Human mouth-level transfer rate of menthol, 1.8-cineole and nicotine from Swedish pouched snus

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2. Imperial Tobacco Limited, PO Box 525, Winterstoke Road, Bristol BS99 1LQ, U.K.
Why conduct a consumer study?

- Smokeless-oral tobacco product: significant history of use in Sweden

- Aims:
  - measure transfer rates of nicotine and selected ingredients
  - be able to compare consumer derived and in vitro derived data

- Consumer study facilitates the development of a transfer model which can be used to estimate exposure to flavour and tobacco constituents
Snus study

Milestones

consumer study
• before & after 30-min of use
• To measure constituents: menthol, 1.8 cineole and nicotine
• at two flavour application rates
• over 595 samples
• to estimate transfer rate

in vitro tests in tandem
• three extraction media: artificial saliva, water and 1% β-cyclodextrin in water
• three immersion time: 30, 10 and 5 minutes
• two flavour application rates

Comparison & perspectives
Consumer snus study design

- Study size: 198 active Swedish snus consumers (18-65)
- Predominantly males (80:20 split)
- Legal consent.

Panel

- Pouches manufactured specifically (0.8 g)
- Experimental pouch

Study Product

- Snus samples used for 30 minutes. No eating & drinking while using snus.
- Three replicate snus tested per participant = ~ 600 samples
- 15 minutes break between each snus pouch
- Samples on dry ice. Stored at -20°C before analyses.

Sample Collection

<table>
<thead>
<tr>
<th>flavor application rate</th>
<th>nicotine (mg/g)</th>
<th>water</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 % w/w</td>
<td>9.2 (6%)</td>
<td>44%</td>
</tr>
<tr>
<td>6 % w/w</td>
<td>10.3 (4%)</td>
<td>38%</td>
</tr>
</tbody>
</table>
Outcomes & Influencing factors

Transfer Rate (%)

\[
\text{Transfer Rate} = \frac{\text{Total extratable amount} - \text{Residual amount found after consumer extraction}}{\text{Total extractable amount}}
\]

Three sources of variability:

- analytical variability
- intra-human variability
  - 3 replicates per pouch
  - pouch-to-pouch variation
- inter-human variability for the three analytes

Human parameter (gender/ages)

Used pouched snus / robustness of technique
- dichloromethane/methanol extraction
- saliva effect evaluated
- post-sampling storage
Consistency of samples: only 2% of the users stated that they used each pouch for less than 30 min.

The level of extraction is not related to gender (Anova p>0.05) or age of consumer.
Individual Results / 2

<table>
<thead>
<tr>
<th>snus pouch application rate</th>
<th>4%</th>
</tr>
</thead>
<tbody>
<tr>
<td>w/w%</td>
<td>6%</td>
</tr>
</tbody>
</table>

Mean [% , U95] 13 ± 1
N 300

Mean [% , U95] 9 ± 2
N 294

U95 Expanded Uncertainty at 95%
Estimated transfer rates to saliva
30 min usage duration

<table>
<thead>
<tr>
<th></th>
<th>N=593</th>
<th>Mean ± U95</th>
<th>Transfer rate ± U95</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>in mg, per whole 0.8 g pouch basis</td>
<td>in %</td>
</tr>
<tr>
<td><strong>Application rate</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4%</td>
<td>6%</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>menthol</strong></td>
<td>1.50 ± 0.03</td>
<td>2.61 ± 0.03</td>
<td>9 ± 2</td>
</tr>
<tr>
<td><strong>1,8-cineole</strong></td>
<td>0.195 ± 0.005</td>
<td>0.346 ± 0.006</td>
<td>25 ± 2</td>
</tr>
<tr>
<td><strong>nicotine</strong></td>
<td>6.8 ± 0.2</td>
<td>7.9 ± 0.1</td>
<td>22 ± 1</td>
</tr>
</tbody>
</table>

U95 Expanded Uncertainty at 95%

- Low levels of extraction of constituents from pouched snus
- Similar results for the 2 flavour rates for the 3 compounds
Relative transfer rates proportional to their relative water solubility.

- Previous findings:
Results /3: Sources of variability expressed in %

<table>
<thead>
<tr>
<th>Application rate</th>
<th>Total variability</th>
<th>analytical variability</th>
<th>intra-human variability</th>
<th>inter-human variability</th>
</tr>
</thead>
<tbody>
<tr>
<td>menthol</td>
<td>17</td>
<td>15</td>
<td>60</td>
<td>25</td>
</tr>
<tr>
<td>4% 1,8-cineole</td>
<td>23</td>
<td>15</td>
<td>60</td>
<td>25</td>
</tr>
<tr>
<td>nicotine</td>
<td>22</td>
<td>5</td>
<td>35</td>
<td>60</td>
</tr>
<tr>
<td>menthol</td>
<td>10</td>
<td>15</td>
<td>70</td>
<td>15</td>
</tr>
<tr>
<td>6% 1,8-cineole</td>
<td>16</td>
<td>20</td>
<td>75</td>
<td>5</td>
</tr>
<tr>
<td>nicotine</td>
<td>16</td>
<td>5</td>
<td>50</td>
<td>45</td>
</tr>
</tbody>
</table>

Calculation based on the three-Way Anova

- total variability:
  - driven by the intra-subject variability for flavors
  - reverse situation for nicotine

- additional outcome: smaller panel for further studies on flavors
Snus study

Milestones

Consumer study → in vitro tests in tandem → Comparison & perspectives
"in vitro " protocol

1 pouch (at 2 flavours levels) agitate at 37°C (150 rpm) for Xmin immersion time using 30 mL of each media

<table>
<thead>
<tr>
<th>Analyzed compounds</th>
<th>Media</th>
</tr>
</thead>
<tbody>
<tr>
<td>menthol</td>
<td>1 β-cyclodextrin artificial saliva water</td>
</tr>
<tr>
<td>1,8-cineole nicotine</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Flavour application rate</th>
<th>Immersion time with media</th>
</tr>
</thead>
<tbody>
<tr>
<td>4w/w% pouch</td>
<td>05 min</td>
</tr>
<tr>
<td>6w/w% pouch</td>
<td>10 min</td>
</tr>
<tr>
<td></td>
<td>30 min</td>
</tr>
</tbody>
</table>
## Artificial media results

### Menthol transfer rate

<table>
<thead>
<tr>
<th>Immersion time (min.)</th>
<th>BCD Water saliva</th>
<th>artificial saliva</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4%</td>
<td>6%</td>
</tr>
<tr>
<td>5</td>
<td>21 ± 3</td>
<td>21 ± 10</td>
</tr>
<tr>
<td>10</td>
<td>28 ± 10</td>
<td>24 ± 7</td>
</tr>
<tr>
<td>30</td>
<td>27 ± 7</td>
<td>25 ± 3</td>
</tr>
</tbody>
</table>

### Nicotine transfer rate

<table>
<thead>
<tr>
<th>Immersion time (min.)</th>
<th>BCD Water saliva</th>
<th>artificial saliva</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4%</td>
<td>6%</td>
</tr>
<tr>
<td>5</td>
<td>60 ± 8</td>
<td>57 ± 11</td>
</tr>
<tr>
<td>10</td>
<td>70 ± 9</td>
<td>66 ± 7</td>
</tr>
<tr>
<td>30</td>
<td>82 ± 4</td>
<td>78 ± 4</td>
</tr>
</tbody>
</table>

**Consumer study**

- Mean [%], U95: 9 ± 2, 13 ± 1
- 90th percentile (%): 16, 18

- Mean [%], U95: 22 ± 2, 23 ± 1
- 90th percentile (%): 37, 33

Two -way ANOVA and confident limits @95%(n=6).
Similar results for the 2 flavour rates for the 3 compounds

- Overestimation of all artificial media vs. consumer data set
- An extraction time of 5 minutes with artificial saliva or water gave the closest results to the consumer data set
Conclusions /1:

For the two Smokeless-oral tobacco products used in this study and focusing on only nicotine and the ingredients (menthol & 1,8-cineole):

Consumer study:

- Low levels of extraction of constituents from pouched snus
- Sources of variability
  - Intra subject variability has a high contribution relatively to the inter subjects variability for flavors
  - Reverse situation for nicotine
- Relative transfer rates appear to be related to their relative water solubility.
Conclusions /2:

Comparison of consumer derived and in vitro derived data:

✓ Overestimation of all artificial media vs. consumer data set.
✓ Artificial media: an extraction time of 5 minutes with artificial saliva or water gave the best fit to the consumer data set.
✓ The relative extraction of the three constituents reflects consumer derived data.
✓ Nevertheless regarding the 90th Percentile of the consumer derived data, there is still an overestimation of 30% for higher solubility compounds such as nicotine
Perspectives

- Open questions on nicotine request further investigations
- Additional tests to be performed to confirm the *in vitro* model with an enlarged panel of flavours
- Extra study to be conducted including smallest size of the study group
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