
Clinical Study Report

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Nicotine plasma concentrations and pharmacokinetics of single doses of non-tobacco-based nicotine pouches (ZYN®) compared with conventional, tobacco-based Swedish snus and American moist snuff among current, daily snus users

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This clinical study was conducted, and essential study documentation archived, in compliance with company SOPs and standards, which incorporate the requirements of the EU Clinical Studies Directive 2001/20/EC and ICH Guideline for Good Clinical Practice.

2 STUDY SYNOPSIS

Study Title Nicotine plasma concentrations and pharmacokinetics of single doses of non-tobacco-based nicotine pouches (ZYN®) compared with conventional, tobacco-based Swedish snus and American moist snuff among current, daily snus users
Study code SM 18-01
Study period Date of first subject screened: 17DEC2018 Date of last subject completed: 07MAR2019
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Study design Open, randomized, 7-way cross-over, single dose administration
Objectives <u>Primary objective</u> To evaluate each subject's plasma concentrations of nicotine after administration of one single dose of ZYN® Smooth containing 6 mg of nicotine, compared to that of one single dose of 2 pouches of General PSWL Swedish snus (2x8 mg nicotine). <u>Secondary objectives</u> To evaluate pharmacokinetic variables for the remaining products, plasma levels of Methyl salicylate and local tolerability: <ol style="list-style-type: none"> 1. To compare T_{max}, C_{max}, AUC_{inf}, AUC_{0-t} from each dose of the non-tobacco-based nicotine ZYN® Smooth pouches, General PSWL Swedish snus pouches and Longhorn American moist snuff pouches. 2. To assess the effect of the flavor component (Methyl salicylate) on nicotine plasma concentrations for the non-tobacco-based nicotine pouch ZYN® Wintergreen and Longhorn Wintergreen pouch American moist snuff, respectively. 3. To assess if there is a difference in nicotine plasma concentrations between upper lip and lower lip placement of the non-tobacco-based nicotine pouch ZYN® Smooth. 4. To compare the estimated <i>in-vivo</i> extracted amount and rate of extraction of nicotine from each dose. 5. To assess plasma levels of Methyl salicylate for the treatments with pouches containing Wintergreen flavor. 6. To compare adverse events (AEs) from each dose.

Upon analysis of data, it was revealed that ZYN® Smooth 6 mg and ZYN® Wintergreen 6 mg contained a smaller amount of nicotine than intended (approximately 4.5 mg rather than 6 mg) and were hence excluded from all comparisons and analyses. The primary objective and secondary objectives No. 2, 3 and 5 could hence not be evaluated. The other objectives are evaluated based on ZYN® Smooth 8 mg, General PSWL 2x8 mg, Longhorn Natural 18 mg and Longhorn Wintergreen 18 mg. The analysis of PK and extraction data for ZYN® Smooth 6 mg and ZYN® Wintergreen 6 mg will be reported elsewhere.

The primary objective has been clarified. The original wording in the clinical study protocol (CSP) was: To evaluate each subject's plasma concentrations of nicotine after administration of one single dose of ZYN® Smooth containing 6 mg of nicotine, compared to that of one single dose of 2x1 pouches of General PSWL Swedish snus.

Number of subjects

The planned number of subjects was 36.

A total of 49 subjects were screened, 36 were randomized and 32 completed the study. Subjects were randomized to 1 of 4 treatment sequences (A-D). All subjects who were randomized to treatment sequence C (9 subjects) and D (9 subjects) completed the study. Of the 9 subjects who were randomized to treatment sequence A, 2 were lost-to-follow up after Visit 2 and one received one treatment only. Of the 9 subjects who were randomized to treatment sequence B, 2 were discontinued from the study after completing Visit 6 due to CSP non-compliance.

Diagnosis and main eligibility criteria

Healthy male and female subjects aged ≥ 19 years who had used snus for ≥ 1 year, with a minimum weekly consumption of 2 or more snus cans (preferably brands with nicotine content $\geq 1\%$) were considered eligible to participate in the study.

Subjects who were pregnant or breastfeeding were excluded. Subjects had to abstain from snus and all other nicotine containing products from 8.00 pm the night before each investigational product (IP) administration day (minimum abstinence period: 13 hours, maximum abstinence period: 13.5 hours).

Methodology

Subjects reported to the clinic on separate days for the 7 experimental sessions in addition to visits for screening and follow-up. Between each experimental session, a wash-out period of at least 24 hours was required. The subjects were instructed to abstain from snus or other nicotine delivery products from 8.00 p.m. the evening before and to refrain from smoking 24 hours before each experimental session. All sessions were performed during the morning hours to facilitate abstinence. The subjects certified abstinence before each treatment was started.

The treatments were administered as single doses in a pre-determined randomized order. The subject kept the pouch(es) still between the upper lip (lower lip for treatment 4) and the gum for 60 minutes and were instructed not to manipulate the pouch with the tongue or lips. The subjects were instructed not to eat, drink, chew chewing gum or brush teeth from 30 minutes before application of treatment, during application of investigational products and 30 minutes after the IP had been taken out. After 60 minutes the pouches were collected and frozen (-20°C) pending analyses of nicotine. Blood samples for assessment of plasma levels of nicotine and Methyl salicylate were collected at pre-defined time points from pre-dose to 6 hours after IP administration.

The treatment sequences were:

- A: ZYN® Smooth 6 mg
- ZYN® Smooth 8 mg
- ZYN® Wintergreen 6 mg
- ZYN® Smooth 6 mg lower lip
- General PSWL 2x8 mg

<p>Longhorn Natural 18 mg Longhorn Wintergreen 18 mg</p> <p>B: General PSWL 2x8 mg Longhorn Natural 18 mg Longhorn Wintergreen 18 mg ZYN[®] Smooth 6 mg ZYN[®] Smooth 8 mg ZYN[®] Wintergreen 6 mg ZYN[®] Smooth 6 mg lower lip</p> <p>C: ZYN[®] Smooth 6 mg Longhorn Natural 18 mg ZYN[®] Smooth 8 mg Longhorn Wintergreen 18 mg General PSWL 2x8 mg ZYN[®] Wintergreen 6 mg ZYN[®] Smooth 6 mg lower lip</p> <p>D: ZYN[®] Smooth 6 mg lower lip General PSWL 2x8 mg ZYN[®] Smooth 6 mg ZYN[®] Wintergreen 6 mg Longhorn Wintergreen 18 mg ZYN[®] Smooth 8 mg Longhorn Natural 18 mg</p>
<p>Investigational Products (IP), dosage and mode of administration, batch numbers</p> <p>Test products</p> <p>1= ZYN[®] Smooth containing 6 mg nicotine per portion (Batch number: B1180711) 2= ZYN[®] Smooth containing 8 mg nicotine per portion (Batch number: B1180927) 3= ZYN[®] Wintergreen containing 6 mg nicotine per portion (Batch number: B1180730) 4= ZYN[®] Smooth containing 6 mg nicotine per portion (lower lip) (Batch number: B1180711)</p> <p>Reference products</p> <p>5= Swedish portion snus, General PSWL (8 mg nicotine/g) 2x1.0 g (Batch number: 11097K6013) 6= American moist snuff, Longhorn Pouch Natural (12 mg nicotine/g) 1.5 g (Batch number: E269 0900) 7= American moist snuff, Longhorn Pouch Wintergreen (12 mg nicotine/g) 1.5 g (Batch number: E279 0913)</p> <p>All IPs were administered in pouches between the upper lip and the gum for 60 min except test product 4, which was administered between the lower lip and the gum.</p> <p>Upon analysis of data, it was revealed that ZYN[®] Smooth 6 mg and ZYN[®] Wintergreen 6 mg contained a smaller amount of nicotine than intended (approximately 4.5 mg rather than 6 mg) and were hence excluded from all comparisons and analyses.</p>
<p>Non-Investigational Products (NIMPs), dosage and mode of administration, batch number</p> <p>Not applicable.</p>

Duration of treatment

The treatments were administered as single doses in a pre-determined randomized order. The subjects kept the pouch still between the upper/lower lip and the gum for 60 minutes.

Duration of subject's involvement in the study

Each subject who completed the study participated in the study for 26 to 59 days. The 4 subjects who discontinued early participated in the study for 15 to 45 days.

Pharmacokinetic (PK) and other assessments

Nicotine plasma concentrations were determined at pre-set timepoints: before (0) and at 5, 10, 15, 30, 60, 90, 120, 240 and 360 minutes after administration of each IP. Pharmacokinetic parameters were calculated using the WinNonlin computer program (Certara Corp. USA).

The extracted dose of nicotine from each pouch was calculated by subtracting the residual amount after use from the mean of 10 unused pouches.

Safety assessments

AEs and serious AEs (SAEs) were recorded from the first IP administration until the last follow-up visit. AEs were coded using the Medical Dictionary of Regulatory Activities (MedDRA).

Statistical methods

Previous studies had showed that ZYN[®] Smooth 6 mg has an AUC_{inf} of 3581 min ng/mL and 2 pouches of 8 mg Swedish snus have an AUC_{inf} of 5586 min ng/mL (assumed AUC_{inf} based on the AUC_{inf} following 60 min administration of 1 pouch of Swedish snus) with a common standard deviation of 1920 min ng/mL.

The hypothesis for this study was that ZYN[®] Smooth 6 mg has a significantly lower AUC compared to 2 pouches 8 mg Swedish snus. With a power of 80% and a significance level of 2.5%, the number of subjects needed was 32. Assuming 10% drop out rate, 36 subjects were included.

The mean \pm SD of AUC_{inf}, AUC_{0-t}, AUC_{inf}, C_{max}, T_{max}, and T_{1/2} based on plasma concentrations of nicotine after administration of each pouch were calculated, described using summary statistics and analyzed using the signed Wilcoxon rank sum test for between treatment differences for all pairwise comparisons.

The mean \pm SD extracted dose of nicotine from each pouch were calculated. The extracted dose of nicotine was analyzed using the signed Wilcoxon rank sum test.

The correlation between the AUC and the total amount of nicotine extracted from the pouch was analyzed using Proc corr.

All statistical analyses were performed using SAS version 9.4 (SAS institute, Cary, NC).

EFFICACY/PHARMACOKINETIC RESULTS

The mean AUC_{inf} of General PSWL 2x8 mg was significantly higher than the mean AUC_{inf} of ZYN[®] Smooth 8mg, Longhorn Natural 18 mg and Longhorn Wintergreen 18 mg, respectively.

Corresponding results were observed for the extracted dose of nicotine, i.e. the mean extracted dose of nicotine from General PSWL 2x8 mg was significantly higher than the mean extracted dose from ZYN[®] Smooth 8 mg, Longhorn Natural 18 mg and Longhorn Wintergreen 18 mg, respectively. In addition, the mean extracted dose of nicotine from ZYN[®] Smooth 8 mg was significantly higher than the mean amount extracted from Longhorn Natural 18 mg. For all products but General PSWL 2x8 mg, there was a strong correlation between the AUC_{inf} and the extracted amount of nicotine.

The mean nicotine rate of extraction from ZYN[®] Smooth 8 mg was significantly higher than the rate of extraction from General PSWL 2x8 mg, Longhorn Natural 18 mg and Longhorn Wintergreen 18 mg. In addition, the rate of extraction of nicotine from General PSWL 2x8 mg was

significantly higher than the rate of extraction from Longhorn Natural 18 mg and Longhorn Wintergreen 18 mg.

Mean AUC_{0-t} and mean C_{max} of General PSWL 2x8 mg were significantly higher than mean AUC_{0-t} and mean C_{max} of ZYN[®] Smooth 8mg, Longhorn Natural 18 mg and Longhorn Wintergreen 18 mg, respectively.

Mean T_{max} and mean $T_{1/2}$ of ZYN[®] Smooth 8 mg was significantly shorter than the mean T_{max} and mean $T_{1/2}$ of Longhorn Wintergreen 18 mg. For baseline adjusted data, mean T_{max} of ZYN[®] Smooth 8 mg was also significantly shorter than the mean T_{max} of General PSWL 2x8 mg.

There were no statistically significant differences in mean AUC_{inf} , AUC_{0-t} , mean C_{max} or extracted dose of nicotine between ZYN[®] Smooth 8 mg and either of Longhorn Natural 18 mg or Longhorn Wintergreen 18 mg or between Longhorn Natural 18 mg and Longhorn Wintergreen 18 mg. In addition, there were no statistically significant differences in rate of extraction between Longhorn Natural 18 mg and Longhorn Wintergreen 18 mg or in T_{max} between most of the IPs.

SAFETY RESULTS

Administration of single doses of nicotine-containing products was safe and well tolerated by the healthy subjects in this study.

Ten subjects (28%) reported a total of 18 AEs during the 24 hours following each administration of ZYN[®] Smooth 8 mg, General PSWL 2x8 mg, Longhorn Natural 18 mg and Longhorn Wintergreen 18 mg. There were no deaths, other SAEs or withdrawals due to AEs. All AEs were of mild to moderate in intensity. The most common AE was nausea. Overall, a majority of the AEs were AEs that are usually associated with nicotine exposure.

CONCLUSION

As the intended ZYN 6 mg product only contained approximately 4.5 mg nicotine, the conclusion is based on comparison between the ZYN 8 mg product and General PSWL 2x8 mg, Longhorn Natural 18 mg and Longhorn Wintergreen 18mg.

In line with the overall aim of the study, it was shown that ZYN[®] Smooth 8 mg did not entail a higher nicotine exposure than General PSWL 2x8 mg, Longhorn Natural 18 mg and Longhorn Wintergreen 18 mg, representing commercially available tobacco-based snus and snus-like products that are currently common on the Scandinavian and U.S. markets.

Single doses of ZYN[®] Smooth 8 mg, General PSWL 2x8 mg, Longhorn Natural 18 mg and Longhorn Wintergreen 18 mg were in general well tolerated by the current daily snus users participating in the study and no safety concerns were observed.

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4 LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

Abbreviation or term	Explanation
AE	Adverse event
ATC	Anatomical therapeutic chemical
AUC	Area under the plasma concentration time curve
AUC _{inf}	Area under the curve from 0 to infinity
AUC _{0-t}	Area under the curve from 0 to t hours where t is the last measured concentration
C _{max}	Maximum (peak) concentration
CO	Carbon monoxide
CSP	Clinical study protocol
CSR	Clinical study report
CTC	Clinical Trial Consultants AB
CV	Coefficient of variation
eCRF	Electronic case report form
EEA	European Economic Area
EU	European Union
FAS	Full analysis set
FPI	First patient in
FU	Follow-up
GCP	Good clinical practice
GDPR	General Data Protection Regulation
HIV	Human immunodeficiency virus
ICF	Informed consent form

ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
IEC	Independent ethics committee
IP	Investigational product
LPO	Last patient out
MedDRA	Medical dictionary for regulatory activities
N	number
NCA	Non-compartmental analysis
NRT	Nicotine replacement therapy
OTC	Over-the-counter
PI	Principal Investigator
PK	Pharmacokinetic
PPS	Per protocol set
PSWL	Pouched snus white portion large
PT	Preferred term
SAE	Serious adverse event
SAP	Statistical analysis plan
SD	Standard deviation
SDV	Source data verification
SOC	System organ class
SOP	Standard operating procedures
T _{max}	Time after drug administration when the maximum plasma concentration is reached
T _{1/2}	Half life
WHO	World Health Organization

5 ETHICAL AND REGULATORY REQUIREMENTS

5.1 Ethical conduct of the study

The study was performed in accordance with ethical principles that have their origin in the Declaration of Helsinki and are consistent with International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH)/Good Clinical Practice (GCP), European Union (EU) Clinical Trials Directive, and applicable local regulatory requirements.

5.2 Ethics and regulatory review

The Principal Investigator (PI) was responsible for submission of the clinical study protocol (CSP), the subject information and Informed Consent Form (ICF), any other written information to be provided to the subjects and any advertisements used for recruitment of subjects to an applicable Independent Ethics Committee (IEC) for approval.

The study and the CSP version 1.0 dated 14SEP2018 were approved in writing by the IEC in Uppsala on 28NOV2018 ([Appendix 16.1.3](#)), before recruitment of the first subject.

There were administrative changes done to the CSP at two occasions. In association with the implementation of the administrative changes, the CSP was updated to version 2.0 on 30NOV2018 and to version 3.0 on 16JAN2019.

The CSP (version 1.0, 2.0 and 3.0) are provided in [Appendix 16.1.1](#).

5.3 Subject information and consent

It was the responsibility of the Investigator or an authorized associate to give each potential study subject adequate verbal and written information before any study specific assessments were performed.

The information included the objectives and the procedures of the study as well as any risks or inconvenience involved. It was emphasized that participation in the study was voluntary and that the subject could withdraw from participation at any time and for any reason, without any prejudice. All subjects were given the opportunity to ask questions about the study and were given sufficient time to consider participation before signing the ICF.

Before performing any study-related procedures the ICF had to be signed and personally dated by the subject and by the Investigator. A copy of the subject information including the signed ICF was provided to the subject.

Documentation of the discussion and the date of informed consent were recorded in the source documentation and in the electronic case report form (eCRF). The subject information sheet and the signed ICF were filed by the Investigator for possible future audits and/or inspections.

The final approved version of the subject information and ICF could not be changed without approval from the Sponsor and the applicable IEC.

The written Subject Information and ICF are included in [Appendix 16.1.3](#).

5.4 Subject data protection

The ICF included information that the data that were recorded, collected and processed could be transferred to European Economic Area (EEA) or non-EEA countries. In accordance with the European Union Data Protection Directive (95/46/EC) and General Data protection Regulation (GDPR), the data do not identify any persons taking part in the study.

The potential study subject was informed that by signing the ICF he/she approved that authorized representatives from Sponsor and CTC and the concerned IEC had direct access to his/her medical records for verification of clinical study procedures. This agreement was substantiated in a separate document, according to local requirements.

The subject had the right to request access to his/her personal data and the right to request rectification of any data that was not correct and/or complete in accordance with the EU Data Protection Directive (95/46/EC).

The Investigator had a Subject Identification List which includes sufficient information to link records, i.e. the eCRF and clinical records. This list will be preserved for possible future inspections/audits but will not be made available to the Sponsor except for monitoring or auditing purposes.

Personal data that were collected in the study such as health information and ethnicity are considered as sensitive personal data. This data was pseudoanonymized, i.e. personally identifiable information (PII) was removed and replaced by a unique subject ID and was processed by the Sponsor and other involved parties during the study. After the study end, only anonymized data, i.e. aggregated data sets, have been used.

For this study, the Sponsor Swedish Match AB is the data controller of all data processed during the study (e.g. TMF, study reports) and CTC AB is the data processor. Any subcontractors used in the study, ABS laboratories, are also data processors.

For data that was processed at the clinic (e.g. medical records and ISF), CTC AB is the data controller.

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7 INTRODUCTION

7.1 Project background

Sweden has developed the lowest prevalence of smoking in Europe, particularly among males. It is widely accepted that one contributory factor to this trend is that snus has replaced cigarettes as the tobacco product of choice among many males, and some female, smokers.

Oral tobacco, like snus, is capable of rapidly delivering nicotine to the venous bloodstream through diffusion over the oral mucosa (Fant *et al.*, 1999). It may therefore be more satisfactory to smokers than currently available pharmaceutical nicotine replacement therapies (NRT).

Use of smokeless tobacco is by definition unassociated with exposure to the many thousands of combustion products found in tobacco smoke (many of which are highly carcinogenic and may induce a state of systemic, chronic inflammation), or chronic irritation in the upper and lower airways resulting from the inhalation of tobacco smoke. Therefore, it is generally accepted that use of smokeless tobacco products has substantially lower health risks than cigarette smoking. However, smokeless tobacco typically entails a systemic exposure to nicotine that is comparable to that among cigarette smokers.

Nicotine is the substance that is thought to contribute the most to the addictive properties of using any type of tobacco product. Also, the nicotine exposure may help to explain the adverse pregnancy outcomes that have been observed among pregnant women who continue to use tobacco after the first trimester of their pregnancy. Such effects have been documented both for cigarette smoking and use of snus.

Traditionally there has been no non-tobacco-based nicotine product on the Swedish market intended for recreational use. Despite the vast risk differential between snus and cigarettes in terms of adverse long-term health effects, snus remains a controversial product as it contains tobacco, is intended for recreational use, and is potentially addictive. The tobacco component of snus explains why it contains measurable amounts of unwanted, potentially carcinogenic constituents, albeit at very low concentrations.

Recently, a novel, non-tobacco-based nicotine product (ZYN[®]) has been developed and is now commercially available both in Sweden and in the U.S. It has some features that are similar to snus. It comes in pouches that are intended to be placed under the upper lip. In contrast to snus, the product contains no nitrosamines or polycyclic hydrocarbons, which are the two main classes of unwanted substances in snus. In ZYN[®] products, the matrix used for nicotine delivery consists of microcrystalline cellulose and maltitol, two inert substances frequently used in food stuffs. ZYN[®] does contain some unwanted substances but they are present in concentrations comparable to or lower than in many commonly used food stuffs or snus. The toxicological safety profile of ZYN[®] thus represents a significant improvement over snus. However, the nicotine content is comparable to that in snus and many other smokeless tobacco products that are currently common on the market in Scandinavia and the U.S.

When comparing the nicotine content of different nicotine-delivery products it is important to consider that the nicotine uptake varies considerably depending on product type (tobacco versus a non-tobacco-based matrix) and product formulation (pouch geometry, water content, particle size, pH, etc.). In addition, there is a substantial inter-individual variation in uptake with products used orally. This is probably related to constitutional differences in saliva production and a resulting wide variation in nicotine extraction from the product. It has also been suggested that some commonly used additives may affect nicotine uptake through an

effect on saliva surface tension. For instance, the flavoring agent methyl salicylate (“wintergreen”) has been hypothesized to facilitate oral nicotine uptake.

Some consumers, particularly smokeless tobacco users in the U.S., place the product under the lower lip. The placement of the pouch under the upper versus the lower lip may theoretically affect nicotine extraction and hence uptake. There are to date no data on whether pouch placement is a relevant factor but, theoretically, placement under the upper lip implies that the pouch is located at a distance from the orifices of large salivary glands. On the other hand, placement under the lower lip implies that the pouch may be more soaked in saliva (which is typically more abundant in the lower part of the mouth) and hence that nicotine extraction may be more rapid and/or complete.

Commercially available snus products have a nicotine content ranging between 1-2%. Previous studies ([Lunell and Curvall, 2011](#)), have indicated that on average about 15-20% of the total nicotine content is extracted and absorbed although with large inter-individual variation. 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 more important determinants of nicotine uptake than pouch weight.

The nicotine delivery profile of a product is probably one main determinant of its efficacy to decrease nicotine craving and, thus, its ability to function as an alternative to cigarettes among current smokers. At the same time, it probably also helps to explain the product’s addictive properties.

A previous in vivo nicotine extraction study compared the ZYN® 3 mg and ZYN® 6 mg products to a conventional snus product (1.0-gram pouch) with 8 mg of nicotine (17-01). Nicotine extraction was measured after 15 and 60 minutes. The study showed that the 3 mg ZYN® product was associated with a nicotine extraction that was less compared to the 8 mg snus product (General), whereas slightly more nicotine was extracted from the 6 mg ZYN® product. As extraction probably is a proxy for nicotine uptake, it is reasonable to assume that also the uptake is slightly higher with the 6 mg product. This hypothesis was confirmed in a formal nicotine uptake study (17-03). A limitation of the mentioned studies was that they did not include the newly developed ZYN® 8 mg product. That product was not commercially available at the time of study initiation.

When comparing nicotine uptake and exposure from currently available nicotine delivery products it is important to consider that many snus brands have a nicotine content that is higher than the 8 mg/pouch product used in the mentioned in vivo extraction study. Also, according to a consumer survey more than 10% of all snus users frequently use two or more pouches simultaneously. Users of loose snus typically use pinches weighing 2-2.5 grams. So, the mentioned results with a 1.0-gram pouch may underestimate and not adequately reflect the level of nicotine exposure experienced by current snus users.

In view of these circumstances, it was justified to study the nicotine delivery and uptake profile of the ZYN® (6 and 8 mg) products in comparison with some commercially available snus products on the Scandinavian and U.S. markets (see [Table 7.1-1](#)). These brands typically have a higher nicotine content and/or larger pouch size than the comparator snus product used in the mentioned in vivo extraction study. Given that approximately 10% of snus users often use two or more pouches simultaneously, it was considered reasonable to study the nicotine uptake with such an exposure. Also, it was considered relevant to assess whether the commonly used flavoring compound methyl salicylate (“wintergreen”) affected nicotine uptake, and to address the putative effect of pouch placement (upper versus lower lip).

Table 7.1-1 Product characteristics

<i>Test article</i>	<i>Pouch size (g)</i>	<i>Nicotine content (mg/g)</i>	<i>Nicotine content (mg per unit)</i>	<i>pH</i>
ZYN Smooth 6 mg	0.4	15	6	8.3
ZYN Wintergreen 6 mg	0.4	15	6	8.3
ZYN Smooth (lower lip) 6 mg	0.4	15	6	8.3
ZYN Smooth 8 mg	0.5	15	8	8.3
General PSWL (2 x 1.0 g)	1.0	8	16 (2 units)	8.7
Longhorn Pouch Natural	1.5	12	18	8.0
Longhorn Pouch Wintergreen	1.5	12	18	8.0

Note: The cited product characteristics represent production target values. This implies that there can be some batch to batch variation.

The overarching aim of the study was to ensure that the ZYN[®] products do not entail a higher nicotine exposure than is the case with commercially available tobacco-based snus or snus-like products that are currently common on the Scandinavian and U.S. markets.

7.2 Investigational Products

Test and reference products were delivered in identical containers labelled with unique identification numbers. The ZYN[®] Smooth test products were to contain 6 and 8 mg of nicotine, respectively, in each pouch while ZYN[®] Wintergreen was to contain 6 mg of nicotine in each pouch. As detailed in Section 9.8, the ZYN[®] products were shown to contain approximately 4.5 mg nicotine rather than 6 mg. The 8 mg product contained 8 mg nicotine as intended.

The reference product with Swedish snus, 2 x 1 g General pouched snus white portion large (PSWL) pouch, contained 8 mg of nicotine/g while the reference products with American moist snuff, 1.5 g Longhorn Natural Pouch and Longhorn Wintergreen Pouch, contained 12 mg nicotine/g.

Administration of the pouch was between the upper lip and the gum except for one treatment when the ZYN[®] Smooth 6 mg pouch was placed between the lower lip and the gum.

For further information on the investigational products (IPs) used in this study, refer to Section 9.4.

7.3 Risk/benefit assessment

It may be considered problematic to expose research subjects to a novel nicotine delivery product, the properties of which are not yet fully known. However, all research subjects were required to be daily snus users since at least one year (with an average or above snus consumption) so the participants were well acquainted with, and used to, the effects of nicotine. Preliminary data from previous studies indicated that the amount of nicotine extracted from the test articles was comparable to that from tobacco-based snus, despite the fact that the overall nicotine content and content of free nicotine in the ZYN[®] pouches were lower than in conventional tobacco-based snus (8 mg). This suggested that adverse effects from the nicotine exposure from the test and reference articles were unlikely to occur among the research subjects. At the time of study initiation, no adverse effects had been reported associated with the use of ZYN[®] apart from effects likely to be related to the nicotine exposure (such as salivation, nausea, and dyspepsia).

Aside from the nicotine, all ingredients used in the test products are food-approved (similar to ingredients in conventional snus). The nicotine in ZYN[®] is of pharmaceutical grade, i.e. the same as the nicotine in nicotine replacement products (gum, lozenges, mouth spray etc.). ZYN[®] is currently commercially available on the U.S. and Swedish markets.

The study did not involve invasive procedures, beside the collection of venous blood samples.

Pregnant women or individuals with a history of hypertension or any cardiovascular disease, who may be particularly vulnerable to nicotine exposure, were excluded from participation.

The potential adverse effects of the study procedures, which were likely to be minor and/or clinically insignificant, were from a research ethics perspective counterbalanced by the potential positive effects of the novel nicotine pouch as a reduced toxicity alternative to conventional snus. As the nicotine delivery profile of a product was likely to be central to its acceptability among current tobacco users, it was reasonable to conduct formal clinical studies to assess this feature in more detail.

Subjects remained in the research clinic for at least 6 hours after the administration of the IPs and were closely monitored by medical staff.

8 STUDY OBJECTIVES AND ENDPOINTS

8.1 Primary objective

To evaluate each subject's plasma concentrations of nicotine after administration of one single dose of ZYN[®] Smooth containing 6 mg of nicotine, compared to that of one single dose of 2 pouches of General PSWL Swedish snus (2x8 mg nicotine).

8.1.1 Primary endpoint

AUC_{inf} based on plasma concentrations of nicotine after administration of a single dose of ZYN[®] Smooth containing 6 mg of nicotine, compared to that of two doses of General PSWL Swedish snus pouches.

8.2 Secondary objectives

The secondary objectives of the study were to evaluate pharmacokinetic (PK) variables for the remaining products, plasma levels of Methyl salicylate and local tolerability:

1. To compare T_{max}, C_{max}, AUC_{inf}, AUC_{0-t} from each dose of the non-tobacco-based nicotine ZYN[®] Smooth pouches, General PSWL Swedish snus pouches and Longhorn American moist snuff pouches.
2. To assess the effect of the flavor component (Methyl salicylate) on nicotine plasma concentrations for the non-tobacco-based nicotine pouch ZYN[®] Wintergreen and Longhorn Wintergreen pouch American moist snuff, respectively.
3. To assess if there was a difference in nicotine plasma concentrations between upper lip and lower lip placement of the non-tobacco-based nicotine pouch ZYN[®] Smooth.
4. To compare the estimated in-vivo extracted amount and rate of extraction of nicotine from each dose.
5. To assess plasma levels of Methyl salicylate for the treatments with pouches containing Wintergreen flavor.
6. To compare Adverse events from each dose.

8.2.1 Secondary endpoints

1. T_{max}, C_{max}, AUC_{inf}, AUC_{0-t} and terminal half-life of the non-tobacco-based nicotine ZYN[®] Smooth pouches, General PSWL Swedish snus pouches and Longhorn American moist snuff pouches.
2. Nicotine plasma concentrations for the ZYN[®] Wintergreen and Longhorn Wintergreen treatments compared to the corresponding non-wintergreen containing product, respectively.
3. Nicotine plasma concentrations for the upper lip and lower lip placement of the non-tobacco-based nicotine pouch ZYN[®] Smooth.
4. In-vivo extracted amount of nicotine from all products.
5. Pairwise analysis of in-vitro extracted nicotine and rate of extraction.
6. Plasma levels of Methyl salicylate for the treatments with pouches containing Wintergreen flavor.
7. Collection and comparison of adverse events

Upon analysis of data, it was revealed that ZYN[®] Smooth 6 mg and ZYN[®] Wintergreen 6 mg contained a smaller amount of nicotine than intended (approximately 4.5 mg rather than 6 mg) and were hence excluded from all comparisons and analyses. The primary objective and secondary objectives No. 2, 3 and 5 could hence not be evaluated. The other objectives are evaluated based on ZYN[®] Smooth 8 mg, General PSWL 2x8 mg, Longhorn Natural 18 mg and Longhorn Wintergreen 18 mg.

9 INVESTIGATIONAL PLAN

9.1 Overall study design and schedule of events

The study was conducted as an open, randomized, 7-way cross-over, single dose administration study and included 36 subjects.

Subjects in the study were healthy males and females aged ≥ 19 years who had used tobacco-based snus for ≥ 1 year with a weekly consumption of two or more snus cans (preferably brands with nicotine content $\geq 1\%$).

Subjects reported to the clinic on separate days for the 7 experimental sessions in addition to visits for screening and follow-up. Between each experimental session, a wash-out period of at least 24 hours was required. The subjects were instructed to abstain from snus or other nicotine delivery products from 8.00 p.m. the evening before (minimum abstinence period: 13 hours, maximum abstinence period: 13.5 hours) and to refrain from smoking 24 hours before each experimental session. All sessions were performed during the morning hours to facilitate abstinence. The subjects certified abstinence before each treatment was started.

The treatments were administered as single doses in a pre-determined randomized order. The subject kept the pouch(es) still between the upper lip (lower lip for treatment 4) and the gum for 60 minutes and were instructed not to manipulate the pouch with the tongue or lips. The subjects were instructed not to eat, drink, chew chewing gum or brush teeth from 30 minutes before application of treatment, during application of investigational products and 30 minutes after the IP had been taken out. After 60 minutes the pouches were collected and frozen (-20°C) pending analyses of nicotine.

Blood samples for assessment of plasma levels of nicotine and Methyl salicylate were collected at pre-defined time points from pre-dose to 6 hours after IP administration.

Table 9.1-1 Schedule of events

Visit	Visit 1 ¹ Screening	Visit 2-8 ² Cross-over	Visit 9 Telephone FU
Assessments / Study days	-28 to -1		7 days (-3/+7) after last dose
Informed consent	x		
Demographics	x		
Medical/surgical history	x		
Inclusion/exclusion criteria	x		
Physical examination	x		
Weight, height	x		
HIV, Hepatitis B and C	x		
Pregnancy test ³	x		
Drugs of abuse	x	x ⁴	
Alcohol screen	x	x ⁴	
Carbon monoxide (CO) measurement		x	
Randomization		x ⁵	
IP (pouch) administration		x	
PK blood sampling		x ⁶	
Collection of pouches		x	
Treatment-emergent adverse events (AEs) ⁸		x	x
Baseline events	x	x ⁷	
Prior and concomitant medications ⁹	x	x	x

¹ Visit 1 could be performed during 2 days.

² Refer to Table 9.1-2 for details.

³ Female subjects only.

⁴ Drug and alcohol tests during the treatment period may be performed randomly.

⁵ Only on visit 2.

⁶ Before and 5, 10, 15, 30, 60, 90, 120, 240 and 360 min after application of the IPs.

⁷ Only prior to dose administration at Visit 2.

⁸ From first administration of IP.

⁹ For definitions of prior and concomitant medication, see the CSP.

Table 9.1-2 Detailed schedule of events for each treatment period

Visit	Visit 2 to 8											
Assessment/time-point	Admission	Pre-dose	0	5 min	10 min	15 min	30 min	60 min	90 min	2 h	4 h	6h
Inclusion/exclusion criteria	x ¹											
CO measurement	x											
Drugs of abuse	x ²											
Randomization		x ³										
IP (pouch) administration			x									
IP (pouch) collection								x ⁴				
PK blood sampling		x		x	x	x	x	x	x	x	x	x
Treatment-emergent AEs			x									
Prior and concomitant medications	x											

¹ Confirmation of inclusion/exclusion criteria (Visit 2 only).

² Drug tests during the treatment period may be performed randomly

³ Only on visit 2

⁴ Collection of pouch +/- 1 (one) minute from specified time.

9.2 Rationale for study design and dose groups

A crossover design was chosen to yield a more efficient comparison of treatments than a parallel study design, i.e., fewer subjects were required since each subject served as his own control. To avoid carryover effects, a wash-out period of at least 24 hours was incorporated between administrations.

Randomization was used to minimize bias in the assignment of subjects to a treatment sequence and to increase the likelihood that known and unknown subject attributes (e.g., demographic and baseline characteristics) were evenly balanced across treatment groups.

9.3 Study population

9.3.1 Recruitment

The subjects were recruited from a database of healthy volunteers at CTC and from advertising in media.

9.3.2 Number of subjects

36 subjects were included in the study.

9.3.3 Inclusion criteria

For inclusion in the study, subjects had to fulfil the following criteria:

1. Snus user who had used snus for ≥ 1 year, with a minimum weekly consumption of two or more snus cans (preferably brands with nicotine content $\geq 1\%$).
2. Willing and able to give written informed consent for participation in the study.
3. Healthy male or female subject aged ≥ 19 years of age inclusive.
4. Willing and able to comply with study procedures.

9.3.4 Exclusion criteria

Subjects could not enter the study if any of the following exclusion criteria were fulfilled:

1. Smoker, defined as "smoking during the last 24 hours according to self-report and CO in exhaled air > 10 ppm at clinical visits".
2. A history or presence of diagnosed hypertension or any cardiovascular disease.
3. Surgery within 6 months of the screening visit that, in the opinion of the investigator, could negatively impact on the subject's participation in the clinical study.
4. Any surgical or medical condition, which, in the judgment of the clinical investigator, might interfere with the absorption, distribution, metabolism or excretion of the investigational product.
5. History of any clinically significant disease or disorder which, in the opinion of the Investigator, could either put the subject at risk because of participation in the study, or influence the results or the subject's ability to participate in the study.
6. Breast feeding, pregnancy or planning to get pregnant during the study.

7. Female use of systemic contraceptives (such as oral contraceptives, implants, injectable steroids, vaginal ring, transdermal patch).
8. Any positive result on screening for serum hepatitis B surface antigen, hepatitis C antibody and Human Immunodeficiency Virus (HIV).
9. Positive screen for drugs of abuse or alcohol at screening or on admission to the unit prior to administration of the IP.
10. Current or history of alcohol abuse and/or use of anabolic steroids or drugs of abuse.
11. Plasma donation within one month of screening or blood donation (or corresponding blood loss) during the three months prior to screening.
12. Investigator considered the subject unlikely to comply with study procedures, restrictions and requirements.

9.3.5 ***Restrictions during the study***

9.3.5.1 General restrictions

The subjects had to be willing to comply with the below restrictions during the entire study duration *i.e.*, from screening to the last follow-up visit.

- Subjects were to abstain from snus and all other nicotine containing products from 8.00 p.m. the night before each study day.
- Subjects were to abstain from smoking the last 24 hours before each study day.
- Subjects were not allowed to eat or drink or use any other mouth related procedure (e.g. tooth brushing) 30 minutes before dose administration, during application of IPs and 30 minutes after the IP had been taken out.
- The female volunteers were expected to be sexually abstinent or to use non-systemic contraceptives to prevent pregnancy during the study period.
- Subjects were to abstain from use of Methyl salicylate containing products, e.g. ointments, mouth wash and chewing gum.
- Subjects were to abstain from drugs of abuse.
- Subjects could not donate blood or plasma during the study until 3 months after the follow-up visit.
- Subjects were not allowed to participate in any other clinical study.

9.3.5.2 Prior and concomitant therapy

Female systemic contraceptives e.g. birth control pills, injectable steroids, vaginal ring, transdermal patch and contraceptive implants were not allowed in the study.

Other concomitant medications or therapies, including herbal remedies, vitamin supplements and over-the-counter (OTC) products, were allowed during participation in the study.

9.3.6 **Criteria for subject withdrawal**

Subjects were free to discontinue their participation in the study at any time and for whatever reason without affecting their right to an appropriate follow-up investigation or their future care. If possible, the reason for withdrawal of consent were to be documented.

Subjects could be discontinued from the study at any time at the discretion of the Investigator for any of the following reasons:

- Severe non-compliance to study protocol procedures, as judged by the Investigator and/or Sponsor.
- Subject was lost to follow-up. A subject was considered lost to follow-up if he/she failed to come for 2 of the scheduled visits and if he/she was not possible to contact by site staff despite several attempts.
- Significant AEs posing a risk for the subject, as judged by the Investigator and/or Sponsor.

9.3.7 **Subject replacement**

Subjects who were prematurely withdrawn from the study were not replaced.

9.3.8 **Randomization**

On study Day 1 (Visit 2), the subjects were randomized to a treatment sequence. As this was an open study, the treatments to which each subject was allocated for the first dose administrations were recorded in the eCRF. A computer-generated randomization list was created using SAS Proc Plan, SAS Version 9.4. The randomization list contained subject number, treatment sequence, period, and treatment.

The randomization list was generated by CTC. The original randomization list was kept by the randomizer. A copy of the randomization list was provided to the clinic. The randomization list is provided in [Appendix 16.1.7](#).

9.3.9 **Blinding**

The present study was an open, randomized study. Subjects were administered each treatment by the study personnel according to the randomization list.

9.4 **Treatments**

The IPs were supplied by Swedish Match AB. Upon analysis of data, it was revealed that ZYN[®] Smooth 6 mg and ZYN[®] Wintergreen 6 mg contained a smaller amount of nicotine than intended (approximately 4.5 mg rather than 6 mg) and were hence excluded from all comparisons and analyses.

9.4.1 **Test products**

- 1= ZYN[®] Smooth containing 6 mg nicotine per portion
- 2= ZYN[®] Smooth containing 8 mg nicotine per portion
- 3= ZYN[®] Wintergreen containing 6 mg nicotine per portion
- 4= ZYN[®] Smooth containing 6 mg nicotine per portion (lower lip)

9.4.2 **Reference products**

5= Swedish portion snus, General PSWL (8 mg nicotine/g) 2x1.0 g

6= American moist snuff, Longhorn Pouch Natural (12 mg nicotine/g) 1.5 g

7= American moist snuff, Longhorn Pouch Wintergreen (12 mg nicotine/g) 1.5 g

9.4.3 **Treatment administration**

Each subject was given a single dose of the respective IP in the morning of each study day. The treatment sequences to which the subjects were randomized are displayed in Table 9.4-1.

Table 9.4-1 Definition of treatment sequences

Description of Element	Description of Planned Arm			
	Treatment Sequence A	Treatment Sequence B	Treatment Sequence C	Treatment Sequence D
ZYN [®] Smooth 6 mg	1	4	1	3
ZYN [®] Smooth 8 mg	2	5	3	6
ZYN [®] Wintergreen 6 mg	3	6	6	4
ZYN [®] Smooth 6 mg lower lip	4	7	7	1
General PSWL 2x8 mg	5	1	5	2
Long horn Natural 18 mg	6	2	2	7
Long horn Wintergreen 18 mg	7	3	4	5

SM18_01 treatment definitions, SAS program: treatment_definitions.sas. Run by: Lars Norberg, lars.norberg@ctc-ab.se 2019-05-08T11:19:24

9.4.4 **Treatment compliance**

All IP was administered at the research clinic under medical supervision to ensure compliance.

9.4.5 **Continuation of treatment with investigational product**

There was no treatment with test or reference products after end of study participation.

9.5 **Study assessments**

9.5.1 **Demographics and other baseline characteristics**

9.5.1.1 **Informed consent**

Signed informed consent was obtained before any screening procedures were initiated. The informed consent procedure is further described in Section 5.3.

9.5.1.2 **Eligibility criteria**

Eligibility criteria were checked during screening and verified before randomization/IP administration. The criteria are specified in Sections 9.3.3 and 9.3.4.

9.5.1.3 Demographic information

The following demographic data were recorded: gender, age, ethnicity, race and preferred snus placement.

9.5.1.4 Weight and height

Weight and height were measured without shoes. BMI was calculated from the height and weight recorded and rounded off to the nearest whole number.

9.5.1.5 Physical examination

A complete physical examination included assessments of general appearance, throat, thyroid, lungs, cardiac, abdomen (liver and spleen), lymph nodes and extremities.

9.5.1.6 Medical/surgical history

Medical/surgical history was obtained by subject interview in order to verify that the eligibility criteria were met.

9.5.1.7 Prior and concomitant medication

Prior medication was obtained by subject interview in order to verify that the eligibility criteria were met.

Medications were classified as prior if the stop date was before or on the day of the first dose administration (pre-dose) and as concomitant if ongoing at the day of the first dose administration, stopped after the first dose administration or started after the first dose administration.

Any use of concomitant medication from screening until the last follow-up visit was documented in the subject's eCRF. Relevant information (*i.e.* name of medication, total daily dose, unit, start and stop dates, reason for use if consistent with the definition of an AE) were recorded. All changes in medication were noted in the CRF.

9.5.1.8 Baseline symptoms

A baseline symptom was defined as an event that occurred between subject's signing of the ICF until the first administration of IP (*i.e.* an event that occurred during the screening period). Such events are not AEs and were recorded in the medical history log in the eCRF.

9.5.1.9 HIV and Hepatitis B/C

Subjects were tested for HIV and hepatitis B/C prior to inclusion into the study in order to protect personnel handling the blood samples.

9.5.1.10 Pregnancy test

All females of childbearing potential took a pregnancy test (urine dipstick) at screening.

9.5.1.11 Urine drug screen

The subjects' urine was screened for drugs of abuse at screening using the Alere™ Drug Screen Test Panel. Additional random tests could be performed during the study period.

9.5.1.12 Alcohol breath test

An alcohol breath test was performed at screening. Additional random tests could be performed during the study period.

9.5.1.13 Carbon monoxide measurement

Measurement of carbon monoxide (CO) in exhaled air was performed at visits to the clinic.

9.5.2 ***Assessments related to primary and secondary PK endpoints for nicotine***

9.5.2.1 Pharmacokinetic samples and analysis

Venous blood samples (approximately 5 mL) for the determination of plasma concentrations of nicotine after administration of the IP, were collected through an indwelling venous catheter at the pre-specified time-points (see [Table 9.1-2](#) in Section 9.1). The date and time of collection of each sample were recorded in the eCRF.

Plasma samples for determination of plasma concentrations of nicotine were analyzed by ABS Laboratories Ltd by means of a validated using LC-MS/MS method. Samples from all evaluable subjects were analyzed.

9.5.3 ***Assessments related to secondary endpoints***

9.5.3.1 Samples for analysis of Methyl salicylate

Plasma samples for determination of plasma concentrations of nicotine were also to be analyzed for plasma levels of salicylic acid which is a proxy for plasma levels of the flavor component Methyl salicylate. The levels of salicylic acid were used for read-across to assess the plasma levels of Methyl salicylate.

The analysis of salicylic acid was only performed for samples drawn during the experimental session when the IP was containing the flavor component i.e. ZYN® Wintergreen 6 mg and the Longhorn Wintergreen 18 mg pouch.

Plasma samples for determination of plasma concentrations of salicylic acid were analyzed by ABS Laboratories Ltd by means of a validated using LC-MS/MS method. Samples from all evaluable subjects excluding withdrawn or dropout subjects were analyzed.

9.5.3.2 Collection of used pouches

Used pouches were collected after 60 minutes (+/- 1 minute) of use for the determination of residual nicotine in the IPs.

All the collected pouches were frozen immediately at -20°C. Pouches for extraction of nicotine were analyzed by Swedish Match. Pouches from all evaluable subjects were analyzed.

9.5.3.3 Adverse Events

AEs were collected from the first administration of IP until the last visit in the study.

All AEs, non-serious as well as serious, were registered in the eCRF. At all visits following the first administration, subjects were asked about the presence of AEs since the previous visit to the clinic. The information could also be obtained from signs and symptoms detected during an examination, laboratory test results, direct observations by study personnel and spontaneous reports from the subjects.

AEs were reported with onset date and time, intensity (mild, moderate, severe), action taken and outcome and were assessed for seriousness and causality in relation to the IP (unlikely related, possibly related, probably related).

For further details on AE definitions, AE recording and AE reporting, refer to the CSP in [Appendix 16.1.1](#).

9.5.1 ***Appropriateness of measurements***

Measurements of nicotine plasma concentration and nicotine extraction are standard assessments in nicotine research. Standardized methods for measurements of safety and tolerability (AE reporting) were used.

9.6 **Data quality assurance**

The study was performed in compliance with GCP, applicable regulations and CTCs Standard Operating Procedures (SOPs).

Before inclusion of the first subject into the study, a study initiation visit was performed by CTC in order to inform and train relevant study staff. The Investigator was thereafter responsible for providing appropriate study related training to new staff and to forward any new information of relevance to the performance of this study to the staff involved.

An eCRF was completed for each subject included. A sample of the eCRF is included as [Appendix 16.1.2](#).

The study site was periodically visited by a Monitor from CTC. The Monitor had direct access to medical records and original data for Source Data Verification (SDV). For all subjects, verification against source data was performed as detailed in the monitoring plan.

All personnel involved in the study were listed on a signature and delegation list kept and updated by the Investigator.

No audits or inspections were performed during the study.

9.7 **Statistical methods planned in the protocol and determination of sample size**

The statistical analyses performed in this study were initially specified in the CSP, see [Appendix 16.1.1](#). Further details of the planned statistical analysis are provided in the

Statistical Analysis Plan (SAP) which was finalized prior to the Clean File meeting and database lock, see [Appendix 16.1.9](#). Any changes in the SAP compared to the CSP are described in Section [9.8](#).

9.7.1 **General**

PK parameters are presented using summary statistics. Data are presented in terms of number (N), arithmetic mean, standard deviation (SD), median, minimum and maximum value, geometric mean and coefficient of variation (CV).

Categorical data are presented as counts and percentages. When applicable, summary data are presented by treatment, and by assessment time. Individual subject data are listed by subject number, treatment, and, where applicable, by assessment time.

9.7.2 **Determination of sample size**

Previous studies had showed that ZYN[®] Smooth 6 mg has an AUC_{inf} of 3581 min ng/mL and 2 pouches of 8 mg Swedish snus have an AUC_{inf} of 5586 min ng/mL (assumed AUC_{inf} based on the AUC_{inf} following 60 min administration of 1 pouch of Swedish snus) with a common standard deviation of 1920 min ng/mL.

The hypothesis for this study was that ZYN[®] Smooth 6 mg has a significantly lower AUC_{inf} compared to 2 pouches 8 mg Swedish snus. With a power of 80% and a significance level of 2.5%, the number of subjects needed was 32. Assuming 10% drop out rate, 36 subjects were included.

9.7.3 **Analysis data sets**

The Full Analysis Set (FAS) consisted of all subjects who were randomized and received at least one dose of IP. This population was used as the Safety analysis set.

The Per Protocol Set (PPS) consisted of all subjects who were randomized and completed the study without any major protocol deviations that were judged to compromise the analysis of the data. All protocol violations were judged as major or minor at the clean file meeting. This population was used as the PK analysis set.

9.7.4 **Description of study population**

9.7.4.1 Demographics and baseline characteristics

Descriptive statistics for demographics and baseline characteristics were summarized by treatment sequence.

9.7.4.2 Treatment compliance

IP application data were listed by treatment sequence and subject.

9.7.4.3 Prior and concomitant medications

Prior and concomitant medication data were listed and tabulated by Anatomic Therapeutic Chemical (ATC) code. Prior and concomitant medications were coded according to the World Health Organization (WHO) ATC classification system.

9.7.5 **Analysis of primary endpoint**

AUC_{inf} based on plasma concentrations of nicotine after administration of one single dose of ZYN[®] Smooth 6 mg of nicotine, compared to that of one single dose of 2 pouches of 8 mg Swedish snus was to be described using summary statistics and non-parametric signed Wilcoxon rank sum test for between treatment difference.

Upon analysis of data, it was revealed that ZYN[®] Smooth 6 mg and ZYN[®] Wintergreen 6 mg contained a smaller amount of nicotine than intended (approximately 4.5 mg rather than 6 mg) and were hence excluded from all comparisons and analyses. The intended comparisons of AUC_{inf} were only performed between the other IP:s, see Section 9.8.2.

9.7.6 **Analysis of secondary endpoints**

9.7.6.1 Calculation of PK parameters and comparison of PK profiles between IPs

The PK parameters were calculated by non-compartmental analysis (NCA) using the software Phoenix WinNonlin[®] version 8.1 (Pharsight Corporation, U.S.A.).

The following non-compartmental PK parameters were assessed:

- T_{max} (sampling time at which C_{max} occurred)
- C_{max} (maximum observed concentration)
- AUC_{inf} (area under the curve from 0 to infinity)
- AUC_{0-t}; AUC_{last} (area under the curve from 0 to t hours where t is the last measured concentration)
- T_{1/2} (half-life)

Plasma concentrations below the quantification limit (0.5 ng/mL) were set to 0 before T_{max} and to missing thereafter. The area under the plasma concentration versus time curve were calculated according to the linear up- log down method. The elimination constant (Lambda Z) was calculated and the applied threshold for acceptance of regression was $R^2 \geq 0.85$, the threshold for % residual AUC $\leq 30\%$ and the threshold for half-life span was ≥ 1.0 . Subjects not fulfilling all 3 acceptance criteria were excluded from the analyses.

All subjects and all elements were qualified in accordance with the Lambda Z acceptance criteria except Subject 102. Three Elements for Subject 102 showed non-acceptable Lambda Z criteria (*viz.* Element General PSWL 2x8mg, ZYN[®] Smooth 6 mg lower lip and ZYN[®] Wintergreen 6 mg). Subject 102 was excluded from the PK analyses.

For Subject 136 (Element=Longhorn Wintergreen 18 mg and ZYN[®] Smooth 8 mg), the 6 h time-point (364 min for Longhorn Wintergreen 18 mg, and 360 min for ZYN[®] Smooth 8 mg) was excluded for best fit Lambda Z calculations.

The mean \pm SD of AUC_{inf}, AUC_{0-t}, AUC_{inf}, C_{max}, T_{max}, and T_{1/2} were calculated based on plasma concentrations of nicotine after administration of each pouch.

AUC_{0-t}, AUC_{inf}, C_{max}, T_{max}, and T_{1/2} of the IPs were described using summary statistics and analyzed using signed Wilcoxon rank sum test for between treatment differences for all pairwise comparisons.

9.7.6.2 In-vivo extracted amount of nicotine

The extracted dose of nicotine was calculated by using the average reference concentration of nicotine by weight (in mg/g) and multiplying this value with the individual measured weight of each pouch used in the study. From this value, the remaining used pouch nicotine content is subtracted to get a value for the extracted amount of nicotine. The mean \pm SD extracted dose of nicotine from each pouch, were calculated. The extracted dose of nicotine was analyzed using the signed Wilcoxon rank sum test and students t-test.

9.7.6.3 Analysis of plasma levels of Methyl salicylate

Plasma levels of Methyl salicylate were summarized using descriptive statistics.

9.7.6.4 Adverse events

AE data were summarized by System Organ Class (SOC) and PT. All AE data were listed and include the verbatim term entered by the Investigator. AEs were coded using the Medical Dictionary of Regulatory Activities (MedDRA).

9.7.7 **Statistical/analytical issues**

9.7.7.1 Adjustments for covariates

No adjustment for covariates was performed.

9.7.7.2 Significance level

The primary endpoint investigated a one-sided hypothesis that ZYN[®] 6 mg has a lower AUC compared to 2 pouches of 8 mg Swedish snus, using a significance level of 2.5%. All other tests were two-sided with a significance level of 5%.

9.7.7.3 Handling of dropouts or missing data

Outliers were included in listings and were handled separately in any analyses. No imputation of data was performed.

9.7.7.4 Multiple comparison/multiplicity

No adjustment for multiple comparison/multiplicity was performed, all significant statistical findings were reviewed for medical relevance.

9.7.7.5 Examination of subgroups

No examination of subgroups was performed.

9.8 Changes in the conduct of the study or planned analyses

9.8.1 *Changes not described in a formal protocol amendment*

The CSP v1.0 (approved by the Ethics committee on 28NOV2018) was updated once before the study start (v2.0; 30NOV2018) and once during the conduct of the study (v3.0; 16JAN2019). All updates were considered administrative changes. All CSP versions are included in [Appendix 16.1.1](#) to this clinical study report (CSR).

9.8.2 *Changes in the planned statistical analyses*

Upon analysis of data, it was revealed that ZYN® Smooth 6 mg and ZYN® Wintergreen 6 mg contained a smaller amount of nicotine than intended (approximately 4.5 mg rather than 6 mg) and were hence excluded from all comparisons and analyses. The primary objective and secondary objectives No. 2, 3 and 5 could hence not be evaluated.

The initial analysis of the ZYN 6 mg samples showed a somewhat higher nicotine content compared to the target value. At preparation of samples for the study it was discovered that the filling weight was somewhat low, however since the nicotine content was shown to be on the higher end, the products were still calculated to be within specification.

At the analysis of extraction data, after the study was completed, it was revealed that the nicotine content was lower than the initial analysis had shown, which unfortunately implied that products with a smaller amount of nicotine, than intended, was used in the study.

10 STUDY SUBJECTS

10.1 Disposition of subjects

10.1.1 *Number of subjects and study discontinuations*

(b) (4)



10.2 Protocol deviations

(b) (4)



10.3 Data sets analyzed

(b) (4)



(b) (4)



10.4 Demographics and other baseline characteristics

10.4.1 *Demographics*

(b) (4)



(b) (4)



10.4.2 *Medical history*

(b) (4)



10.4.3 *Prior and concomitant medication*

(b) (4)



10.5 *Measurements of treatment compliance*

All IPs were administered at the research clinic under supervision by study staff to ensure compliance. Thirty-one subjects took the IP in accordance with the protocol. For details on the subjects who did not, refer to Section [11.1](#).

Individual IP administration data are provided in [Appendix 16.2.5](#).

11 EVALUATION OF RESULTS

11.1 Extent of exposure

Pouches containing nicotine were administered as single doses. The subjects kept the pouch(es) between the upper lip and the gum for 60 min (ZYN[®] Smooth 6 mg was also kept between the lower lip and the gum for the same amount of time).

A total of 31 subjects took all doses of IP but for one subject, blood sampling was not possible at Visit 8 (last dose), hence the subject was excluded from the PPS (Table 10.3-1).

Details on subjects who did not take all doses of IP are presented in Section 10.3.

The plasma concentrations of nicotine vs time for all treatments analyzed are shown in Figure 11.3-1. Individual IP administration data and plasma concentration data are provided in Appendix 16.2.5.

11.2 Evaluation of primary endpoint

The primary endpoint: “AUC_{inf} based on plasma concentrations of nicotine after administration of a single dose of ZYN[®] Smooth containing 6 mg of nicotine, compared to that of two doses of General PSWL Swedish snus pouches” could not be evaluated since the ZYN[®] products contained less nicotine than anticipated, see Section 9.8.2. The remaining products were evaluated in terms of difference in AUC_{inf}.

11.2.1 *Comparison of AUC_{inf} between products*

The highest mean AUC_{inf} was observed following a single dose of General PSWL 2x8 mg and the lowest following a single dose of ZYN[®] Smooth 8 mg (Table 11.2-1). The difference was statistically significant (p=0.0001, Table 14.3-1).

The mean AUC_{inf} following a single dose of General PSWL 2x8 mg was also significantly higher than the mean AUC_{inf} of both Longhorn Natural 18 mg (p=0.0023, Table 14.3-4) and Longhorn Wintergreen 18 mg (p=0.0091, Table 14.3-5).

There were no statistically significant differences in mean AUC_{inf} between ZYN[®] Smooth 8 mg and either of Longhorn Natural 18 mg and Longhorn Wintergreen 18 mg (Table 14.3-2 and Table 14.3-3) or between Longhorn Natural 18 mg and Longhorn Wintergreen 18 mg (Table 14.3-6).

Corresponding results were found for the baseline adjusted comparisons (Table 14.3-7 to Table 14.3-12). Individual data are listed in Appendix 16.2.6.

Table 11.2-1 AUC_{inf} by treatment (Per Protocol Set)

(b) (4)

11.3 Evaluation of secondary endpoints

11.3.1 *Nicotine extraction*

11.3.1.1 Comparisons between products - extracted dose of nicotine

The mean extracted dose of nicotine from General PSWL 2x8 mg pouches (5.04 mg) was significantly higher than the mean extracted dose from ZYN[®] Smooth 8 mg (3.79 mg; $p < 0.0001$), Longhorn Natural 18 mg (3.00 mg; $p < 0.0001$) and Longhorn Wintergreen 18 mg (3.28 mg; $p < 0.0001$), see Table 11.3-1, [Table 14.3-13](#), [Table 14.3-16](#) and [Table 14.3-17](#).

In addition, the mean extracted dose of nicotine from ZYN[®] Smooth 8 mg (3.79 mg) was significantly higher than the mean amount extracted from Longhorn Natural 18 mg (3.00 mg; $p = 0.0042$) whereas there were no statistically significant differences in the extracted dose of nicotine between ZYN[®] Smooth 8 mg and Longhorn Wintergreen 18 mg or between Longhorn Natural 18 mg and Longhorn Wintergreen 18 mg see Table 11.3-1, [Table 14.3-14](#), [Table 14.3-15](#) and [Table 14.3-18](#).

The extracted doses from the unused pouches used for the calculations are listed and summarized in [Table 14.3-19](#). Individual extraction data are provided in [Appendix 16.2.6](#).

Table 11.3-1 Extracted nicotine (PPS)

(b) (4)



11.3.1.2 Correlations – AUC_{inf} and extracted nicotine

Scatter plots of mean AUC_{inf} against extracted nicotine are presented for ZYN[®] Smooth 8 mg, General PSWL 2x8 mg, Longhorn Natural 18 mg a and Longhorn Wintergreen 18 mg in [Figure 14.3-1](#) to [Figure 14.3-4](#) (non-baseline adjusted data) and in [Figure 14.3-5](#) to [Figure 14.3-8](#) (baseline adjusted data).

For all treatment groups, the regression lines seem to demonstrate a correlation. All correlations which are above 0.5, i.e. all IPs but General PSWL 2x8 mg; both non-baseline adjusted and baseline adjusted data, shows strong relationship between AUC_{inf} and extracted amount of nicotine, i.e. high AUC_{inf} values correspond to high amounts of extracted nicotine.

11.3.1.3 Comparisons between products - rate of extraction

The mean nicotine rate of extraction from ZYN[®] Smooth 8 mg (50.37%) was significantly higher than the rate of extraction from General PSWL 2x8 mg (32.55 %; $p < 0.0001$), Longhorn Natural 18 mg (18.94%; $p < 0.0001$) and Longhorn Wintergreen 18 mg (20.53%; $p < 0.0001$), see [Table 14.3-20](#), [Table 14.3-21](#) and [Table 14.3-22](#)).

In addition, the rate of extraction of nicotine from General PSWL 2x8 mg (32.55%) was significantly higher than the rate of extraction from Longhorn Natural 18 mg (18.94%; $p < 0.0001$, [Table 14.3-23](#)) and Longhorn Wintergreen 18 mg (20.53%; $p < 0.0001$, [Table 14.3-24](#)).

There were no statistically significant differences in rate of extraction between Longhorn Natural 18 mg and Longhorn Wintergreen 18 mg ([Table 14.3-25](#)).

Individual rate of extraction data are provided in [Appendix 16.2.6](#).

11.3.2 **Nicotine plasma concentrations**

The plasma concentration vs time curves were similar for all IP:s with the highest concentrations of nicotine observed at 1 hour after start of IP administration in association with removal of the IP.

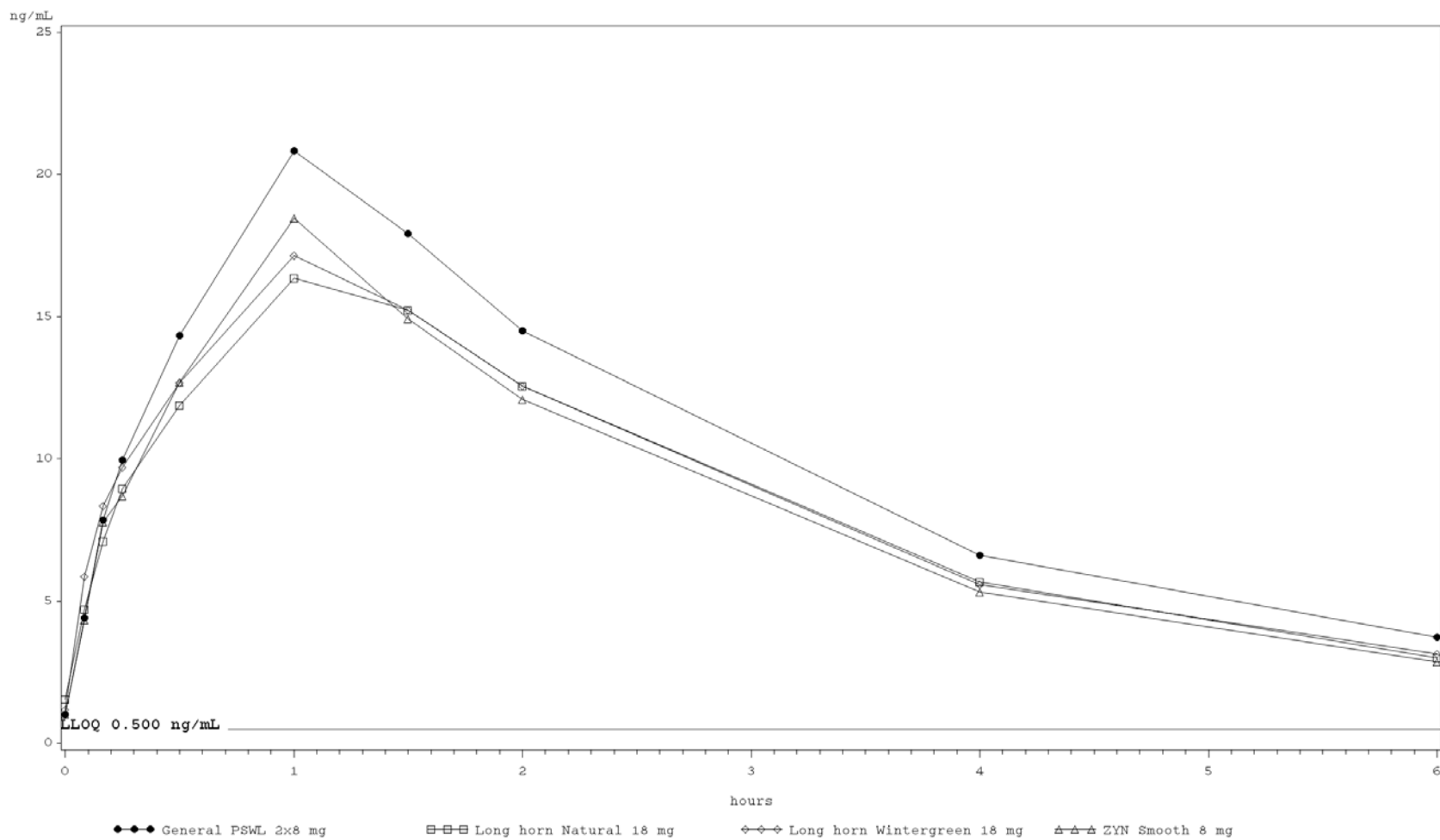
Nicotine plasma concentrations are summarized by treatment for ZYN[®] Smooth 8 mg, General PSWL 2x8 mg, Longhorn Natural 18 mg and Longhorn Wintergreen 18 mg in [Table 14.3-26](#) and are displayed in [Figure 11.3-1](#) (before and 5, 10, 15, 30, 60, 90, 120, 240 and 360 minutes after administration of IP). Individual data are displayed by treatment in [Figure 14.3-9](#) to [Figure 14.3-12](#).

Individual data for all treatments are listed in [Appendix 16.2.5](#).

Figure 11.3-1

Plot of mean plasma concentrations of nicotine (ng/mL) vs time for all treatments (Per protocol set)

Graph 14.2.7.5 Plot of mean plasma concentrations of nicotine vs time for all treatments (Per protocol set)



SM18_01 PK conc mean figure, SAS program: pc_graphs_mean.sas. Run by: Lars Norberg, lars.norberg@ctc-ab.se 2019-05-27T15:43:24

11.3.3 **Pharmacokinetic parameters**

A summary of the PK parameters is given per treatment in Table 11.3-2 (non-baseline adjusted data) and in [Table 14.3-28](#) (baseline adjusted data). Individual subject data are provided in [Appendix 16.2.6](#).

Table 11.3-2 Pharmacokinetic parameters by treatment (Per Protocol Set)

(b) (4)



11.3.3.1 Comparisons between products - pharmacokinetic parameters

AUC_{0-t}

As for AUC_{inf}, the highest mean AUC_{0-t} was observed following a single dose of General PSWL 2x8 mg and the lowest following a single dose of ZYN[®] Smooth 8 mg (Table 11.3-2). The difference was statistically significant (p=0.0002, [Table 14.3-29](#)).

The mean AUC_{inf} following a single dose of General PSWL 2x8 mg was also significantly higher than the mean AUC_{inf} of both Longhorn Natural 18 mg (p=0.0017, [Table 14.3-32](#)) and Longhorn Wintergreen 18 mg (p=0.0035, [Table 14.3-33](#)).

There were no statistically significant differences in mean AUC_{0-t} between ZYN[®] Smooth 8 mg and either of Longhorn Natural 18 mg and Longhorn Wintergreen 18 mg or between Longhorn Natural 18 mg and Longhorn Wintergreen 18 mg ([Table 14.3-30](#), [Table 14.3-31](#) and [Table 14.3-34](#)).

Corresponding results were found for baseline adjusted data ([Table 14.3-53](#) to [Table 14.3-58](#))

C_{max}

The highest mean C_{max} was observed following a single dose of General PSWL 2x8 mg (21.23 ng/mL) and the lowest following a single dose of Longhorn Natural 18 mg (16.95 ng/mL; [Table 11.3-2](#)). The difference was statistically significant ($p < 0.0001$, [Table 14.3-38](#)). The mean C_{max} of General PSWL 2x8 mg was also significantly higher than the mean C_{max} of ZYN[®] Smooth 8 mg ($p = 0.0084$; [Table 14.3-35](#)) and Longhorn Wintergreen 18 mg ($p = 0.0002$; [Table 14.3-39](#)).

There were no statistically significant differences in mean C_{max} between ZYN[®] Smooth 8 mg and either of Longhorn Natural 18 mg and Longhorn Wintergreen 18 mg, respectively, or between Longhorn Natural 18 mg and Longhorn Wintergreen 18 mg ([Table 14.3-36](#), [Table 14.3-37](#) and [Table 14.3-40](#)).

Corresponding results were found for baseline adjusted data ([Table 14.3-59](#) to [Table 14.3-64](#))

T_{max}

Mean T_{max} was around 1 hour for all products (range: 57 min [ZYN[®] Smooth 8 mg] to 64 min [Longhorn Wintergreen 18 mg]). The mean T_{max} of ZYN[®] Smooth 8 mg was significantly shorter than the mean T_{max} of Longhorn Wintergreen 18 mg ($p = 0.0247$; [Table 14.3-43](#)).

Otherwise there were no statistically significant differences between the products in terms of T_{max} ([Table 14.3-41](#) to [Table 14.3-42](#) and [Table 14.3-44](#) to [Table 14.3-46](#)).

Corresponding results were found for baseline adjusted data except that the mean T_{max} of ZYN[®] Smooth 8 mg was also significantly shorter than the mean T_{max} of General PSWL 2x8 mg ([Table 14.3-65](#) to [Table 14.3-70](#)).

T_{1/2}

The mean T_{1/2} of ZYN[®] Smooth 8 mg was significantly shorter than mean T_{1/2} of Longhorn Wintergreen 18 mg ($p = 0.0114$; [Table 14.3-49](#)). Otherwise there were no statistically significant differences between the products in terms of T_{1/2} ([Table 14.3-47](#), [Table 14.3-48](#) and [Table 14.3-50](#) to [Table 14.3-52](#)).

Corresponding results were found for baseline adjusted data ([Table 14.3-71](#) to [Table 14.3-76](#)).

11.4 Plasma levels of methyl salicylate for products containing Wintergreen flavor

The plasma levels of methyl salicylate for Longhorn Wintergreen 18 mg are summarized in [Table 14.3-27](#). Quite strikingly, approximately half of the subjects for whom salicylate was analyzed had measurable concentrations of salicylate prior to intake of both Longhorn Wintergreen 18 mg and ZYN[®] Wintergreen 6 mg (4.5 mg) ([Appendix 16.2.5](#)). However, none of the subjects reported that they had taken any concomitant medications containing acetyl salicylic acid substances ([Appendix 16.2.4](#)).

11.5 Summary of efficacy and PK results

The mean AUC_{inf} of General PSWL 2x8 mg was significantly higher than the mean AUC_{inf} of ZYN[®] Smooth 8 mg, Longhorn Natural 18 mg and Longhorn Wintergreen 18 mg, respectively.

Corresponding results were observed for the extracted dose of nicotine, i.e. the mean extracted dose of nicotine from General PSWL 2x8 mg was significantly higher than the

mean extracted dose from ZYN[®] Smooth 8 mg, Longhorn Natural 18 mg and Longhorn Wintergreen 18 mg, respectively. In addition, the mean extracted dose of nicotine from ZYN[®] Smooth 8 mg was significantly higher than the mean dose extracted from Longhorn Natural 18 mg. For all products but General PSWL 2x8 mg, there was a strong correlation between the AUC_{inf} and the extracted amount of nicotine.

The mean nicotine rate of extraction from ZYN[®] Smooth 8 mg was significantly higher than the rate of extraction from General PSWL 2x8 mg, Longhorn Natural 18 mg and Longhorn Wintergreen 18 mg. In addition, the rate of extraction of nicotine from General PSWL 2x8 mg was significantly higher than the rate of extraction from Longhorn Natural 18 mg and Longhorn Wintergreen 18 mg.

Mean AUC_{0-t} and mean C_{max} of General PSWL 2x8 mg were significantly higher than mean AUC_{0-t} and mean C_{max} of ZYN[®] Smooth 8mg, Longhorn Natural 18 mg and Longhorn Wintergreen 18 mg, respectively.

Mean T_{max} and mean T_{1/2} of ZYN[®] Smooth 8 mg was significantly shorter than the mean T_{max} and mean T_{1/2} of Longhorn Wintergreen 18 mg. For baseline adjusted data, mean T_{max} of ZYN[®] Smooth 8 mg was also significantly shorter than the mean T_{max} of General PSWL 2x8 mg.

There were no statistically significant differences in mean AUC_{inf}, AUC_{0-t}, mean C_{max} or extracted dose of nicotine between ZYN[®] Smooth 8 mg and either of Longhorn Natural 18 mg or Longhorn Wintergreen 18 mg or between Longhorn Natural 18 mg and Longhorn Wintergreen 18 mg. In addition, there were no statistically significant differences in rate of extraction between Longhorn Natural 18 mg and Longhorn Wintergreen 18 mg or in T_{max} between most of the IPs.

12 ADVERSE EVENTS

12.1 Brief summary of adverse events

(b) (4)



(b) (4)



(b) (4)



12.2 Display and analysis of adverse events

(b) (4)



(b) (4)

12.3 Listing of adverse events by subject

Adverse events are listed by subject in [Appendix 16.2.7](#).

12.4 Deaths, other serious adverse events and other significant adverse events

12.4.1 *Listing of deaths, other serious adverse events and significant adverse events*

There were no deaths, other SAEs or other significant AEs during the study, see [Table 12.1-1](#) and Table 12.2-1.

12.5 Summary of safety results

Administration of single doses of nicotine-containing products was safe and well tolerated by the healthy subjects in this study.

Ten subjects (28%) reported a total of 18 AEs during the 24 hours following each administration of ZYN[®] Smooth 8 mg, General PSWL 2x8 mg, Longhorn Natural 18 mg and Longhorn Wintergreen 18 mg. There were no deaths, other SAEs or withdrawals due to AEs. All AEs were of mild to moderate in intensity. The most common AE was nausea. Overall, a majority of the AEs were AEs that are usually associated with nicotine exposure.

13 DISCUSSION AND OVERALL CONCLUSIONS

13.1 DISCUSSION

This open-label, randomized, 7-way cross-over study compared the PK, extracted dose of nicotine, and the nicotine rate of extraction of a single dose of the non-tobacco-based nicotine product (ZYN[®]) to single doses of General PSWL 2x8 mg, Longhorn Natural 18 mg and Longhorn Wintergreen 18 mg. A total of 36 healthy subjects who were regular tobacco-based snus users were included in the study.

The study intended to investigate 3 different ZYN[®] products: ZYN[®] Smooth 6 mg, ZYN[®] Smooth 8 mg and ZYN[®] Wintergreen 6 mg. However, since it was discovered that both ZYN[®] Smooth 6 mg and ZYN[®] Wintergreen 6 mg used in the study contained less nicotine than intended (approximately around 4.5 mg rather than 6 mg), the PK and extraction data following administration of these IP:s were not included in summaries and analyses and were listed only. Safety data were both summarized and listed. The analysis of PK and extraction data for ZYN[®] Smooth 6 mg and ZYN[®] Wintergreen 6 mg will be reported elsewhere.

The main aim of the study was to evaluate the nicotine uptake, measured as AUC_{inf}, of ZYN[®], versus conventional snus pouches and moist snuff. In addition, the in vivo extraction of nicotine was assessed. The study also aimed to evaluate other PK parameters, to assess the effect of the flavor component (methyl salicylate) on nicotine plasma levels for ZYN[®] Wintergreen 6 mg and Longhorn Wintergreen 18 mg, to assess the effect of the placement of the pouch (upper versus lower lip) and to assess safety in terms of AEs. The effect of methyl salicylate or pouch placement could not be analyzed since both these analyses would have involved ZYN[®] 6 mg products.

The IPs compared in this study had a nicotine content that ranged between 8 mg (ZYN[®] Smooth) and 18 mg (Longhorn Natural and Longhorn Wintergreen). Many commercially available snus products on the Scandinavian and U.S. markets have a nicotine content that is higher than 8 mg, hence the 18 mg products were used in the study. In addition, as more than 10% of all snus users frequently use two or more pouches simultaneously ([Digard et al., 2009](#)), 2 pouches of General PSWL, each containing 8 mg nicotine, were used as a comparison to the ZYN[®] products.

Use of General PSWL 2x8 mg resulted in a significantly higher mean AUC_{inf} compared to ZYN[®] Smooth 8 mg and both Longhorn products. Consistently, the extracted dose of nicotine from General PSWL 2x8 mg was significantly higher than the extracted dose from ZYN[®] Smooth 8 mg and the Longhorn products. The rate of extraction, however, was highest from the ZYN[®] Smooth 8 mg pouches and lowest from the Longhorn pouches, which likely explains why there were no statistically significant differences in mean AUC_{inf}, mean AUC_{0-t} and mean C_{max} between ZYN[®] Smooth 8 mg and Longhorn Natural 18 mg and Longhorn Wintergreen 18 mg containing more than twice the amount of nicotine. A high ZYN[®] rate of extraction compared to other products has been observed also in previous studies (17-01 and

17-03) and is likely explained by the geometry of the ZYN[®] pouch, which is believed to facilitate a more efficient saliva penetration of the pouch.

In general, corresponding results to those obtained for AUC_{inf} and extracted amount of nicotine were also obtained for AUC_{0-t} and C_{max}. There were less apparent differences between the products in terms of T_{max} and T_{1/2}.

Most reported AEs were events that are usually associated with nicotine exposure such as nausea, salivation and dizziness. Overall, no safety concerns were identified.

13.2 OVERALL CONCLUSIONS

As the intended ZYN 6 mg product only contained approximately 4.5 mg nicotine, the conclusion is based on comparison between the ZYN 8 mg product and General PSWL 2x8 mg, Longhorn Natural 18 mg and Longhorn Wintergreen 18 mg.

In line with the overall aim of the study, it was shown that ZYN[®] Smooth 8 mg did not entail a higher nicotine exposure than General PSWL 2x8 mg, Longhorn Natural 18 mg and Longhorn Wintergreen 18 mg, representing commercially available tobacco-based snus and snus-like products that are currently common on the Scandinavian and U.S. markets.

Single doses of ZYN[®] Smooth 8 mg, General PSWL 2x8 mg, Longhorn Natural 18 mg and Longhorn Wintergreen 18 mg were in general well tolerated by the current daily snus users participating in the study and no safety concerns were observed.

14 TABLES, FIGURES AND GRAPHS REFERRED TO BUT NOT INCLUDED IN THE TEXT

14.1 Study subjects

14.2 Demographic data and other baseline characteristics

(b) (4)



Table 14.2-2 Baseline characteristics and demographics (PPS)

(b) (4)



(b) (4)



Table 14.2-3 Nicotine abstinence

(b) (4)



Table 14.2-4 Medical history (FAS)

(b) (4)



(b) (4)



Table 14.2-5 Prior medications (FAS)

(b) (4)



(b) (4)



(b) (4)

14.3 Endpoints

14.3.1 *Difference in AUC_{inf} (non-baseline adjusted data)*

(b) (4)

Table 14.3-2 *Difference in AUC_{inf}: ZYN Smooth 8 mg vs. Longhorn Natural 18 mg*

(b) (4)

Table 14.3-3 *Difference in AUC_{inf}: ZYN Smooth 8 mg vs. Longhorn Wintergreen 18 mg*

(b) (4)

Table 14.3-4 *Difference in AUC_{inf}: General PSWL 2x8 mg vs. Longhorn Natural 18 mg*

(b) (4)

SM18_01 Analysis of primary endpoint - AUCinf, SAS program: Difference_in_AUCinf.sas. Run by:
Linnea Eriksson, linnea.eriksson@ctc-ab.se 2019-06-04T09:08:52

Table 14.3-5 Difference in AUC inf: General PSWL 2x8 mg vs. Longhorn Wintergreen 18 mg

(b) (4)

Table 14.3-6 Difference in AUC inf: Longhorn Natural 18 mg vs. Longhorn Wintergreen 18 mg

(b) (4)

14.3.2 **Difference in AUCinf (baseline adjusted data)**

Table 14.3-7 Difference in AUC inf, with baseline adjustment: ZYN Smooth 8 mg vs. General PSWL 2x8 mg

(b) (4)

Table 14.3-8 Difference in AUC inf, with baseline adjustment: ZYN Smooth 8 mg vs. Longhorn Natural 18 mg

(b) (4)

Table 14.3-9 Difference in AUC inf, with baseline adjustment: ZYN Smooth 8 mg vs. Longhorn Wintergreen 18 mg

(b) (4)

(b) (4)



Table 14.3-10 *Difference in AUC inf, with baseline adjustment: General PSWL
2x8 mg vs. Longhorn Natural 18 mg*

(b) (4)



Table 14.3-11 *Difference in AUC inf, with baseline adjustment: General PSWL
2x8 mg vs. Longhorn Wintergreen 18 mg*

(b) (4)



Table 14.3-12 *Difference in AUC inf, with baseline adjustment: Longhorn Natural
18 mg vs. Longhorn Wintergreen 18 mg*

(b) (4)



14.3.3 **Extraction**

14.3.3.1 Extracted dose

Table 14.3-13 *Difference in extracted nicotine: ZYN Smooth 8 mg vs. General PSWL 2x8 mg*

(b) (4)

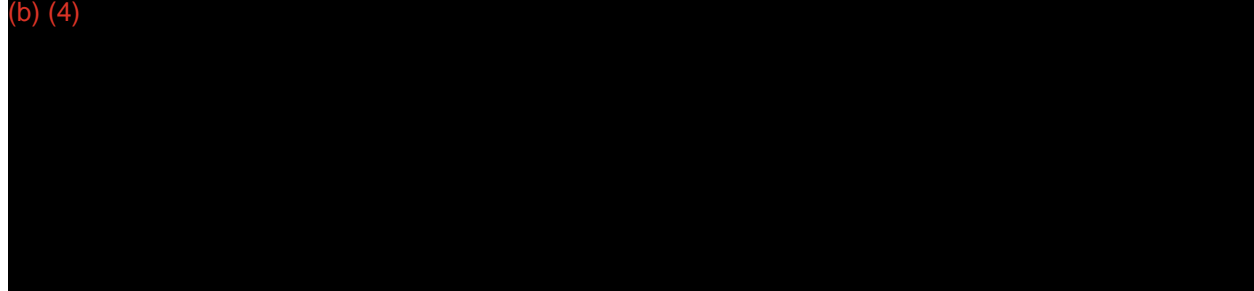


Table 14.3-14 *Difference in extracted nicotine: ZYN Smooth 8 mg vs. Longhorn Natural 18 mg*

(b) (4)

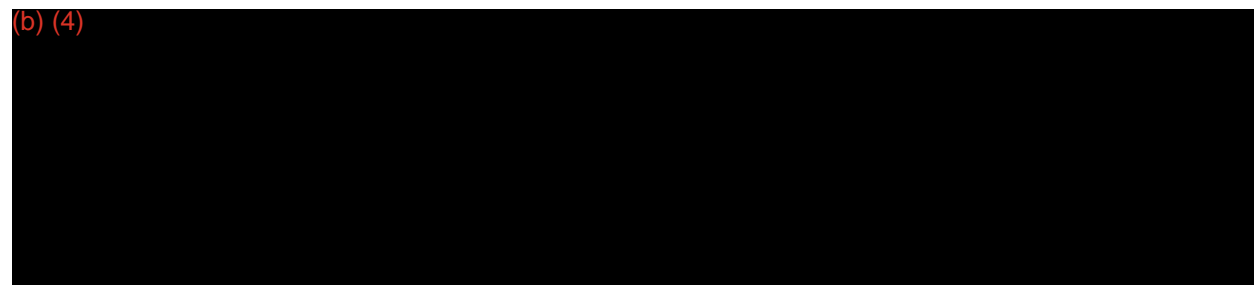


Table 14.3-15 *Difference in extracted nicotine: ZYN Smooth 8 mg vs. Longhorn Wintergreen 18 mg*

(b) (4)

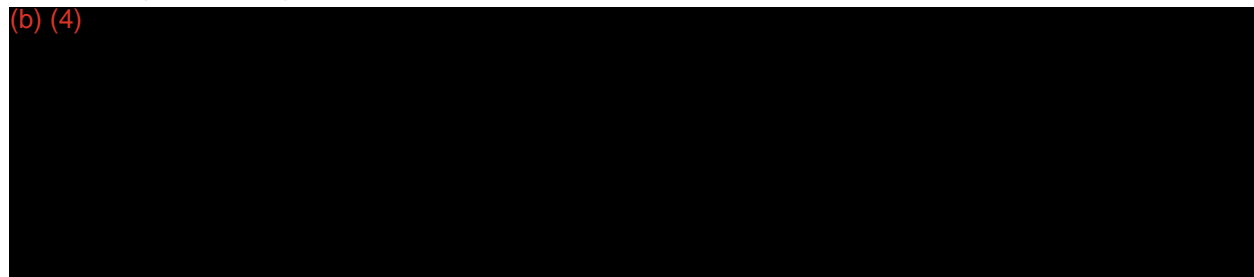


Table 14.3-16 *Difference in extracted nicotine: General PSWL 2x8 mg vs. Longhorn Natural 18 mg*

(b) (4)

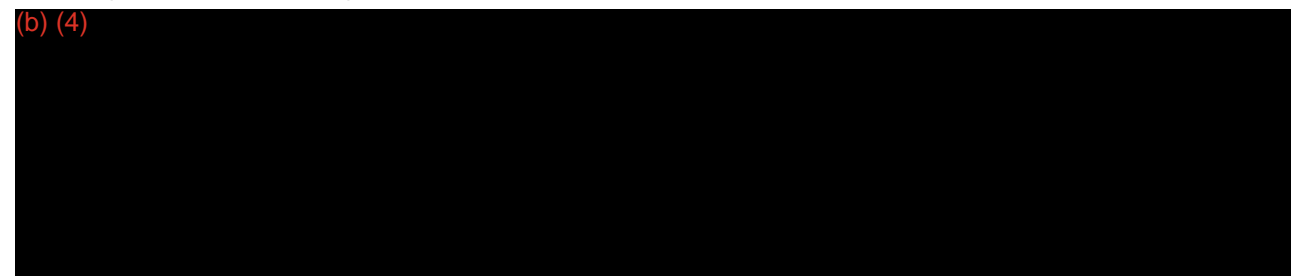


Table 14.3-17 *Difference in extracted nicotine: General PSWL 2x8 mg vs. Longhorn Wintergreen 18 mg*

(b) (4)



*Table 14.3-18 Difference in extracted nicotine: Longhorn Natural 18 mg vs.
Longhorn Wintergreen 18 mg*

(b) (4)



Table 14.3-19 Reference samples nicotine content and weight

(b) (4)



(b) (4)



(b) (4)



14.3.3.2 AUC vs extracted amount of nicotine (non-baseline adjusted data)

(b) (4)



(b) (4)



(b) (4)



(b) (4)



Swedish Match

(b) (4)



CONFIDENTIAL

69 (97)

(b) (4)



(b) (4)



(b) (4)



14.3.3.4 Rate of extraction

Table 14.3-20 Difference in rate of extraction (%): ZYN Smooth 8 mg vs. General
PSWL 2x8 mg

(b) (4)

Table 14.3-21 Difference in rate of extraction (%): ZYN Smooth 8 mg vs.
Longhorn Natural 18 mg

(b) (4)

Table 14.3-22 Difference in rate of extraction (%)e: ZYN Smooth 8 mg vs.
Longhorn Wintergreen 18 mg

(b) (4)

Table 14.3-23 Difference in rate of extraction (%): General PSWL 2x8 mg vs.
Longhorn Natural 18 mg

(b) (4)

Table 14.3-24 *Difference in rate of extraction (%): General PSWL 2x8 mg vs. Longhorn Wintergreen 18 mg*

(b) (4)



Table 14.3-25 *Difference in rate of extraction (%): Longhorn Natural 18 mg vs. Longhorn Wintergreen 18 mg*

(b) (4)



14.3.4 **Plasma concentrations of nicotine**

Table 14.3-26 *Plasma concentrations of nicotine by treatment (PPS)*

(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



(b) (4)



14.3.5 ***Plasma concentrations of methyl salicylate***

Table 14.3-27 *Plasma concentrations of methyl salicylate by treatment (Per protocol set)*

(b) (4)



14.3.6 ***Pharmacokinetic parameters***

Table 14.3-28 *Pharmacokinetic parameters by treatment, with baseline adjustment (Per Protocol Set)*

(b) (4)



14.3.7 *Difference in pharmacokinetic parameters (non-baseline adjusted data)*

Table 14.3-29 *Difference in AUC to Last Nonzero Conc: ZYN Smooth 8 mg vs. General PSWL 2x8 mg*

(b) (4)



Table 14.3-30 *Difference in AUC to Last Nonzero Conc: ZYN Smooth 8 mg vs. Longhorn natural 18 mg*

(b) (4)



Table 14.3-31 *Difference in AUC to Last Nonzero Conc: ZYN Smooth 8 mg vs. Longhorn wintergreen 18 mg*

(b) (4)



Table 14.3-32 *Difference in AUC to Last Nonzero Conc: General PSWL 2x8 mg vs. Longhorn Natural 18 mg*

(b) (4)



Table 14.3-33 *Difference in AUC to Last Nonzero Conc: General PSWL 2x8 mg vs. Longhorn Wintergreen 18 mg*

(b) (4)



SM18_01 Analysis of secondary endpoint - PK, SAS program: sec_endpoint_auc.sas. Run by: Linnea Eriksson, linnea.eriksson@ctc-ab.se 2019-06-10T11:51:26

Table 14.3-34 *Difference in AUC to Last Nonzero Conc: Longhorn Natural 18 mg vs. Longhorn Wintergreen 18 mg*

(b) (4)

Table 14.3-35 *Difference in Max Concentration: ZYN Smooth 8 mg vs. General PSWL 2x8 mg*

(b) (4)

Table 14.3-36 *Difference in Max Concentration: ZYN Smooth 8 mg vs. Longhorn Natural 18 mg*

(b) (4)

Table 14.3-37 *Difference in Max Concentration: ZYN Smooth 8 mg vs. Longhorn Wintergreen 18 mg*

(b) (4)

Table 14.3-38 *Difference in Max Concentration: General PSWL 2x8 mg vs. Longhorn Natural 18 mg*

(b) (4)

(b) (4)

Table 14.3-39 *Difference in Max Concentration: General PSWL 2x8 mg vs. Longhorn Wintergreen 18 mg*

(b) (4)

Table 14.3-40 *Difference in Max Concentration: Longhorn Natural 18 mg vs. Longhorn Wintergreen 18 mg*

(b) (4)

Table 14.3-41 *Difference in Time of Cmax: ZYN Smooth 8 mg vs. General PSWL 2x8 mg*

(b) (4)

Table 14.3-42 *Difference in Time of Cmax: ZYN Smooth 8 mg vs. Longhorn Natural 18 mg*

(b) (4)

Table 14.3-43 *Difference in Time of Cmax: ZYN Smooth 8 mg vs. Longhorn Wintergreen 18 mg*

(b) (4)

Table 14.3-44
Natural 18 mg

Difference in Time of C_{max}: General PSWL 2x8 mg vs. Longhorn

(b) (4)

Table 14.3-45
Wintergreen 18 mg

Difference in Time of C_{max}: General PSWL 2x8 mg vs. Longhorn

(b) (4)

Table 14.3-46
Longhorn Wintergreen 18 mg

Difference in Time of C_{max}: Longhorn Natural 18 mg vs.

(b) (4)

Table 14.3-47
PSWL 2x8 mg

Difference in Half-Life Lambda z: ZYN Smooth 8 mg vs. General

(b) (4)

Table 14.3-48
Natural 18 mg

Difference in Half-Life Lambda z: ZYN Smooth 8 mg vs. Longhorn

(b) (4)



Table 14.3-49
Wintergreen 18 mg

Difference in Half-Life Lambda z: ZYN Smooth 8 mg vs. Longhorn

(b) (4)



Table 14.3-50
Longhorn Natural 18 mg

Difference in Half-Life Lambda z: General PSWL 2x8 mg vs.

(b) (4)



Table 14.3-51
Longhorn Wintergreen 18 mg

Difference in Half-Life Lambda z: General PSWL 2x8 mg vs.

(b) (4)



Table 14.3-52
Longhorn Wintergreen 18 mg

Difference in Half-Life Lambda z: Longhorn Natural 18 mg vs.

(b) (4)



(b) (4)

14.3.8 ***Difference in pharmacokinetic parameters (baseline adjusted data)***

Table 14.3-53 *Difference in AUC to Last Nonzero Conc, with baseline adjustment: ZYN Smooth 8 mg vs. General PSWL 2x8 mg*

(b) (4)

Table 14.3-54 *Difference in AUC to Last Nonzero Conc, with baseline adjustment: ZYN Smooth 8 mg vs. Longhorn Natural 18 mg*

(b) (4)

Table 14.3-55 *Difference in AUC to Last Nonzero Conc, with baseline adjustment: ZYN Smooth 8 mg vs. Longhorn Wintergreen 18 mg*

(b) (4)

Table 14.3-56 *Difference in AUC to Last Nonzero Conc, with baseline adjustment: General PSWL 2x8 mg vs. Longhorn Natural 18 mg*

(b) (4)

Table 14.3-57 *Difference in AUC to Last Nonzero Conc, with baseline adjustment: General PSWL 2x8 mg vs. Longhorn Wintergreen 18 mg*

(b) (4)



Table 14.3-58 *Difference in AUC to Last Nonzero Conc, with baseline adjustment: Longhorn Natural 18 mg vs. Longhorn Wintergreen 18 mg*

(b) (4)



Table 14.3-59 *Difference in Max Concentration, with baseline adjustment: ZYN Smooth 8 mg vs. General PSWL 2x8 mg*

(b) (4)



Table 14.3-60 *Difference in Max Concentration, with baseline adjustment: ZYN Smooth 8 mg vs. Longhorn Natural 18 mg*

(b) (4)



Table 14.3-61 *Difference in Max Concentration, with baseline adjustment: ZYN Smooth 8 mg vs. Longhorn Wintergreen 18 mg*

(b) (4)



Table 14.3-62 *Difference in Max Concentration, with baseline adjustment:*
General PSWL 2x8 mg vs. Longhorn Natural 18 mg

(b) (4)

Table 14.3-63 *Difference in Max Concentration, with baseline adjustment:*
General PSWL 2x8 mg vs. Longhorn Wintergreen 18 mg

(b) (4)

Table 14.3-64 *Difference in Max Concentration, with baseline adjustment:*
Longhorn Natural 18 mg vs. Longhorn Wintergreen 18 mg

(b) (4)

Table 14.3-65 *Difference in Time of Cmax, with baseline adjustment: ZYN*
Smooth 8 mg vs. General PSWL 2x8 mg

(b) (4)

Table 14.3-66 *Difference in Time of Cmax, with baseline adjustment: ZYN*
Smooth 8 mg vs. Longhorn Natural 18 mg

(b) (4)

(b) (4)

Table 14.3-67

Difference in Time of C_{max}, with baseline adjustment: ZYN

(b) (4)

Table 14.3-68

*Difference in Time of C_{max}, with baseline adjustment: General
PSWL 2x8 mg vs. Longhorn Natural 18 mg*

(b) (4)

Table 14.3-69

*Difference in Time of C_{max}, with baseline adjustment: General
PSWL 2x8 mg vs. Longhorn Wintergreen 18 mg*

(b) (4)

Table 14.3-70

*Difference in Time of C_{max}, with baseline adjustment: Longhorn
Natural 18 mg vs. Longhorn Wintergreen 18 mg*

(b) (4)

Table 14.3-71 *Difference in Half-Life Lambda z, with baseline adjustment: ZYN*
Smooth 8 mg vs. General PSWL 2x8 mg

(b) (4)

Table 14.3-72 *Difference in Half-Life Lambda z, with baseline adjustment: ZYN*
Smooth 8 mg vs. Longhorn Natural 18 mg

(b) (4)

Table 14.3-73 *Difference in Half-Life Lambda z, with baseline adjustment: ZYN*
Smooth 8 mg vs. Longhorn Wintergreen 18 mg

(b) (4)

Table 14.3-74 *Difference in Half-Life Lambda z, with baseline adjustment:*
General PSWL 2x8 mg vs. Longhorn Natural 18 mg

(b) (4)

Table 14.3-75 *Difference in Half-Life Lambda z, with baseline adjustment:*
General PSWL 2x8 mg vs. Longhorn Wintergreen 18 mg

(b) (4)

Table 14.3-76 *Difference in Half-Life Lambda z, with baseline adjustment:*
Longhorn Natural 18 mg vs. Longhorn Wintergreen 18 mg

(b) (4)

14.4 Adverse events

14.4.1 *Displays of adverse events*

Table 14.4-1 *Adverse events: severity and relation, with patient identification*

(b) (4)

(b) (4)



Table 14.4-2 Adverse events occurring during wash-out by system organ class and preferred term

(b) (4)



(b) (4)



Table 14.4-3 Adverse events occurring during wash-out: severity and relation, with patient identification

(b) (4)



(b) (4)



Table 14.4-4 Overview of adverse events, ZYN 6 mg

(b) (4)



(b) (4)



Table 14.4-5 Adverse events by system organ class and preferred term, ZYN 6 mg

(b) (4)



Table 14.4-6 Adverse events, ZYN 6 mg: severity and relation, with patient identification

(b) (4)



14.4.2 **Listings of deaths, other serious adverse events and significant adverse events**

There were no deaths, other SAEs or significant events in the study.

15 REFERENCE LIST

Digard H et al Patterns and behaviors of snus consumption in Sweden, Nicotine and Tobacco Research 2009 11:1175-1181

Fant RV, Henningfield JE, Nelson RA and Pickworth WB. Pharmacokinetics and pharmacodynamics of moist snuff in humans. Tob. Control, 8, 387-392 (1999)

Lunell E and Curvall M. Nicotine Delivery and Subjective Effects of Swedish Portion Snus Compared With 4 mg Nicotine Polacrilex Chewing Gum. Nicotine Tob Res, 13 (7), 573-578 (2011)

16 APPENDICES

16.1 Study information

- 16.1.1 Protocol and protocol amendments
- 16.1.2 Sample CRF (unique pages only)
- 16.1.3 IEC approval including list of IEC members. Representative written subject information and sample consent form.
- 16.1.4 List and description of Investigators and other important participants in the study, including brief (1 page) CVs.
- 16.1.5 Signatures of the Sponsor, Statistician and Principal Investigator
- 16.1.6 Listing of subjects receiving IP from specific batches, where more than one batch was used.
- 16.1.7 Randomization scheme and codes (subject identification and treatment assigned)
- 16.1.8 Audit certificates (not applicable)
- 16.1.9 Documentation of statistical methods (*Statistical Analysis Plan*)
- 16.1.10 Documentation of inter-laboratory standardization methods and quality assurance procedures if used (not applicable)
- 16.1.11 Publications based on the study (not applicable)
- 16.1.12 Important publications referenced in the report (available upon request)

16.2 Subject data listings

- 16.2.1 Discontinued subjects
- 16.2.2 Protocol deviations
- 16.2.3 Subjects excluded from the (efficacy) analysis
- 16.2.4 Demographic data and other baseline characteristics
- 16.2.5 Compliance and/or drug concentration data
- 16.2.6 Pharmacokinetic and extraction data
- 16.2.7 AE listings
- 16.2.8 Listing of individual laboratory measurements by subject

16.3 Case report forms

- 16.3.1 CRFs for deaths, other SAEs and withdrawals for AE (not applicable)
- 16.3.2 Other CRFs submitted (not applicable)

16.4 Individual subject data listings (US archival listings) (available upon request)

Appendix 16 is provided as a separate document to the CSR.