Clinical Study Protocol

Investigational product	Nicotine pouches
Study code	SM23-01
Protocol version and date	Final version; 29SEP2023

NICOTINE PLASMA CONCENTRATIONS, PHARMACOKINETICS, AND PHARMACODYNAMICS FOLLOWING SINGLE DOSES OF NICOTINE POUCHES WITH A NEW FORMAT IN CURRENT, DAILY ORAL TOBACCO/NICOTINE POUCH USERS

Test products and dose

Nicotine pouch (NP) 1 - Unflavored 3 mg
NP 2 - Unflavored 6 mg
NP 3 - Flavor A 6 mg
NP 4 - Flavor B 6 mg
NP 5 - Flavor C 6 mg
NP 6 - Flavor D 6 mg
NP 7 - Flavor E 6 mg
NP 8 - Flavor F 6 mg
NP 9 - Flavor G 6 mg
NP 10 - Flavor H 6 mg

Comparator product and dose

Longhorn Natural 18 mg

Sponsor signatory

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Swedish Match
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1 STUDY SYNOPSIS

Study title	
Nicotine plasma concentrations, pharmacokinetics, and pharmacodynamics following single doses of nicotine pouches with a new format in current, daily oral tobacco/nicotine pouch users.	
Study code	**Planned study period**
SM23-01	Q4 2023 to Q3 2024
Coordinating/Principal Investigator	
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Study design	
This is a multi-center, open-label, randomized, 11-way cross-over, single dose administration study, designed to assess nicotine exposure from new format nicotine pouch (NP) products. The investigational products (IPs) include 2 unflavored products (3 mg and 6 mg) and 8 flavored products (Flavor A-H 6 mg). The comparator product is Longhorn Natural 18 mg.	
Objectives Primary objective To demonstrate that the new format unflavored NP 6 mg product does not result in substantially higher nicotine exposure compared to the comparator product, Longhorn Natural 18 mg. Secondary objectives 1. To compare the *in vivo* extracted amount (mg/unit) and extracted fraction (%) of nicotine between the unflavored NP 3 mg and 6 mg products and the comparator product, Longhorn Natural 18 mg. 2. To compare the pharmacokinetic (PK) profile between the unflavored NP 3 mg and 6 mg products and the comparator product, Longhorn Natural 18 mg. 3. To assess the pharmacodynamic (PD) effects, measured as pulse rate and subjective outcome measures, of the unflavored NP 3 mg and 6 mg products and the comparator product, Longhorn Natural 18 mg. 4. To compare the *in vivo* extracted amount (mg/unit) and extracted fraction (%) of nicotine between the unflavored NP 6 mg product and the flavored NP 6 mg products. 5. To compare the PK profile between the unflavored NP 6 mg product and the flavored NP 6 mg products. 6. To assess the PD effects, measured as pulse rate and subjective outcome measures, of the unflavored NP 6 mg products and the flavored NP 6 mg products. 7. To evaluate the safety and tolerability of new format NP products by administering single doses of unflavored and flavored NP products and the comparator product, Longhorn Natural 18 mg, in current, daily oral tobacco/nicotine pouch users. Exploratory objectives 1. To evaluate the impact on "product-liking" *vs*. the subjects' usual oral tobacco/nicotine product of choice by administering single doses of unflavored NP 3 mg and 6 mg products and the comparator product, Longhorn Natural 18 mg. 2. To compare the nicotine extraction-normalized PK parameters area under the plasma concentration *vs*. time curve from 0 to infinity ($AUC_{0\text{-}inf}$) and maximum observed	

concentration (C_{max}) between the unflavored NP 3 mg and 6 mg products and the comparator product, Longhorn Natural 18 mg.

3. To evaluate the impact on "product-liking" *vs*. the subjects' usual oral tobacco/nicotine product of choice by administering single doses of unflavored NP 6 mg and flavored NP 6 mg products.
4. To compare the nicotine extraction-normalized PK parameters $AUC_{0\text{-}inf}$ and C_{max} between the unflavored NP 6 mg and the flavored NP 6 mg products.
5. To compare the PK profile and the extracted amount and fraction of nicotine between the flavored NP 6 mg product with the highest concentrations and the comparator product, Longhorn Natural 18 mg.

Endpoints

Primary endpoint

The primary endpoint of this study is to compare nicotine exposure, as measured by baseline-adjusted $AUC_{0\text{-}inf}$ based on nicotine plasma concentrations, between the new format unflavored NP 6 mg product and the comparator product, Longhorn Natural 18 mg. The goal is to demonstrate that the upper bound of the 90 % confidence interval of the ratio for nicotine exposure of the new format unflavored NP 6 mg product and the comparator product is below 1.25.

Secondary endpoints

1. Extraction from pouches: The difference in *in vivo* extracted amount (mg/unit) and extracted fraction (%) of nicotine between the unflavored NP 3 mg and 6 mg products and the comparator product, Longhorn Natural 18 mg.
2. PK of nicotine in plasma: The difference between the unflavored NP 3 mg and 6 mg products and the comparator product, Longhorn Natural 18 mg, in the non-adjusted and baseline-adjusted PK parameters based on plasma concentrations of nicotine:
   - $AUC_{0\text{-}inf}$,
   - C_{max},
   - time of occurrence of C_{max} (T_{max}),
   - AUC from 0 to 1.5 hours ($AUC_{0\text{-}1.5h}$),
   - AUC from 0 to time of last measurable time point ($AUC_{0\text{-}last}$),
   - Terminal elimination half-life ($T_{1/2}$).

3a. PD (pulse rate): The difference between the unflavored NP 3 mg and 6 mg products and the comparator product, Longhorn Natural 18 mg, for the highest recorded increase (E_{max}) in pulse rate from baseline, measured using a pulse oximeter after IP administration.

3b. PD parameters: The difference between the unflavored NP 3 mg and 6 mg products and the comparator product, Longhorn Natural 18 mg, for the highest recorded value (E_{max}) in the subjective parameters "craving" and "satisfaction", measured using a 100 mm visual analogue scale (VAS) after IP administration.

3c. PD (subjective outcome parameters): The difference between the unflavored NP 3 mg and 6 mg products and the comparator product, Longhorn Natural 18 mg, for the subjective parameters "product-liking" and "intent to use again", measured using a 100 mm VAS 30 minutes after IP administration.

4. Extraction from pouches: The difference in *in vivo* extracted amount (mg/unit) and extracted fraction (%) of nicotine between the unflavored NP 6 mg and the flavored NP 6 mg products.

5a. PK of nicotine in plasma: The equivalence between the unflavored NP 6 mg and flavored NP 6 mg products in the non-adjusted and baseline-adjusted PK parameters $AUC_{0\text{-}inf}$ and C_{max} based on plasma concentrations of nicotine.

5b. PK of nicotine in plasma: The difference between the unflavored NP 6 mg and flavored NP 6 mg products in the non-adjusted and baseline-adjusted PK parameters T_{max}, $AUC_{0\text{-}1.5h}$, and $AUC_{0\text{-}last}$ and $T_{1/2}$.

6a. PD (pulse rate): The difference between the unflavored NP 6 mg and flavored NP 6 mg products for the highest recorded increase (E_{max}) in pulse rate from baseline, measured using a pulse oximeter after IP administration.

6b. PD parameters: The difference between the unflavored NP 6 mg and flavored NP 6 mg products for the highest recorded value (E_{max}) in the subjective parameters "craving" and "satisfaction", measured using a 100 mm VAS after IP administration.

6c. PD (subjective outcome parameters): The difference between the unflavored NP 6 mg and flavored NP 6 mg products for the subjective parameters "product-liking" and "intent to use again", measured using a 100 mm VAS 30 min after IP administration.

7. Frequency, intensity, and seriousness of adverse events (AEs).

Exploratory endpoints

1. The difference in "product-liking" *vs*. the subjects' usual oral tobacco/nicotine product of choice between the unflavored NP 3 mg and 6 mg products and the comparator product, Longhorn Natural 18 mg, measured on a 3-point scale using a multiple-choice question (MCQ) 30 min after IP administration.
2. The difference in nicotine extraction normalized $AUC_{0\text{-}inf}$ and C_{max} between the unflavored NP 3 mg and 6 mg products and the comparator product, Longhorn Natural 18 mg.
3. The difference in "product-liking" *vs*. the subjects' usual oral tobacco/nicotine product of choice between the unflavored NP 6 mg and flavored NP 6 mg products, measured on a 3-point scale using a MCQ 30 minutes after IP administration.
4. The difference in nicotine extraction normalized $AUC_{0\text{-}inf}$ and C_{max} between the unflavored NP 6 mg and flavored NP 6 mg products.
5. The difference in the PK parameters $AUC_{0\text{-}inf}$ and C_{max} and the extracted amount and fraction of nicotine between the flavored NP 6 mg product with the highest concentrations and the comparator product, Longhorn Natural 18 mg.

The results of the exploratory objectives may not be reported in the clinical study report (CSR).

Number of subjects planned

Approximately 66 subjects are planned to be screened to achieve 36 randomized subjects and at least 32 evaluable subjects, *i.e.*, subjects who have received all 11 IPs.

An effort will be made to randomize at least 14 female subjects (approximately 40%).

Diagnosis and main eligibility criteria

Healthy male or female subjects aged ≥21 to ≤60 years who have used oral tobacco/nicotine pouch products for ≥1 year, with a minimum daily consumption of 5 or more pouches, who are willing and able to use both tobacco-based moist snuff and NPs with high nicotine content, may be considered to be eligible for participation in the study. All subjects must be willing to comply with study procedures and give written informed consent. Female subjects of child-bearing potential must practice abstinence from heterosexual intercourse (only allowed when this is the preferred and usual lifestyle of the subject) or must agree to use a highly effective method of contraception with a failure rate of <1 % to prevent pregnancy for the duration of the study.

Subjects who intend to stop using nicotine-containing products, and/or subjects who are pregnant, breastfeeding, or intend to become pregnant during the study, and/or subjects with a history or presence of diagnosed hypertension or cardiovascular disease or other medical condition that may interfere with the evaluation of the IPs or may put the subject at risk because of participation in the study, will be excluded from the study.

Methodology

Subjects will report to the study site for a screening visit (Visit 1) followed by 11 treatment visits (Visits 2-12) on separate days. Screening will take place within 4 weeks prior to Visit 2 and will include an eligibility check, including evaluations of smoking and oral tobacco/nicotine use, a brief physical examination, serology tests, electrocardiogram (ECG) and collection of medical history, vital signs (pulse rate and blood pressure), height, weight, and body mass index (BMI). At the end of the screening visit, the subjects will complete a product familiarization session. During this session, they will be given the comparator product, Longhorn 18 mg nicotine, to ensure the acceptability of the product. The subjects will use the product in the same way they would normally use a snus or NP product (*i.e.*, placing it under the upper lip). The duration of product use will be a minimum of 20 minutes, starting from the moment the subject places it in their mouth until they remove it. Subjects who successfully complete this familiarization session, tolerating the product without significant unexpected adverse effects, will be allowed to continue in the study.

Prior to each treatment visit (Visits 2-12), subjects will abstain from tobacco/nicotine products as well as smoking (cigarettes or e-cigarettes) for >12 hours. All treatment sessions will be performed during the morning hours (08:00 to 12:00) to facilitate abstinence.

The IPs will be administered as single pouches in a pre-determined randomized order. Subjects will keep the pouch still between the upper lip and gum for 30 minutes and will be instructed not to manipulate the pouch with the tongue or lips. They will also be instructed not to eat, drink, chew gum, or brush their teeth for 30 minutes before, during, or 30 minutes after IP administration.

After 30 minutes, each used pouch will be collected and frozen (-20°C) pending analysis of residual nicotine content. Unused pouches from the same batch will serve as references and will be stored at - 20 °C pending analyses.

Blood samples for the assessment of nicotine plasma levels and PK parameters will be collected at pre-defined time points from pre-administration to 6 hours after each IP administration. The PD effects of the IPs will be determined using pulse rate measurements and subjective parameters (using VAS questions) at the same pre-defined time points as well as an MCQ 30 minutes after IP administration.

AEs will be collected through subject interviews and will also include any AEs reported spontaneously by the subjects, starting from the initiation of IP administration (Visit 2) and continuing until the last treatment visit (Visit 12).

Investigational Products and dosage

IP	Nicotine content per pouch
NP 1 - Unflavored	3 mg
NP 2 - Unflavored	6 mg
NP 3 - Flavor A	6 mg
NP 4 - Flavor B	6 mg
NP 5 - Flavor C	6 mg
NP 6 - Flavor D	6 mg
NP 7 - Flavor E	6 mg
NP 8 - Flavor F	6 mg
NP 9 - Flavor G	6 mg
NP 10 - Flavor H	6 mg
Longhorn Natural (comparator)	18 mg

Duration of treatment

The participating subjects will receive IPs on 11 occasions, in a cross-over fashion, with 30 minutes of treatment per occasion.

Duration of each subject's involvement in the study

Each subject will participate in the study for a period of approximately 5 weeks, not including the preceding 4-week screening period.

Pharmacokinetic assessments

Blood samples for analysis of PK parameters will be collected pre-administration, and at 5 min, 10 min, 15 min, 20 min, 30 min, 40 min, 60 min, 1 h:30 min, 2 h, 4 h and 6 h post-administration. The PK parameters in the study will include $AUC_{0\text{-}inf}$, $AUC_{0\text{-}last}$, $AUC_{0\text{-}1.5h}$, C_{max}, T_{max}, and $T_{1/2}$.

Pharmacodynamic assessments

The PD effects will be assessed by measuring pulse rate and subjective parameters (using VAS) from pre-administration (15 minutes prior to IP administration) to 6 hours post-administration, at pre-defined time points. The subjective parameters that will be assessed are "craving", "satisfaction", "product-liking", and "intent to use again". In addition, "product-liking" *vs*. the subjects' usual oral tobacco/nicotine product of choice will be assessed using a 3-point scale questionnaire at 30 minutes post-administration.

Nicotine extraction assessment

Used pouches will be collected after 30 minutes of use for the determination of residual nicotine in the IPs. The extracted amount (mg/unit) and extracted fraction (%) of nicotine will be assessed**.**

Safety assessments

AEs will be collected through subject interviews and will also include any AEs reported spontaneously by the subjects, starting from the initiation of IP administration (Visit 2) and continuing until the last treatment visit (Visit 12).

Statistical methods

The sample size has been calculated considering the secondary endpoint: to show equivalence between the unflavored NP 6 mg and flavored NP 6 mg products. A lower number of subjects will be needed for the primary endpoint. Using a coefficient of variation (CV) of 29 %, based on previous studies, a power of 80 %, a significance level of 10%, lower and upper equivalence bounds of 0.8 and 1.25, and a null ratio of 1, 32 evaluable subjects will be needed. Assuming a dropout rate of 10 %, a total of 36 subjects will be randomized.

Continuous data will be presented in terms of evaluable and missing observations, arithmetic mean, standard deviation (SD), median, minimum, and maximum value, Q1-Q3 (interquartile range [IQR]). In addition, for the parameters AUC and C_{max} the geometric mean and CV will be presented.

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

All descriptive summaries and statistical analyses will be performed using SAS Version 9.4 or later (SAS Institute, Inc., Cary, NC).

Baseline will be defined as the last data collection time point prior to each IP administration. No adjustment for multiple comparisons will be performed. All formal comparisons will be made towards a designated reference product and all significant findings will be reviewed for medical relevance.

Generally, no imputation of data will be performed. In case of missing start and stop times of AEs that cannot be investigated further, missing data will be imputed according to a worst-case scenario, *i.e.*, start time will be imputed as the closest time point post intake of IP and end time as 23:59, resulting in the longest possible treatment emergent duration of the AE. Spurious data will be evaluated continuously through data validation and, if connected to protocol deviations, evaluated case-by-case at the latest prior to declaration of clean file and database lock. Deviations from the original statistical analysis plan (SAP) will be described in the CSR. Methods for handling of missing, unused, and spurious data will be further specified in the SAP.
Study reporting After completion of the study, an International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) E3 guideline-compliant CSR will be prepared.

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3 LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

Abbreviation	Explanation
ADL	Activities of daily living
AE	Adverse event
AUC	Area under the plasma concentration *vs.* time curve
$AUC_{0\text{-inf}}$	AUC from 0 to infinity
$AUC_{0\text{-last}}$	AUC from 0 to time of last measurable plasma concentration
$AUC_{0\text{-1.5h}}$	AUC from time 0 to time 1.5 h
BMI	Body mass index
C_{max}	Maximum observed concentration
CRO	Contract research organization
CSP	Clinical study protocol
CSR	Clinical study report
CTC	Clinical Trial Consultants AB
CTCAE	Common terminology criteria for adverse events
CV	Coefficient of variation
ECG	Electrocardiogram
eCRF	Electronic case report form
EDC	Electronic data capture
EEA	European Economic Area
E_{max}	Highest recorded change from baseline
EU	European Union
FDA	United States Food and Drug Administration
GCP	Good clinical practice
GDPR	General data protection regulation
HIV	Human immunodeficiency virus
HR	Heart rate
ICF	Informed consent form
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
IEC	Independent ethics committee
IP	Investigational product
ISF	Investigator site file
LLOQ	Lower limit of quantification

Abbreviation	Explanation
ADL	Activities of daily living
LSMeans	Least-squares means
MCQ	Multiple-choice question
NP	Nicotine pouch
NRT	Nicotine replacement therapies
PD	Pharmacodynamics
PI	Principal Investigator
PK	Pharmacokinetics
PKAS	PK analysis set
QC	Quality control
RBM	Risk based monitoring
SAE	Serious adverse event
SAP	Statistical analysis plan
SAS	Statistical analysis software
SD	Standard deviation
SDV	Source data verification
SOC	System organ class
SOP	Standard operating procedures
$T_{1/2}$	Terminal elimination half-life
T_{max}	Time of occurrence of C_{max}
TMF	Trial master file
US	United States (of America)
VAS	Visual Analogue Scale
WHO	World Health Organization

4 IMPORTANT MEDICAL PROCEDURES TO BE FOLLOWED BY THE INVESTIGATOR

4.1 Medical emergencies contact

The Principal Investigator (PI) is responsible for ensuring that procedures and expertise are available to handle medical emergencies during the study. A medical emergency usually constitutes a serious adverse event (SAE) and is to be reported as such. Detailed SAE reporting procedures are described in Section 11.4.4.10.

In the case of a medical emergency, the Investigator may, during office hours, contact the Sponsor's medically responsible person (Table 4.1-1).

Table 4.1-1 Medical emergencies contact

Name	Function in the study	Contact information
[redacted]	[redacted]	[redacted]

5 INVESTIGATOR AND STUDY ADMINISTRATIVE STRUCTURE

Sponsor

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Sponsor's Medical Representative

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Clinical Research Manager

[redacted]

Biostatistician

[redacted]

Pharmacokineticist

[redacted]

Medical Writer

[redacted]

Laboratory (virology)	[redacted]
Laboratory (bioanalysis)	[redacted]
Investigational product (IP) manufacturing	Swedish Match Europe Division
IP packaging and labelling	[redacted]
Electronic data capture (EDC) system provider	[redacted]

Signatures are provided in Section 19.

6 INTRODUCTION

6.1 Background

Sweden has 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 females.

The nicotine delivery profile of a product is probably one of the main determinants of its efficacy to decrease nicotine craving and, thus, its ability to function as an alternative to cigarettes among current smokers. Oral smokeless tobacco, such as Swedish snus and moist snuff, is capable of effectively delivering nicotine to the bloodstream through diffusion over the oral mucosa [1]. It may therefore be more satisfactory to smokers than some currently available pharmaceutical nicotine replacement therapies (NRTs) with a slower nicotine-delivery profile.

Use of oral tobacco is by definition unassociated with exposure to the many thousands of combustion compounds 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 oral tobacco products has substantially lower health risks than cigarette smoking.

Traditionally there has been no non-tobacco-based nicotine product 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. Nicotine pouch (NP) products have been commercially available for a few years. They have some features that are similar to snus as they come in pouches that are intended to be placed under the upper lip. However, in contrast to snus, these products contain no nitrosamines or polycyclic hydrocarbons, which are the two main classes of unwanted substances in snus. The nicotine content in the NPs in this study is 3 mg and 6 mg and comparable to that in snus and many other oral tobacco products that are currently common on the market in Scandinavia and the United States (US), which have nicotine contents ranging from 3 mg/unit to more than 20 mg/unit.

When comparing the nicotine content of various nicotine-delivery products, it is essential to take into account that nicotine extraction and uptake can vary considerably depending on the product type (tobacco-based *vs*. a non-tobacco-based matrix) and product formulation (including factors like pouch geometry, solubility, water content, particle size, pH, *etc*.). Furthermore, there exists considerable interindividual variation in the uptake of orally consumed products, which is likely attributed to constitutional differences in saliva production. This variation results in a wide range of nicotine extraction levels.

While numerous NP products are currently commercially available, only a limited number of these products have, thus far, been scientifically evaluated. It has been suggested that some flavors may enhance nicotine uptake, which has not been fully scientifically investigated for this product category. Similarly, there is a lack of scientific data regarding any possible impact of flavors on pharmacodynamics (PD). Further, the addition of flavors to tobacco products and e cigarettes have been discussed by regulatory agencies during the last years. The World Health Organization (WHO) seventh report on the scientific basis of tobacco product regulation included a chapter on flavors [2]. Additionally, the US Food and Drug

Administration (FDA) is stressing the need to investigate the effect of flavors on the pharmacokinetics (PK) and PD of nicotine products, as well as a guidance [3].

This study will evaluate the new format of both unflavored and flavored NP products. While the general PK characteristics of nicotine are known, the nicotine delivery, uptake and subsequent exposure associated with use of the new format NP are not. The study enables a solid scientific evaluation of the PK and PD properties of the new format NP, which will have implications for the assessment of the overall safety profile associated with this new format NP *vs*. a commercially available moist snuff product. The results of the study will be made available in the public domain to enhance awareness among key stakeholders.

The new format NP product offers a smaller pouch size and contains a reduced amount of nicotine compared to currently available ZYN Slim products on the Swedish market. In accordance with FDA regulations, the new format NP product will be subjected to comparative testing against a tobacco product within the same category and currently available on the US market. The tobacco product in this study is the US moist snuff Longhorn Natural 18 mg, previously used in another clinical study, and for which only around 5.4 mg of the nicotine was found to be released after 60 minutes of usage. The current study will investigate the nicotine delivery and uptake profile of the new format NP, including 2 unflavored products (3 mg and 6 mg) and 8 flavored products (6 mg) in comparison with Longhorn Pouch Natural 18 mg.

The results from this study are intended to be used as a basis in a premarket tobacco product application to the FDA. Prior to introduction and marketing of novel nicotine-based products in the US, the FDA specifically requires data on nicotine uptake and nicotine effects in order to assess health risks and addiction potential of the new tobacco/nicotine-containing products as compared to existing ones.

6.2 Study rationale

The overall aim of the study is to evaluate nicotine exposure, PK and PD in a new format of unflavored and flavored NP products. The rationale for the study design is presented in Section 8.2.

6.3 Risk/benefit assessment

6.3.1 Risk assessment

It may be considered problematic to expose research subjects to a novel nicotine delivery product with properties that are not yet fully known. However, all research subjects are required to be daily oral tobacco/nicotine pouch users for at least one year. Thus, the participants are well acquainted with and used to the effects of nicotine, and there will be no risk for the development of any novel nicotine dependency among the participants.

The nicotine in the NP products is of pharmaceutical grade, same as the nicotine used in NRT products (*e.g*., gum, lozenges, mouth spray *etc*.). Aside from nicotine, all ingredients used in the NP products are approved for use in food.

The potential adverse effects of the study procedures are likely to be minor and/or clinically insignificant, based on experience from clinical trials on similar products [4-9]. The evidence on the health effects of nicotine exposure is based on existing tobacco products. In addition, any potential subject who intends to change their nicotine consumption habit or stop using nicotine products will not be offered the opportunity to participate in the study. Consequently, the present study is not perceived to confer any societal burden in terms of increased use of tobacco or NP products.

Subjects who intend to stop using nicotine-containing products and/or are pregnant, breastfeeding, or intend to become pregnant during the study, and/or subjects with a history or presence of diagnosed hypertension or cardiovascular disease or other medical condition who may be particularly vulnerable to nicotine exposure, are also excluded from participation.

Each unit of IP (one pouch) will be used for 30 minutes per study day (see Section 8.1). The subjects will not be exposed to nicotine levels from the IPs that are higher than those they are usually exposed to during their daily consumption of nicotine. Therefore, acute risks related to IP administration are anticipated to be low. So far, no adverse events (AEs) have been reported in previous clinical studies with similar products, apart from effects likely to be related to the nicotine exposure (such as salivation, nausea, and dyspepsia) [8-10] .

The PIs at the study sites will ascertain that adequate facilities and procedures are available to handle emergency situations should they occur during the study. The medical staff at CTC have extensive experience in clinical studies, and there are adequate procedures in place to handle unexpected and expected adverse reactions in the study subjects.

Aside from the risks related to the IPs, as detailed above, there may also be risks related to the medical devices used in the study (*e.g.*, indwelling venous catheters). However, these devices are used in routine medical care and the risk associated with their use is considered low and ethically justifiable. The repeated blood-sampling for PK analysis will be conducted during a 5-week period to give sufficient time between each blood sample occasion. Study-specific evaluations and sampling procedures, such as blood-pressure measurements using a blood pressure cuff and frequent blood-sampling, may cause transient discomfort but the risk is deemed to be low and ethically justifiable. Overall, the combined safety data from previous pre-clinical and clinical studies have not revealed any safety issues that would outweigh the expected benefits of the study.

6.3.2 Benefit assessment

In analogy with a regular phase I study in healthy volunteers, there is no direct benefit for the subjects to participate in the study, aside from a brief medical examination, which may provide them with information on their general state of health.

The development of new, nicotine-containing products takes place both in the pharmaceutical industry and in the tobacco industry. Parts of the tobacco industry today are moving towards reducing the presence of known harmful substances, other than nicotine, in the products that are being developed. NP products are an example of such a development, and the use, prevalence and variety of these products has increased globally in recent years. NPs constitute a substitute to both combusted or non-combusted tobacco/nicotine containing inhalation products (*e.g.*, conventional and electronic cigarettes) and to oral tobacco products (*e.g.*, tobacco-based snus and moist snuff).

The potential AEs of the study procedures, which are likely to be minor and/or clinically insignificant, are from a research ethics perspective, counterbalanced by substantially reduced harm and risk of tobacco-related diseases of the novel NPs. As the nicotine delivery profile of a product is likely to be central to its acceptability among current nicotine users, it is reasonable to conduct formal clinical studies to assess this feature in more detail.

6.3.3 Risk/benefit conclusions

It is reasonable to assume that NP products will have less harmful effects on health than tobacco-based nicotine products. From that perspective, the aim of the present study is consistent with society’s overall goal of reducing the harm caused by tobacco use.

While keeping the above-mentioned risk factors at a minimum level in order to not expose the subjects participating in the study to risks that would not be ethically justifiable, it is concluded that the planned study assessments are considered sufficient to meet the scientific and medical goals for the study. It is therefore concluded that the potential benefits from the study will outweigh the potential risks for the subjects.

7 STUDY OBJECTIVES AND ENDPOINTS

7.1 Primary objective

To demonstrate that the new format unflavored NP 6 mg product does not result in substantially higher nicotine exposure compared to the comparator product, Longhorn Natural 18 mg.

7.1.1 Primary endpoint

The primary endpoint of this study is to compare nicotine exposure, as measured by baseline-adjusted area under the plasma concentration *vs*. time curve from 0 to infinity ($AUC_{0\text{-}inf}$) based on nicotine plasma concentrations, between the new format unflavored NP 6 mg product and the comparator product, Longhorn Natural 18 mg. The goal is to demonstrate that the upper bound of the 90 % confidence interval of the ratio for nicotine exposure of the new format unflavored NP 6 mg product and the comparator product is below 1.25.

7.2 Secondary objectives

1. To compare the *in vivo* extracted amount (mg/unit) and extracted fraction (%) of nicotine between the unflavored NP 3 mg and 6 mg products and the comparator product, Longhorn Natural 18 mg.
2. To compare the PK profile between the unflavored NP 3 mg and 6 mg products and the comparator product, Longhorn Natural 18 mg.
3. To assess the PD effects, measured as pulse rate and subjective outcome measures, of the unflavored NP 3 mg and 6 mg products and the comparator product, Longhorn Natural 18 mg.
4. To compare the *in vivo* extracted amount (mg/unit) and extracted fraction (%) of nicotine between the unflavored NP 6 mg product and the flavored NP 6 mg products.
5. To compare the PK profile between the unflavored NP 6 mg product and the flavored NP 6 mg products.
6. To assess the PD effects, measured as pulse rate and subjective outcome measures, of the unflavored NP 6 mg products and the flavored NP 6 mg products.
7. To evaluate the safety and tolerability of new format NP products by administering single doses of unflavored and flavored NP products and the comparator product, Longhorn Natural 18 mg, in current, daily oral tobacco/nicotine pouch users.

7.2.1 Secondary endpoints

1. Extraction from pouches: The difference in *in vivo* extracted amount (mg/unit) and extracted fraction (%) of nicotine between the unflavored NP 3 mg and 6 mg products and the comparator product, Longhorn Natural 18 mg.
2. PK of nicotine in plasma: The difference between the unflavored NP 3 mg and 6 mg products and the comparator product, Longhorn Natural 18 mg, in the non-adjusted and baseline-adjusted PK parameters based on plasma concentrations of nicotine:
    - $AUC_{0\text{- inf}}$,
    - maximum observed concentration (C_{max}),
    - time of occurrence of C_{max} (T_{max}),
    - AUC from 0 to 1.5 hours ($AUC_{0\text{-}1.5h}$),
    - AUC from 0 to time of last measurable time point ($AUC_{0\text{-last}}$),

- terminal elimination half-life ($T_{1/2}$)

3a. PD (pulse rate): The difference between the unflavored NP 3 mg and 6 mg products and the comparator product, Longhorn Natural 18 mg, for the highest recorded increase (E_{max}) in pulse rate from baseline, measured using a pulse oximeter after IP administration.

3b. PD parameters: The difference between the unflavored NP 3 mg and 6 mg products and the comparator product, Longhorn Natural 18 mg, for the highest recorded value (E_{max}) in the subjective parameters "craving" and "satisfaction", measured using a 100 mm visual analogue scale (VAS) after IP administration.

3c. PD (subjective outcome parameters): The difference between the unflavored NP 3 mg and 6 mg products and the comparator product, Longhorn Natural 18 mg, for the subjective parameters "product-liking" and "intent to use again", measured using a 100 mm VAS 30 minutes after IP administration.

4. Extraction from pouches: The difference in *in vivo* extracted amount (mg/unit) and extracted fraction (%) of nicotine between the unflavored NP 6 mg and the flavored NP 6 mg products.

5a. PK of nicotine in plasma: The equivalence between the unflavored NP 6 mg and flavored NP 6 mg products in the non-adjusted and baseline-adjusted PK parameters $AUC_{0\text{-}inf}$ and C_{max} based on plasma concentrations of nicotine.

5b. PK of nicotine in plasma: The difference between the unflavored NP 6 mg and flavored NP 6 mg products in the non-adjusted and baseline-adjusted PK parameters T_{max}, $AUC_{0\text{-}1.5h}$, $AUC_{0\text{-}last,}$ and $T_{1/2}$.

6a. PD (pulse rate): The difference between the unflavored NP 6 mg and flavored NP 6 mg products for the highest recorded increase (E_{max}) in pulse rate from baseline, measured using a pulse oximeter after IP administration.

6b. PD parameters: The difference between the unflavored NP 6 mg and flavored NP 6 mg products for the highest recorded value (E_{max}) in the subjective parameters "craving" and "satisfaction", measured using a 100 mm VAS after IP administration.

6c. PD (subjective outcome parameters): The difference between the unflavored NP 6 mg and flavored NP 6 mg products for the subjective parameters "product-liking" and "intent to use again", measured using a 100 mm VAS 30 min after IP administration.

7. Frequency, intensity, and seriousness of AEs.

7.3 Exploratory objectives

1. To evaluate the impact on "product-liking" *vs*. the subjects' usual oral tobacco/nicotine product of choice by administering single doses of unflavored NP 3 mg and 6 mg products and the comparator product, Longhorn Natural 18 mg.
2. To compare the nicotine extraction-normalized PK parameters $AUC_{0\text{-}inf}$ and C_{max} between the unflavored NP 3 mg and 6 mg products and the comparator product, Longhorn Natural 18 mg.
3. To evaluate the impact on "product-liking" *vs*. the subjects' usual oral tobacco/nicotine product of choice by administering single doses of unflavored NP 6 mg and flavored NP 6 mg products.
4. To compare the nicotine extraction-normalized PK parameters $AUC_{0\text{-}inf}$ and C_{max} between the unflavored NP 6 mg and the flavored NP 6 mg products.

5. To compare the PK profile and the extracted amount and fraction of nicotine between the flavored NP 6 mg product with the highest concentrations and the comparator product, Longhorn Natural 18 mg.

7.3.1 Exploratory endpoints

1. The difference in "product-liking" *vs*. the subjects' usual oral tobacco/nicotine product of choice between the unflavored NP 3 mg and 6 mg products and the comparator product, Longhorn Natural 18 mg, measured on a 3-point scale using a multiple-choice question (MCQ) 30 minutes after IP administration.
2. The difference in nicotine extraction normalized $AUC_{0\text{-}inf}$ and C_{max} between the unflavored NP 3 mg and 6 mg products and the comparator product, Longhorn Natural 18 mg.
3. The difference in "product-liking" *vs*. the subjects' usual oral tobacco/nicotine product of choice between the unflavored NP 6 mg and flavored NP 6 mg products , measured on a 3-point scale using a MCQ 30 minutes after IP administration.
4. The difference in nicotine extraction normalized $AUC_{0\text{-}inf}$ and C_{max} between the unflavored NP 6 mg and flavored NP 6 mg products.
5. The difference in the PK parameters $AUC_{0\text{-}inf}$ and C_{max} and the extracted amount and fraction of nicotine between the flavored NP 6 mg product with the highest concentrations and the comparator product, Longhorn Natural 18 mg.

8 STUDY DESIGN

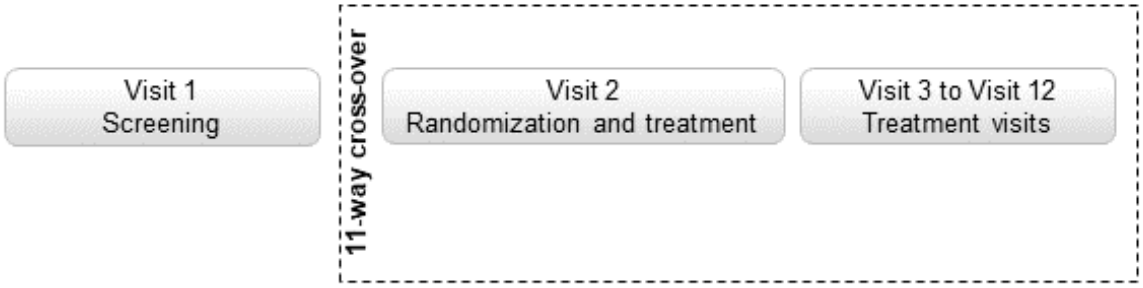
8.1 Overall study design and schedule of events

This is a multi-center, open-label, randomized, 11-way cross-over, single dose administration study, designed to assess nicotine exposure from new format NP products. The IPs include 2 unflavored products (3 mg and 6 mg) and 8 flavored products (Flavor A-H 6 mg). The comparator product is Longhorn Natural 18 mg.

The study will include 36 randomized subjects with the aim of achieving 32 evaluable subjects. The subjects are healthy male or female oral tobacco/nicotine pouch users aged 21 to 60 years, inclusive, who have used oral tobacco/nicotine products for ≥1 year, with a minimum daily consumption of 5 or more pouches. An effort will be made to randomize at least 14 female subjects (approximately 40%). Each subject will participate in the study for approximately 5 weeks, not including the preceding 4-week screening period.

An overview of the study design is shown in Figure 8.1-1.

Figure 8.1-1 Overview of the study design



Subjects will report to the study sites for a screening visit (Visit 1) followed by 11 treatment visits (Visits 2-12) on separate days. Screening will take place within 4 weeks prior to Visit 2 and will include an eligibility check, including evaluations of smoking and oral tobacco/nicotine use, a brief physical examination, serology tests, electrocardiogram (ECG) and collection of medical history, vital signs (pulse rate and blood pressure), height, weight, and body mass index (BMI) and a product familiarization session. For details, refer to Table 8.1-1.

At the end of the screening visit, the subjects will complete a product familiarization session. During this session, they will be provided with the comparator product, Longhorn 18 mg nicotine, to ensure the acceptability of the product. The subjects will use the product in the same way they would normally use a snus or NP product (*i.e.*, placing it under the upper lip). The duration of product use will be a minimum of 20 minutes, starting from the moment the subject places it in their mouth until they remove it. Subjects who successfully complete this familiarization session, tolerating the product without significant unexpected adverse effects, will be allowed to continue in the study.

Subjects shall abstain from tobacco/nicotine products as well as smoking (cigarettes or e-cigarettes) for 12 hours prior to each treatment visit (Visits 2-12). All treatment sessions will be performed during the morning hours (08:00 to 12:00) to facilitate abstinence.

The IPs will be administered as single pouches in a pre-determined randomized order. Subjects will keep the pouch still between the upper lip and gum for 30 minutes and will be instructed not to manipulate the pouch with the tongue or lips. They will also be instructed not

to eat, drink, chew gum, or brush their teeth for 30 minutes before, during, or 30 minutes after IP administration.

After 30 minutes, each used pouch will be collected and frozen (-20°C) pending analysis of residual nicotine content. Unused pouches from the same batch will serve as references and will be stored at -20°C pending analyses.

Blood samples for the assessment of nicotine plasma levels and PK parameters will be collected at pre-defined time points from pre-administration to 6 hours after each IP administration. The PD effects of the IPs will be determined using pulse rate measurements and subjective parameters (using VAS questions) at the same pre-defined time points as well as a MCQ 30 minutes after IP administration.

AEs will be collected through subject interviews and will also include any AEs reported spontaneously by the subjects, starting from the initiation of IP administration (Visit 2), and continuing until the last treatment visit (Visit 12). An overall schedule of event is presented in Table 8.1-1 and a detailed schedule of events for each treatment visit is presented in Table 8.1-2. Study assessments are described in Section 11.

Table 8.1-1 Schedule of events

Events	CSP section	Visit 1 Screening	Visit 2 First treatment visit	Visits 3-12 Treatment visits
Informed consent	11.2.1	X		
Demographics	11.2.2	X		
Medical/surgical history	11.2.3	X		
History of nicotine use	11.2.4	X		
Inclusion/exclusion criteria	9.4/9.5	X	X[1]	
Weight, height, and BMI	11.2.6	X		
Physical examination	11.2.7	X		
Vital signs (blood pressure and pulse rate)	11.2.8	X		
Electrocardiogram	11.2.9	X		
HIV, Hepatitis B and C	11.2.10	X		
Product familiarization session	11.2.11	X		
Pregnancy test[2]	11.2.12	X	X	X[3]
Urine drug screen[4]	11.2.13	X	X	
Alcohol screen[4]	11.2.14	X	X	
Randomization	9.9		X	
IP (pouch) administration	10.5		X[5]	X[5]
PK blood sampling (plasma)	11.3.1		X[5]	X[5]
Subjective effects (VAS questions and MCQ)	11.4.2.2 11.5.1.1		X[5]	X[5]
Pulse rate (pulse oximeter)	11.4.2.1		X[5]	X[5]
IP (pouch) collection	11.4.3		X[5]	X[5]
Baseline symptoms	11.2.15	X	X	
AEs	11.4.4		X	X
Prior and concomitant medications	11.2.16	X	X	X

AE=adverse event, BMI= body mass index, HIV=human immunodeficiency virus, IP=investigational product, MCQ=multiple-choice question, VAS=visual analogue scale.

1. Confirmation of eligibility criteria.
2. Only subjects of child-bearing potential.
3. Only at the discretion of the Investigator on Visits 3-12.
4. Additional drug and alcohol tests may be performed at the discretion of the Investigator during treatment visits.
5. The detailed timing of assessments is outlined in Table 8.1-2.

Table 8.1-2 Detailed schedule of events for each treatment visit (Visits 2-12)

Visits 2-12																
Assessment / Time point	**Admission**	**-00:15**	**-00:10**	**-00:01**	**00:00**	**00:05**	**00:10**	**00:15**	**00:20**	**00:30**	**00:40**	**01:00**	**01:30**	**02:00**	**04:00**	**06:00**
Inclusion/exclusion criteria	X[1]															
Urine drug screen	X[2]															
Alcohol screen	X[2]															
Pregnancy test	X[3]															
Randomization	X[4]															
IP (pouch) administration					X											
IP (pouch) collection										X						
PK blood sampling (plasma)			X[5]			X	X	X	X	X	X	X	X	X	X	X
VAS question (“craving”)			X			X	X	X	X	X	X	X	X	X	X	X
VAS question (“satisfaction”)						X	X	X	X	X	X	X	X	X	X	X
VAS (“product-liking” and “intent-to-use-again”)										X						
MCQ (“product liking” *vs.* usual product)										X						
Pulse rate (pulse oximeter)		X				X	X	X	X	X	X	X	X	X	X	X
Baseline symptoms	X[6]															
AEs	X[7]															
Prior and concomitant medications	X															

1. Only at Visit 2. Confirmation of eligibility before randomization.
2. Only at Visit 2. Additional drug and alcohol tests may be performed during treatment visits at the discretion of the Investigator.
3. Only subjects of child-bearing potential. Additional tests at the discretion of the Investigator on Visits 3-12.
4. Randomization occurs only on Visit 2.
5. Pre-administration PK-sample taken at any time within 10 min to -1 min prior to IP administration, except in direct association with the pre-administration pulse rate assessment at -15 min.
6. Only on Visit 2. Baseline symptoms experienced prior to first IP administration.
7. AEs experienced from first IP administration.

8.2 Rationale for study design

This is a multi-center, open-label, randomized, 11-way cross-over, single dose administration study, designed to assess nicotine exposure from new format NP products.

A cross-over design was chosen to yield a more efficient evaluation of NPs than a parallel study design, *i.e.*, fewer subjects are required since each subject will serve as its own control. To avoid carryover effects, subjects will abstain from oral tobacco/nicotine products as well as smoking (cigarettes or e-cigarettes) for at least 12 hours prior to each treatment visit (Visits 2-12).

Randomization will be used to minimize bias in the assignment of subjects to an IP administration sequence and to increase the likelihood that known and unknown subject attributes (*e.g.*, demographic and baseline characteristics) are evenly balanced.

9 STUDY POPULATION

Prospective approval of deviations from the eligibility criteria, also known as protocol waivers or exemptions, is not permitted.

9.1 Recruitment

Subjects will be recruited from CTC's database of healthy volunteers, as well as from strategic marketing campaigns. Advertisements in social media and other media (newspapers, internet, radio, local distribution of flyers *etc*.) will be used to reach the target audience. The advertisement texts approved by the independent ethics committee (IEC) will be used to create all materials (digital, radio and/or print) for recruitment.

9.2 Screening and enrolment log

Investigators must keep a record of all screened subjects even if they were not subsequently included in the study. This information is necessary to verify that subjects were selected without bias. The reason for screening failure should be stated for all subjects screened but not included. The reason for withdrawal should be stated for all subjects that were included but did not complete the study.

A screening number generated automatically in the electronic case report form (eCRF) will be allocated to each subject in connection with the informed consent process at the screening visit (Visit 1). The screening number will allow identification of subjects irrespective of their possible eligibility for the study.

Eligible subjects will be assigned a 3-digit randomization number prior to the first IP administration. If a subject is unable to receive the planned initial IP administration within 28 days after screening (*i.e.*, the time interval between signing informed consent and the first IP administration) the subject should undergo rescreening before continuing in the study.

9.3 Number of subjects

Approximately 66 subjects will be screened with the aim of achieving 36 randomized subjects and 32 fully evaluable subjects. Evaluable subjects are subjects that have received all 11 IPs and completed all study visits.

For the replacement of subjects who discontinue the study, see Section 9.8.3.

An effort will be made to randomize at least 14 female subjects (~40%).

9.4 Inclusion criteria

For inclusion in the study, the subjects must fulfil the following criteria:

1. Willing and able to give written informed consent for participation in the study.
2. Subjects who have used oral tobacco/nicotine products for ≥1 year, with a minimum daily consumption of 5 or more pouches who are willing and able to use both tobacco-based moist snuff and NPs with high nicotine content.
3. Healthy male or female subject aged 21 to 60 years, inclusive.
4. Medically healthy subject without abnormal clinically significant medical history, physical findings, vital signs, ECG, and hepatitis B/C and human immunodeficiency virus (HIV) results at the time of the screening visit, as judged by the Investigator.

5. Successful completion of the product familiarization session for the comparator product use is required before the first IP administration. The subject should be able to follow the instructions, tolerate the product, and not experience any adverse effects different from what is expected during typical smokeless pouch use in the training session.
6. **Female subjects of child-bearing potential** must practice abstinence from heterosexual intercourse (only allowed when this is the preferred and usual lifestyle of the subject) or must agree to use a highly effective method of contraception with a failure rate of <1 % to prevent pregnancy for the duration of the study.

   The following are considered highly effective methods of contraception:

   - combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation (oral, intravaginal, transdermal),
   - progestogen-only hormonal contraception associated with inhibition of ovulation (oral, injectable, implantable),
   - intrauterine device or intrauterine hormone-releasing system.

9.5 Exclusion criteria

Subjects must not enter the study if any of the following exclusion criteria are fulfilled:

1. A history of diagnosed hypertension or any cardiovascular disease, or ongoing manifestations of hypertension or any cardiovascular disease as judged by the Investigator.
2. Any surgical or medical condition, including abnormal salivation (also pharmaceutically induced), or history thereof, which, in the judgment of the Investigator, might interfere with the absorption, distribution, metabolism or excretion of the IP or may either put the subject at risk because of participation in the study, influence the results, or the subject's ability to participate in the study.
3. A history of diagnosed severe allergy/hypersensitivity or ongoing manifestations of severe allergy/hypersensitivity to aroma compounds (including fragrances and/or flavorings), as judged by the Investigator.
4. Subjects with poor venous access or being scared of needles.
5. Any planned major surgery within the duration of the study.
6. Subjects who are pregnant, currently breastfeeding, or intend to become pregnant during the course of the study.
7. Any positive result at the screening visit for serum hepatitis B surface antigen, hepatitis C antibodies and/or HIV.
8. Positive screening result for drugs of abuse or alcohol at the screening visit or on admission to the study site prior to IP administration. Positive results that are expected given the subject's medical history and prescribed medications can be disregarded as judged by the Investigator.
9. History of alcohol abuse or excessive intake of alcohol, as judged by the Investigator.
10. Presence or history of drug abuse, as judged by the Investigator.
11. History of, or current use of anabolic steroids, as judged by the Investigator.

12. Current, ongoing use of beta-adrenergic blocking agents (beta blockers), including *pro re nata* (as needed) use.
13. Plasma donation within 1 month of screening or blood donation (or corresponding blood loss) during the last 3 months prior to screening.
14. Subjects who intend to change their nicotine consumption habit, including the intention to stop using nicotine products, within the next 3 months of the screening visit, as judged by the Investigator.
15. The Investigator considers the subject unlikely to comply with study procedures, restrictions, and requirements.

9.6 Restrictions during the study

Subjects must be willing to comply with the restrictions as outlined in Section 9.6.1 and 9.6.2.

9.6.1 General restrictions

1. Contraception requirements: Subjects of child-bearing potential are expected to use contraceptive methods in accordance with inclusion criterion #6 or practice abstinence from heterosexual intercourse (only allowed when this is the preferred and usual lifestyle of the subject) during the clinical study.
2. Tobacco/nicotine products: Subjects shall abstain from any self-administered oral tobacco/nicotine products as well as smoking (cigarettes and e-cigarettes) for at least 12 hours prior to treatment visits and during each treatment visit (Visits 2-12).
3. Mouth related procedures: Subjects shall abstain from eating, drinking, or conducting any other mouth related procedure (*e.g*., tooth brushing) for 30 minutes prior to IP administration, during IP use, and for 30 minutes after IP removal (Visits 2-12).
4. Drugs of abuse: Subjects shall abstain from any drugs of abuse during the study, *i.e*., from screening (Visit 1) to the last treatment visit (Visits 12).
5. Alcohol: Subjects shall abstain from alcohol for at least 12 hours prior to each treatment visit (Visits 2-12).
6. Blood donation: The subjects must not donate blood or plasma within 1 month of screening until 3 months after the last treatment visit (Visits 12).
7. Participation in other clinical studies: The subjects are not allowed to participate in any other clinical study from the screening visit (Visit 1) until the last treatment visit (Visit 12).

9.6.2 Prior and concomitant therapy

Use of any prescribed medication that includes beta-adrenergic blocking agents (beta blockers), including *pro re nata* use, is not allowed from admission to the study sites at Visit 2 until the last treatment visit (Visit 12). As outlined in exclusion criterion #12, subjects currently using beta-adrenergic blocking agents will be excluded from participation in the study.

There will be no other restrictions concerning concomitant medications or therapies, as long as the subject is on a stable course of medication from the screening visit to the last treatment visit (Visit 12). Prescribed medications taken *pro re nata* may be a reason for exclusion as judged by the Investigator if they affect the subject's general condition and salivation.

As detailed in exclusion criterion # 8, a positive drug screen will exclude a subject from participation in the study. However, positive results that are expected given the subject's medical history and prescribed medications (*e.g.*, opioid analgesics or attention deficit hyperactivity disorder [ADHD] medications) can be disregarded as judged by the Investigator. This does not include positive drug screens resulting from the use of beta-adrenergic blocking agents.

9.7 Screen failures

Screen failures are defined as subjects who consent to participate in the clinical study but do not fulfil all eligibility criteria and are not subsequently randomized in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure subjects. Minimal information includes documentation of signed and dated informed consent form (ICF) and reason(s) for screening failure.

Re-screening can be performed if any of the following were reasons for screening failure or non-randomization, as judged by the Investigator:

- Practical reasons.
- Non-significant medical conditions (*e.g.*, influenza, nasopharyngitis).
- Plasma or blood donation outside of the allowed time windows.
- Reserve subjects.

For subjects who are re-screened, a new screening number will be assigned and new, signed ICF must be collected.

9.8 Subject withdrawal

9.8.1 General withdrawal criteria

Subjects are 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 should be documented.

Subjects may be discontinued from the study at any time at the discretion of the Investigator.

Reasons for discontinuation can include:

- Withdrawal of consent (subject decision).
- Severe non-compliance to CSP procedures, as judged by the Investigator and/or Sponsor.
- Subject is lost to follow-up.
- Significant AEs posing a risk for the subject, as judged by the Investigator and/or Sponsor.
- Pregnancy.

9.8.2 Procedures for discontinuation of a subject from the study

A subject who prematurely discontinues participation in the study will always be asked about the reason(s) for discontinuation and the presence of any AEs. Any ongoing AEs will be followed-up as described in Section 11.4.4.11.

The primary reason for discontinuation/early withdrawal must be specified in the eCRF and final drug accountability must be performed. If the reason for discontinuation was an AE, the AE must be specified in the eCRF.

9.8.3 Subject replacement

Subjects who are prematurely withdrawn from the study for any reason except the occurrence of AEs assessed as possibly or probably related to the IPs may be replaced at the decision of the Sponsor. For the replacement numbers, the number "1" will be added to the end of the screening number.

9.9 Randomization

At Visit 2, subjects will be randomized to one of 11 administration sequences. As this is an open-label study, the IP administration sequence to which each subject is allocated will be recorded in the eCRF. Computer-generated randomization lists for each site will be created using the statistical analysis software (SAS) Proc Plan, SAS Version 9.4. The randomization lists will contain site, subject number, randomization sequence, day, and product.

The randomization list will be generated by CTC. The original randomization list will be kept by the randomizer and copies of the randomization list will be provided to each site and to the IP packing company.

10 STUDY TREATMENTS

The IPs are supplied by Swedish Match.

10.1 Identity of test and comparator products

The test and comparator products that will be used in the study are detailed in Table 10.1-1. The new format NP product offers a smaller pouch size and contains a reduced amount of nicotine compared to currently available ZYN Slim products on the Swedish market.

Table 10.1-1 Identity of test and comparator products

IP	Nicotine content per pouch
NP 1 - Unflavored	3 mg
NP 2 - Unflavored	6 mg
NP 3 - Flavor A	6 mg
NP 4 - Flavor B	6 mg
NP 5 - Flavor C	6 mg
NP 6 - Flavor D	6 mg
NP 7 - Flavor E	6 mg
NP 8 - Flavor F	6 mg
NP 9 - Flavor G	6 mg
NP 10 - Flavor H	6 mg
Longhorn Natural (comparator)	18 mg

10.2 Manufacturing, packaging, and labelling

All IPs are manufactured and packaged by Swedish Match in compliance with the Swedish law on food production. Production sites and batch IDs for the IPs will be documented in the trial master file (TMF).

IPs will be transferred from the original container, weighed, and individually packaged in identical sealed food approved test containers at the Swedish Match analytical laboratory. The containers will be labelled with unique identification numbers by Swedish Match in accordance with the randomization lists. IPs will be shipped by Swedish Match directly to CTC.

10.3 Conditions for storage

IPs will be stored in access-controlled storage areas at the study sites under refrigerator temperature (4-8°C).

10.4 Preparation and accountability

The IPs will be dispensed according to the randomization list by the site personnel. The Investigator will maintain a storage and accountability log as well as a dispensing log detailing the dates and quantities of study IPs received, and used by each subject, as well as any IPs destroyed at the end of the study. Products deliberately and/or accidentally destroyed by the site or the subject must be accounted for.

10.5 Treatment administration

A single dose will be given in the morning of each treatment visit (Visits 2-12). Subjects will keep the pouch still between the upper lip and gum for 30 minutes and are instructed not to manipulate the pouch with the tongue or lips. The subjects will also be instructed not to eat or drink, chew gum, or brush their teeth for 30 minutes before, during use, or 30 minutes after the administration of IP. Subjects shall abstain from oral tobacco/nicotine products as well as smoking (cigarettes or e-cigarettes) for at least 12 hours prior to each treatment visit. To this end, subjects will be instructed to abstain from such products from approximately 20:00 (8 pm) the day before treatment visits (Visits 2-12). All IP administrations will be performed during the morning hours (08:00 to 12:00) to facilitate abstinence.

10.6 Treatment compliance

Any IP not used, as well as all empty containers, will be destructed at the site upon confirmation from the Sponsor. The Monitor will perform a final IP accountability reconciliation at the end of the study to verify that all unused IP is adequately destroyed and documented.

11 STUDY ASSESSMENTS

The study assessments are described in the sections below and the timing of assessments are detailed in the schedule of events (Table 8.1-1 and Table 8.1-2).

11.1 Recording of data

The PIs will provide the Sponsor with all data produced during the study from the scheduled assessments. They will ensure the accuracy, completeness, legibility, and timeliness of the data reported to Sponsor in the eCRF and in all required reports.

It is important that IP application and collection of PK blood sampling occurs as close as possible to scheduled time points in Table 8.1-2. In order to achieve this, the timing priority order at a particular timepoint is:

1. Blood samples for PK
2. Pulse rate assessment
3. VAS questions
4. MCQ

Allowed deviations from actual time points are outlined in Table 11.1-1 below.

Table 11.1-1 Allowed deviations from actual time points

Time point	IP administration and collection	PK blood sampling (plasma)	Pulse rate (pulse oximeter)	VAS questions "craving" and "satisfaction"	VAS questions "product-liking" and "intent to use again"	MCQ
-00:15			± 5 min			
-00:10		Pre-administration sample can be taken at any time within 10 min prior to IP administration[1].		± 3 min[2]		
-00:01						
00:00	± 0 min (IP admin.)					
00:05		± 2 min	± 2 min	± 3 min		
00:10		± 2 min	± 2 min	± 3 min		
00:15		± 2 min	± 2 min	± 3 min		
00:20		± 2 min	± 2 min	± 3 min		
00:30	±1 min (IP collection)	± 5 min	± 5 min	± 5 min	± 10 min	± 10 min
00:40		± 5 min	± 5 min	± 5 min		
01:00		± 10 min	± 10 min	± 10 min		
01:30		± 10 min	± 10 min	± 10 min		
02:00		± 10 min	± 10 min	± 10 min		
04:00		± 10 min	± 10 min	± 10 min		
06:00		± 10 min	± 10 min	± 10 min		

1. Pre-administration PK blood sample should not be taken in direct association with the pre-administration pulse rate assessment at -15 min.
2. Only "craving" at -10 min.

11.2 Demographics and other baseline characteristics

11.2.1 Informed consent

Signed informed consent must be obtained before any screening procedures are initiated. The informed consent procedure is further described in Section 14.3.

11.2.2 Demographic information

The following demographic data will be recorded: gender, age, ethnicity, and race.

11.2.3 Medical/surgical history

Medical/surgical history will be obtained by subject interview in order to verify that the eligibility criteria are met.

11.2.4 History of nicotine use

History of oral tobacco/nicotine products use in terms of brands, average consumption per day during the last 30 days, and duration of use (years, months), history of smoking in terms of number of cigarettes per day during the last 30 days, as well as history of vaping in terms of number of days during last 30 days, will be obtained by subject interview.

11.2.5 Eligibility criteria

Eligibility criteria should be checked during screening and verified before randomization and administration of the first IP on Visit 2. The criteria are specified in Sections 9.4 and 9.5.

11.2.6 Height, weight, and body mass index

Weight and height will be measured without shoes. BMI will be calculated, with one decimal, from the recorded height and weight.

11.2.7 Physical examination

A brief physical examination will include assessments of the head, eyes, ears, nose, throat, skin, thyroid, neurological, lungs, cardiovascular, abdomen (liver and spleen), lymph nodes, and extremities.

11.2.8 Vital signs

Systolic and diastolic blood pressure, and pulse will be measured at screening in supine position after 10 minutes of rest.

Pulse rate assessments will be considered as ”normal” if within the following ranges:

-At screening (Visit 1): 40 to 90 bpm

-During treatment visits (Visit 2 to Visit 12): 40 to100 bpm. Refer to Section 11.4.2.

11.2.9 Electrocardiogram

Single 12-lead ECGs will be recorded at screening in supine position after 10 minutes of rest using an ECG machine. The resting heart rate (HR) and PQ/PR, QRS, QT and QTcF intervals will be recorded.

ECGs will be reviewed and interpreted on-site by the Investigator.

11.2.10 HIV and hepatitis B/C

Subjects will be tested for HIV-1 and HIV-2 antibodies and HIV-1 p24 antigen, hepatitis B virus surface antigen, and hepatitis C virus antibodies prior to inclusion into the study. Any positive result will exclude the subject from participating in the study.

11.2.11 Product familiarization session

At the end of the screening visit, a familiarization session will be conducted with the comparator product (Longhorn 18 mg nicotine). Subjects will not be required to fast prior to this training session. Extensive experience indicate that snus/nicotine pouch users can assess the effects of nicotine exposure and their ability to tolerate snus/nicotine pouch products within 20 to 30 minutes of use. Subjects will use the product once and there will be a minimum of 20 minutes duration of product use from when the subject places it in their mouth until they remove it. Subjects who successfully complete this familiarization session, meaning they can tolerate the product without any adverse effects different from what is typical for smokeless pouch use, will be eligible to continue participating in the study.

Used pouches from the familiarization session will not be retained for residual nicotine analysis and should be disposed of in compliance with local regulations.

11.2.12 Pregnancy test

All females of childbearing potential will do a urine pregnancy test at the screening visit and at visits specified in Table 8.1-1 (urine dipstick).

11.2.13 Urine drug screen

Urine will be screened for drugs of abuse at time points outlined in the schedule of events (Table 8.1-1) using the Drug-Screen Multi-15 Dip Test. Additional random tests can be performed during the study period, at the discretion of the Investigator.

11.2.14 Alcohol test

An alcohol test will be performed at time points outlined in the schedule of events (Table 8.1-1). Additional random tests can be performed during the study period, at the discretion of the Investigator.

11.2.15 Baseline symptoms

A baseline symptom is defined as an event that occurs between the subject's signing of the ICF until the administration of IP (*i.e.*, an event that occurs during the screening period). Such events are not AEs but will be recorded as baseline symptoms in the AE Log in the eCRF.

11.2.16 Prior and concomitant medication

Prior medications taken within 2 weeks prior to screening will be obtained by subject interview in order to verify that the eligibility criteria are met.

Medications are classified as prior if the stop date was before or on the day of the first IP administration (prior to IP administration) and as concomitant if ongoing on the day of the first IP administration, stopped after the first IP administration, or started after the first IP administration. To distinguish between prior and concomitant medications on Day 1 (*i.e.*, the first treatment visit), the start time of any newly introduced medication or the stop time of any previously ongoing medication must be recorded in the eCRF.

Any use of prior/concomitant medication from the screening visit until the last treatment visit (Visit 12) must be documented appropriately in the subject's eCRF. Relevant information (*i.e.*, name of medication, dose, dose form, unit, route, frequency, start and stop dates, reason for use) must be recorded. All changes in medication must be noted in the eCRF.

11.3 Assessments related to primary endpoints

11.3.1 Nicotine plasma concentration and pharmacokinetic sampling and analysis

Venous blood samples (approximately 3 mL) for the determination of plasma concentrations of nicotine after IP administration will be collected through an indwelling venous catheter at the pre-specified visits and time points detailed in Table 8.1-1 and Table 8.1-2. It is important that blood PK sampling does not deviate from the planned time points more than the allowed time deviations, as outlined in Table 11.1-1.

Pre-PK sampling should be conducted -10 minutes to -1 minute before the first IP administration.

The date and time of collection of each sample will be recorded in the eCRF.

The blood samples will be collected in pre-labelled tubes. All the collected blood samples will be centrifuged for 10 minutes at 20°C (± 4°C) to separate the plasma within 60 minutes from when the sample was drawn. The separated plasma from each blood sample will be divided into 2 aliquots in pre-labelled cryotubes and frozen at -20°C within 1 hour after the centrifugation. Further details will be described in a separate laboratory manual.

Plasma samples for determination of plasma concentrations of nicotine will be analyzed by Lablytica Life Science AB, Uppsala, Sweden, by means of a validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) method. The details of the analytical method used will be described in a separate bioanalytical report.

11.4 Assessments related to secondary endpoints

11.4.1 Pharmacokinetic sampling related to secondary endpoints

PK sampling related to secondary endpoints 2, 5a and 5b is part of the procedure described in Section 11.3.1 above.

11.4.2 Pharmacodynamic effects

The PD effects of the IPs will be assessed by measuring pulse rate and subjective parameters (using VAS and MCQ) at pre-defined time points as described below and in Table 8.1-2.

11.4.2.1 Pulse rate

The pulse rate will be monitored using a pulse-oximeter and will be spot-assessed at -15 min pre-administration, as well as at 5 min, 10 min, 15 min, 20 min, 30 min, 40 min, 60 min, 1 h:30 min, 2 h, 4 h, and 6 h post-administration (see Table 8.1-2). Any post-IP measurements of pulse rate outside of normal ranges will be judged as clinically significant or not clinically significant. The assessment will be recorded in the eCRF. Abnormal post-IP administration findings assessed by the Investigator as clinically significant will be reported as AEs. Refer to Section 11.2.8

Allowed deviations from actual time points are outlined in Table 11.1-1.

11.4.2.2 Subjective parameters

Subjective parameters ("craving", "satisfaction", "product-liking", and "intent to use again") will be rated using VAS, anchored with "not at all" to "extremely", or "very likely" for the "intent to use again" parameter.

The "craving" parameter will be assessed with the question "Right now, how strong is your urge to snus?" at the same pre-defined time points as the pulse-rate assessments: -10 min prior to IP administration, and 5 min, 10 min, 15 min, 20 min, 30 min, 40 min, 60 min, 1 h:30 min, 2 h, 4 h, and 6 h post-administration.

The "satisfaction" parameter will be assessed with the question "Right now, is the product satisfying?" at the pre-defined time points: 5 min, 10 min, 15 min, 20 min, 30 min, 40 min, 60 min, 1 h:30 min, 2 h, 4 h, and 6 h post-administration.

In addition, "product-liking" and "intent to use again" parameters will be assessed at 30 minutes post-administration with the questions "How much did you like the product?" and "How likely are you to use this product again in the future?", respectively.

Time points for each of the VAS questions are outlined in Table 8.1-2, and allowed deviations from actual time are outlined in Table 11.1-1.

11.4.3 Nicotine extraction from pouches

Used pouches will be collected after 30 minutes (± 1 min) of use for the determination of residual nicotine in the IPs.

All the collected pouches will be frozen within 60 minutes at -20°C. Pouches for extraction of nicotine will be analyzed by Swedish Match.

11.4.4 Adverse events

The PIs are responsible for ensuring that all medical staff involved in the study are familiar with the content of this section and the content of the CTC standard operating procedures (SOPs) regarding emergencies.

AEs will be handled in accordance with applicable regulations and guidelines [12].

For the purpose of this study, AEs will be assessed in relation to the IPs and comparator.

11.4.4.1 Definition of adverse event

An AE is defined as any untoward medical occurrence in a subject to whom a medicinal product is administered, and which does not necessarily have a causal relationship with this treatment.

An AE can be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

11.4.4.2 Definition of serious adverse event

An SAE is any untoward medical occurrence that at any dose:

- results in death,
- is life-threatening,

  NOTE: The term "life-threatening" in the definition of "serious" refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.
- requires inpatient hospitalization or prolongation of existing hospitalization,
- results in persistent or significant disability/incapacity, or
- is a congenital anomaly/birth defect.

Medical and scientific judgement should be exercised in deciding whether expedited reporting is appropriate in various situations. These situations may include "important medical events" that, while not immediately life-threatening or resulting in death or hospitalization could jeopardize the subject's well-being or require intervention to prevent any of the other outcomes listed in the definition above. These should also usually be considered serious.

Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization; or development of drug dependency or drug abuse.

11.4.4.3 Time period and frequency for collecting adverse events

All AEs (including SAEs) will be collected from the start of first IP administration until the last visit (Visit 12).

Any AE with start date on the day of each IP administration must be recorded with start time.

On the last treatment visit, information on new AEs or SAEs, if any, and stop dates for ongoing events must be recorded as applicable.

Investigators will not be obliged to actively seek AEs or SAEs after conclusion of the study participation. However, if the Investigator learns of any SAE, including a death, at any time after a participant has been discharged from the study, and they consider the event to be reasonably related to the study intervention or study participation, the Investigator must promptly notify the Sponsor.

11.4.4.4 Collection of adverse events

AEs identified using any of the following methods will be recorded:

- AEs spontaneously reported by the subject.
- AEs observed by the Investigator or medical personnel.
- AEs elicited based on non-leading questions from the Investigator or medical personnel.

11.4.4.5 Recording of adverse events

AEs must be recorded in the AE Log of the eCRF. The Investigator must provide information on the AE, preferably as a diagnosis or at least as signs and symptoms; start and end dates,

start and end time; intensity; causal relationship to IP and comparator, action taken, and outcome. If the AE is serious, this must be indicated in the eCRF.

AEs, including out-of-range clinically significant clinical safety laboratory values, must be recorded individually, except when considered manifestations of the same medical condition or disease state; in such cases, they must be recorded under a single diagnosis.

11.4.4.6 Assessment of seriousness

The Investigator must assess and document the seriousness (serious or non-serious) of each AE using the definitions in Section 11.4.4.2. If the event is assessed as serious it must be reported as an SAE by the Investigator to the Sponsor according to Section 11.4.4.10.

For the seriousness criteria of inpatient hospitalization or prolongation of existing hospitalization to be fulfilled, the AE requires at least an overnight admission (24 hours) or prolongs a hospitalization beyond the expected length of stay. Emergency room visits that do not result in admission to the hospital should be evaluated for one of the other serious outcomes.

Planned hospitalizations or surgical interventions for a condition that existed before the subject signed the ICF, and that did not change in intensity, are not SAEs.

If there is any doubt as to whether an AE meets the definition of an SAE, a conservative approach will be taken, and the AE will be reported as an SAE.

11.4.4.7 Assessment of intensity

The grading of the intensity of AEs will follow the common terminology criteria for adverse events (CTCAE) v5.0 [11]. Grade refers to the severity of the AE. The CTCAE displays Grades 1 through 5 with unique clinical descriptions of severity for each AE based on this general guideline.

The Investigator must assess the intensity of an AE using the following definitions, and record it in the AE Log of the eCRF:

Grade 1 Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.

Grade 2 Moderate; minimal, local, or non-invasive intervention indicated; limiting age-appropriate instrumental activities of daily living (ADL)*.

Grade 3 Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self- care ADL**.

Grade 4 Life-threatening consequences: urgent intervention indicated.

Grade 5 Death related to AE.

*Instrumental ADL refers to preparing meals, shopping for groceries or clothes, using the telephone, managing money, *etc.*

**Self-care ADL refers to bathing, dressing, and undressing, feeding self, using the toilet, taking medications, and not bedridden.

11.4.4.8 Assessment of causal relationship

The Investigator must assess the causal relationship between an AE and the use of the IP and comparator using the definitions below. Each assessment should be recorded in the AE log of the eCRF.

Probable	The event has a strong temporal relationship to the IP and comparator, or recurs on re-challenge, and another etiology is unlikely or significantly less likely.
Possible	The event has a suggestive temporal relationship to the IP and comparator, and an alternative etiology is equally or less likely.
Unlikely	The event has no temporal relationship to the IP and comparator or is due to underlying or concurrent illness or effect of another drug (*i.e.*, there is no causal relationship between the IP, and the event).

An AE is considered causally related to the use of the IP and comparator when the causality assessment is probable or possible.

11.4.4.9 Outcome of adverse event

The Investigator must assess the outcome of an AE using the definitions below and record it on the AE log of the eCRF.

Recovered/resolved	The subject has recovered completely, and no symptoms remain.
Recovering/resolving	The subject's condition is improving, but symptoms still remain.
Recovered/resolved with sequelae	The subject has recovered, but some symptoms remain (*e.g.*, the subject had a stroke and is functioning normally, but has some motor impairment).
Not recovered/not resolved	The subject's condition has not improved, and the symptoms are unchanged (*e.g.*, atrial fibrillation has become chronic).
Fatal	
Unknown	

11.4.4.10 Reporting of serious adverse events

The Investigator must report SAEs within **24 hours** of awareness to the Sponsor or its designee, this includes both initial information and any subsequent relevant/significant follow up information to a previously reported SAE. The primary mechanism for reporting an SAE will be via the eCRF. When the Investigator classifies the event as "serious" in the eCRF, and signs off the event, an automatic e-mail alert is sent to the Sponsor or its designee, and any other pre-defined recipients.

The backup procedure for reporting an SAE in case the eCRF is unavailable, will be via the paper SAE form provided in the Investigator Site File (ISF). The Investigator must fill in the SAE form and send it Sponsor or its designee. The study site must notify the site Monitor via phone or e-mail about the submission of the SAE report. As soon as the site personnel have access to the eCRF, the SAE must be reported electronically as well. The completed, signed, and dated paper SAE form should, within 24 hours, be scanned and delivered via encrypted e-mail or secure file transfer to:

[redacted]

A copy of the SAE paper form must also be delivered via encrypted e-mail or secure file transfer to CTC at: [redacted]

All available information regarding the SAE must be entered in the AE log for the specific subject, *i.e.*, AE term, intensity, causality, outcome, seriousness criteria, action taken with IP, a narrative including the Investigators rationale for the causality assessment.

The SAE report will be reviewed by the Sponsor or its designee to ensure that the report is valid. The Sponsor or its designee will acknowledge receipt of the SAE report to the reporting Investigator. For SAEs where important or relevant information is missing, follow-up queries to the site are raised promptly to keep the regulatory reporting timelines.

Sponsor will perform an independent assessment of causality, including a rationale for the assessment. The causality assessment given by the Investigator should not be downgraded by the Sponsor. If the Sponsor disagrees with the Investigator's causality assessment, the opinion of both the Investigator and the Sponsor should be provided with the report.

If any additional information or documentation (*e.g.*, autopsy report) on the SAE is required for Sponsor's assessment of the SAE, the Sponsor or its designee will request this information from the Investigator, and the Investigator is required to promptly respond to the request.

Any subsequent relevant/significant follow-up information to a previously reported SAE must be entered in the AE log for the specific subject. If the Investigator makes any changes to the assessment of the case *e.g.*, changes in seriousness, causality, or intensity, a justification for the change should be provided in the case narrative. If the SAE report in the eCRF is updated, a new automatic e-mail alert is sent to Sponsor or its designee.

Detailed information on the SAE handling will be described in a study specific safety management plan.

11.4.4.11 Treatment and follow-up of adverse events

Subjects with AEs that occur during the study must be treated according to daily clinical practice at the discretion of the Investigator.

AEs must be followed up until resolution or to the last treatment visit (Visit 12), whichever comes first. On the last treatment visit, information on new AEs, if any, and stop dates for previously reported AEs must be recorded (if known). AEs assessed as stable by the Investigator at the last treatment visit will not have to be followed-up until resolution.

It is the responsibility of the Investigator to follow-up on all SAEs until the subject has recovered, stabilized, or recovered with sequelae, and to report to the Sponsor all relevant new information using the same procedures and timelines as those for the initial report. Relevant information includes discharge summaries, autopsy reports, and medical consultation.

11.4.4.12 Procedures in case of pregnancy

In case of pregnancy or suspicion of possible pregnancy, the study treatment must be stopped immediately, and the subject should be withdrawn from the study.

Pregnancy itself will not be regarded as an AE unless there is a suspicion that the IP may have interfered with the effectiveness of the contraceptive medication. However, the outcome of all pregnancies (spontaneous miscarriage, elective termination, normal birth, or congenital abnormality) must be followed-up by the Investigator and the Sponsor and documented even after the subject was discontinued from the study.

All pregnancies must immediately be reported by the Investigator to the Sponsor or designee using the Pregnancy Report Form provided in the ISF. Once known, information on the outcome of the pregnancy must also be reported to the Sponsor or designee using the Pregnancy Report Form.

All events of congenital abnormalities, birth defects and spontaneous miscarriages are SAEs and must be handled and reported as such as described in Section 11.4.4.10.

11.4.4.13 Treatment of overdose

An overdose is a dose in excess of the dose specified for subject in this clinical study protocol (CSP).

Overdosing is not likely to occur in this study since all IPs will be administered as single doses by site personnel under medical surveillance. In cases of accidental overdose, standard supportive measures will be adopted as required.

Overdoses must be documented in the eCRF. An overdose associated with AE will be recorded as the AE diagnosis/symptoms in the AE log of the eCRF. An overdose without associated symptoms will only be reported in the subject's medical records and documented in the PD log.

11.5 Assessments related to exploratory endpoints

11.5.1.1 Exploratory subjective parameters

The subjective parameter "product-liking" *vs*. the subject's usual snus/nicotine product of choice will be assessed at 30 minutes with the MCQ "How much did you like the product compared with your usual snus or nicotine product of choice?" on a 3-point scale:

"1 = To a lesser extent, 2 = To the same extent, 3 = To a greater extent."

The allowed deviation from actual time for the MCQ is outlined in Table 11.1-1.

11.5.1.2 Pharmacokinetic sampling related to exploratory endpoints

PK sampling related to exploratory endpoints 2, 4 and 5 is part of the procedure described in Section 11.3.1 above.

11.5.1.3 Nicotine extraction related to exploratory endpoints

Nicotine extraction related to exploratory endpoints 2, 4 and 5 is part of the procedure described in Section 11.4.3 above.

11.6 Appropriateness of measurements

All methods used are commonly used in standard medical care and in phase I clinical studies. Non-compartmental analysis of PK parameters is standard for phase I clinical studies.

12 PROCEDURES FOR BIOLOGICAL SAMPLES

12.1 Sample collection

The sample collection procedure for PK analysis is described in Section 11.3.1.

12.2 Volume of blood

The anticipated volume of blood samples collected during the 5-week study from each subject will be approximately 400 mL (Table 12.2-1). For reference, a regular blood donation consists of between 350 mL to 450 mL (±10 %) and is typically collected in a single occasion for persons weighing at least 45-50 kg [13]. Additional blood samples for safety evaluation may be collected at the discretion of the Investigator.

Table 12.2-1 Estimated blood volumes

Assessment	Estimated number of sampling occasions	Estimated volume per occasion (mL)	Total (mL)
PK blood sampling	132	3	396
HIV, Hepatitis B/C	1	4	4 mL
		Total:	**400**

12.3 Handling, storage, and destruction of laboratory samples

All biological samples will be registered in a biobank at CTC (Swedish Health and Social Care Inspectorate biobank registry number 893).

Any remains from the laboratory samples will be disposed of after analyses.

The samples for analyses of PK parameters will be stored at ≤ -20 °C until analyzed. The samples will be disposed of after the clinical study report (CSR) has been finalized.

12.4 Chain of custody of biological samples

A full chain of custody is maintained for all samples throughout their lifecycle.

CTC keeps full traceability of collected biological samples from the study subjects while in storage at the study site until shipment and keeps documentation of receipt of arrival. The sample receiver (the analytical laboratory) will keep full traceability of the samples while in their storage and during use until used or disposed of.

The Sponsor will keep oversight of the entire lifecycle of the samples through internal procedures, monitoring of study sites and auditing of external laboratory providers.

12.5 Withdrawal of informed consent for donated biological samples

If a subject withdraws consent to the use of biological samples donated, the samples will be disposed of/destroyed, if not already analyzed and documented.

The PI will ensure that:

1. Subject withdrawal of consent is notified immediately to the Sponsor.
2. Biological samples from the subject, if stored at the study site, are immediately identified, disposed of/destroyed and the action is documented.

The Sponsor has to ensure that the laboratory/laboratories holding the samples is/are informed about the withdrawn consent immediately and that samples are disposed of/destroyed or returned to the study site and the action is documented.

13 QUALITY MANAGEMENT, QUALITY ASSURANCE AND QUALITY CONTROL

13.1 Quality management: critical processes, systems, and data identification

During CSP development, the Sponsor will identify those processes, systems (facilities, computerized systems) and data that are critical to ensure human subject protection and the reliability of trial results according to applicable SOPs and the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) E6(R2) guideline [14].

Identified risks will be categorized separately from the CSP.

13.2 Quality assurance and quality control

The Sponsor has delegated the responsibilities outlined below to CTC whilst maintaining overall study oversight:

1. Implementing and maintaining quality assurance and quality control systems with written SOPs with regard to management of identified risks, CSP compliance, good clinical practice (GCP) compliance and applicable regulatory requirements.
2. Securing agreements with involved subcontractors and performing regular subcontractor oversight to ensure CSP compliance, GCP compliance and compliance with applicable regulatory requirements.
3. Implementing a risk-based validated EDC system and maintain SOPs for the whole life cycle of the system.
4. QC application to each stage of data handling to ensure that all data are reliable and have been processed correctly.

14 ETHICAL AND REGULATORY REQUIREMENTS

14.1 Ethical conduct of the study

The study will be performed in accordance with ethical principles that have their origin in the Declaration of Helsinki [15] and are consistent with the ICH E6 (R2) guideline for GCP [14], applicable sections of the Clinical Trials Regulation European Union (EU) no.536/2014 [16], and applicable local regulatory requirements.

14.2 Ethics and regulatory review

The coordinating PI is responsible for submission of the CSP, the subject information and ICF, any other written information to be provided to the subjects and any advertisements used for recruitment of subjects to applicable IEC for approval.

Approval must be obtained in writing from the IEC before the first subject can be recruited.

The Sponsor will provide the IEC and PI with safety updates/reports according to local requirements.

14.3 Subject information and consent

It is 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 are performed.

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

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

Documentation of the discussion and the date of informed consent must be recorded in the source documentation and in the eCRF. The subject information sheet and the signed and dated ICF should be filed by the Investigator for possible future audits and/or inspections.

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

14.4 Subject privacy and data protection

The clinical personnel affirm and uphold the principle of the subject’s right to privacy during and after the study.

The ICF includes information that data will be recorded, collected, and processed and information related to potential transfer to European Economic Area (EEA) or non-EEA countries. In accordance with the General Data Protection Regulation (GDPR [EU] 2016/679) [17], these pseudo-anonymized data will not identify any persons taking part in the study. If any part of the data is handled by any other organization, inside or outside the EU, appropriate agreements and/or other documentation will be established, to ensure that the data processing is performed in accordance with the provisions of the GDPR and other relevant legislation before any data transfer takes place.

The potential subject should be informed that by signing and dating the ICF they approve that the authorized representatives from the Sponsor and CTC, as well as the concerned IEC, have direct access to their medical records for verification of clinical study procedures. For further details on the subject information and ICF process, refer to Section 14.3.

The subject has the right to request access to their personal data and the right to request rectification of any data that is not correct and/or complete in accordance with GDPR [17] and the request will be raised to the PIs.

The Investigator must file a subject identification list which includes sufficient information to link records, *i.e.*, the eCRF and clinical records. This list must be preserved for possible future inspections/audits but must not be made available to the Sponsor except for monitoring or auditing purposes by the authorized representatives from the Sponsor.

Personal data that are collected in the study such as health information and ethnicity are considered as sensitive personal data. This data will be pseudo-anonymized, *i.e.*, personally identifiable information will be removed and replaced by a unique subject ID and will be processed by the Sponsor and other involved parties during the study. After the study ends, only pseudo-anonymized data, *i.e.*, aggregated data sets, can be used.

For this study, the Sponsor 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 are also data processors.

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

14.5 Changes to the approved clinical study protocol

Any proposed change to the approved final CSP, including appendices, will be documented in a written and numbered CSP amendment. All substantial amendments to the CSP must be approved by the appropriate IEC before implementation according to applicable regulations.

14.6 Audits and inspections

Authorized representatives of the Sponsor, or an IEC may perform audits or inspections at the study site, including source data verification (SDV). The purpose of an audit or inspection is to examine all investigation-related activities and documents systematically and independently, to determine whether these activities were conducted, and data were recorded, analyzed, and accurately reported according to the CSP, ICH-GCP guidelines, and any applicable regulatory requirements.

14.7 Insurance

Subjects will be covered under the Sponsor’s liability insurance policy through IF insurances. The certificate of insurance and an information leaflet containing essential information about the insurance coverage can be provided upon request. The participating subjects are also protected in accordance with national regulations, as applicable. CTC has a company insurance covering medical procedures and services performed by CTC. The certificate of insurance can be provided upon request.

15 STUDY MANAGEMENT

15.1 Training of study site personnel

Before inclusion of the first study subject, a Sponsor representative or delegate will perform a study initiation visit at the study sites. The requirements of the CSP and related documents will be reviewed and discussed, and the investigational staff will be trained in any study specific procedures and system(s) utilized.

It is the responsibility of the Investigator to ensure that all personnel involved in the study are fully informed of all relevant aspects of the study and have a detailed knowledge of and training in the procedures that are to be executed by them. Any new information of relevance to the performance of this study must be forwarded to the staff involved in a timely manner.

The Investigator will keep a list of all personnel involved in the study together with their function and study related duties delegated. A Curriculum Vitae will be available for all staff delegated study-specific duties.

15.2 Clinical monitoring

The Sponsor is responsible for securing agreement from all involved parties to ensure direct access to all participating sites, source data/documents, and reports for the purpose of monitoring and auditing by the Sponsor, and inspection by domestic and foreign regulatory authorities.

As defined in the risk-based monitoring (RBM) plan, approved by the Sponsor and provided separately, the responsible Monitor will periodically visit the study sites at times agreed upon by the Investigator and the Monitor. At each monitoring visit, the role of the Monitor is (but not limited to) the following:

- provide information and support to the investigational team,
- confirm that facilities and resources remain acceptable,
- confirm that the investigational team is adhering to the CSP, applicable SOPs, guidelines, manuals, and regulatory requirements,
- verify that data are being accurately and timely recorded in the eCRFs and that IP accountability checks are being performed,
- verify that data in the eCRF are consistent with the clinical records (SDV) in accordance with the RBM plan,
- verify that the correct informed consent procedure has been adhered to for participating subjects,
- ensure that withdrawal of informed consent to the use of the subject’s biological samples will be reported and biological samples are identified and disposed of/destructed accordingly, and that this action is documented and reported to the subject,
- verify that AEs are recorded and reported in a timely manner and according to the CSP,
- raise and escalate any serious quality issues, serious GCP breach and any data privacy breach to the Sponsor.

Centralized monitoring will also be performed continuously by project team members at CTC in accordance with the RBM plan.

When the study has been completed, all queries have been resolved and the database has been locked, the Monitor will perform a close-out visit.

15.3 Source data documents

A separate origin of source data list will be generated before the start of enrolment, specifying the location of the source of derived information appearing in the eCRF. This document must be signed by the PI and the Monitor to confirm agreement before the start of recruitment.

Source documents are all documents used by the Investigator or hospital that relate to the subject's medical history, and that verify the existence of the subject, the inclusion and exclusion criteria, and all records covering the subject's participation in the study. They include laboratory notes, memoranda, material dispensing records, subject files, *etc*. The eCRF may constitute source data if clearly defined in the origin of source data list.

The Investigator must guarantee access to source documents to the Monitor, and the IECs, if required.

15.4 Study agreements

This study is fully financed by the Sponsor, Swedish Match. The management and conduct of the clinical study have been outsourced to the contract research organization (CRO), CTC. The coordinating PI and the PI at each site are employees of CTC.

The agreements between Sponsor and CTC must be in place before any study-related procedures can take place, or subjects be enrolled.

The Sponsor and CRO responsibility and duty split is regulated in a separate clinical study agreement. The PIs must comply with all the terms, conditions, and obligations of the clinical study agreement for this clinical study.

15.5 Study timetable and end of study

The study is expected to start in Q4 2023 and to be completed by Q3 2024.

A subject is considered to have completed the study if they have completed all visits in the study including the last visit.

The end of the study is defined as the date of the last visit of the last subject in the study.

15.6 Termination of the study

The Investigator or the Sponsor may terminate this study prematurely for any reasonable cause. The IEC must be informed promptly. Conditions that may warrant study termination include, but are not limited to, a decision by the Sponsor to suspend or discontinue the development of the IP.

If the study is prematurely terminated or suspended for any reason, the Investigator must promptly inform the study subjects and must assure appropriate follow-up for the subjects.

15.7 Reporting and publication

15.7.1 Clinical study report

After completion of the study, an ICH E3 [18] guideline-compliant CSR describing the conduct of the study, any statistical analyses performed, and the results obtained will be prepared by the Sponsor or their designee. The CSR will be reviewed and approved by, as a minimum, the PI, the Statistician, and the Sponsor.

All results obtained from any exploratory analyses may be reported separately.

15.7.2 Confidentiality and ownership of study data

Any confidential information relating to the IPs or the study, including any data and results from the study, will be the exclusive property of the Sponsor. The Investigator and any other persons involved in the study are responsible for protecting the confidentiality of this proprietary information.

15.7.3 Publication

The results from this study may be submitted for publication at the discretion of the Sponsor.

15.8 Archiving

The PI is responsible for maintaining essential documents, (as defined in ICH E6(R2), Section 8 [14]) for 25 years after finalization of the CSR. This includes any original source documents related to the study, the subject identification list (providing the sole link between named subject source records and pseudonymous eCRF data), the original signed ICFs and detailed records of IP disposition.

It is the responsibility of the Sponsor to inform the Investigator/institution as to when these documents no longer need to be retained.

The Sponsor will archive the TMF in accordance with the ICH E6(2) guideline, Section 8 [14], and applicable regulatory requirements.

The data from the eCRFs will be sent to the Sponsor and a copy will be sent to the study site and filed in the ISF for archiving for 25 years after finalization of the CSR.

The completed original eCRFs are the sole property of the Sponsor and must not be made available in any form to third parties, except for authorized representatives of appropriate health/regulatory authorities, without written permission from the Sponsor.

16 DATA MANAGEMENT

The data management routines include procedures for handling of the eCRF, database set-up and management, data entry and verification, data validation, QC of the database, and documentation of the performed activities including information of discrepancies in the process. The database, data entry screens, and program will be designed in accordance with the CSP.

Data validation/data cleaning procedures are designed to assure validity and accuracy of clinical data. These procedures consist of computerized online edit checks identifying *e.g.*, data values that are outside the allowed range and SAS-programmed batch checks on data exports. All study-specific and standard data validation programming will be tested prior to being used on final data.

Detailed information on data management will be described in a study-specific Data Management Plan.

16.1 The web-based eCRF

Clinical data will be entered into a 21 CFR Part 11-compliant eCRF (Viedoc™) provided by Viedoc Technologies AB. The eCRF includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the source documents or at bedside (if the eCRF data constitutes source data). Source data are to be defined at the sites before inclusion of the first subject (Section 15.3).

Authorized site personnel designated by the Investigator will complete data collection. Appropriate training and security measures will be completed with the Investigator and all authorized trial site personnel prior to the trial being initiated and any data being entered into the system for any study subject.

16.2 The entering of data into the eCRF

All entries, corrections, and alterations are to be made by the Investigator or designee. Neither the Monitor nor any other study team member besides site staff can enter data in the eCRF. All data should be entered in English. The eCRFs should be completed as soon as possible during or after the subject's visit. To avoid inter-observer variability, every effort should be made to ensure that preferably same individual who made the initial baseline determinations completes all corresponding follow-up evaluations. The Investigator must verify that all data entries in the eCRFs are accurate and correct. If some assessments are not done, or if certain information is not available, not applicable, or unknown, the Investigator or assigned clinical staff will record such information in the eCRF.

The Investigator will be required to electronically sign off on the clinical data. This will be performed by means of the Investigator's unique User ID and password; date and time stamps will be added automatically at time of electronic signature.

16.3 The query process

The Monitor will review the eCRFs and evaluate them for completeness and consistency. Data in the eCRF will be compared with the respective source documents to ensure that there are no discrepancies for critical data as described in the RBM plan. All entries, corrections, and alterations are to be made by the Investigator or designee.

If corrections are needed, queries will be raised within the eCRF, either as a result of built-in edit checks or manually raised by the Monitor. An appropriate member of the site staff will answer the queries in the eCRF either by correcting the data or by entering a response to the query.

16.4 Audit trail

All entries in the eCRF will be fully recorded in a protected audit trail. Once clinical data have been saved, corrections to the data fields will be audit trailed, meaning that the reason for change, the name of the person who made the change, together with time and date will be logged.

16.5 External data

External data consists of data that are not recorded in the eCRF. Data may be received in electronic format or as a paper printout. Key variables are defined in order to uniquely identify each sample record. File and data formats are agreed with the external data provider.

16.6 Medical coding

Medical coding will be performed by trained personnel at CTC. AEs and medical/surgical history verbatim terms are coded using the medical dictionary of regulatory activities (MedDRA, latest version available at eCRF setup).

Prior and concomitant medications will be coded according to the WHO anatomic therapeutic chemical (ATC) classification system. All coding will be approved by the Sponsor prior to database lock.

16.7 Database lock

When all data has been entered and discrepancies solved, clean file will be declared, the database will be locked, and the data will be analyzed.

17 STATISTICAL METHODS AND DETERMINATION OF SAMPLE SIZE

The principal features of the statistical analysis to be performed are described in this section. A more technical and detailed elaboration of the principal features will be presented in a separate statistical analysis plan (SAP), which will be signed and approved prior to database lock.

17.1 General

Continuous data will be presented in terms of evaluable and missing observations, arithmetic mean, standard deviation (SD), median, minimum, and maximum value, Q1-Q3 (interquartile range [IQR]).

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

All descriptive summaries and statistical analyses will be performed using SAS Version 9.4 or later (SAS Institute, Inc., Cary, NC). The PK parameters will be calculated by non-compartmental analysis using the software Phoenix WinNonlin® version 8.3 or later (Certara, U.S.A.).

Baseline will be defined as the last data collection point prior to each IP administration.

No adjustment for multiple comparisons will be performed. All formal comparisons will be made towards a designated reference product and all significant findings will be reviewed for medical relevance.

17.1.1 Missing, unused and spurious data

Generally, no imputation of data will be performed. In case of missing start and stop times of AEs that cannot be investigated further, missing data will be imputed according to a worst-case scenario, *i.e.*, start time will be imputed as the closest time point post intake of IP and end time as 23:59, resulting in the longest possible treatment emergent duration of the AE.

Spurious data will be evaluated continuously through data validation (see Section 16) and, if connected to protocol deviations, evaluated case-by-case at the latest prior to declaration of clean file and database lock.

Deviations from the original SAP will be described in the CSR.

Methods for handling of missing, unused, and spurious data will be further specified in the SAP.

17.2 Determination of sample size

About 66 subjects will be screened to achieve 36 randomized subjects and 32 fully evaluable subjects. A fully evaluable subject is defined as a subject who has received all 11 IPs and who has completed all study visits. An effort will be made to randomize at least 14 female subjects (approximately 40 %).

The sample size has been calculated considering the secondary endpoint: to show equivalence between the unflavored NP 6 mg and flavored NP 6 mg products. A lower number of subjects will be needed for the primary endpoint. Using a CV of 29 %, based on previous studies, a power of 80 %, a significance level of 10 %, lower and upper equivalence bounds of 0.8 and 1.25, and a null ratio of 1, 32 evaluable subjects will be needed. Assuming a dropout rate of 10 %, a total of 36 subjects will be randomized.

17.3 Analysis data sets

17.3.1 Full analysis set

The Full Analysis Set (FAS) will consist of all subjects who have been randomized and received at least 1 IP administration and who have at least 1 post-baseline data point. This population will be used for the safety analysis set.

17.3.2 PK analysis set

The PK analysis set (PKAS) will consist of all subjects who received at least 1 IP administration and provided at least 1 evaluable PK-profile and no major deviation or AEs judged to compromise the PK analysis. Individual PK values and/or full profiles for explicit IPs may be excluded from the analysis as specified in the SAP.

17.4 Pharmacokinetic analysis

The PK analysis will be based on the PKAS and performed by CTC. The PK parameters will be calculated by non-compartmental PK analysis using the software Phoenix WinNonlin® version 8.3 or later (Certara Inc, Princeton, New Jersey, U.S.A.). In addition to $AUC_{0\text{-}inf}$ and C_{max} parameters, other non-compartmental PK parameters will be determined in relation to secondary endpoints (see Section 17.7.2 below). Where possible, both baseline-adjusted and non-adjusted PK parameters will be calculated.

For $AUC_{0\text{-}inf}$, the area under the plasma concentration *vs*. time curve will be calculated to the time point of the last quantifiable plasma concentration of nicotine and then extrapolated to infinity using the concentration in the last quantifiable sample and the estimated terminal elimination rate constant ($Lambda_z$).

PK data will be presented for each IP using summary statistics. This data will be presented in terms of N, arithmetic mean, median, SD, minimum and maximum value. For AUC and C_{max} parameters, the geometric mean and CV % will be presented. Categorical data will be presented as counts and percentages, as applicable.

17.5 Description of study population

17.5.1 Demographics and baseline characteristics

Descriptive statistics for demographics, weight and height, BMI as well as history of oral tobacco/nicotine use, and smoking will be presented for all subjects. All data will be listed by subject number.

17.5.2 Medical/surgical history and prior/concomitant medication

Medical/surgical history will be presented by system-organ-class (SOC) and preferred term (PT). Prior/concomitant medications will be presented by ATC level 4 and 5. All data will be listed by subject number.

17.5.3 Treatment compliance

The number of subjects treated with each IP will be presented through listings.

17.5.4 Physical examination

Physical examination parameters will be specified as "normal", "abnormal, not clinically significant", or "abnormal, clinically significant (as judged by the Investigator at the screening visit) and summarized.

All data will be listed by subject.

17.5.5 Vital signs

Vital signs (systolic/diastolic blood pressure, pulse rate) will be summarized by IP. If applicable, data will be presented with absolute and percent change from baseline.

All data will be listed by subject.

17.5.6 Electrocardiogram

All ECGs will be categorized as" normal"," abnormal, not clinically significant", or" abnormal, clinically significant" (as judged by the Investigator at the screening visit) and summarized.

All data will be listed by subject.

17.6 Analysis of primary endpoint

17.6.1 Comparison of the new format unflavored NP 6 mg and comparator ($AUC_{0\text{-}inf}$)

The primary endpoint of this study is to compare nicotine exposure, as measured by baseline-adjusted $AUC_{0\text{-}inf}$ based on nicotine plasma concentrations, between the new format unflavored NP 6 mg product and the comparator product, Longhorn Natural 18 mg.

$AUC_{0\text{-}inf}$ will be calculated as described in Section 17.4 above.

The comparison of $AUC_{0\text{-}inf}$ between the new format unflavored NP 6 mg and the comparator Longhorn Natural 18 mg will be assessed using the following null (H_0) and alternative (H_1) hypotheses:

$$H_0: \frac{\mu_T}{\mu_R} \geq 1.25$$

$$H_1: \frac{\mu_T}{\mu_R} < 1.25$$

where μ_T is the least-squares geometric mean of $AUC_{0\text{-}inf}$ for the new format unflavored NP 6 mg and where μ_R is the least-squares geometric mean of $AUC_{0\text{-}inf}$ for the comparator Longhorn Natural 18 mg.

In the analysis, a mixed model will be used with the natural log of $AUC_{0\text{-}inf}$ as the dependent variable, treatment as a fixed effect and subject as a random effect. The improved Kenward-Roger's approximation for degrees of freedom will be used. The estimated least-squares means (LSMeans) difference between the new format unflavored NP 6 mg and the comparator Longhorn Natural 18 mg will be back transformed into the original scale to present the ratio of geometric LSMeans as well as the corresponding 90 % confidence interval. If the upper bound of the 90 % confidence interval of this ratio is below 1.25, the null hypothesis will be rejected, and it will be concluded that the new format unflavored NP 6 mg product does not result in substantially higher nicotine exposure compared to the comparator product. The model will be estimated for both non-adjusted and baseline-adjusted $AUC_{0\text{-}inf}$.

17.7 Analysis of secondary endpoints

17.7.1 In vivo extracted amount and fraction of nicotine

The difference between the nicotine content of an unused reference pouch and the used study pouch will be used to calculate the *in vivo* extracted amount of nicotine for each IP. The mean of the extracted amount (mg/unit) and extracted fraction (%) of nicotine between unflavored NP 3 mg, the unflavored NP 6 mg and flavored 6 mg products and the comparator product Longhorn Natural 18 mg will be calculated. The amount of nicotine in the reference pouches and in used pouches will be presented through descriptive statistics.

17.7.2 Pharmacokinetics of nicotine in plasma

The following non-compartmental PK parameters will be determined for each IP: $AUC_{0\text{-}inf}$, $AUC_{0\text{-}last}$, $AUC_{0\text{-}1.5h}$, C_{max}, T_{max} and $T_{1/2}$. Where possible, both baseline-adjusted and non-adjusted PK parameters will be calculated.

C_{max} and T_{max} will be derived from the observed nicotine plasma concentration data. $AUC_{0\text{-}1.5h}$ and $AUC_{0\text{-}last}$ will be calculated using log-linear trapezoidal interpolation. Calculations will be based on the actual sampling times recorded during the study. Concentrations below the lower limit of quantification (LLOQ) occurring before C_{max} will be treated as zero. Concentrations below LLOQ occurring after C_{max} will be omitted from the analysis. All baseline-adjusted PK parameters will be corrected for nicotine plasma concentrations at baseline (pre-administration).

$AUC_{0\text{-}inf}$ will be calculated as described in Section 17.4 above.

The equivalence in $AUC_{0\text{-}inf}$ and C_{max} between the unflavored NP 6 mg and each flavored NP 6 mg will be assessed using a mixed model with the natural log of AUC_{inf} as the dependent variable, treatment as a fixed effect and subject as a random effect. The improved Kenward-Roger's approximation for degrees of freedom will be used. The estimated LSMeans difference between unflavored NP 6 mg and flavored NP 6 mg will be back transformed into the original scale to present the ratio of geometric LSMeans as well as the corresponding 90 % confidence interval. If the 90% confidence interval of this ratio falls within the range 0.8-1.25, the IPs will be considered equivalent. The model will be estimated for both non-adjusted and baseline adjusted $AUC_{0\text{-}inf}$.

17.7.3 Pharmacodynamic effects

The analyses of pulse rate as a measure of the PD effect of nicotine will be further described in the SAP. Data will be presented as summary tables of descriptive statistics and plots of pulse rates over time for each IP.

The subjective parameters "craving", "satisfaction", "product-liking" and "intent to use again", measured by VAS, will be summarized for each IP using descriptive statistics as a total score during the IP administration phase. The relative and absolute change from baseline will also be calculated for the subjective parameter "craving" for each IP.

To determine the maximum PD effect attributable to the IP, the highest recorded increase (E_{max}) as well as time to first instance of E_{max} will be calculated for the pulse rates as well as for the subjective parameter "craving". For the subjective parameter "satisfaction", which does not have a baseline, the maximum value will be recorded as E_{max} along with time to E_{max}.

17.7.4 Adverse events

An overview of all AEs, including SAEs, intensity, and deaths will be presented by IP. The incidence of AEs and SAEs will be summarized by SOC and PT by IP.

All AE data will be listed by subject number and include the verbatim term entered by the Investigator.

17.8 Analysis of exploratory objectives

17.8.1 Exploratory subjective parameter

The subjective parameter "product-liking" *vs*. the subjects' usual snus/nicotine product of choice, measured through MCQ, will be summarized for each IP using descriptive statistics.

17.8.2 Extraction normalized $AUC_{0\text{-}inf}$ and C_{max}

Nicotine extraction-normalized PK parameters $AUC_{0\text{-}inf}$ and C_{max} (with and without baseline-adjustment) will be determined for the unflavored NP 3 mg and 6 mg products and the comparator product, Longhorn Natural 18 mg as well as for the unflavored NP 6 mg and flavored NP 6 mg products. The difference in $AUC_{0\text{-}inf}$ and C_{max} and the extracted amount and fraction of nicotine between the flavored NP 6 mg product with the highest concentration and Longhorn Natural 18 mg will be analyzed. Extraction-normalized $AUC_{0\text{-}inf}$ and C_{max} data will be presented for each IP using summary statistics.

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19 SIGNATURES

19.1 Principal Investigator statement

I, the undersigned, have read and understood this CSP and agree to conduct the trial accordingly and to comply with the Investigator obligations stated in this CSP, GCP and applicable regulatory requirements.

19.2 Approval of the clinical study protocol

I, the undersigned, approve this CSP.

Sponsor signatory

Coordinating Principal Investigator