- mice
  − Carcinogenicity study with A/J mice
Many HPHC levels in *HeatStick* aerosols are reduced compared to reference cigarette smoke. However, consuming 10 *HeatSticks* exposes users to levels of mercury, ammonia, acrylamide, butyraldehyde, acetamide, pyridine, formaldehyde, catechol, propylene oxide, and acetaldehyde that are comparable to smoking 1-3 reference cigarettes.

Source: Section 6.1., Pg. 20, Fig. 1
Many HPHC levels in HeatStick aerosols are reduced compared to reference cigarette smoke. However, consuming 10 HeatSticks exposes users to levels of mercury, ammonia, acrylamide, butyraldehyde, acetamide, pyridine, formaldehyde, catechol, propylene oxide, and acetaldehyde that are comparable to smoking 1-3 reference cigarettes.

A number of the HPHCs found in HeatStick aerosols are carcinogenic or possibly carcinogenic to humans.

Source: Section 6.1., Pg. 20, Fig. 1
TOXICOLOGICAL EVALUATION OF REFERENCE CIGARETTE SMOKE AND HEATSTICK AEROSOLS

The applicant has recently submitted data identifying at least 12 possibly carcinogenic or genotoxic chemicals that are found at higher levels in HeatStick aerosols than in reference cigarette smoke.

- For carcinogens that are mutagenic, cancer potency is assessed using a linear extrapolation from the low-dose region of the dose-response curve. Using this model, any increased exposure increases cancer risk.

<table>
<thead>
<tr>
<th>CAS#</th>
<th>Chemical</th>
<th>Fold Increase over 3R4F Cigarette</th>
<th>Designation</th>
</tr>
</thead>
<tbody>
<tr>
<td>98-00-0</td>
<td>2-Furanmethanol</td>
<td>6.8</td>
<td>Possibly Carcinogenic</td>
</tr>
<tr>
<td>96-24-2</td>
<td>3-chloro-1,2-Dihydroxyropane</td>
<td>5.7</td>
<td>Possibly Carcinogenic</td>
</tr>
<tr>
<td>556-52-5</td>
<td>Glycidol</td>
<td>3.2</td>
<td>Probably Carcinogenic</td>
</tr>
<tr>
<td>98-01-1</td>
<td>Furfural</td>
<td>1.6</td>
<td>Possibly Carcinogenic</td>
</tr>
<tr>
<td>128-37-0</td>
<td>Butylated hydroxytoluene</td>
<td>23.4</td>
<td>Possibly Genotoxic</td>
</tr>
<tr>
<td>25395-31-7</td>
<td>Diacetin</td>
<td>5.9</td>
<td>Possibly Genotoxic</td>
</tr>
<tr>
<td>930-60-9</td>
<td>2-Cyclopentene-1,4-dione</td>
<td>5.0</td>
<td>Possibly Genotoxic</td>
</tr>
<tr>
<td>106-61-6</td>
<td>Glyceryl 1-acetate</td>
<td>4.0</td>
<td>Possibly Genotoxic</td>
</tr>
<tr>
<td>102-62-5</td>
<td>1,2-Diacylglycerol</td>
<td>2.4</td>
<td>Possibly Genotoxic</td>
</tr>
<tr>
<td>765-87-7</td>
<td>1,2-Cyclohexanedione</td>
<td>1.8</td>
<td>Possibly Genotoxic</td>
</tr>
<tr>
<td>28564-83-2</td>
<td>2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one</td>
<td>1.6</td>
<td>Possibly Genotoxic</td>
</tr>
<tr>
<td>487-06-9</td>
<td>5,7-Dimethoxycoumarin</td>
<td>1.1</td>
<td>Possibly Genotoxic</td>
</tr>
</tbody>
</table>

The applicant submitted study reports for several in vitro cytotoxicity and mutagenicity assays for regular and menthol HeatStick aerosols and compared the results to reference cigarette smoke.

- Among these was the Ames test, which detects mutagenicity in bacteria.
- In the Ames test, reference cigarette total particulate matter (TPM; +S9) was mutagenic in 3 of the 5 bacterial strains, while HeatStick TPM was not mutagenic under any of the conditions tested.
The applicant submitted study reports for several in vitro cytotoxicity and mutagenicity assays for regular and menthol HeatStick aerosols and compared the results to reference cigarette smoke.

- While both reference cigarette smoke and HeatStick aerosols contain chemicals that are carcinogenic or possibly carcinogenic to humans (e.g., acetaldehyde, formaldehyde, benzene), HeatStick aerosols did not produce a positive response under conditions of the Ames test conducted by the applicant.

- The study reports did not contain information from an Ames test with the gas vapor phase (GVP) of the HeatStick aerosol. An Ames test with GVP would provide additional information about the mutagenic potential of HeatStick aerosol.
The applicant submitted study reports for several in vitro cytotoxicity and mutagenicity assays for regular and menthol *HeatStick* aerosols and compared the results to reference cigarette smoke.

- Study reports for neutral red uptake tests (NRU), which use a mammalian cell line to detect cytotoxicity, were also submitted.
- The NRU results indicate that *HeatStick* aerosols can be cytotoxic, but these effects are generally less severe and require higher levels of exposure than reference cigarette smoke.
- In the NRU test, total particulate matter (TPM) from *HeatStick* aerosols was 90% less cytotoxic than TPM from reference cigarette smoke.
The applicant also submitted study reports from the mouse lymphoma assay (MLA), a test that detects mutagenicity in a mammalian cell line.
HeatStick aerosols were mutagenic in the MLA, with toxicity occurring at higher concentrations compared to reference cigarette smoke. (↑15-30 fold for TPM, ↑8-24 fold for GVP).
The MLA study reports did not contain information about clastogenicity, or the capacity to cause damage to chromosomes.

Also, while the MLA study, which is an in vitro test, indicates that HeatStick aerosols are mutagenic, there was no in vivo mutagenicity information which could have further clarified the mutagenic potential of the products.

Source: Section 7.2, multiple files
The applicant submitted data from five separate in vitro organotypic studies assessing the effects of aerosols from regular *HeatSticks* compared to reference cigarette smoke on human gingival, buccal, nasal, bronchial, and coronary arterial epithelium cultures.

- The results indicate that *HeatStick* aerosols generally produced fewer pathophysiological changes and adverse effects than reference cigarette smoke.
- For example, reference cigarette smoke produced significant cytotoxicity and histological changes in the bronchial epithelium that persisted for at least 72 hours, while *HeatStick* aerosols produced fewer effects, and those effects were less severe.
• Similarly, *HeatStick* aerosols can have pro-inflammatory effects as well as adverse pathophysiological effects in buccal cell cultures, and alter responses to oxidative stress in gingival cell cultures, but those changes are less pronounced than effects from the 3R4F reference cigarette smoke and generally occur at higher concentrations.

• Also, *HeatStick* aerosols increased cell adhesion and reduced monocyte migration in coronary artery cell cultures, but only at higher concentrations than 3R4F reference cigarette smoke.
The applicant submitted study reports from two separate 90-day nose-only rat inhalation studies with a 42-day post-exposure recovery period using regular and menthol HeatSticks, as well as reference cigarettes.

- In general, repeated exposure to HeatStick aerosols produced fewer or less severe pathophysiological changes in the respiratory tract than exposure to reference cigarette smoke.

- However, some degeneration was observed in the larynx of rats exposed to either reference smoke or menthol HeatStick aerosols.

- Also, concentration-dependent increases in the epithelial thickness of the floor of the larynx and vocal cords occurred to a lesser extent in rats exposed to HeatStick aerosols than to those exposed to reference cigarette smoke.
For squamous metaplasia, a potentially precancerous lesion, the response produced in the larynx by HeatStick aerosols was similar to that of the reference cigarette smoke after the 90-day exposure period.

Source: Section 7.2, “15006 THS SR Part 5.pdf”
8-month Switching and Cessation Study with ApoE -/- Mice

- The ApoE-/- mouse model is well established for studying atherosclerosis, as mice develop hypercholesterolemia on a standard chow diet.
- Groups included:
  - Sham (filtered air for 8 months)
  - Reference cigarette smoke for 8 months
  - HeatStick aerosol for 8 months
  - Cessation (2 months reference cigarette smoke and 6 months filtered air)
  - Switching (2 months reference cigarette smoke and 6 months HeatStick aerosol)
8-month Switching and Cessation Study with ApoE -/- Mice

- Histopathological findings indicate that 8 months of reference cigarette smoke exposure increased mean cord length, destructive index, and alveolar emphysema score, and decreased the number of bronchiolar attachments compared to other groups.

Source: Section 7.5, “15015_CVD_Resp_ApoE_SW_SR_Part 5.pdf”
8-month Switching and Cessation Study with ApoE -/- Mice

- Mice that were only exposed to the *HeatStick* aerosol, that switched from reference cigarette smoke to *HeatSticks* aerosol, that underwent cessation, and that were only exposed to sham conditions all had similar histopathological characteristics.

Source: Section 7.5, "15015_CVD_Resp_ApoE_SW_SR_Part 5.pdf"
8-month Switching and Cessation Study with ApoE -/- Mice

- Biomarkers of exposure for carbon monoxide, acrolein (shown), NNK (shown), benzene, and acrylonitrile, as well as some biomarkers of oxidative stress and inflammation were elevated in the ApoE -/- mice exposed to reference cigarette smoke, but not in the HeatStick-exposed or sham control mice.

Source: Section 7.5, “15015_CVD_Resp_ApoE_SW_SR_Part 1.pdf”; Pg. 65, Fig. 12
Preliminary data indicate that after 10 months of exposure, neoplastic lesions (e.g., bronchioloalveolar adenoma) were found in the lungs of female mice exposed to reference cigarette smoke and HeatStick aerosols.

- The study with male mice was terminated at 15 months due to a high number of deaths.

Source: Section 7.2, “15020 THS SR Part 5.pdf” Pg. 132-133, Fig. 19-20
SUMMARY

- *HeatStick* aerosols demonstrated potential toxicity under the conditions tested by the applicant, but the adverse effects were generally fewer and less severe than what was observed with reference cigarette smoke.
- When *HeatStick* aerosols induced toxicity in the in vitro and in vivo studies, toxicity occurred at higher concentrations compared to reference cigarette smoke.
- *HeatStick* aerosols did not produce any additional adverse effects beyond those observed in test groups exposed to reference cigarette smoke.
- Based on the studies submitted, however, it is unclear if the effects observed in treatment groups exposed to *HeatStick* aerosols translate to a potential risk reduction for noncancer-related effects when chronically used by humans.
CLARIFYING QUESTIONS?