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

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

1. SEITA, Imperial Tobacco Group, 48 rue Danton, 45404 Fleury-les-Aubrais, France
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
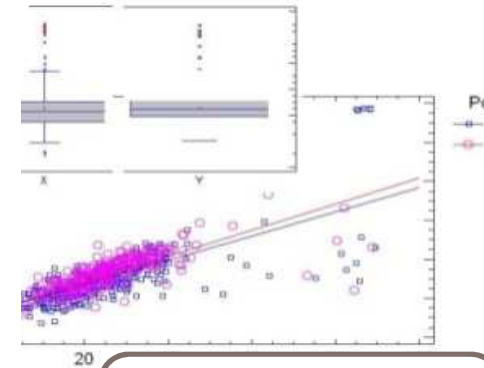
Snus study
Milestones
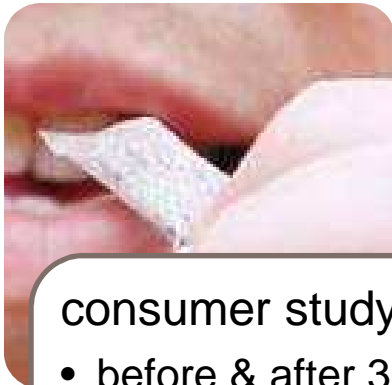
consumer study

- 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*

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

Study Product
- Pouches manufactured specifically (0.8 g)
- Experimental pouch

Sample Collection
- 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.

flavor application rate	nicotine (mg/g)	water
4 % w/w	9.2 (6%)	44%
6 % w/w	10.3 (4%)	38%

Outcomes & Influencing factors

$Transfer\ Rate\ (\%)$

$$= \frac{Total\ extratable\ amount - Residual\ amount\ found\ after\ consumer\ extraction}{Total\ extractable\ amount}$$

Used pouched snus / robustness of technique
-dichloromethane/methanol extraction
-saliva effect evaluated
-post-sampling storage

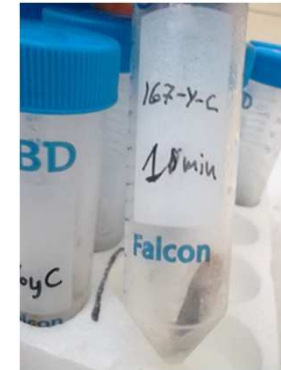➡ Amount of used snus and transfer rates

➡ 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)

Results /1
panel

❑ Consistency of samples:
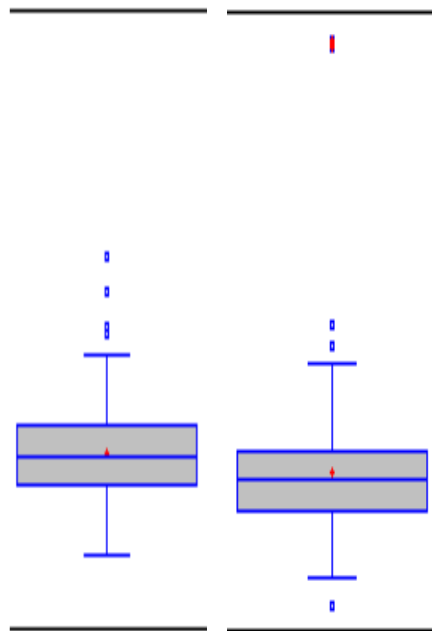only 2% of the users stated that they used
each pouch for less than 30 min.

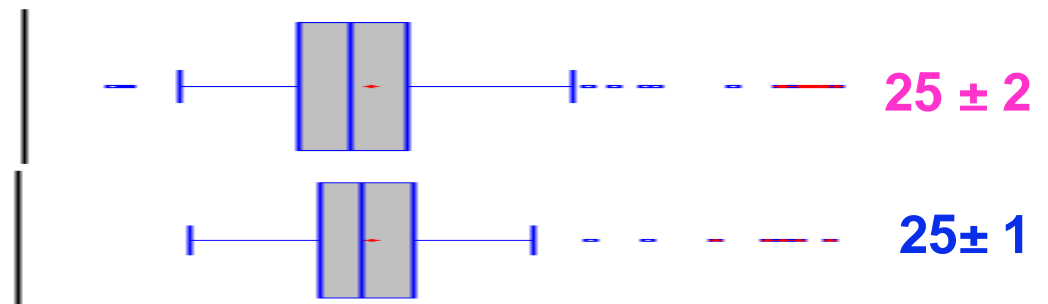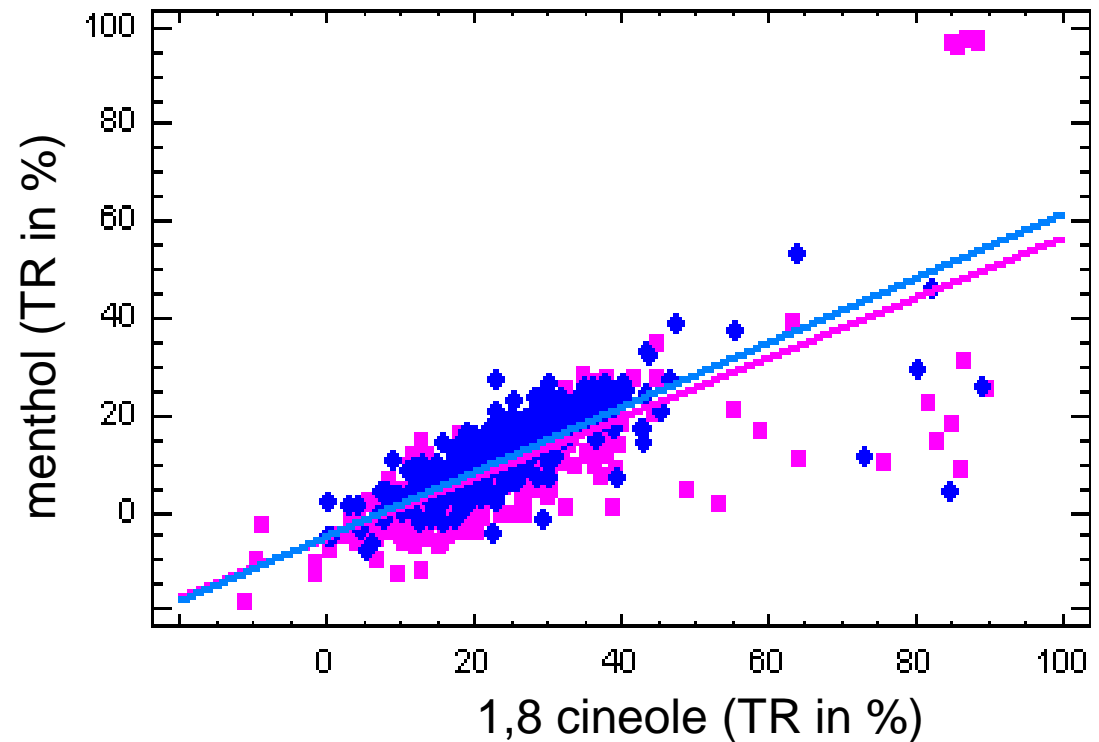❑ The level of extraction is not related to gender (Anova
p>0.05) or age of consumer.

snus pouch

application rate	**4%**
w/w%	**6%**

menthol (TR in %)

1,8 cineole (TR in %)

Mean [%, U95]	**13 ± 1**	**9 ± 2**
N	**300**	**294**

25 ± 2

25± 1

U95 Expanded Uncertainty at 95%

Estimated transfer rates to saliva

30 min usage duration

| N=593	Mean ± U95		Transfer rate ± U95	
	in mg, per whole 0.8 g pouch basis		in %	
Application rate	**4%**	**6%**	**4%**	**6%**
menthol	1.50 ± 0.03	2.61 ± 0.03	9 ± 2	13 ± 1
1,8-cineole	0.195 ± 0.005	0.346 ± 0.006	25 ± 2	25 ± 1
nicotine	6.8 ± 0.2	7.9 ± 0.1	22 ± 1	23 ± 1

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

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Transfer rate *vs*. water solubility

❑ Relative transfer rates proportional to their relative water solubility.

Water solubility mg/L @25°C	Transfer rate [%]		
		4%	**6%**
490	**menthol**	9 ± 2	13 ± 1
3500	**1,8-cineole**	25 ± 2	25 ± 1
100000	**nicotine**	22 ± 1	23 ± 1

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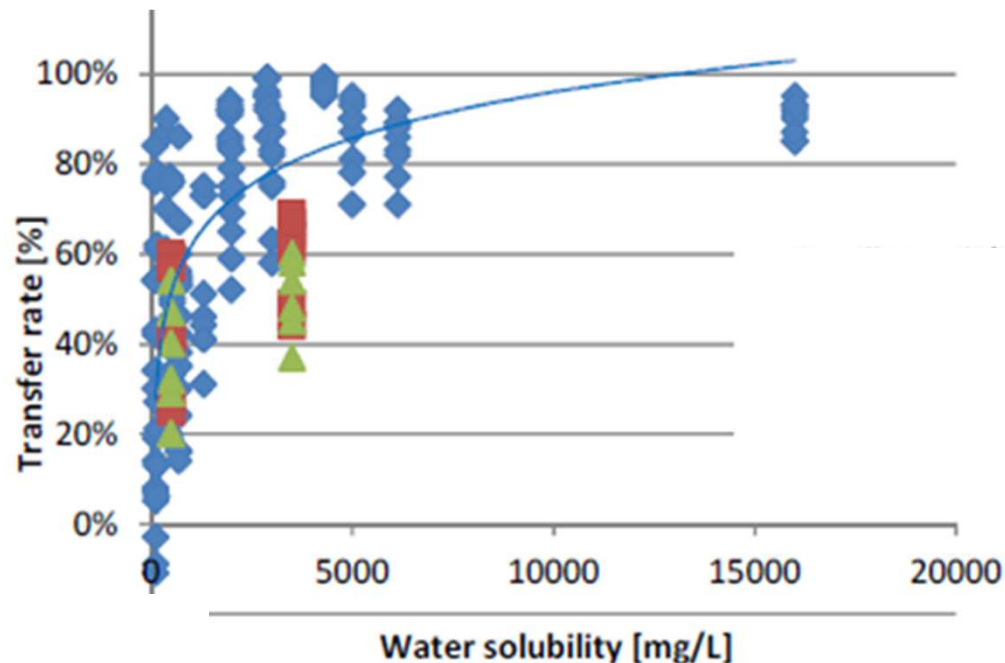
Results /3 : Sources of variability expressed in %

Application rate		Total variability	analytical variability	intra-human variability	inter-human variability
	menthol	17	15	60	25
4%	1,8-cineole	23	15	60	25
	nicotine	22	5	35	60
	menthol	10	15	70	15
6%	1,8-cineole	16	20	75	5
	nicotine	16	5	50	45

Calculation based on the three–Way Anova

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

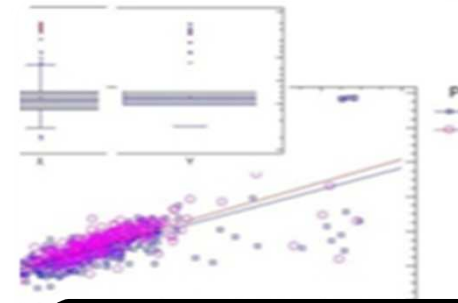❑ additional outcome: smaller panel for further studies on flavors

Snus study
Milestones

Consumer study

in vitro tests in tandem

Comparison & perspectives

"in vitro " protocol

3-factor study
108 tests

In vitro-system:

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

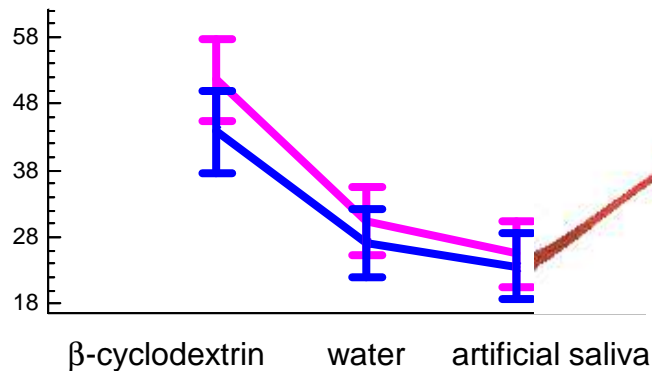| Analyzed compounds	Media
menthol 1,8-cineole nicotine	1 β-cyclodextrin artificial saliva water
Flavour application rate	**Immersion time with media**
4w/w% pouch 6w/w% pouch	05 min 10 min 30 min

Artificial media

results

menthol TR

artificial saliva

immersion time (min.)	menthol transfer rate [%,U95]	
	4%	**6%**
5	21 ± 3	21 ± 10
10	28 ± 10	24 ± 7
30	27 ± 7	25 ± 3

Consumer study

Mean [%, U95]		**9 ± 2**	**13 ± 1**
90th percentile (%)		**16**	**18**
Mean [%, U95]		**22 ± 2**	**23 ± 1**
90th percentile (%)		**37**	**33**

nicotine TR

Two –way Anova and confident limits @95%(n=6).
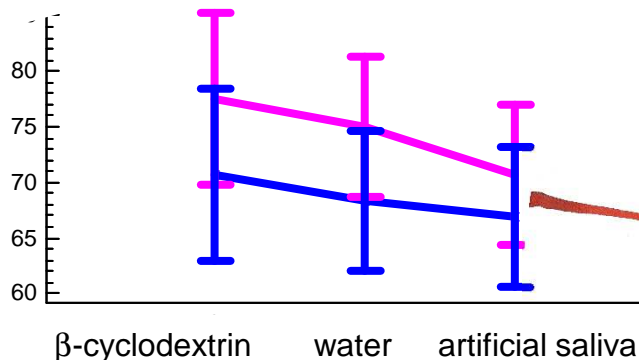
artificial saliva

immersion time (min.)	nicotine transfer rate [%, U95]	
	4%	**6%**
5	60 ± 8	57 ± 11
10	70 ± 9	66 ± 7
30	82 ± 4	78 ± 4

Artificial media *overview*
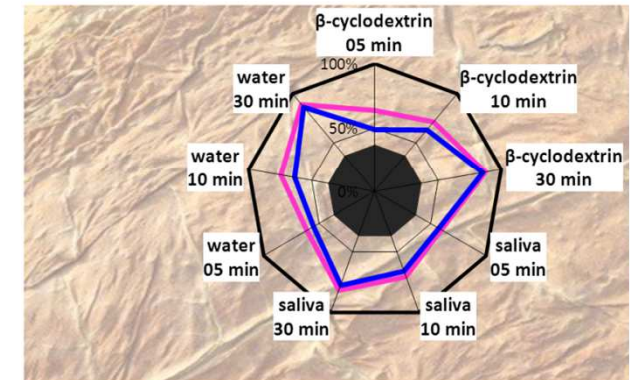
menthol (TR in %)
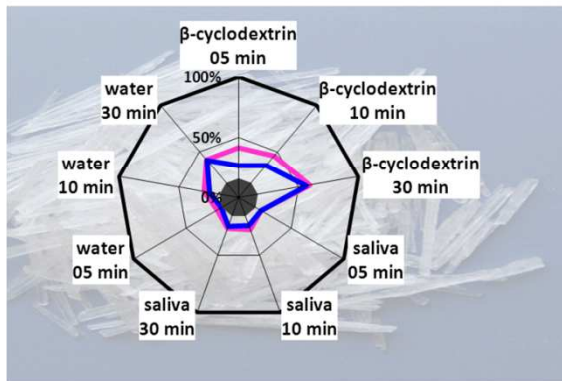
1,8 cineole (TR in %)
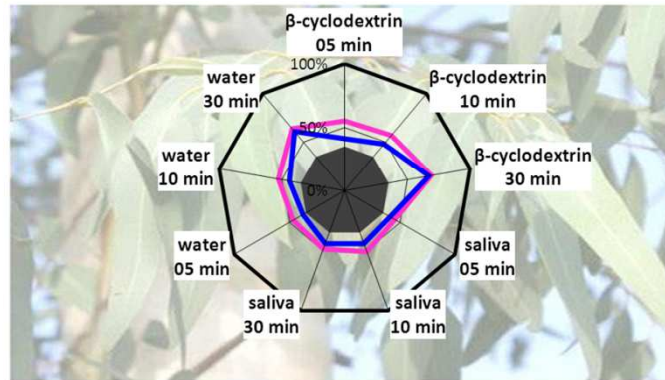
nicotine (TR in %)

—— 4%   —— 6% application rate   —— TR=100%   ■ 90th Percentile from consumer study

❑ 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 focussing 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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