Influence of usage time on exposure of snus users to nicotine, NNN and NNK from snus pouches

Nathan Gale, Helena Digard, Kevin McAdam and Justine Williamson

British American Tobacco, Group Research and Development, Regents Park Road, Southampton, SO15 8TL, United Kingdom

Correspondence: kevin_mcadam@bat.com

INTRODUCTION

Measuring the amount of constituents extracted by snus consumers during use is a valuable step in estimating exposure to tobacco constituents. A factor potentially influencing the extent of exposure is the length of time that individual consumers keep snus pouches in their mouths during use. A 2007/2008 survey of snus use in Sweden established that, on average, use of pouched snus extends from approximately 30 minutes to just less than 120 minutes[1]. The objective of this study therefore was to quantify the importance of use duration on constituent transfer from pouched snus to the consumer over this timescale. The constituents selected for evaluation were nicotine and the two tobacco-specific nitrosamines (TSNAs) classified by IARC as Group 1 (sufficient evidence of carcinogenicity in humans)[2], 4-(N-Methylnitrosamino)-1-(3-pyridinyl)-1-butanone (NNK) and N-Nitrosonornicotine (NNN).

METHODS

User Trial

30 volunteer pouched snus users gave informed consent and took part in a central location study split between Stockholm and Lund, Sweden. The following recruitment criteria were employed:

- Males, aged 19 – 65 years old
- Users with a minimum of 6 months snus use
- Daily users of "Brown", 1g pouched snus products
- Users of a minimum of 8 pouches per day, for one hour or more per use on average
- Users who place the pouch in their mouth, under their upper lip
- Users with no dislike for the "Lucky Strike" brand

Each volunteer used a 1g pouch of the commercial "Lucky Strike Original" snus product, for ten different duration periods (from 5 to 120 minutes), randomly ordered over 3 sessions. The same 30 volunteers were therefore investigated for each time point of the time-course study. Throughout their sessions, volunteers were required not to consume food, beverages or tobacco products, other than as directed. Drinking water was allowed during breaks of 15-30 minutes which were provided between each pouch use.

After use each used pouch was collected in an individual glass vial, and a corresponding unused pouch taken from the same tin of snus was collected in a second glass vial. At the end of each session, all portions were stored frozen (-20°C) at the study site prior to shipping to the BAT Analytical Laboratories in the UK in cool boxes containing ice packs. On arrival at the laboratories all portions were stored in a freezer (-20°C) for a minimum of 24 hours and a maximum of 11 weeks.

Sample Analysis

Each used and unused pouch was thawed at room temperature for 1 hour, prior to extraction *in situ* with methanol (20mL). This multi-constituent method based on methanol extraction has been described in a previous presentation[3]. **Nicotine** analysis was performed using a HP6890 gas chromatograph fitted with an autosampler and 5973 mass selective detector. 1µL of solution was injected into a splitless injector at 250°C. A J&W HP-5MS 30M x 0.25mm ID x 0.25µm film column was employed with a temperature programme of 70°C to 230°C over 44 minutes. Helium was used as carrier gas at 1.5mL/minute. The mass selective detector operated in SIM mode with a source temperature of 230°C and quadrupole temperature of 150°C.

TSNAs were quantified using a triple quadrupole LC/MS/MS. Deuterated equivalents of NNN and NNK were added to the extraction aliquot prior to analysis. Analysis was conducted with an Applied Biosystems API 5000 LC/MS/MS in positive ESI mode, with a mass range of m/z of 5 to 1250. An Agilent 1200 series LC system, consisting of 1200 series binary pump, autosampler, vacuum degasser, column compartment and control module, was used with a Phenomenex Luna 3µ C18(2) 100A 100*2.00mm column. A SecurityGuard cartridge kit was used as guard column. The mobile phases were 5mM aqueous ammonium acetate solution and a 5mM ammonium acetate solutiuon in 95% acetonitrile/5% water. An LC flow rate of 0.2mL/min was used.

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METHODS – Calculation of Exposure

From the wet-weight basis, per-portion, analytical data, values for amount extracted and for percentage transfer of each constituent to the volunteer were calculated for each pair (used and unused) of samples using the equations below:

Amount extracted = Quantity in unused pouch – Quantity in used pouch

Transfer (%) = 100 * (Amount extracted / Quantity in unused pouch)

RESULTS

Table 1 below shows descriptive statistics for the amount of nicotine, NNK and NNN in unused pouches analysed in the study. One extreme outlier for nicotine content (2.99mg/portion) was removed from the data set. The variability described in the table encompasses both the product and analytical method variability, and in the case of each constituent the standard deviation is less than 9% of the mean amount per pouch.

Table 1: Descriptive statistics for unused pouches analysed in the study

Constituent (units)	Mean Amount	Standard Deviation	Minimum	Median	Maximum	Number of pouches
Nicotine (mg/pouch)	10.89	0.95	8.83	10.91	14.23	299
NNK (ng/pouch)	158.7	11.4	122.7	158.7	193.3	300
NNN (ng/pouch)	580.2	35.6	462.2	580.0	702.2	300

One value for nicotine transfer, corresponding to -126% transfer after 75 minutes' use, was identified as an extreme outlier and was removed from the results for nicotine. Figures 1a, 1b and 1c show fitted line plots of nicotine, NNK and NNN transfer, respectively, with the red broken lines corresponding to 95% confidence intervals and the green broken lines corresponding to 95% prediction intervals. Transfer of nicotine, NNK and NNN was found to increase with increasing usage duration, with a mean transfer of 5-6% after 5 minutes use, increasing to around 50% after 120 minutes use. At no point during the study was full and complete transfer of these tobacco constituents observed. Similar mean percentage transfer was observed for nicotine and both TSNAs at a given usage duration, regardless of the substantial difference in magnitudes at which these constituents are present in the product. Regression analysis confirmed a statistically significant ($p<0.001$) relationship between usage duration and percentage transfer of nicotine, NNK and NNN.

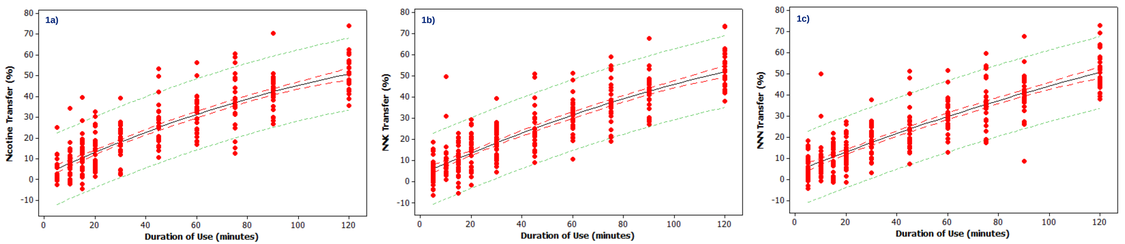

Figure 1: Fitted line plots of individual values for % transfer after each usage duration of a) nicotine, b) NNK and c) NNN.

At each timepoint the standard deviation of the mean percentage transfer of each constituent is in the range of 6-12%. At the shorter usage durations (5, 10, and to a lesser extent 15mins) the standard deviation is therefore similar to the mean itself, although this is not the case for longer durations. There is very little difference between constituents in terms of the standard deviation associated with mean percentage transfer. The highest values for nicotine transfer at all but the 15min timepoint, and for TSNA transfer at seven of the timepoints, were obtained from the same individual, suggesting that this volunteer extracted more from each pouch than the other volunteers.

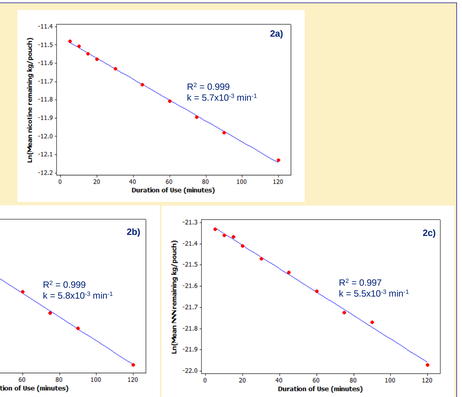

Figure 2: 1st order kinetic plots of mean amount remaining per pouch for a) nicotine, b) NNK and c) NNN.

Figures 2a, 2b and 2c show that a first order kinetic analysis fits the experimental data. The rate constant (k) for transfer of each of the constituents is similar, and as a result the half life time is just in excess of 2 hours for each constituent. This would suggest that only prolonged use (in excess of 12 hours) by a typical user of a snus pouch would result in near-complete transfer of these constituents from the pouch.

Constituent transfer is also consistent with concentration-gradient driven Fickian diffusional processes if the concentrations of nicotine, NNK and NNN in saliva are low.

The findings are consistent with those from recent pharmacokinetic studies[4,5] which showed that t_{max} for peak plasma nicotine concentration appears to be linked to duration of snus use.

CONCLUSIONS

- Exposure of snus users to tobacco constituents such as nicotine, NNK and NNN increased significantly with the duration of use.
- For usage durations of 5-120minutes, complete transfer of these tobacco constituents to users did not occur in this study.

ACKNOWLEDGEMENT

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