Pharmacokinetic comparison of a novel non-tobacco-based nicotine pouch (ZYN®) with conventional, tobacco-based Swedish snus and American moist snuff.

Erik Lunell, MD, PhD †
CROEL AB, Slottsvägen 21
SE 25284 Helsingborg, Sweden
croelab@gmail.com

Karl Fagerström, PhD
Framnäsvägen 8
SE 18531 Vaxholm, Sweden

John Hughes MD, PhD
Department of Psychiatry, University of Vermont
UHC Campus, Mailstop #482 OH4 Burlington, VT 05401, USA
Robert Pendrill, PhD

Research Scientist, Product Science, Swedish Match

SE-Box 17037, 104 62 Stockholm, Sweden

† Correspondence

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Abstract

The single dose pharmacokinetics of a novel, non-tobacco-based nicotine pouch, ZYN®, 3 and 6 mg, were compared to 8 mg General snus (Part 1) and ZYN® 8 mg was compared to 18 mg Longhorn moist snuff (Part 2).

Methods. A single-dose randomized cross-over design was used. In-vivo extraction and pharmacokinetic parameters were determined.

Results. Part 1. The AUCinf of ZYN® 3 mg was 27% smaller than that of 8 mg General and the AUCinf of ZYN® 6 mg was 34% larger than that of 8 mg General. Less nicotine was extracted from ZYN® 3 mg (1.5 mg) and more from ZYN® 6 mg (3.5 mg) than from 8 mg General (2.4 mg). The extracted fractions of nicotine for both ZYN® products (56% and 59%) were significantly larger than for 8 mg General (32%).

Results. Part 2. Close to identical plasma nicotine curves, AUCinf and Cmax were found for ZYN® 8 mg and Longhorn Natural 18 mg moist snuff. The extracted amount of nicotine from ZYN® 8 mg (3.8 mg) was larger than the amount extracted from Longhorn Natural 18 mg (3.0 mg), but smaller than the extracted amount of nicotine from General 2x8 mg snus pouches (5.0 mg). The extracted fraction of nicotine for ZYN® 8 mg (50%) was larger than for Longhorn Natural (19%) and General 2x8 mg snus pouches (33%).

Conclusions. The two higher doses of ZYN (6 mg and 8 mg) deliver nicotine as quickly and to a similar extent as existing smokeless products, with no significant adverse effects.

Implications. The present study demonstrates the characteristics of three strengths of a novel tobacco-free oral snus, ZYN®, viz. the extraction of nicotine from the oral cavity and its uptake into the systemic blood circulation. Comparison is made to Swedish General® snus and American Longhorn® moist snuff and from literature 4 mg Nicorette® gum and 13 brands of e-cigarettes.
Introduction

Nicotine is the main driving force that motivates use of tobacco products. Several types of nicotine products have been marketed to deliver nicotine either for recreational use, smoking cessation or relief of withdrawal. Cigarettes are the most efficient nicotine delivery system due to their very fast uptake and high levels of nicotine (1). Smokeless tobacco products also contain and deliver nicotine enough to be self-administered. Pharmaceutical nicotine replacement therapy (NRT) products, like gum, lozenges and patches are effective but they are less acceptable to smokers in part because they deliver nicotine more slowly and to a lower level than cigarettes and Swedish snus (2,3). Recently, however, a higher strength of Nicorette Gum, 6 mg, has been introduced that produced higher Cmax and AUCinf but only marginally improved craving control (4) since a common problem with NRT has been underdosing (5,6).

Recently a novel non-tobacco oral nicotine product category seems to be established. The first product in this category was ZYN® marketed by Swedish Match North America. ZYN® comes in three strengths, 3, 6 and 8 mg, and is packed in a white pouch that is put under the upper lip. In the US ZYN® appears very popular among smokeless tobacco (SLT) users (7). One possible reason for its appeal may be that it is a non-tobacco product.

The purpose of the present paper is to study the pharmacokinetics (PK) of ZYN®. Because the product has similar features to a SLT product, we compared the PK of ZYN® to the Swedish General snus, the first product to obtain a Modified Risk Tobacco Product order from FDA, and the American Longhorn moist snuff. From literature references comparison was made with 4 mg Nicorette and 13 brands of first-generation e-cigarettes.
Background & rationale

When comparing the nicotine content of different nicotine-containing products it is important to consider that the nicotine extraction and uptake varies considerably depending on product type and formulation (1).

Previous studies have indicated that on average about 15-20% of the total nicotine content is extracted and absorbed, with large inter-individual variation (1-3). Extraction is generally not linear with pouch size: it is larger with small compared to larger pouches, which suggests that surface area, saliva penetration and diffusion factors may be equally important determinants of nicotine uptake as pouch weight. ZYN® is a novel non-tobacco-based nicotine pouch. ZYN® does not contain nitrosamines or polycyclic aromatic hydrocarbons, the two most controversial classes of substances (which potentially may be carcinogenic) that can be found in conventional, tobacco-based snus, albeit at extremely low concentrations.

Swedish snus products with different nicotine content have previously been compared with 2 and 4 mg nicotine chewing gum (2,3). We here extend previous observations on Swedish Snus by comparing the novel non-tobacco-based product (ZYN®) with conventional, tobacco-based Swedish snus and American moist snuff.
Study Objectives

Primary objective

To compare each subject’s area under the plasma concentration time curve from time zero to infinity (AUCinf) after administration of a single dose of a non-tobacco-based nicotine pouch (ZYN®) containing 3 and 6 mg of nicotine, respectively, to that of a single dose of General Swedish snus containing 8 mg of nicotine (part 1) and a single dose of ZYN 8mg to that of American Longhorn moist snuff (18 mg) and 2 pouches General snus (part 2).

Secondary objectives

- To compare AUC60min, maximum concentration (Cmax), time to maximum concentration (Tmax), AUC0-t and terminal half-life.
- To compare the estimated in vivo extracted amount of nicotine
- To compare pulse rate and subjective effects ("head buzz") after study product administration
- Collection of adverse events (AEs)
Material and Methods

Study design

An open, randomized, cross-over, single dose administration trial was performed in two parts that both were approved by the Independent Ethics Committee (IEC) in Uppsala, Sweden. The trials were performed by CTC Clinical Trial Consultants AB, Uppsala and adhered to the requirements of the EU Clinical Studies Directive 2001/20/EC and ICH Guideline for Good Clinical Practice.

Subjects

Healthy subjects aged >19 years who had used tobacco-based snus for >1 year with a weekly consumption of 3 or more snus cans (brands with nicotine content <1%) or 2 or more cans (brands with nicotine content >1%) were considered eligible to participate in the study. Before study entry, subjects signed an informed consent form and subsequently underwent screening evaluations including smoking and snus use, and medical history. Subjects who were pregnant or who had a history of hypertension or any cardiovascular disease were excluded.

Before study entry, subjects signed an informed consent form and subsequently underwent screening evaluations including smoking and snus use, medical history and pulse measurements before/after application of their usual brand of snus.

Part 1. In total, 39 subjects were screened and 18 subjects were enrolled into the study. Each subject participated in the study for 22 to 41 days. One subject withdrew his consent after visit 2, i.e. after 6 days in the study. In summary, the number of subjects in part 1 that compared ZYN® to Swedish snus was 17.

Part 2. In total, 36 subjects were randomized, 6 subjects were discontinued due to protocol
non-compliance and loss-to-follow up. The number examined in part 2 that compared ZYN® to American moist snuff and 2 pouches General snus was 30; one additional subject was excluded from the PK analysis due to non-acceptable Lambda Z criteria, leaving 29 subjects in the PK analysis set.

Methods

Subjects visited the clinic on separate days for each experimental session. The subjects were instructed to abstain from snus, cigarettes or other nicotine delivery products from 8.00 p.m. the evening before. All sessions were performed during the morning hours to facilitate abstinence. Abstinence was determined by asking the subjects prior to each dosing and there was no exclusion of data based on baseline nicotine plasma concentrations. The highest value was 6.9 ng/ml. However, baseline nicotine plasma concentrations generally support the subjects’ self-reported abstinence. Adjustment for baseline nicotine for the calculation of pharmacokinetic parameters gave values between 6-16% lower in study 1 and between 3-8% lower in study 2. Baseline adjustment did not result in any change in which of the differences that were significant (with the exception of Tmax for ZYN 8mg vs General snus 2x8mg, see discussion), nor did adjustment result in any meaningful change in the comparisons. Thus, data without adjustment for baseline concentrations are presented, mirroring the real-life situation.

The investigational products (IPs) were administered as single doses according to a computer-generated randomization list. A Latin square approach was used. All IPs were administered at the research clinic under supervision of the clinical staff to ensure compliance.

The subjects kept the pouch still between the upper lip and the gum for 60 min and were instructed not to manipulate the pouch with the tongue or lips. This is the way ZYN and other
smokeless products are actually used by consumers (8). The subjects were also instructed not to eat, drink, chew chewing gum or brush teeth from 30 min before application of treatment, during application of the IP and 30 min after the IP had been taken out (9). Each used pouch was collected and frozen pending analysis of nicotine.

A telephone follow-up was conducted 1 week after last dose to assess any adverse effects.

Investigational Products (IP) and labeled dosage

In addition to pharmaceutical grade nicotine, microcrystalline cellulose, malitol and gum arabic are used as fillers, sodium carbonate and sodium bicarbonate regulates pH (8.3) and all flavors are food grade.

Part 1.

Test products:
1= ZYN 3 Smooth containing 3 mg nicotine per dose (pouch).
2= ZYN 6 Smooth containing 6 mg nicotine per dose (pouch).

Reference product:
General portion snus 1.0 g (8 mg nicotine/g).

Part 2.

Test products:
1= ZYN 8 Smooth containing 8 mg nicotine per dose (pouch).
2= General portion snus 1.0 g 8 mg nicotine per pouch; 1 dose = 2 pouches
(one on each side of the upper lip)
Reference product:

Longhorn Natural portion snus 18 mg nicotine per dose (pouch).

**Efficacy assessments**

Nicotine plasma concentrations were determined at preset time points, before (0) and at 5, 10, 15, 30 and 60 min, 1.5, 2, 4 and 6 h after administration of each product.

Pharmacokinetic parameters were calculated by non-compartmental analysis (NCA) according to the linear up- log down method using Phoenix WinNonlin ver. 8.1 (Pharsight Corporation, U.S.A.). $AUC_{\text{inf}} =$ Area Under the plasma Concentration vs time curve extrapolated to infinity, $AUC_{0-t} =$ Area Under the plasma Concentration vs time curve to last measuring point, $AUC_{60 \text{min}}$, $C_{\text{max}}$, $T_{\text{max}}$ and terminal half-life were calculated.

The time for the pre-dose sample was set to 0 and plasma concentrations below the quantification limit was set to 0 before $T_{\text{max}}$ and to missing thereafter. Baseline measurement was defined as the latest measurement prior to each dosing.

The elimination constant ($K_e$) was calculated and the threshold for acceptance of regression was defined by: $\geq 0.85$ for $R^2_{\text{adj}}$, $\leq 30\%$ for % residual AUC and $\geq 1.0$ for the half-life span. The elimination constant for subjects not fulfilling all three acceptance criteria was calculated based on the mean calculated eliminations constants for the same subject at other dosing occasions. Subjects not fulfilling acceptable criteria for eliminations constant determination at any dosing occasion were excluded from the PK population.

Vital signs – Heart rate was measured at the following time points: before (0) and at 5, 10, 15, 30 and 60 min after administration of each product. Heart rate was measured using an automatic device in sitting position after 10 min of rest.
Nicotine plasma assays

Frozen plasma samples collected for nicotine determinations were shipped to a certified contract laboratory, ABS Laboratories Ltd, UK. The analysis of the plasma samples was performed by a validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) assay. To quantify nicotine, a multilevel calibration at eight concentrations were performed over a range of 0 to 50 ng/mL. The calibration line was fitted by means of linear regression weighted by 1/concentration\(^2\). The samples were assayed once.

Incurred sample reproducibility was performed according to the European Medicines Agency (EMA) and Food and Drug Administration (FDA) guidelines so that 10% of the analyzed study samples up to 1000 were reanalyzed and then 5% of the number above 1000. The calibration standards must have a back-calculated accuracy within 100 ±15%, and within 100 ±20% at the lower limit of quantification (LLOQ). The standard curve was constructed from at least 3 quarters (i.e. 12) of the calibration standards, excluding the zero concentration calibration standards. Duplicate quality control samples at low, medium and high concentrations were included in each analysis batch. The accuracy of at least two thirds of the quality control samples were within 100 ±15%. Half of the quality control samples at each concentration were within 100 ±15%. At least half of the blank samples with internal standard and half of the blank samples without internal standard, placed immediately before the calibration standards, were free of interference. Internal method on file with ABS Laboratories. The LLOQ is 0.5 ng/mL.

Collection and analysis of pouches

Pouches for the determination of nicotine after administration of the IP were collected after 60± 1 min. All the collected pouches were collected and frozen immediately at -20°C. All pouches were analyzed in Swedish Match laboratories. Used pouches from all evaluable subjects excluding withdrawn or dropout subjects were analyzed. Nicotine was extracted from
the snus using sodium hydroxide and methyl-tert-butyl ether containing quinoline as an internal standard. The nicotine present in the extract was determined by using a gas chromatograph equipped with a flame ionization detector.

Subjective effects

Each subject’s rating of ‘head buzz’ using a Visual Analogue Scale (VAS), anchored with “not at all” to “extremely” were obtained before (0) and at 5, 10, 15, 30 and 60 min after administration of each product.

Safety assessments

Adverse events and serious adverse events (SAEs) were recorded from start of IP administration until the last follow-up visit. Medical events occurring between screening and first treatment with IP were reported separately as baseline events. Adverse events were coded using the Medical Dictionary of Regulatory Activities (MedDRA).

Statistical methods

A previous study (3) made the calculation of sample size possible. In-vivo nicotine extraction from a 1 g Swedish portion snus containing 8 mg nicotine/pouch was estimated at 2.18±0.92 mg per portion. Under the assumption of a complete dissolution and extraction of the 3 and 6 mg ZYN® products, respectively, versus the 2.18±0.92 mg nicotine, and a standard deviation of 5.0 the estimated sample size was 16 with a power of 80% and alpha=0.05 for part 1, and 32 for part 2.

AUCinf based on plasma concentrations of nicotine after administration of 1 single dose of the non-tobacco-based nicotine pouch containing 3 and 6 mg of nicotine, and that of single
doses of a 1 g Swedish snus pouch 8 mg, was described using summary statistics and non-parametric Wilcoxon signed rank sum test for within-subject difference. Summary statistics was used to present all continuous variables and frequency tables for categorical variables. A significance level of 5% with 2-sided tests was used in all comparisons. The test products were compared to the reference product in all analyses. In addition, pairwise comparisons between the test products were performed.

The extracted dose of nicotine was analyzed using the Wilcoxon signed rank sum test within subject difference. AUCinf, AUC60min, AUC0-t, Cmax, Tmax and terminal half-life of nicotine after use of the ZYN® pouch versus the General snus pouch (Part 1) were analyzed using Wilcoxon signed rank sum test for within subject difference. The same statistical methods were applied for the comparisons with Longhorn moist snuff (Part 2). Heart rate and VAS scales for “head buzz” were analyzed using the Wilcoxon signed rank sum test for within subject difference.

All AE data were fully listed by Investigator terms and MedDRA Preferred Term (PT). Adverse event data were summarized by System Organ Class (SOC) and PT.

RESULTS

Part 1.

Less nicotine was extracted from ZYN® 3 mg (1.5 mg, 95% CI: 1.3-1.8 mg, p=0.002) and more from ZYN® 6 mg (3.5 mg, 95% CI: 3.0-4.0 mg, p=0.002) than from 8mg General (2.4 mg, 95% CI: 2.0-2.8 mg). The extracted fractions of nicotine for both ZYN® products (56% and 59%, 95% CI: 46-65% and 50-68%) were significantly larger than for 8mg General (32%, 95% CI: 27-37%). See Table 1 for a summary of nicotine exposure for the different products. The plasma concentrations from the ZYN® product containing 3 mg nicotine were lower than those of 8 mg General snus and the plasma concentrations from ZYN® containing 6 mg were
higher than the 8 mg General snus at almost all timepoints, see Figure 1. See Table 2 for pharmacokinetic parameters for the different products. For comparison, literature data for 4 mg Nicorette gum (3) and mean of 13 brands of e-cigarettes (10) are included in Tables 1 and 2.

The Cmax of ZYN® 3mg was 25% lower (95% CI: 14-37%, p<0.001) than that of 8 mg General snus, whereas the Cmax of ZYN® 6 mg was 42% higher (95% CI: 24-60%, p<0.001) than that of 8 mg General. The AUCinf of ZYN® 3mg was 27% smaller than that of 8mg General (95% CI: 19-36%, p<0.001) and the AUCinf of ZYN® 6mg was 34% larger than that of 8mg General (95% CI: 16-52%, p=0.0056).

For the secondary PK parameters, there were statistically significant differences in AUC0-t, AUC60min and Cmax, ZYN® 3 mg being lower and ZYN® 6 mg higher than 8 mg General snus. No statistically significant differences were seen in terms of terminal half-life and Tmax.

The maximum increase in pulse rate was 2.7 beats per minute larger (95% CI: 0.2-5.2 beats per minute, p=0.027) for the ZYN 6mg product than for the ZYN 3mg product, whereas neither of the ZYN products differed significantly from 8mg General snus. The maximum increase in head buzz for 8mg General snus was larger than for ZYN 3mg; the median difference was 7 mm (interquartile range: 0-27.5 mm, p=0.033), see Table 5.

Part 2.

The extracted amount of nicotine from ZYN® 8 mg (3.8 mg, 95% CI: 3.3-4.3 mg) was larger (p=0.0042) than the amount extracted from Longhorn Natural 18 mg (3.0 mg, 95% CI: 2.1-3.9), but smaller (p<0.001) than the extracted amount of nicotine from General 2x8 mg snus pouches (5.0 mg, 95% CI: 4.7-5.4 mg), see Table 3.
The extracted fraction of nicotine from ZYN® 8 mg (50%, 95% CI: 44-57%) was significantly larger than the fraction extracted nicotine from General snus 2x8 mg (33%, 95% CI: 30-35%, \( p<0.001 \)) and Longhorn Natural 18 mg (19%, 95% CI: 13-25, \( p<0.001 \)), see Table 3. In addition, the extracted fraction of nicotine of General snus 2x8 mg was significantly higher than the extracted fraction nicotine of Longhorn Natural 18 mg (\( p<0.001 \)).

The plasma concentration versus time curves were similar for all test products with the highest concentrations of nicotine observed at 1 hour after start of product administration, in association with removal of the test product. Nicotine plasma concentrations are summarized in Figure 2.

There were no statistically significant differences in mean AUCinf, AUC0-t, mean Cmax or extracted amount of nicotine between ZYN® 8mg and Longhorn Natural 18 mg, see Table 4. ZYN 8mg gave 17% lower AUCinf (95% CI: 9-25%, \( p<0.001 \)) and 12% lower Cmax (95% CI: 3-22%, \( p=0.0084 \)) than General snus 2x8mg. For all products but General snus 2x8 mg, there was a strong correlation between the AUCinf and the extracted amount of nicotine (not shown).

The minor differences in Tmax or terminal half-life between the different products were not statistically significant.

**Safety**

Administration of single doses of nicotine-containing pouches was safe and well tolerated by the healthy subjects in this study. A total of 16 AEs were reported by 8 subjects during the study and 2 AEs (dry mouth) were judged to have a possible or probable relationship to treatment. There were no SAEs or discontinuations due to AEs during the study.
Discussion

These open, randomized, cross-over studies compared the PK and subjective effects of nicotine following a single dose of a non-tobacco-based nicotine pouch (ZYN®) containing either 3 mg, 6 mg or 8 mg nicotine and single or double doses of conventional, tobacco-based Swedish General 8 mg snus and Longhorn Natural 18 mg, respectively. We also looked at the in vivo extraction of nicotine to explain differences in AUCinf and Cmax.

Part 1. The in vivo extracted amount of nicotine from ZYN® 3 mg was significantly lower than from 8 mg General snus and from ZYN® 6 mg significantly higher. This was an unexpected finding. Rather, it was expected that the extraction and absorption of nicotine would correlate with the amount of nicotine in each product. However, these findings may be explained by differences in the extracted fraction of nicotine. Thus, the extracted fraction nicotine for both ZYN® products were significantly larger (56-59%) compared to the 32% of the reference General snus 8 mg. As expected, there was a statistically significant difference in the extracted amount of nicotine between the ZYN® 6 mg and ZYN® 3 mg.

Retrospectively comparing a 6 mg gum formulation to ZYN® 6 mg, the gum was seen to release more nicotine, 4.9 mg vs 3.5 mg, which corresponds to extracted fractions of 82% and 59% respectively (6). Despite the higher nicotine delivery of the 6 mg gum compared with the 4 mg version the effect on craving for cigarettes was small (11).

In line with the extraction data, ZYN® 6 mg resulted in significantly higher nicotine AUCinf, compared to the General portion snus 1.0 g (8 mg nicotine). As for the extraction of nicotine, systemic absorption, AUCinf, did not correlate with the amount of nicotine in each product. Hence, the AUCinf was lower for the ZYN® 3 mg pouches and higher for the ZYN® 6 mg pouches compared to that from the General 8 mg pouch. In general, corresponding results to those obtained for AUCinf and extracted amount of nicotine, were obtained also for AUC0-t, AUC60min and Cmax. There were,
however, no statistically significant differences between the ZYN® products and the General portion snus 1.0 g (8 mg nicotine/g), in terms of terminal half-life and Tmax.

Despite significant differences between each of the ZYN® products and the reference product General snus for all primary and secondary PK endpoints.

The extraction and plasma data were supplemented with assessments of subjective effects of “head buzz” and heart rate measurements, shown in Table 5, both of which constitute proxies for systemic nicotine uptake. Interestingly, the maximum VAS score for “head buzz” was higher following intake of General 8 mg than after intake of any of the ZYN® products (3 mg and 6 mg) despite a higher AUCinf following ZYN® 6 mg, i.e. there was no obvious correlation between nicotine levels and head buzz (although for ZYN 6mg vs General 8mg the difference was not statistically significant). Potentially, another component of conventional snus, e.g. tobacco, could have contributed to the feeling of head buzz. The subjective effects could also have been due to classical conditioning to the long-term use of a specific product, in this case General snus, or in tobacco-based snus in general.

Part 2. The extracted amount of nicotine from General snus 2x8 mg was significantly larger than from the other products. Corresponding results were observed for mean AUC0-t and mean Cmax. The mean AUCinf of General snus 2x8 mg was significantly higher than the mean AUCinf of the other products. The extracted amount of nicotine from ZYN® Smooth 8 mg was significantly larger than the from Longhorn Natural.

As is seen in Figure 2, all plasma nicotine curves except General snus 2x8mg are very similar despite very different nicotine contents in the unused products. The reason is differences in the fraction of nicotine that is extracted upon use. Thus, the extracted fraction of nicotine for ZYN® 8 mg (50%) was significantly larger than Longhorn Natural 18 mg (19%; p<0.001) as well as General sus 2x8 mg (33%;
p<0.001), see Table 3.

For all products but General snus 2x8 mg, there was a strong correlation between the AUCinf and the extracted amount of nicotine. Both AUCinf and Cmax for General snus 2x8 mg were significantly higher than for ZYN® 8mg and Longhorn Natural 18 mg, respectively. See table 4. For baseline-adjusted data, the Tmax for ZYN® 8 mg (59 min) was significantly shorter than for General snus 2x8 mg (64 min, p=0.042).

In general, corresponding results to those obtained for AUCinf and extracted amount of nicotine were obtained also for AUCO-t, AUC60min and Cmax. There were however, no statistically significant differences between the products in terms of Tmax. The nicotine gum and e-cigarette data given here for comparative reasons only are from other studies and thus need to be interpreted with caution. It seems though that the extracted amount of nicotine from 4 mg gum is higher than from ZYN 3 mg but lower than from the ZYN 6 mg and 8 mg doses. The same seems to be true for Cmax, and the 4 mg gum peaks faster, i.e. has a shorter Tmax, although this could be explained by the shorter administration time (30 min). E-cigarettes clearly have a shorter Tmax, but not necessarily a higher Cmax.

Limitations. First the results can only be applied to snus users. The nicotine absorption may be different in smokers not used to nicotine pouches. Secondly, this study has two parts with different subjects and making comparisons across studies should be made with caution. Thirdly, in Part 1 the number of subjects was relatively small.
Conclusions

Despite a lower nicotine content, the non-tobacco-based ZYN® 6 mg product gave rise to a significantly larger uptake of nicotine to the systemic blood circulation, measured as AUCinf than did the conventional, tobacco-based 8 mg General snus.

Conventional, tobacco-based General snus (8 mg) gave rise to significantly larger nicotine extraction and subsequent uptake in the systemic blood circulation than did the non-tobacco-based ZYN® 3 mg product. ZYN 8mg delivers similar amounts of nicotine as the Longhorn Natural moist snuff (18mg), but significantly less than two pouches of General snus (2x8mg).

In summary, the two higher doses of ZYN (6 mg and 8 mg) deliver nicotine as quickly and to a similar concentration compared to existing smokeless products, with no significant adverse effects. This suggests their efficacy in reducing withdrawal symptoms and helping smokers reduce or stop combustible tobacco use should be similar to that for existing smokeless products.
Disclosures

Funding

The present study was funded by Swedish Match AB, Stockholm, Sweden. The funder developed the protocol and an independent CRO conducted the studies. The funder has provided data, figures and tables, but has in no way approved the manuscript.

Declaration of Interests

Dr Lunell has received grants from Swedish Match for a number of clinical trials on nicotine and tobacco products. He has not received any consulting fees from Swedish Match or any other tobacco company. Dr Pendrill is a Research Scientist at Swedish Match.

Dr Fagerström has received consulting fees from many companies that develop or market pharmaceutical and behavioral treatments for smoking cessation. He currently receives consulting fees from Swedish Match and has received fees in the past from BAT and PMI to assist their development of less-risky tobacco products.

Dr Hughes has received consulting and speaking fees from several companies that develop or market pharmacological and behavioral treatments for smoking cessation or harm reduction and from several non-profit organizations that promote tobacco control. He currently receives consulting fees from the pharmaceutical company Achieve. He also receives fees from the tobacco companies of Swedish Match, Altria and Philip Morris International to assist their efforts to develop less risky tobacco products.
Reference list


**Figure Captions**

**Fig 1.** Part 1. Diagram showing mean plasma concentrations (ng/mL) versus time (minutes). The upper curve shows ZYN® 6 mg nicotine per pouch. The middle curves show General snus 8 mg and Nicorette 4 mg gum from reference 3. The lower curve shows ZYN® 3 mg nicotine per pouch. Error bars correspond to 95% CIs.

**Fig 2.** Part 2. Diagram showing mean plasma concentrations (ng/mL) versus time (minutes). The upper curve shows General snus 2x8 mg nicotine per pouch. The middle curves show ZYN® 8 mg nicotine per pouch and Longhorn Natural 18 mg per pouch. The lower curve shows 4 mg Nicorette gum from reference 3. Error bars correspond to 95% CIs.
Table 1. Part 1. *In-vivo* extracted amount of nicotine from each test product (n=17), mean and (95%CI).

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<th>Product</th>
<th>Weight (g)</th>
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<td></td>
<td></td>
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<td>Used</td>
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N.A.=Not Applicable.

*Ranges in brackets are minimum and maximum.

#Wilcoxon signed-rank sum test compared to General 8mg, p<0.05.

%Values for Nicorette 4mg (n=15) based on data from reference 3 and were not included in significance testing.

§Values for E-cigarettes (n=13) taken from reference 10 and were not included in significance testing.

Unused and used exposure correspond to the vaporised and exhaled dose, respectively, during 15 puffs.
Table 2. Part 1. Mean and 95% CI pharmacokinetics results of each test product (n=17).

<table>
<thead>
<tr>
<th>Product</th>
<th>AUCinf (ng/mL*h)</th>
<th>Cmax (ng/mL)</th>
<th>Tmax (min)</th>
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<td>Mean</td>
<td>95% CI</td>
</tr>
<tr>
<td>ZYN 3mg</td>
<td>32.0*</td>
<td>23.3–40.7</td>
<td>7.7*</td>
<td>6.3–9.0</td>
</tr>
<tr>
<td>ZYN 6mg</td>
<td>57.7*</td>
<td>43.9–71.6</td>
<td>14.7*</td>
<td>12.3–17.1</td>
</tr>
<tr>
<td>General snus 8mg</td>
<td>45.9</td>
<td>29.8–62.1</td>
<td>10.6</td>
<td>8.9–12.3</td>
</tr>
<tr>
<td>Nicorette 4mg</td>
<td>52.5</td>
<td>40.0–65.1</td>
<td>12.8</td>
<td>11.1–14.5</td>
</tr>
<tr>
<td>E-cigarettes</td>
<td>14.1</td>
<td>8.8–19.3</td>
<td>8.4</td>
<td>5.4–11.5</td>
</tr>
</tbody>
</table>

*Wilcoxon signed-rank sum test compared to General snus 8mg, p<0.05.

#Values for Nicorette 4mg (n=15) based on data from reference 3 and were not included in significance testing.

§Values for E-cigarettes (n=13) taken from reference 10 and were not included in significance testing.
Table 3. Part 2. *In vivo* extracted amount of nicotine from each test product (n=30), mean and (95%CI).

<table>
<thead>
<tr>
<th>Product</th>
<th>Weight (g)</th>
<th>Nicotine (mg/dose)</th>
<th>Extracted nicotine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Unused</td>
<td>Used</td>
</tr>
<tr>
<td></td>
<td></td>
<td>mg/dose</td>
<td>% of total</td>
</tr>
<tr>
<td>ZYN® 8mg</td>
<td>0.530</td>
<td>7.52 [6.95–7.83]*</td>
<td>3.74 (3.23–4.25)</td>
</tr>
<tr>
<td></td>
<td>[0.511–0.544]*</td>
<td>3.79 (3.29–4.29)</td>
<td>50.4 (43.6–57.1)</td>
</tr>
<tr>
<td>General 2x8mg</td>
<td>2.009</td>
<td>15.43 [15.03–16.04]*</td>
<td>10.44 (10.05–10.83)</td>
</tr>
<tr>
<td>(16mg)</td>
<td>[1.909–2.081]*</td>
<td>5.04# (4.66–5.42)</td>
<td>32.6# (30.1–35.0)</td>
</tr>
<tr>
<td></td>
<td>[1.449–1.549]*</td>
<td>2.99# (2.06–3.93)</td>
<td>18.9# (13.1–24.8)</td>
</tr>
</tbody>
</table>

*Ranges in brackets are minimum-maximum.

#Wilcoxon signed-rank sum test compared to ZYN 8mg, p<0.05.
Table 4. Part 2. Mean and 95% CI pharmacokinetics results of each test product (n=29).

<table>
<thead>
<tr>
<th>Product</th>
<th>$AUC_{\text{inf}}$ (ng/mL*h)</th>
<th>$C_{\text{max}}$ (ng/mL)</th>
<th>$T_{\text{max}}$ (min)</th>
<th>$T_{\frac{1}{2}}$ (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>95% CI</td>
<td>Mean</td>
<td>95% CI</td>
</tr>
<tr>
<td>ZYN 8mg</td>
<td>58.4</td>
<td>50.7–66.1</td>
<td>18.5</td>
<td>16.1–20.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>General snus 8mg x 2</td>
<td>70.3*</td>
<td>63.9–76.6</td>
<td>21.2*</td>
<td>19.0–23.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Longhorn Natural 18mg</td>
<td>60.6</td>
<td>50.9–70.2</td>
<td>16.9</td>
<td>14.4–19.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Wilcoxon signed-rank sum test compared to ZYN 8mg, p<0.05.
Table 5. Part 1. Maximum “head buzz” and Maximum Change in Heart Rate.

<table>
<thead>
<tr>
<th>Product</th>
<th>Maximum “head buzz” VAS (mm)</th>
<th>Median (Q1; Q3)</th>
<th>Range</th>
<th>Maximum Change in Heart Rate (bpm)</th>
<th>Median (Q1; Q3)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZYN® 3mg</td>
<td></td>
<td>9 (4; 19)</td>
<td>0–59</td>
<td></td>
<td>8.5 (5.5; 14.5)</td>
<td>4.0–18.0</td>
</tr>
<tr>
<td>ZYN® 6mg</td>
<td></td>
<td>11 (5; 26)</td>
<td>0–63</td>
<td></td>
<td>10.5* (9.5; 16.5)</td>
<td>4.5–22.5</td>
</tr>
<tr>
<td>General® snus 8mg</td>
<td></td>
<td>24* (12; 47)</td>
<td>0–62</td>
<td></td>
<td>11.0 (4.0; 15.0)</td>
<td>0.0–22.0</td>
</tr>
</tbody>
</table>

*Wilcoxon signed-rank sum test compared to ZYN 3mg, p<0.05. All other comparisons had p>0.05.
Figure 1.

![Graph showing plasma nicotine levels over time for different products]

- ZYN® 6 mg
- Nicorette® 4 mg
- General 8 mg
- ZYN® 3 mg
Figure 2.