Analytical method to model human mouth-level transfer of ingredients from Swedish pouched snus

S. Grapton[1], S. Gadois-Pommereul[1], V.Troude[1], M. Blanchet[1], A. Clarke[2], L. Simms[2]

1-SEITA Imperial Tobacco, 48 rue Danton 45404 Fleury les Aubrais France. 2- Imperial Tobacco Ltd, Bristol, United Kingdom

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Objective

- To support the development of a mouth-level transfer model, levels of target compounds (nicotine and the ingredients menthol and 1,8 cineole) from Swedish pouched snus were compared following human use and *in vitro* extraction.
  Unused pouched snus were extracted with 3 different media (1% β-cyclodextrin solution, artificial saliva and water) and with 3 different immersion times for each media (5, 10 or 30 min).
- The aim was to rank analytically the products with an *in vitro* extraction in a similar order than the human extraction average levels.

Consumer study protocol

- 2 flavour application rates (4 and 6w/w%)
- Panel: 200 Swedish consenting adults
- 3 pouches (~0,8g) per volunteer (600 samples)
- Time in mouth = 30 minutes
- Snus transferred into a flask for analysis

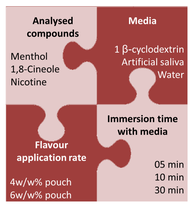
Introduction

- **The first step of the study** was based on panel consumption of 2 pouched snus samples with 2 different flavour application rates. The aim was to calculate the human mouth-level transfer rate of nicotine and the ingredients menthol and 1,8-cineole. The transfer rate (TR%) was calculated from residual levels of target compound remaining in snus after consumption versus the amount present in unused snus. Human mouth-level extraction transfer rates were then compared with the results from the *in vitro* extraction study.

Analytical approach

Extraction with the media

Parameters



Protocol

Snus sample: 1 pouch weighed

Media: pre-heated at 37°C for 3 hours

***In vitro* extraction**:

- volume: 30mL in a 50mL falcon tube
- stirred at 150 rpm in a water bath at 37°C
- immersion time: 5, 10 or 30 minutes

After *in vitro* extraction, pouches are isolated from media and transferred into an other tube

Solvent extraction step

- Pouch was slit open and 25mL of dichloromethane/methanol (1:1 v/v) containing 10µL/L tetradecane (ISTD) was added.
- Extraction for 24 hours (lab shaker = 220 rpm).
- Supernatant filtered into a vial with a syringe filter (0,45µm PTFE).
- Extraction efficiency confirmed with a second extraction (< LOQ).

Analytical method

Gas Chromatography Agilent GC 7890A: Solgel Wax column (30m x 0,25mm x 0,25µm) - 1µL injected in hot needle (250°C) - Split mode (1:100).

Mass Spectrometry: Electronic impact *EI* source - Single Ion Monitoring *SIM* detection mode.

Results

$$\text{Transfer rate TR (\%)} = 100 \times \frac{\text{Total extractable amount - Residual amount found after } \textit{in vitro} \text{ extraction}}{\text{Total extractable amount}}$$

Total extractable results and water solubility

Application rate	Menthol[a] Mean amount [mg/pouch]	CV [%]	1,8-Cineole[a] Mean amount [mg/pouch]	CV [%]	Nicotine[a] Mean amount [mg/pouch]	CV [%]
Water Solubility (mg/L)	490 @25°C		3500 @21°C		1000000	
4%	1,75	5%	0,238	8%	9,2	6%
6%	2,99	3%	0,399	13%	10,3	4%

[a] Values based on n=6 individual replicates
Results on wet weight based of unused snus

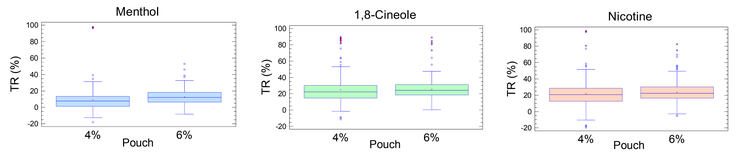
Consumer panel results

- *Figures obtained from the consumer study*

Application rate	n replicates		Menthol[a] Mean amount [mg/pouch]	CV [%]	Mean TR[b] [%]	1,8-Cineole[a] Mean amount [mg/pouch]	CV [%]	Mean TR[b] [%]	Nicotine[a] Mean amount [mg/pouch]	CV [%]	Mean TR[b] [%]
4%	294	Mean	1,50	16%	9%	0,195	18%	25%	6,8	19%	22%
		90th Percentile	1,66		16%	0,224		35%	8,0		37%
6%	300	Mean	2,61	6%	13%	0,346	11%	25%	7,9	13%	23%
		90th Percentile	2,81		18%	0,389		34%	9,0		33%

[a] Results on wet weight based of used snus
[b] TR calculated with the total extractable amount from the panel study

- *Box plot*



Artificial media results (*in vitro*)

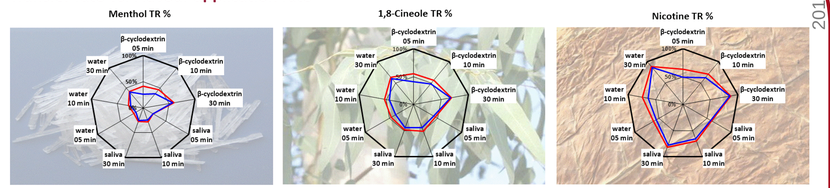
- *Figures obtained from the transfer in artificial media*

Application rate	Artificial Media	Contact Time [min]	Menthol[a] Mean amount [mg/pouch]	CV [%]	Mean TR [%]	1,8-Cineole[a] Mean amount [mg/pouch]	CV [%]	Mean TR [%]	Nicotine[a] Mean amount [mg/pouch]	CV [%]	Mean TR [%]
4%	1% β-Cyclodextrin	5	1,02	11%	42%	0,107	12%	55%	3,3	14%	64%
		10	0,95	8%	45%	0,102	10%	57%	2,6	16%	71%
		30	0,71	18%	60%	0,073	26%	69%	1,2	26%	87%
	Artificial Saliva	5	1,38	5%	21%	0,130	18%	45%	3,7	23%	60%
		10	1,26	12%	28%	0,117	19%	51%	2,7	26%	70%
		30	1,28	11%	27%	0,122	16%	49%	1,7	20%	82%
	Water	5	1,39	7%	20%	0,127	10%	47%	3,6	13%	61%
		10	1,24	7%	29%	0,112	10%	53%	2,4	20%	74%
		30	1,03	8%	41%	0,086	12%	64%	1,0	14%	89%
6%	1% β-Cyclodextrin	5	2,19	12%	27%	0,235	17%	41%	5,3	20%	49%
		10	1,94	13%	35%	0,207	16%	48%	3,8	26%	63%
		30	1,30	29%	57%	0,131	35%	67%	1,5	45%	85%
	Artificial Saliva	5	2,35	11%	21%	0,235	18%	41%	4,4	27%	57%
		10	2,26	12%	24%	0,223	19%	44%	3,5	23%	66%
		30	2,24	6%	25%	0,223	9%	44%	2,3	18%	78%
	Water	5	2,50	8%	16%	0,249	12%	38%	4,6	21%	55%
		10	2,26	9%	24%	0,224	13%	44%	3,8	23%	64%
		30	1,78	12%	40%	0,155	26%	61%	1,4	58%	86%

[a] Values based on n=6 individual replicates, except for 5 and 10 min with 1% β-Cyclodextrin where n=3
Results on wet weight based

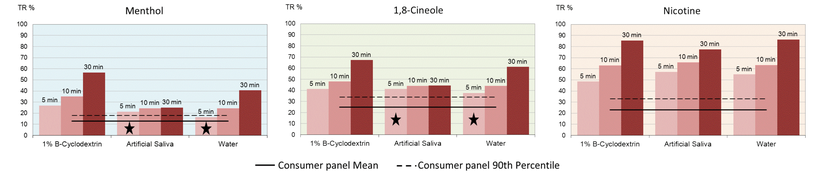
Discussion

Transfer rate vs. Flavour application rate



- The transfer rates of nicotine and the ingredients menthol and 1,8-cineole were similar, independent of application rates used (no statistically significant difference between 4% and 6%, $p \geq 0{,}05$).

Transfer rate vs. Immersion time in media (6% Pouch)



- The *in vitro* transfer rate of nicotine and the ingredients menthol and 1,8-cineole after 30 min extraction in all 3 media overestimated the potential mouth-level transfer rate in consenting adults using the product for 30 min. Nevertheless, artificial saliva provides the result closest to that of the consumer panel.
- The *in vitro* transfer rate of nicotine increased with increasing immersion time with all 3 media.
- The *in vitro* transfer rate of menthol and 1,8-cineole increased with increasing immersion time in water and 1%-β-cyclodextrin, whereas the transfer rate with artificial saliva was largely independent of immersion time.
- The human-mouth level transfer rate of menthol and 1,8-cineole (after 30 min of use) was the closest to the results obtained with *in vitro* extraction of 5 min in water or artificial saliva.

Conclusions

- Nicotine transfer was overestimated for all media and all immersion times. Closest results to the human data for a immersion time of 5 minutes in 1% β-cyclodextrin solution.
- *In vitro* transfer rates for menthol and 1,8-cineole: Closest results to the human data for a immersion time of 5 minutes in water or artificial saliva (★).
- A similar order for flavour compounds (menthol and 1,8-cineole) in human extraction and *in vitro* extraction.

Perspectives

- Additional tests to be performed to confirm the *in vitro* results with an enlarged panel of flavours and the specificity of the Nicotine.
- Determination of a multi-analyte approach to analyse several tobacco constituents from different kinds of pouched snus (such as different recipes, flavours, flavour application rates…).

Reference Saskia van Ruth: "Evaluation of the influence of mastication on temporal aroma release of ripe and unripe bananas, using a model mouth system and gas chromatography-olfactometry"; European Food Res. Technol. 217, 291-295, 2003.