
Clinical Study Protocol

Investigational product	N/A
Study code	SM22-03
Protocol version and date	Final version 1.0; 09NOV2022

**ASSESSING BIOMARKERS OF EXPOSURE IN PLASMA AND URINE IN
CURRENT, DAILY USERS OF NICOTINE POUCHES, TOBACCO-BASED
SNUS, OR COMBUSTIBLE CIGARETTES, OR NONUSERS OF
TOBACCO/NICOTINE PRODUCTS.**

Test product and dose

N/A

Sponsor signatory

(b) (6)

Swedish Match
Maria Skolgata 83
SE-118 53 Stockholm
Sweden
Phone: (b) (6)

(b) (6)

Coordinating/Principal Investigator

(b) (4), (b) (6)

Clinical study conduct and management

(b) (4)

This clinical trial protocol is the property of Swedish Match and is a confidential document. It is not to be copied or distributed to other parties without written approval from Swedish Match.

1 STUDY SYNOPSIS

Study title	
Assessing biomarkers of exposure in plasma and urine in current, daily users of nicotine pouches, tobacco-based snus, or combustible cigarettes, or nonusers of tobacco/nicotine products.	
Study code	Planned study period
SM22-03	Q1 2023 to Q2 2023
Coordinating/Principal Investigator	
(b) (6)	
(b) (4)	
Study design	
This is a cross-sectional, 4-group, non-randomized study, designed to assess biomarkers of exposure (BoE) and biomarkers of potential harm (BoPH) in current, daily users of nicotine pouches, tobacco-based snus, or combustible cigarettes, or nonusers of tobacco/nicotine products. The subjects in the 3 nicotine user groups will use their product of choice <i>ad libitum</i> throughout the 14 days study period.	
Objectives	
<u>Primary objective</u>	
<ul style="list-style-type: none"> To compare plasma concentrations of nicotine, cotinine, 3'-trans-hydroxycotinine (OH-cotinine), 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), and N-nitrosonornicotine (NNN) between users of nicotine pouches, tobacco-based snus, or combustible cigarettes, and nonusers of tobacco/nicotine products. 	
<u>Secondary objectives</u>	
<ol style="list-style-type: none"> To compare urine concentrations of nicotine and its metabolites and tobacco-specific nitrosamines (TSNAs) between users of nicotine pouches, tobacco-based snus, or combustible cigarettes, and nonusers of tobacco/nicotine products. To compare urine concentrations of anatabine, anabasine, and 3-hydroxybenzo(a)pyrene (3-OH-BaP) between users of nicotine pouches, tobacco-based snus, or combustible cigarettes, and nonusers of tobacco/nicotine products. To compare urine concentrations of eicosanoids in urine between users of nicotine pouches, tobacco-based snus, or combustible cigarettes, and nonusers of tobacco/nicotine products. To compare plasma concentrations of soluble intercellular adhesion molecule-1 (sICAM-1) and growth differentiation factor 15 (GDF-15) between users of nicotine pouches, tobacco-based snus, or combustible cigarettes, and nonusers of tobacco/nicotine products. To compare the extracted amounts and fractions of nicotine, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), and NNN from nicotine pouches and tobacco-based snus. To evaluate the safety and tolerability of nicotine pouches, tobacco-based snus, and combustible cigarettes in current users of these nicotine products. 	
<u>Exploratory objective</u>	
<ol style="list-style-type: none"> To correlate the extracted amounts of nicotine, NNN, and NNK, multiplied by the used number of pouches, with plasma and urine concentrations of BoE. 	

2. To analyze the pattern of use between users of nicotine pouches, tobacco-based snus, and combustible cigarettes.

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

Endpoints

Primary endpoint

- Difference in plasma concentrations of nicotine, cotinine, OH-cotinine, NNAL, and NNN between users of nicotine pouches, tobacco-based snus, or combustible cigarettes, and nonusers of tobacco/nicotine products.

Secondary endpoints

1. Difference in urine concentrations of total nicotine equivalents and TSNAs (NNAL, NNN, N'-nitrosoanabasine [NAB], and N'-nitrosoanatabine [NAT]) between users of nicotine pouches, tobacco-based snus, or combustible cigarettes, and nonusers of tobacco/nicotine products.
2. Difference in urine concentrations of anatabine, anabasine, and 3-OH-BaP between users of nicotine pouches, tobacco-based snus, or combustible cigarettes, and nonusers of tobacco/nicotine products.
3. Difference in urine concentrations of eicosanoids (8-iso prostaglandin F2 α , 11-dehydrothromboxane B2, 2,3-dinor-thromboxane B2, and leukotriene E4) between users of nicotine pouches, tobacco-based snus, or combustible cigarettes, and nonusers of tobacco/nicotine products.
4. Difference in plasma concentrations of sICAM-1 and GDF-15 between users of nicotine pouches, tobacco-based snus, or combustible cigarettes, and nonusers of tobacco/nicotine products.
5. Difference in the extracted amounts (mg/unit) and fractions (%) of nicotine, NNK, and NNN from nicotine pouches and tobacco-based snus.
6. Frequency, seriousness, and intensity of adverse events (AEs).

Exploratory endpoint

1. The correlation of the extracted amounts (mg/unit) of nicotine, NNN, and NNK, multiplied by the used number of pouches, with plasma and urine concentrations of BoE for users of nicotine pouches and tobacco-based snus.
2. Difference in the pattern of use between users of nicotine pouches, tobacco-based snus, and combustible cigarettes.

Number of subjects planned

The study will include a total of approximately (b) (4) subjects: (b) (4) subjects who are exclusive nicotine pouch users, (b) (4) subjects who are exclusive tobacco-based snus users, (b) (4) subjects who are exclusive users of combustible cigarettes, and (b) (4) subjects who are nonusers of tobacco/nicotine products. An effort will be made to include at least (b) (4) female subjects ((b) (4)) in each group, however a minimum of (b) (4) female subjects ((b) (4)) will be considered acceptable.

Diagnosis and eligibility criteria

Healthy male and female subjects aged ≥ 25 to ≤ 45 years meeting the criteria for each group, respectively, will be considered to be eligible for participation in the study. The criteria for the 4 groups are: A) exclusive users of a Swedish Match brand nicotine pouch product, with a nicotine content between 3 and 16 mg per pouch, for ≥ 1 year, with a minimum daily consumption of 4 or more pouches, prior to screening; B) exclusive user of a Swedish tobacco-based snus product, with a nicotine content between 4 and 20 mg per pouch, for ≥ 1 year, with a minimum daily consumption of 4 or more pouches, prior to screening; C) exclusive user of a commercially manufactured combustible cigarette product, for ≥ 1 year, with a minimum daily consumption of 4 or more combustible cigarettes, prior to screening; and D) nonusers of tobacco/nicotine products who have

used <100 units of tobacco/nicotine products during their lifetime, with no usage during last 1 year. If the nicotine product user groups (nicotine pouches, tobacco-based snus, or combustible cigarettes) use different brand, type, flavor, and nicotine strength, only one type of product should be used during the 14-day study period. No exposure to passive smoking (from living with someone who smokes at home) may occur in any of the study groups, except for the smokers.

All subjects must be willing to comply with study procedures and give written informed consent. 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 BoE or may put the subject at risk because of participation in the study, and/or intend to stop using nicotine-containing products, will be excluded from the study.

Methodology

This is a multi-center, cross-sectional, 4-group, non-randomized study, designed to assess BoE and BoPH in current, daily users of nicotine pouches, tobacco-based snus, or combustible cigarettes, or nonusers of tobacco/nicotine products. The subjects in the 3 nicotine user groups will use their product of choice *ad libitum* throughout the 14 days study period.

All subjects will provide informed consent prior to study procedures. The subjects will report to the study sites for a screening visit (Visit 1), followed by 1 (nonusers) or 2 (users of nicotine pouches, tobacco-based snus, and combustible cigarettes) study visits (Visit 2 and Visit 3).

Screening (Visit 1) will take place within 4 weeks prior to Visit 2 and will include an eligibility check, including evaluations of smoking and oral tobacco/nicotine product use, a brief physical examination, laboratory tests, electrocardiograms (ECG) and collection of medical history, vital signs (pulse rate and blood pressure), height, weight, body mass index (BMI) and lung function test/spirometry. The subjects will not be allowed to eat within 1 hour prior to spirometry assessments, nor will subjects be allowed to use any kind of tobacco/nicotine product within 1 hour prior to these assessments. Compliance with the present criteria in terms of nicotine use (Group A, B, C) and abstinence (Group D), respectively, will be assessed by urinary cotinine strip test (cotinine cut off: ≥ 200 ng/mL for tobacco/nicotine use; < 200 ng/mL for nonusers of tobacco/nicotine products). During screening, subjects using tobacco/nicotine products (Group A, B, C) will choose 1 product which they will exclusively use during the study. This shall be the product brand that they have mostly used in the past month in case they are not exclusive users of 1 product brand. Note that this also implies nicotine strength and flavor variations of the same brand. The brand, including nicotine strength and flavor, will be documented in the electronic case report form (eCRF) during screening, at Visit 2, as well as at the end-of study visit (Visit 3).

During the screening visit, all subjects (including the nonusers of tobacco/nicotine products) will be informed how to collect the first morning urine void and be provided with a urine sample collection container and a cooling bag for transportation to the study sites. Users of nicotine pouches and tobacco-based snus will also be provided with collection containers and another cooling bag for their used pouches.

All subjects will report to the study sites for Visit 2. Blood will be collected for the analysis of plasma concentrations of nicotine, cotinine, OH-cotinine, NNAL, and NNN in users of nicotine pouches, tobacco-based snus, and combustible cigarettes. From this visit, the users of nicotine pouches, tobacco-based snus, and combustible cigarettes will exclusively use their product of choice *ad libitum*, following their regular pattern of use, and document their consumption via an electronic diary during the 14-day study period (once per day). The product of choice will be documented in the eCRF. Also, the users of nicotine pouches and tobacco-based snus will collect 4 used pouches on 2 separate days the first week (Samples A) and on 2 separate days the second week (Samples B) and store these in a freezer ($\leq -18^{\circ}\text{C}$). In total, 8 pouches will be collected per week. For the nonusers of tobacco/nicotine products blood and urine for all analysis of BoE and BoPH will be collected at 1 study visit (Visit 2). Thus, this group of subjects will also bring their morning urine void, collected

by the subject in the provided container and placed in the cooling bag, at the time of this study visit (Visit 2) and will not need to report to the study sites for Visit 3.

After 14 days, the users of nicotine pouches, tobacco-based snus, and combustible cigarettes report to the study sites for Visit 3. The subjects will bring their morning urine void to the study sites, collected in the container, and placed in the cooling bag provided during screening (Visit 1). The subjects will be interviewed about experienced AEs, used brand, nicotine strength and flavor and there will be a compliance check of the electronic diary. Also, the users of nicotine pouches and tobacco-based snus will bring their used and frozen pouches collected on 4 separate days (in a separate cooling bag to avoid cross contamination with the urine sample). Blood will be collected from all subjects (users of nicotine pouches, tobacco-based snus, and combustible cigarettes) for analysis of BoE and BoPH.

If the subjects forget to bring the collected morning urine void, they shall inform the study sites and a new appointment will be made as soon as possible (preferably the next day). If the nicotine pouch and tobacco-based snus users forget to bring their used pouches to the study site at Visit 3, they shall re-visit the study sites as soon as possible (preferably the same day) after performing the assessments.

Based on the information in the product use diary, the Sponsor will purchase the applicable products used by the subjects in Group A and B for chemical characterization of the unused pouches.

Investigational Product, dosage, and mode of administration

There will be no investigational nor test product provided or examined in this study.

- Subjects in Group A will be required to use exclusively one brand of Swedish Match nicotine pouch product (3-16 mg nicotine per pouch) throughout the study.
- Subjects in Group B will be required to use exclusively one brand of tobacco-based snus product (4-20 mg nicotine per pouch) throughout the study.
- Subjects in Group C will be required to use exclusively one brand of commercially manufactured combustible cigarettes product throughout the study.
- Subjects in Group D will be required to continue to not use tobacco/nicotine products from screening to Visit 2.

Duration of treatment

Fourteen (14) days of *ad libitum* use of the study products by the nicotine product user groups (nicotine pouches, tobacco-based snus, and combustible cigarettes). The nonusers will remain abstaining from tobacco/nicotine products.

Duration of each subject's involvement in the study

Each subject (tobacco/nicotine products users) is expected to participate in the study for 14 days, not including the up to 28-day screening period. The nonusers of tobacco/nicotine products will participate in the study for one day, not including the preceding screening period.

Analysis of biomarkers

- Analysis of baseline plasma concentrations of nicotine, cotinine, OH-cotinine, NNAL and NNN in blood.
- Analysis of plasma and urine concentrations of BoE and BoPH after 14 days of use of the study products.

Chemical analysis of study products

The content of nicotine, NNK, and NNN in the unused study products will be subjected to chemical analysis.

Nicotine extraction assessment

The extracted amount and fraction of nicotine, NNK, and NNN will be calculated by subtracting the average of the pouches used by the nicotine pouch and tobacco-based snus users on (b) (4)

Safety assessments

AEs will be collected from Visit 2 and up until Visit 3 (end-of study visit).

Statistical methods

Descriptive statistics will be provided overall for the parameters collected during the study based on the analysis population (group). Arithmetic mean (mean), geometric mean (GM), standard deviation (SD), coefficient of variation (CV), median, minimum (min), maximum (max) and interquartile range (IQR) will be calculated for metric parameters, additionally graphical presentation of data using box plots where applicable. Categorical and ordinal parameters will be summarized using the number and percentages of subjects in each group.

Analyses regarding group differences will be performed using a significance level of 5% ($p < 0.05$).

Individual subject data will be listed by subject number, study group, 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 collected prior to the start of the 14 days *ad libitum* usage period.

No adjustment for multiple comparisons will be made. No imputation of missing data will be performed.

Study reporting

After completion of the study, an International Council for Harmonization (ICH) E3 guideline-compliant CSR will be prepared.

2 TABLE OF CONTENTS

1	STUDY SYNOPSIS.....	2
2	TABLE OF CONTENTS.....	7
3	LIST OF ABBREVIATIONS AND DEFINITION OF TERMS.....	12
4	IMPORTANT MEDICAL PROCEDURES TO BE FOLLOWED BY THE INVESTIGATOR.....	14
4.1	Medical emergencies contact.....	14
5	INVESTIGATOR AND STUDY ADMINISTRATIVE STRUCTURE.....	15
6	INTRODUCTION.....	17
6.1	Background.....	17
6.2	Study rationale.....	17
6.3	Risk/benefit assessment.....	18
6.3.1	General risk/benefit assessment	18
6.3.2	Risk/benefit conclusion	19
6.3.3	Risk assessment with regard to the Covid-19 pandemic.....	19
7	STUDY OBJECTIVES AND ENDPOINTS	21
7.1	Study objectives	21
7.1.1	Study endpoints	21
8	STUDY DESIGN	23
8.1	Overall study design and schedule of events.....	23
8.2	Rationale for study design	27
9	STUDY POPULATION.....	28
9.1	Recruitment	28
9.2	Screening and enrolment log	28
9.3	Number of subjects.....	28
9.4	Inclusion criteria	29
9.4.1	Additional inclusion criteria for Group A (Users of Swedish Match brand nicotine pouch products)	29
9.4.2	Additional Inclusion Criteria for Group B (Users of tobacco-based snus products).....	29
9.4.3	Additional Inclusion Criteria for Group C (Users of combustible cigarettes)	29
9.4.4	Additional Inclusion Criteria for Group D (Nonusers)	30
9.5	Exclusion criteria.....	30
9.5.1	Additional Exclusion Criteria for users of nicotine pouches (Group A):	31
9.5.2	Additional Exclusion Criteria for users of tobacco-based snus (Group B):.....	31
9.5.3	Additional Exclusion Criteria for users of combustible cigarettes (Group C):.....	31
9.5.4	Additional Exclusion Criteria for nonusers of tobacco/nicotine products (Group D):	31

9.6	Restrictions during the study	31
9.6.1	General restrictions	31
9.6.2	Prior and concomitant therapy	32
9.7	Screen failures	32
9.8	Subject withdrawal	32
9.8.1	General withdrawal criteria	32
9.8.2	Procedures for discontinuation of a subject from the study	33
9.8.3	Subject replacement	33
10	STUDY TREATMENTS	34
10.1	Identity of investigational products	34
10.2	Treatment administration	34
10.3	Continuation of treatment with investigational product	34
10.4	Treatment compliance	34
10.5	Return and destruction of investigational product	34
11	STUDY ASSESSMENTS.....	35
11.1	Recording of data	35
11.2	Demographics and other baseline characteristics	35
11.2.1	Informed consent.....	35
11.2.2	Eligibility criteria	35
11.2.3	Demographic information	35
11.2.4	Height, weight, and body mass index	35
11.2.5	Medical/surgical history	35
11.2.6	History of tobacco/nicotine product use.....	35
11.2.7	HIV and hepatitis B/C	35
11.2.8	Pregnancy test	35
11.2.9	Urine drug screen	36
11.2.10	Alcohol test	36
11.2.11	Urine cotinine screen.....	36
11.2.12	Lung function and spirometry	36
11.2.13	Baseline symptoms.....	36
11.2.14	Prior and concomitant medication.....	36
11.3	Assessments related to primary and secondary endpoints.....	37
11.3.1	Assessment of biomarkers.....	37
11.3.2	Analysis of selected biomarkers.....	37
11.3.3	Analysis of BoE and BoPHs	37
11.4	Assessment related to secondary endpoints.....	38
11.4.1	Nicotine, NNK, and NNN extraction from pouches	38

11.4.2	Adverse events	38
11.4.3	Vital signs.....	42
11.4.4	Electrocardiogram	42
11.4.5	Laboratory assessments.....	42
11.4.6	Physical examinations	43
11.5	Assessments related to exploratory endpoints.....	43
11.6	Appropriateness of measurements	43
12	PROCEDURES FOR BIOLOGICAL SAMPLES.....	44
12.1	Sample collection	44
12.2	Blood sampling	44
12.3	Morning urine void	44
12.4	Volume of blood.....	44
12.5	Handling, storage, and destruction of laboratory samples.....	45
12.6	Chain of custody of biological samples.....	45
12.7	Withdrawal of informed consent for donated biological samples	45
12.8	Collection of used pouches.....	45
13	QUALITY MANAGEMENT, QUALITY ASSURANCE AND QUALITY CONTROL.....	46
13.1	Quality management: critical process, system, and data identification.....	46
13.2	Quality assurance and quality control	46
14	ETHICAL AND REGULATORY REQUIREMENTS	47
14.1	Ethical conduct of the study	47
14.2	Ethics and regulatory review	47
14.3	Subject information and consent	47
14.4	Subject privacy and data protection.....	47
14.5	Changes to the approved clinical study protocol.....	48
14.6	Audits and inspections	48
14.7	Insurance.....	48
15	STUDY MANAGEMENT	49
15.1	Training of study sites personnel	49
15.2	Clinical monitoring	49
15.3	Source data documents	50
15.4	Study agreements	50
15.5	Study timetable and end of study.....	50
15.6	Termination of the study	50
15.7	Reporting and publication.....	50
15.7.1	Clinical study report	50

15.7.2	Annual safety report	51
15.7.3	Confidentiality and ownership of study data.....	51
15.7.4	Publication.....	51
15.8	Archiving.....	51
16	DATA MANAGEMENT	52
16.1	The web-based eCRF	52
16.2	The entering of data into the eCRF	52
16.3	Electronic patient reported outcome	52
16.4	The query process.....	53
16.5	Audit trail.....	53
16.6	External data	53
16.7	Medical coding.....	53
16.8	Database lock	53
17	STATISTICAL METHODS AND DETERMINATION OF SAMPLE SIZE	54
17.1	General	54
17.2	Determination of sample size	54
17.3	Analysis data sets.....	54
17.4	Description of study population	54
17.4.1	Demographics and baseline characteristics	54
17.4.2	Medical/surgical history and prior/concomitant medication.....	55
17.4.3	Treatment compliance	55
17.4.4	Analysis of biomarkers.....	55
17.4.5	Analysis of extracted amount of nicotine, NNK, and NNN.....	55
17.4.6	Adverse events	56
17.4.7	Vital signs.....	56
17.4.8	Electrocardiogram	56
17.4.9	Laboratory analysis	56
17.4.10	Physical examinations	56
17.5	Analysis of exploratory objectives	56
17.5.1	Correlation analysis.....	56
17.5.2	Pattern of use	58
18	REFERENCES	59
19	SIGNATURES	61
19.1	Principal Investigator statement.....	61
19.2	Approval of the clinical study protocol	62

List of tables

Table 4.1-1	Medical emergencies contact	14
Table 8.1-1	Schedule of events	25
Table 11.3-1	BoEs and BoPHs to be analyzed in plasma and urine.	37
Table 11.4-1	Laboratory parameters	43
Table 12.4-1	Estimated blood volumes, Group A - C	44
Table 12.4-2	Estimated blood volumes, Group D	44
Table 17.5-1	Correlation analysis models	57

3 LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

Abbreviation	Explanation
ADL	Activities of daily living
AE	Adverse event
ATC	Anatomical therapeutic chemical
BMI	Body mass index
BoE	Biomarker of exposure
BoPH	Biomarker of potential harm
CA	Competent authority
CSP	Clinical study protocol
CSR	Clinical study report
(b) (4)	(b) (4)
CTCAE	Common terminology criteria for adverse events
CV	Coefficient of variation
CVD	Cardiovascular disease
ECG	Electrocardiogram
eCRF	Electronic case report form
EDC	Electronic data capture
EEA	European Economic Area
EU	European Union
GCP	Good clinical practice
GDF-15	Growth differentiation factor 15
GDPR	General data protection regulation
HIV	Human immunodeficiency virus
ICF	Informed consent form
ICH	International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use
IEC	Independent ethics committee
LTE ₄	Leukotriene E ₄
NAB	N-Nitrosoanabasin
NAT	N- Nitrosoanatabin
NNAL	4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanol
NNK	4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone
NNN	N-Nitrosornicotine
OH-Cot	3'-trans-Hydroxycotinine

Abbreviation	Explanation
PI	Principal Investigator
QC	Quality control
QRS interval	(ECG) The time required for stimulus to spread through the heart's ventricles
QT interval	(ECG) The time from the beginning of the QRS complex to the end of the T wave
QTcF	(ECG) Corrected QT interval by Fredericia
SAE	Serious adverse event
sICAM-1	Soluble intercellular adhesion molecule-1
SOC	System organ class
SOP	Standard operating procedures
TMF	Trial master file
TSNA	Tobacco-specific nitrosamine
WHO	World Health Organization
WOCBP	Women of childbearing potential

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 11.4.2.9.

In the case of a medical emergency, the Investigator may, contact the medically responsible person at Swedish Match AB (Table 4.1-1).

Table 4.1-1 Medical emergencies contact

Name	Function in the study	Contact information
(b) (6)	Medically responsible person at Swedish Match AB	(b) (6)

5 INVESTIGATOR AND STUDY ADMINISTRATIVE STRUCTURE

Sponsor

Swedish Match
Maria Skolgata 83
SE-118 53 Stockholm
Sweden

Sponsor's Medical Representative

(b) (6)

Phone: (b) (6)

E-mail: (b) (6)

Sponsor's Project Manager

(b) (6)

Phone: (b) (6)

E-mail: (b) (6)

Clinical conduct

(b) (4)

Coordinating /Principal Investigator

(b) (6)

Phone: (b) (6)

E-mail: (b) (6)

Study management

(b) (4)

Clinical Research Manager

(b) (6)

Phone: (b) (6)

E-mail: (b) (6)

Biostatistician

(b) (6)

Phone: (b) (6)

E-mail: (b) (6)

Medical Writer (Author of the clinical study protocol [CSP])

(b) (6)

Phone: (b) (6)

E-mail: (b) (6)

Laboratory (clinical chemistry and microbiology)

(b) (4)

Laboratory (bioanalysis)

(b) (4)

Laboratory (extraction analysis)

Analytical, Product & Regulatory Science
Swedish Match North Europe AB
Maria Skolgata 83
SE-118 53 Stockholm, Sweden

**Electronic data capture (EDC) system
provider**

(b) (4)

Signatures are provided in Section [19](#).

6 INTRODUCTION

6.1 Background

Tobacco use, particularly smoking of combustible cigarettes, is associated with an increased risk for diseases such as cancer, cardiovascular diseases (CVDs), and chronic obstructive pulmonary diseases [1]. The combustion of cigarettes results in numerous smoke toxicants which are inhaled and rapidly taken in by the smoker leading to the increasing health risks which correlate with the duration of smoking and number of cigarettes smoked per day. As of today, smoking remains the number one preventable death with more than 8 million deaths globally each year [2]. The World Health Organization (WHO) set effective tobacco control measures which have been implemented – at least in parts – in 24 countries leading to a substantial reduction in cigarette sales [3]. Yet, with smoking rates over 30% of the adult population in some regions/countries in the world, smoking of combustible cigarettes will remain a great public health risk in the coming decades.

The concept of tobacco harm reduction embraced alternative nicotine and tobacco products of potentially reduced risk as a tool to reduce toxicant exposure. In 2011, the US Institute of Medicine defined tobacco harm reduction as a concept to decrease total mortality without completely eliminating tobacco and nicotine use, calling for the development of product alternatives that raise less risk to the consumer [4]. Sweden plays an outstanding role in the fight against the worldwide smoking epidemic with the lowest prevalence of smoking and less smoking-related deaths within the whole European Union (EU) [5]. In 2015, only 11 % of the adult population in Sweden smoked, which is partly attributed to the growing prevalence of snus use as an alternative tobacco product [6]. Numerous studies support the benefits for public health in Sweden due to the switch in tobacco use from smoking to snus. An extensive review citing more than 250 studies supporting the reduced health risks associated with snus use concluded that Swedish snus bears a reduced risk compared to most other tobacco products, including other forms of traditional smokeless tobacco [7].

Oral nicotine pouches as another emerging smokeless product category gained popularity over the past years. In contrast to snus, these products contain no tobacco and as such generally have a low burden (if any) from tobacco-derived toxicants such as tobacco-specific nitrosamines (TSNAs) [8]. Hence, these products have the potential to further reduce tobacco-related harm. However, in order to substantiate these findings and to categorize these products in the risk continuum of tobacco products, an exposure assessment in exclusive users is indispensable [9].

6.2 Study rationale

To better assess the health risks attributed to different types of nicotine delivery products, it is important to analyze the chemical composition of the products as well as the consumers actual exposure to these substances. This is influenced by product usage as well as by the uptake of substances in these products and can be quantified by assessing adequate biomarkers of exposure (BoE).

Use of traditional smokeless tobacco products exposes the consumer to TSNAs like 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and N-nitrosornicotine (NNN), which are human carcinogens. These are of particular importance in terms of harm reduction with respect to smokeless and oral tobacco use as these (tobacco-derived) constituents may cause oral, esophageal, and pancreatic cancer in smokeless tobacco users [9, 10, 11, 12].

Swedish snus shows the lowest TSNA concentrations reported for tobacco-based smokeless tobacco products known so far, partly due to the use of pasteurization as the primary tobacco-

processing method [13]. Tobacco-free, oral nicotine pouches have the potential to further reduce the risk from TSNA exposure as suggested from recent chemical characterization studies [8].

In order to explore the actual exposure to TSNA in users of nicotine pouches related to Swedish snus users as well as smokers of combustible cigarettes and nonusers of tobacco/nicotine products, applicable BoE for TSNA exposure will be assessed in plasma and urine. In addition, this study includes the measurement of biomarkers of potential harm (BoPH) in order to investigate the potentially reduced risk of these products with respect to cardiovascular disease (CVD) and cancer. Such data are needed to categorize the products on the risk continuum scale of tobacco and nicotine use.

This study aims to: 1) assess BoE in plasma and urine in current, daily users of nicotine pouches, tobacco-based snus, or combustible cigarettes, and nonusers of tobacco/nicotine products; 2) investigate the potential pathophysiological impact of the exposure from the different types of nicotine delivery products by measuring BoPH related to CVD and cancer in plasma and urine, and 3) assess the extracted amount and fraction of nicotine and TSNA from pouches used by nicotine pouch and tobacco-based snus users.

6.3 Risk/benefit assessment

6.3.1 General risk/benefit assessment

Participants in the study will not, within the ramification of the study design, be exposed to any new form or dose of a nicotine product. The subjects in the study groups that will use nicotine products (nicotine pouches, tobacco-based snus, or combustible cigarettes) are required to have been daily users of these products for at least 1 year to be eligible for participation in this study. Thus, these subjects will be 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 these subjects. Overdosing is not likely to occur as the subjects in these study groups are current, daily nicotine users that are experienced to nicotine exposure. The nonusers of tobacco/nicotine products will remain abstaining from tobacco/nicotine products and the above-mentioned risks do thus not apply.

Pregnant and breastfeeding subjects, and individuals with a history of hypertension or any CVD, who may be particularly vulnerable to nicotine exposure, will be excluded from participation. 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 nicotine products.

The potential adverse events (AEs) of the study procedures, which are likely to be minor and/or clinically insignificant, will from a research ethics perspective be counterbalanced by increasing the knowledge about the exposure of nicotine pouches, tobacco-based snus, and combustible cigarettes users to some key biomarkers which may impact their health.

Urine will be collected non-invasively and is thus not expected to be associated with any risks for the subjects. Collection of blood for analysis of BoE and BoPH in plasma will be performed using an indwelling venous catheter. This device is used in routine medical care and the risk associated with its use is considered low and ethically justifiable.

The PI at the study sites will ascertain adequate facilities and procedures are available to handle any emergency situations that may occur during the study. The medical staff at (b) (4)

have extensive experience in clinical studies and there are adequate procedures in place to handle unexpected and expected AEs.

In analogy with a regular Phase I study in healthy subjects, there will be 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. Hence, the safety and wellbeing of the subjects are of outmost importance.

Smoking causes many diseases, such as cancer, pulmonary disease, and CVD and reduces overall general health. Use of tobacco-based snus is, by definition, not associated 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 tobacco-based snus products has substantially lower health risks than cigarette smoking. However, tobacco-based snus products typically contain low levels of TSNAs. So, although the health effects are substantially smaller for tobacco-based snus compared to cigarette smoking, some adverse effects cannot be ruled out.

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. Nicotine pouch products are an example of such a development, and the use, prevalence and variety of these products has increased globally in recent years. Nicotine pouches constitute a substitute to both combusted or non-combusted tobacco/nicotine-containing inhalation products (*e.g.*, conventional cigarettes, heated tobacco vaporizers or electronic cigarettes) and to oral tobacco products (*e.g.*, tobacco-based snus and moist snuff).

Limited data are currently present regarding the exposure to BoE and BoPH in users of various nicotine products. As nicotine pouches are a new category, clinical exposure data are scarce, and it is of importance to characterize the exposure to BoE, attributed to nicotine pouch use and link these results to product composition and nicotine extraction. As tobacco-based snus use seems to define a relatively safe level of nicotine exposure it is also relevant to compare the level of nicotine uptake between nicotine pouch and tobacco-based snus users. Thus, the results generated from this study should be of interest not only for the tobacco industry and consumers, but also for lawmakers and the relevant regulatory authorities.

6.3.2 Risk/benefit conclusion

The potential AEs of the study procedures, which are likely to be minor and/or clinically insignificant, will from a research ethics perspective be counterbalanced by increasing the knowledge about the exposure of users of nicotine pouches, tobacco-based snus, and combustible cigarettes to some key biomarkers which may impact their health. Hence, potential benefit of this study is considered to outweigh the minimal risks that the subjects are exposed to in the study.

6.3.3 Risk assessment with regard to the Covid-19 pandemic

Current recommendations from the authorities will be considered on a day-to-day basis and a continuous risk evaluation will be made to assess how the Covid-19 pandemic may affect the study conduct, quality/integrity of data and the safety of the study subjects. The risks and mitigating actions are documented in a risk log as part of the Sponsor's trial master file (TMF). The present study will be performed in accordance with clinical practice. Hence,

participation in the study is not considered to confer increased risks for the subjects in terms of Covid-19.

The recommendations from the European Medicines Agency [[14](#), [15](#)] regarding the conduct and management of clinical trials during the Covid-19 pandemic will be taken into consideration throughout the study.

7 STUDY OBJECTIVES AND ENDPOINTS

The study objectives and endpoints are included below.

7.1 Study objectives

Primary objective

To compare plasma concentrations of nicotine, cotinine, 3'-trans-hydroxycotinine (OH-cotinine), 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), and NNN between users of nicotine pouches, tobacco-based snus, or combustible cigarettes, and nonusers of tobacco/nicotine products.

Secondary objectives

1. To compare urine concentrations of nicotine and its metabolites and TSNAs between users of nicotine pouches, tobacco-based snus, or combustible cigarettes, and nonusers of tobacco/nicotine products.
2. To compare urine concentrations of anatabine, anabasine, and benzo(a)pyrene (BaP) between users of nicotine pouches, tobacco-based snus, or combustible cigarettes, and nonusers of tobacco/nicotine products.
3. To compare urine concentrations of eicosanoids in urine between users of nicotine pouches, tobacco-based snus, or combustible cigarettes, and nonusers of tobacco/nicotine products.
4. To compare plasma concentrations of soluble intercellular adhesion molecule-1 (sICAM-1) and growth differentiation factor 15 (GDF-15) between users of nicotine pouches, tobacco-based snus, or combustible cigarettes, and nonusers of tobacco/nicotine products.
5. To compare the extracted amounts and fractions of nicotine, NNK, and NNN from nicotine pouches and tobacco-based snus.
6. To evaluate the safety and tolerability of nicotine pouches, tobacco-based snus, and combustible cigarettes in current users of these nicotine products.

Exploratory objective

1. To correlate the extracted amounts of nicotine, NNN, and NNK, multiplied by the used number of pouches, with plasma and urine concentrations of BoE.
2. To analyze the pattern of use between users of nicotine pouches, tobacco-based snus, and combustible cigarettes.

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

7.1.1 Study endpoints

Primary endpoint

Difference in plasma concentrations of nicotine, cotinine, OH-cotinine, NNAL, and NNN between users of nicotine pouches, tobacco-based snus, or combustible cigarettes, and nonusers of tobacco/nicotine products.

Secondary endpoints

1. Difference in urine concentrations of total nicotine equivalents and TSNAs (NNAL, NNN, N'-nitrosoanabasine (NAB), and N'-nitrosoanatabine (NAT)) between users of nicotine pouches, tobacco-based snus, or combustible cigarettes, and nonusers of tobacco/nicotine products.
2. Difference in urine concentrations of anatabine, anabasine, and BaP between users of nicotine pouches, tobacco-based snus, or combustible cigarettes, and nonusers of tobacco/nicotine products.
3. Difference in urine concentrations of eicosanoids (8-iso prostaglandin F2 α , 11-dehydrothromboxane B2, 2,3-dinor-thromboxane B2, and leukotriene E4) between users of nicotine pouches, tobacco-based snus, or combustible cigarettes, and nonusers of tobacco/nicotine products.
4. Difference in plasma concentrations of sICAM-1 and GDF-15 between users of nicotine pouches, tobacco-based snus, or combustible cigarettes, and nonusers of tobacco/nicotine products.
5. Difference in the extracted amounts (mg/unit) and fractions (%) of nicotine, NNK, and NNN from nicotine pouches and tobacco-based snus.
6. Frequency, seriousness, and intensity of AEs.

Exploratory endpoints

1. The correlation of the extracted amounts (mg/unit) of nicotine, NNN, and NNK, multiplied by the used number of pouches, with plasma and urine concentrations of BoE for users of nicotine pouches and tobacco-based snus.
2. Difference in the pattern of use between users of nicotine pouches, tobacco-based snus, and combustible cigarettes.

8 STUDY DESIGN

8.1 Overall study design and schedule of events

This is a multi-center, cross-sectional, 4-group, non-randomized study, designed to assess BoE and BoPH in current, daily users of nicotine pouches, tobacco-based snus, or combustible cigarettes, or nonusers of tobacco/nicotine products. The subjects in the 3 nicotine user groups will use their product of choice *ad libitum* throughout the 14 days study period.

The study will include approximately (b) (4) healthy adult male and female subjects ≥ 25 to ≤ 45 years of age. Four (4) separate study groups will be recruited:

- A) exclusive users of Swedish Match brand nicotine pouch product ((b) (4); Group A)
- B) exclusive users of a Swedish tobacco-based snus product ((b) (4); Group B)
- C) exclusive users of a commercially manufactured combustible cigarette product ((b) (4); Group C)
- D) nonusers of tobacco/nicotine products ((b) (4); Group D).

All subjects will provide informed consent prior to study procedures. The subjects will report to the study sites for a screening visit (Visit 1), followed by 1 (nonusers) or 2 (users of nicotine pouches, tobacco-based snus, and combustible cigarettes) study visits (Visit 2 and Visit 3).

Screening (Visit 1) will take place within 4 weeks prior to Visit 2 and will include an eligibility check, including evaluations of smoking and oral tobacco/nicotine product use, a brief physical examination, laboratory tests, electrocardiograms (ECG) and collection of medical history, vital signs (pulse rate and blood pressure), height, weight, body mass index (BMI) and lung function test/spirometry. The subjects will not be allowed to eat within 1 hour prior to spirometry assessments, nor will subjects be allowed to use any kind of tobacco/nicotine product within 1 hour prior to these assessments. Compliance with the present criteria in terms of nicotine use (Group A, B, C) and abstinence (Group D), respectively, will be assessed by urinary cotinine strip test (cotinine cut off: ≥ 200 ng/mL for tobacco/nicotine use; < 200 ng/mL for nonusers of tobacco/nicotine products). During screening, subjects using tobacco/nicotine products (Group A, B, C) will choose 1 product which they will exclusively use during the study. This shall be the product brand that they have mostly used in the past month in case they are not exclusive users of one product brand. Note that this also implies nicotine strength and flavor variations of the same brand. This brand, including nicotine strength and flavor, will be documented in the electronic case report form (eCRF) during screening, at Visit 2, as well as at the end-of study visit (Visit 3).

During the screening visit, all subjects (including the nonusers of tobacco/nicotine products) will be informed how to collect the first morning urine void and be provided with a urine sample collection container and a cooling bag for transportation to the study sites (refer to Section 12.3). Users of nicotine pouches and tobacco-based snus will also be provided with prelabelled collection containers and another cooling bag for their used pouches (refer to Section 12.8).

All subjects will report to the study sites for Visit 2. Blood will be collected for the analysis of plasma concentrations of nicotine, cotinine, OH-cotinine, NNAL, and NNN in users of nicotine pouches, tobacco-based snus, and combustible cigarettes. From this visit, the users of nicotine pouches, tobacco-based snus, and combustible cigarettes will exclusively use their

product of choice ad libitum, following their regular pattern of use, and document their consumption via an electronic diary during the 14-day study period (once per day). The product of choice will be documented in the eCRF. Also, the users of nicotine pouches and tobacco-based snus, (b) (4)

For the nonusers of tobacco/nicotine products blood and urine for all analysis of BoE and BoPH will be collected at 1 study visit (Visit 2, Table 8.1-1) Thus, this group of subjects will also bring their morning urine void, collected by the subject in the provided container and placed in the cooling bag, at the time of this study visit (Visit 2) and will not need to report to the study sites for Visit 3.

After 14 days, the users of nicotine pouches, tobacco-based snus, and combustible cigarettes report to the study sites for Visit 3. The subjects will bring their morning urine void to the study sites, collected in the container, and placed in the cooling bag provided during screening (Visit 1). The subjects will be interviewed about experienced AEs and there will be a compliance check of the electronic diary. Also, the users of nicotine pouches and tobacco-based snus will bring their used and frozen pouches collected on 4 separate days (in a separate cooling bag to avoid cross contamination with the urine sample). Blood will be collected from all subjects (users of nicotine pouches, tobacco-based snus, and combustible cigarettes) for analysis of BoE and BoPH (see Table 8.1-1).

If the subjects forget to bring the collected morning urine void, they shall inform the study sites and a new appointment will be made as soon as possible (preferably the next day). If the nicotine pouch and tobacco-based snus users forget to bring their used pouches to the study sites at Visit 3, they shall re-visit the study sites as soon as possible (preferably the same day) after performing the assessments.

Based on the information in the product use diary, the Sponsor will purchase the applicable products used by the subjects in group A and B for chemical characterization of the unused pouches.

The users of nicotine pouches, tobacco-based snus, and combustible cigarettes (group A, B, C) will participate in the study for 14 days, and the nonusers of tobacco/nicotine products will participate in the study for 1 day, excluding the preceding screening period. A study schedule is illustrated in Table 8.1-1.

Table 8.1-1 Schedule of events

(b) (4)

(b) (4)

8.2 Rationale for study design

This is a cross-sectional, 4-group, non-randomized study, designed to assess BoE and BoPH in current, daily users of nicotine pouches, tobacco-based snus, or combustible cigarettes, or nonusers of tobacco/nicotine products.

To better assess the health risks attributed to distinct types of nicotine delivery products, it is important to analyze the chemical composition of the products as well as the consumers actual exposure to these substances. This is influenced by product usage as well as by the uptake of substances in these products and can be quantified by assessing adequate BoE.

This study aims to: 1) assess BoE in plasma and urine in current, daily users of nicotine pouches, tobacco-based snus, or combustible cigarettes, and nonusers of tobacco/nicotine products; 2) investigate the potential pathophysiological impact of the exposure from the different types of nicotine delivery products by measuring BoPH in plasma and urine, related to CVD and cancer, and 3) assess the extracted amount and fraction of nicotine and TSNAs from pouches used by nicotine pouch and tobacco-based snus users.

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 (b) (4) 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 subject number at Visit 2.

If a subject cannot return to the study sites for Visit 2 within 28 days after screening (*i.e.*, the time interval between signing informed consent until Visit 2) the subject should be rescreened before proceeding in the study.

9.3 Number of subjects

Approximately (b) (4) subjects are planned to be screened to achieve (b) (4) included subjects (50 subjects per group). The study will include:

- (b) (4) subjects who are exclusive users of a Swedish Match brand nicotine pouch product,
- (b) (4) subjects who are exclusive users of a Swedish tobacco-based snus product,
- (b) (4) subjects who are exclusive users of commercially manufactured combustible cigarettes, and
- (b) (4) subjects who are nonusers of tobacco/nicotine products.

An effort will be made to include at least (b) (4) female subjects ((b) (4)) in each group, however a minimum of (b) (4) female subjects ((b) (4)) will be considered acceptable. Based on previous experiences with other study groups, (b) (4) subjects ((b) (4) subjects per study group) are considered to generate sufficient data for the purpose of this study; also, with an estimated dropout rate of 10% per study group.

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

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. Healthy male or female subject aged ≥ 25 to ≤ 45 years.
3. Clinically normal medical history, physical findings, vital signs, ECG, lung function assessment/spirometry and laboratory values at the time of screening, as judged by the investigator.
4. No exposure to passive smoking (from living with someone who smokes at home) may occur in any of the study groups, except for the users of combustible cigarettes.
5. Women of child-bearing potential (WOCBP) must be willing to use a sufficient contraceptive method for the duration of the study, this includes mechanical barrier (*e.g.*, a male condom or a female diaphragm), 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], intra uterine device or intra uterine system. Sexual abstinence is allowed when this is the preferred and usual lifestyle of the subject.

9.4.1 Additional inclusion criteria for Group A (Users of Swedish Match brand nicotine pouch products)

1. Exclusive user of a Swedish Match brand nicotine pouch product, with a nicotine content between 3 and 16 mg per pouch, for ≥ 1 year, with a minimum daily consumption of 4 or more pouches, prior to screening.
2. Used <100 units of combustible cigarette products during their lifetime, with no usage during the last 1 year.
3. Urinary cotinine levels ≥ 200 ng/mL on Visit 1.
4. Willingness to use only one specific Swedish Match brand nicotine pouch (type, flavor, and nicotine strength) product during the conduct of this study (total of 14 days).

9.4.2 Additional Inclusion Criteria for Group B (Users of tobacco-based snus products)

1. Exclusive user of a Swedish tobacco-based snus product, with a nicotine content between 4 and 20 mg per pouch, for ≥ 1 year, with a minimum daily consumption of 4 or more pouches, prior to screening.
2. Used <100 units of combustible cigarette products during their lifetime, with no usage during the last 1 year.
3. Urinary cotinine levels ≥ 200 ng/mL on Visit 1.
4. Willingness to use only one specific tobacco-based snus product (brand, type, flavor, and nicotine strength) during the conduct of this study (total of 14 days).

9.4.3 Additional Inclusion Criteria for Group C (Users of combustible cigarettes)

1. Exclusive user of a commercially manufactured combustible cigarette product, for ≥ 1 year, with a minimum daily consumption of 4 or more combustible cigarettes, prior to screening.
2. Urinary cotinine levels ≥ 200 ng/mL on Visit 1.
3. Willingness to use only one specific commercially manufactured combustible cigarette product (brand, type, flavor, and nicotine strength) during the conduct of this study (total of 14 days).

9.4.4 Additional Inclusion Criteria for Group D (Nonusers)

1. Nonusers of tobacco/nicotine products who have used <100 units of tobacco/nicotine products during their lifetime, with no usage during the last 1 year.
2. Urinary cotinine levels <200 ng/mL on Visit 1.

9.5 Exclusion criteria

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

1. History of any clinically significant disease or disorder which, in the opinion of the Investigator, may either put the subject at risk because of participation in the study, or influence the results or the subject's ability to participate in the study.
2. A history of diagnosed hypertension or any CVD, or chronic respiratory disease like asthma, chronic obstructive pulmonary diseases, chronic bronchitis, or ongoing manifestations of hypertension or any CVD or chronic respiratory disease as judged by the Investigator.
3. 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 nicotine products 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.
4. Subjects who are pregnant, breastfeeding, or intend to become pregnant during the course of the study.
5. Any positive result on screening for serum hepatitis B surface antigen, hepatitis C antibody and Human Immunodeficiency Virus (HIV).
6. 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.
7. Positive screen for drugs of abuse or alcohol at screening or on the study visits. Positive results that are expected given the subject's medical history and prescribed medications can be disregarded as judged by the Investigator.
8. Current or history of alcohol abuse and/or use of anabolic steroids or drugs of abuse, as judged by the Investigator.
9. BMI ≤ 18 and ≥ 33 kg/m².
10. Regular use of any medication, especially those which may interfere with the cyclooxygenase pathway (*e.g.*, anti-inflammatory drugs including aspirin and ibuprofen) or drugs known to be strong inducers/inhibitors of CYP450 enzymes within 14 days prior to screening or during the study; use of hormonal contraceptives (females) and non-prescription pain medication [paracetamol] are permitted.
11. 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.
12. The Investigator considers the subject unlikely to comply with study procedures, restrictions, and requirements.

13. Planned treatment or treatment with an investigational drug within 3 months prior to Visit 2. Subjects consented and screened but not dosed in previous Phase I studies are not to be excluded.

9.5.1 Additional Exclusion Criteria for users of nicotine pouches (Group A):

1. Use of other tobacco/nicotine products, including any other Swedish Match brand or other brand of nicotine pouch products, instead of or in addition to the Swedish Match nicotine pouch product used at the study start.
2. No use of the product on one or more days during the study.
3. Exposure to passive smoking in the household.

9.5.2 Additional Exclusion Criteria for users of tobacco-based snus (Group B):

1. Use of any other tobacco/nicotine products, including any other tobacco-based snus product instead of or in addition to the tobacco-based snus product used at study start.
2. No use of the product on one or more days during the study.
3. Exposure to passive smoking in the household.

9.5.3 Additional Exclusion Criteria for users of combustible cigarettes (Group C):

1. Use of any other tobacco/nicotine products, including any other combustible cigarette brand instead of or in addition to the combustible cigarette product used at study start.
2. No use of the product on one or more days during the study.

9.5.4 Additional Exclusion Criteria for nonusers of tobacco/nicotine products (Group D):

1. Initiation of use of any tobacco/nicotine product use since study start.
2. Exposure to passive smoking in the household.

9.6 Restrictions during the study

Subjects must be willing to comply with the restrictions as outlined in [9.6.1](#) and [9.6.2](#).

9.6.1 General restrictions

1. The subjects will be asked to avoid exposure to passive smoking at any place in addition to the strict prohibition of passive smoke exposure at home.
2. Contraception requirements: Subjects are expected to use contraceptive methods in accordance with inclusion criterion #5 or practice abstinence from heterosexual intercourse (only allowed when this is the preferred and usual lifestyle of the subject) during the clinical study.
3. If the nicotine product user groups (nicotine pouches, tobacco-based snus, or combustible cigarettes) use different brands/products, only one should be used during the 14-day study period.
4. Drugs of abuse: Subjects shall abstain from any drugs of abuse during the study, *i.e.*, from screening (Visit 1) to the last study visit (Visit 3).
5. Subjects are not allowed to participate in any other clinical studies during the study period, *i.e.*, from screening (Visit 1) to the last study visit (Visit 3).

9.6.2 *Prior and concomitant therapy*

There will be no restrictions (except for as specified below) concerning concomitant medications or therapies, as long as the subject is on a stable course of medication from the screening visit to the last visit. 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. Use of hormonal contraceptives (females) and non-prescription pain medication (paracetamol) are permitted.

Prohibited medications

Regular use of any medication which may interfere with the cyclooxygenase pathway (*e.g.*, anti-inflammatory drugs including aspirin and ibuprofen) or drugs known to be strong inducers/inhibitors of CYP450 enzymes are prohibited within 14 days prior to screening and during the study.

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 included 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.

Subjects who do not meet the criteria for participation in this study may be rescreened.

Re-screening can be performed once 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).
- Reserve subjects not used in a previous group of 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 study protocol procedures, as judged by the Investigator and/or Sponsor.
- Subject is lost to follow-up. A subject will be considered lost to follow-up if he/she fails to come for consecutive scheduled visits and if he/she is not possible to be contacted by site staff despite several attempts.
- Significant AEs posing a risk for the subject, as judged by the Investigator and/or Sponsor.

- Withdrawal of informed consent to the use of biological samples as detailed in Section 12.7.
- Pregnancy.
- Death.
- Meeting of an exclusion criterion during the study, which, in the opinion of the Investigator, may pose a risk for the subject.

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. If a subject withdraws consent, the Investigator must ask the subject if he/she is willing, as soon as possible, to be assessed according to the procedures scheduled for the last visit. Any ongoing AEs will be followed-up as described in Section 11.4.2.10.

The primary reason for discontinuation/early withdrawal must be specified in the eCRF. 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 tobacco/nicotine product use may be replaced.

10 STUDY TREATMENTS

10.1 Identity of investigational products

There will be no investigational nor test product provided or examined in this study; hence, the users of tobacco/nicotine products will purchase these products themselves. From Visit 2, the users of nicotine pouches, tobacco-based snus, and combustible cigarettes will exclusively use their product of choice *ad libitum*, following their regular pattern of use.

Nicotine pouch users: Swedish Match nicotine pouch product of choice, containing 3-16 mg nicotine per pouch.

Tobacco-based snus users: tobacco-based snus product of choice, containing 4-20 mg nicotine per pouch.

Combustible cigarette users: commercially manufactured combustible cigarette product of choice.

Nonusers of tobacco/nicotine products: No tobacco/nicotine product.

10.2 Treatment administration

There will be no investigational nor test product provided or examined in this study.

- Subjects in Group A will exclusively use one Swedish Match brand nicotine pouch product throughout the study.
- Subjects in Group B will exclusively use one Swedish brand of tobacco-based snus pouch product throughout the study.
- Subjects in Group C will exclusively use one brand of commercially manufactured combustible cigarettes throughout the study.
- Subjects in Group D will remain nonusers of tobacco/nicotine products throughout the study.

It must be assured that no alternative product (also no other nicotine strength or flavor variation of the same product) will be used in the study. The product specifications (nicotine content, brand name, *etc.*) will be documented in the eCRF. The subjects in Group A, B, C will use their product *ad libitum*, following their regular pattern of use, throughout the course of the study. The product usage will be documented in the electronic diary (refer to Section 16.3).

10.3 Continuation of treatment with investigational product

Not applicable.

10.4 Treatment compliance

The subjects in the 3 nicotine user groups will use their product of choice *ad libitum* throughout the 14-day study period and document their consumption in an electronic diary once per day.

10.5 Return and destruction of investigational product

Not applicable.

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](#))

11.1 Recording of data

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

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 Eligibility criteria

Eligibility criteria should be checked at the screening visit (Visit 1) and verified at Visit 2. The criteria are specified in [Sections 9.4](#) and [9.5](#).

11.2.3 Demographic information

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

11.2.4 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.

11.2.5 Medical/surgical history

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

11.2.6 History of tobacco/nicotine product use

The history of nicotine use in terms of tobacco/nicotine product use, brands, average consumption per day during the last 30 days, and duration of use (years), and history of smoking (*e.g.*, combustible cigarettes and e-cigarettes) will be obtained by subject interview.

11.2.7 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.

11.2.8 Pregnancy test

All WOCBP will do a urine pregnancy test at the screening visit as well as at the discretion of the Investigator during the study visits (Visit 2-3).

11.2.9 Urine drug screen

Urine will be screened for drugs of abuse at timepoints outlined in the schedule of events ([Table 8.1-1](#)) using the Drug-Screen Multi-15 Dip Test (nal von minden GmbH or equivalent).

The test screens for 4-methylpentadron, 7-aminoclonazepam, amphetamine, benzodiazepines, buprenorphine, fentanyl, tetrahydro-cannabinoids, cocaine, methadone, methamphetamine, methylenedioxy-methamphetamine (MDMA, ecstasy), morphine, oxycodone, pregabalin and tramadol, along with pH and creatinine.

11.2.10 Alcohol test

An alcohol test will be performed at timepoints outlined in the schedule of events ([Table 8.1-1](#)).

11.2.11 Urine cotinine screen

Subjects will be screened for urine cotinine levels at the screening visit (Visit 1), to determine tobacco/nicotine product use status as part of the inclusion criteria (refer to Section [9.4](#)).

11.2.12 Lung function and spirometry

A spirometry assessment (without bronchodilator) will be performed in compliance with study site practices. Subjects will not be allowed to eat within the 1 hour prior to spirometry assessments, nor will subjects be allowed to use any kind of tobacco/nicotine product within 1 hour prior to these assessments. Spirometry will be used to measure peak flow, forced vital capacity, forced expiratory flow, and forced expiratory volume in 1 second. Only subjects with no clinically significant findings will be enrolled into the study.

11.2.13 Baseline symptoms

A baseline symptom is defined as an event that occurs between the subject's signing of the ICF until Visit 2 (*i.e.*, an event that occurs during the screening period). Such events are not AEs and will be recorded as baseline symptoms in the Medical History Log in the eCRF.

11.2.14 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 (see also Section [9.6.2](#)).

Medications are classified as prior if the stop date was before or on the day of Visit 2 and as concomitant if ongoing on the day of Visit 2. To distinguish between prior and concomitant medications on Visit 2, 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 visit must be documented 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 and secondary endpoints

11.3.1 Assessment of biomarkers

Urine and plasma will be shipped frozen to the contracted bioanalytical laboratory (ABF GmbH, Planegg, Germany) for analysis of BoE and BoPH, using validated bioanalytical methods. Creatinine will also be assessed for urine normalization of BoE and BoPH at the contracted bioanalytical laboratory (ABF GmbH, Planegg, Germany). In addition, pH will also be assessed in the urine samples.

11.3.2 Analysis of selected biomarkers

At Visit 2, plasma will be analyzed in the tobacco/nicotine user groups (Group A-C) for selected BoEs (refer to [Table 8.1-1](#)). The following BoEs will be analyzed: nicotine, cotinine, OH-cotinine, NNAL, and NNN.

11.3.3 Analysis of BoE and BoPHs

The BoEs and BoPHs to be analyzed (at Visit 2 in the nonuser group, Group D, and at Visit 3 in the tobacco/nicotine user groups, Group A-C, refer to [Table 8.1-1](#)) are specified in [Table 11.3-1](#).

Table 11.3-1 BoEs and BoPHs to be analyzed in plasma and urine.

(b) (4)

(b) (4)

11.4 Assessment related to secondary endpoints

11.4.1 Nicotine, NNK, and NNN extraction from pouches

Used pouches will be shipped frozen to Swedish Match for analysis of the extracted amount and fraction of nicotine, NNK, and NNN. Based on the information in the electronic diary, the Sponsor will purchase the applicable products used by the subjects in Group A and B for chemical characterization of the unused pouches at Swedish Match. The extracted amount and fraction of nicotine, NNK, and NNN will be calculated by subtracting the average of the pouches used by the nicotine pouch and tobacco-based snus users from the average of 10 unused pouches.

11.4.2 Adverse events

The PI is responsible for ensuring that all medical staff involved in the study are familiar with the content of this section and the content of the (b) (4) standard operating procedures (SOPs) regarding emergencies.

11.4.2.1 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.

In accordance with the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) E2A guideline [16], 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 tobacco/nicotine products.

11.4.2.2 Definition of serious adverse event

An SAE is any untoward medical occurrence that:

- results in death,
- is life-threatening,

NOTE: The term "life-threatening" in the definition of "serious" refers to an event in which the subject 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 other situations, such as "important medical events" that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one 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.

11.4.2.3 Time period and frequency for collecting adverse events

All AEs (including SAEs) will be collected from Visit 2 until the last visit (Visit 3).

Any AE with the start date on Visit 2 must be recorded with the start time.

At Visit 3, 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.

11.4.2.4 Collecting and recording 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.

AEs must be recorded in the AE Log of the eCRF. The Investigator must provide information on the AE, preferably as a diagnosis, if available, otherwise as signs and symptoms; start and end dates, start and end time; intensity; causal relationship to IMP; action taken, and outcome.

If the AE is serious, this must be indicated in the eCRF.

AEs 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.

11.4.2.5 Assessment of seriousness

The Investigator must assess and document the seriousness (serious or non-serious) of each AE using the definitions in Section 11.4.2.2. If the event is assessed as serious it must be reported as an SAE by the Investigator to the Sponsor according to Section 11.4.2.9.

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.

11.4.2.6 Assessment of intensity

The grading of the intensity of AEs will follow the common terminology criteria for adverse events (CTCAE) v5.0 [17]. 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.

11.4.2.7 Assessment of causal relationship

The Investigator must assess if there is a causal relationship between an AE and the use of the tobacco/nicotine product and record it the AE log of the eCRF using the definitions below:

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

An AE is considered causally related to the use of the tobacco/nicotine product when the causality assessment is probable or possible.

11.4.2.8 Outcome of adverse event

The Investigator must document the outcome of an AE and record it in the AE log of the eCRF using the definitions below:

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 (<i>e.g.</i> , 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 (<i>e.g.</i> , an atrial fibrillation has become chronic).
Fatal	
Unknown	

11.4.2.9 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 predefined 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 to the Sponsor or its designee. The study sites must notify the sites Monitor via phone or e-mail about the submission of the SAE report. As soon as the sites 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 e-mailed to:

(b) (6)
Swedish Match AB
Phone: (b) (6)
E-mail: (b) (6)

A copy of the paper SAE form must also be e-mailed to (b) (4)

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 study drug, 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 sites are raised promptly.

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 (SMP).

11.4.2.10 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 visit, whichever comes first. At the last 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 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.

11.4.3 Vital signs

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

Any vital signs outside of normal ranges will be specified and documented as clinically significant or not clinically significant.

11.4.4 Electrocardiogram

Single 12-lead ECGs will be recorded 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. Safety ECGs will be reviewed and interpreted on-site by the Investigator.

Any abnormalities will be specified and documented as clinically significant or not clinically significant.

11.4.5 Laboratory assessments

Urine samples for the detection of cotinine levels using dipsticks will be taken at the screening visit.

Blood samples for the analysis of clinical chemistry and hematology will be collected through venipuncture or an indwelling venous catheter and sent to the certified clinical chemistry laboratory at Uppsala University Hospital or Synlab Sverige AB and analyzed by routine analytical methods.

The laboratory parameters are defined in [Table 11.4-1](#) and will be assessed at the screening visit as specified in [Table 8.1-1](#).

Any laboratory values outside of normal ranges will be specified and documented as normal, abnormal not clinically significant, or abnormal clinically significant in the eCRF.

Table 11.4-1 Laboratory parameters

(b) (4)

11.4.6 Physical examinations

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

Any abnormalities will be specified and documented as clinically significant or not clinically significant.

11.5 Assessments related to exploratory endpoints

The pattern of use of nicotine pouches, tobacco-based snus, and combustible cigarettes will be recorded by the subjects in the electronic diary once per day.

11.6 Appropriateness of measurements

All methods used for safety assessments are commonly used in standard medical care and in Phase I clinical studies.

12 PROCEDURES FOR BIOLOGICAL SAMPLES

12.1 Sample collection

Blood and urine samples will be collected according to standard procedures.

12.2 Blood sampling

Additional measures will be undertaken by the study staff to avoid contamination of the collected samples. The study staff designated to sample collection will wash their hands before handling samples and wear disposable gloves. As there is a high risk of contamination with clothing as the contamination source, all study staff must wear clothing that has not been exposed to nicotine. Approximately (b) (4) mL blood ((b) (4)) will be taken either by direct venipuncture or from a cannula placed in a forearm vein. Blood samples will be centrifuged for 10 min at 2500 x g to separate the plasma and stored at $\leq -18^{\circ}\text{C}$ until shipment.

12.3 Morning urine void

Subjects will collect the first morning urine void in a urine collection container in the morning of Visit 2 (nonusers only, Group D) or Visit 3 (all subjects in Group A, B, C). It is not necessary to collect the whole void but at least 125 mL shall be collected. The morning urine void will immediately be wrapped into a plastic bag and placed into the cooling bag; all materials for collection and transportation will be provided by the clinic at screening (Visit 1). The urine sample will be stored refrigerated ($2-8^{\circ}\text{C}$) upon arrival of the subject at the clinic and further processed within 1 hour. The urine sample will be thoroughly mixed, aliquoted into 2 separate 50 mL prelabelled high-density polyethylene (HDPE) containers and stored in a freezer ($\leq -18^{\circ}\text{C}$) until shipment.

12.4 Volume of blood

The anticipated volume of blood samples collected during the study from each subject in Group A-C will be approximately (b) (4) mL (Table 12.4-1) and (b) (4) mL for subjects in Group D (Table 12.4-2). For reference, a regular blood donation consists of between 350 mL to 450 mL ($\pm 10\%$) for persons weighing at least 45-50 kg [23].

Table 12.4-1 Estimated blood volumes, Group A - C

(b) (4)

Table 12.4-2 Estimated blood volumes, Group D

(b) (4)

12.5 Handling, storage, and destruction of laboratory samples

All biological samples will be registered in a biobank at (b) (4).
(b) (4).

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

All plasma and urine samples will be shipped to ABF GmbH, analyzed, and disposed of after the CSR has been finalized.

12.6 Chain of custody of biological samples

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

(b) (4) keeps full traceability of collected biological samples from the study subjects while in storage at the research clinic 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.

12.7 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:

- Subject withdrawal of consent is notified immediately to the Sponsor.
- Biological samples from the subject, if stored at the research clinic, 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 research clinic and the action is documented.

12.8 Collection of used pouches

The used pouches will be collected upon arrival at the study sites, checked for completeness by study sites personnel, and immediately placed in a freezer ($\leq -18^{\circ}\text{C}$) until shipment.

13 QUALITY MANAGEMENT, QUALITY ASSURANCE AND QUALITY CONTROL

13.1 Quality management: critical process, system, and data identification

During clinical study protocol (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 study results according to applicable SOPs and the ICH E6 (R2) guideline [19].

Identified risks will be categorized separately from the CSP.

Identified risks, including risks associated with the Covid-19 pandemic, will be categorized separately from the CSP.

Sponsor oversight responsibilities, such as monitoring, AE reporting, safety monitoring, changes in Investigators and key study team staff, and quality assurance activities, may need to be reassessed in relation to the Covid-19 pandemic and temporary, alternative proportionate mechanisms of oversight may be required.

13.2 Quality assurance and quality control

The Sponsor has delegated the responsibilities outlined below to (b) (4) whilst maintaining overall study oversight:

- Implementing and maintaining quality assurance and quality control (QC) systems with written SOPs with regard to management of identified risks, CSP compliance, good clinical practice (GCP) compliance and applicable regulatory requirements.
- Securing agreements with involved subcontractors and performing regular subcontractor oversight to ensure CSP compliance, GCP compliance and compliance with applicable regulatory requirements.
- Implementing a risk-based validated electronic data capture (EDC) system and maintain SOPs for the whole life- cycle of the system.
- 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 [18] and are consistent with the ICH E6 (R2) guideline for GCP [19], applicable sections of the EU Clinical Trials Directive 2001/20/EC [20], and applicable local regulatory requirements.

14.2 Ethics and regulatory review

The 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 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 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 card and the signed 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) [21], these pseudonymized 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 the ICF they approve that authorized representatives from the Sponsor and (b) (4), 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 [21] and the request will be raised to the PI.

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 pseudonymized, *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 end of the study, only pseudonymized data can be used, *i.e.*, aggregated data sets.

For this study, the Sponsor is the data controller of all data processed during the study (*e.g.*, TMF, study reports) and (b) (4) is the data processor. Any subcontractors used in the study are also data processors.

For data that are processed at the study sites (*e.g.*, medical records and ISF), (b) (4) 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 and/or competent authority (CA) 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 clinic, including source data verification. 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. The Investigator will contact the Sponsor immediately if contacted by a CA about an inspection at the study sites.

14.7 Insurance

The study is funded by the Sponsor Swedish Match North Europe, Stockholm, Sweden. Subjects will be covered under Swedish Match AB'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. (b) (4) has a company insurance covering services performed by (b) (4)

15 STUDY MANAGEMENT

15.1 Training of study sites personnel

Before inclusion of the first study subject, a Sponsor representative or delegate will perform study initiation visits at the study clinics. 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.
- verify that data in the eCRF are consistent with the clinical records (source data verification) 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 (b) (4) 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

The study is funded by the Sponsor Swedish Match North Europe, Stockholm, Sweden. The management and conduct of the clinical investigation have been outsourced to the contract research organization (CRO), (b) (4). The PI is an employee of (b) (4).

The agreements between Sponsor and (b) (4) must be in place before any study-related procedures can take place, or enrollment of subjects.

The Sponsor and CRO responsibility and duty split is regulated in a separate clinical study agreement.

The PI 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 Q1 2023 and to be completed by Q2 2023.

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 and CA must be informed promptly. Conditions that may warrant study termination include but are not limited to the discovery of an unexpected, significant, or unacceptable risk to the subjects included in the study or potential study subjects.

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 [22] guideline-compliant CSR describing the conduct of the study, any statistical analyses performed, and the results obtained will be prepared by (b) (4). 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 Annual safety report

If the study duration exceeds 1 year, the Sponsor must submit development safety update report (DSUR) to the IEC. The report must summaries all pertinent safety information collected during the reporting period and contain an update of the risk-benefit evaluation if there has been any change since the approval of the clinical study.

15.7.3 Confidentiality and ownership of study data

Any confidential information relating to 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.

15.7.4 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 [19]) for 10 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), and the original signed ICFs.

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 [19], and applicable regulatory requirements [20].

The data from the eCRFs will be sent to the Sponsor and a copy will be sent to the clinic and filed in the ISF for archiving for 10 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 CA, 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 study sites personnel prior to the study 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 project team member besides the Investigator or clinical staff can enter data in the eCRF. All data will be entered in English. The eCRFs will be completed as soon as possible during or after the subject's visit. To avoid inter-observer variability, every effort will be made to ensure that preferably the 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 Electronic patient reported outcome

Subject reported data will be recorded (product specifications including nicotine content, brand name, *etc.*) using an electronic patient reported outcomes (ePRO) system (ViedocMe™) linked to the eCRF. The ePRO system includes password protection and internal quality checks. Text reminders can be sent to the subject through the ePRO. All data registered in the ePRO are stored together with the eCRF data.

16.4 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. The Investigator or clinical staff will answer the queries in the eCRF either by correcting the data or by entering a response to the query.

16.5 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.

16.6 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.

16.7 Medical coding

Medical coding will be performed by trained personnel at (b) (4). 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.

16.8 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, which will be signed and approved prior to database lock.

The analyses of the primary and secondary endpoints will be performed by (b) (4).

17.1 General

Descriptive statistics will be provided overall for the parameters collected during the study based on the analysis population (group). Arithmetic mean (mean), geometric mean (GM), standard deviation (SD), coefficient of variation (CV), median, minimum (min), maximum (max) and interquartile range (IQR) will be calculated for metric parameters, additionally graphical presentation of data using box plots where applicable. Categorical and ordinal parameters will be summarized using the number and percentages of subjects in each group.

Analyses regarding group differences will be performed using a significance level of 5 % ($p < 0.05$).

Individual subject data will be listed by subject number, study group, 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 collected prior to the start of the 14 days *ad libitum* usage period.

No adjustment for multiple comparisons will be made. No imputation of missing data will be performed.

17.2 Determination of sample size

The primary endpoint in this study is the difference in plasma concentrations of nicotine, cotinine, OH-cotinine, NNAL, and NNN between users of nicotine pouches, tobacco-based snus, or combustible cigarettes, and nonusers of tobacco/nicotine products. No formal sample size calculation has been performed as available data for this study design is lacking. Based on previous experiences with other study groups, (b) (4) subjects (b) (4) subjects per study group) are considered to generate sufficient data for the purpose of this study, also with an estimated dropout rate of 10% per study group.

17.3 Analysis data sets

The Full Analysis Set (FAS) will consist of all subjects who have been included and who have at least 1 post-baseline data point.

17.4 Description of study population

17.4.1 Demographics and baseline characteristics

Descriptive statistics for demographics, weight, and height will be presented for all subjects. All data will be listed by subject number.

17.4.2 Medical/surgical history and prior/concomitant medication

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

All data will be listed by subject number.

17.4.3 Treatment compliance

The number of subjects in each group, and their individual use will be listed.

17.4.4 Analysis of biomarkers

The difference in plasma and urine concentrations of the different biomarkers of nicotine pouches, tobacco-based snus, combustible cigarettes, and nonusers of tobacco/nicotine products will be analyzed using ANOVA. Pairwise comparisons between treatments will be calculated with its corresponding p-value.

In all biomarker formal statistical analyses, the last collected biomarker data will be used, *i.e.*, Visit 2 for nonusers and Visit 3 for users.

17.4.4.1 Analysis of primary endpoint

The difference in plasma concentrations of nicotine, cotinine, OH-cotinine, NNAL (marker for NNK), and NNN between users of nicotine pouches, tobacco-based snus, or combustible cigarettes, and nonusers of tobacco/nicotine products will be analyzed using ANOVA. Pairwise comparisons between groups will be calculated with its corresponding p-value.

17.4.4.2 Analysis of secondary endpoints

The difference in urine and plasma concentrations will be analyzed between users of nicotine pouches, tobacco-based snus, or combustible cigarettes, and nonusers of tobacco/nicotine products. Difference will be analyzed using ANOVA for:

- total nicotine equivalents and TSNA's (NNAL, NNN, NAB, and NAT) in urine
- anatabine, anabasine, and 3-OH-BaP in urine
- eicosanoids (8-iso-PGF2 α , 2,3-dinor-TXB2, 11-dehydro-TXB2 and LTE4) in urine
- sICAM-1 and GDF-15 in plasma

Pairwise comparisons between groups will be calculated with its corresponding p-value.

17.4.5 Analysis of extracted amount of nicotine, NNK, and NNN

Nicotine, NNK, and NNN will be determined in unused and used pouches for Group A and Group B. The difference in contents between unused (as measured by the mean of the corresponding reference pouches, see Section 11.4.1 above) and used pouches will be used to calculate the *in vivo* extraction. The calculated extracted amounts of nicotine, NNK, and NNN per pouch will be averaged per subject and multiplied with the consumption (number of pouches) reported in the electronic diary over the 14 days *ad libitum* usage period, to receive the total exposure.

Total exposure to nicotine, NNK, and NNN will be summarised descriptively.

Average extracted amounts (mg/unit) and fractions (%) of nicotine, NNK, and NNN will also be summarised descriptively.

17.4.6 Adverse events

An overview of all AEs, including SAEs, intensity, relationship to use, and deaths will be presented. The incidence of AEs and SAEs will be summarized by SOC and PT by group and overall. An overview of any tobacco/nicotine product-related AEs will be summarized by SOC and PT if considered appropriate.

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

17.4.7 Vital signs

Vital signs (systolic/diastolic blood pressure and pulse rate) will be summarized by group.

All data will be listed by subject number.

17.4.8 Electrocardiogram

All ECGs will be categorized as "normal", "abnormal, not clinically significant", or "abnormal, clinically significant" (as judged by the Investigator) and summarized by group using frequency tables.

All data will be listed by subject number.

17.4.9 Laboratory analysis

Safety laboratory data will be summarized by group. Abnormal, clinically significant values will be summarized separately, if considered appropriate.

All data will be listed by subject number.

17.4.10 Physical examinations

Clinically significant and non-clinically significant abnormal findings will be specified and presented by subject number and summarized by group.

All data will be listed by subject number.

17.5 Analysis of exploratory objectives

17.5.1 Correlation analysis

Total exposure to nicotine, NNK, and NNN will be calculated as described under Section 17.4.5. The intake will be correlated with plasma and urine concentrations of BoE (nicotine, NNAL, NNN, Cotinine, and 3-OH-Cotinine) for dose-relationship investigations using linear regression with BoE levels as the dependent variable and total exposure to nicotine, NNK, and NNN as a continuous explanatory variable in separate models.

In total the following models will be developed (refer to [Table 17.5-1](#)):

Table 17.5-1 Correlation analysis models

(b) (4)

To all of the models above, study group (A or B) will be fitted to the models as dummy variables together with the interaction effect total exposure to nicotine/NNK/NNN*study group to produce different regression slopes for Group A and Group B. The regression result

will be presented in a graph together with some model fit statistics and a hypothesis test p-value that the beta-coefficient for Group A = Group B, *i.e.*, a test for difference between Group A and B on the total exposure to nicotine/NNK/NNN – BoE correlation.

17.5.2 Pattern of use

The pattern of use, as measured by the total number of pouches/cigarettes taken during the 14 days *ad libitum* usage period, will be summarized descriptively for users of nicotine pouches, tobacco-based snus, and combustible cigarettes. The pattern of use data will also be listed.

18 REFERENCES

1. US Department of Health and Human Services. How Tobacco Smoke Causes Disease: The Biology and Behavioral Basis for Smoking-Attributable Disease. A Report of the Surgeon General; National Library of Medicine Cataloging in Publication, 2010.
2. GBD 2019 Risk Factors Collaborators. Global burden of 87 risk factors in 204 countries and territories, 1990-2019: a systematic analysis for the Global Burden of Disease Study 2019. *Lancet* 2020, 396 (10258), 1223-1249. DOI: 10.1016/s0140-6736(20)30752-2 From NLM.
3. World Health Organization (WHO). WHO report on the global tobacco epidemic 2021: addressing new and emerging products; World Health Organization (WHO), Geneva, 2021.
4. Institute of Medicine (IOM). Scientific standards for studies on modified risk tobacco products. National Academy Press, 2011.
5. Eurostat. 18.4% of EU population smoked daily in 2019 2021. <https://ec.europa.eu/eurostat/web/products-eurostat-news/-/edn-20211112-1> (accessed 12OCT2022).
6. Patja, K.; Hakala, S. M.; Boström, G.; Nordgren, P.; Haglund, M. Trends of tobacco use in Sweden and Finland: Do differences in tobacco policy relate to tobacco use? *Scandinavian Journal of Public Health* 2009, 37 (2), 153-160. DOI: 10.1177/1403494808100277.
7. ENVIRON International Corporation. Review of the Scientific Literature on Snus (Swedish Moist Snuff); 2010.
8. Bundesinstitut für Risikobewertung (BfR). Health Risk Assessment of Nicotine Pouches; 2021. <https://www.bfr.bund.de/cm/349/health-risk-assessment-of-nicotine-pouches.pdf> DOI: 10.17590/20220204-105615.
9. Azzopardi, D.; Liu, C.; Murphy, J. Chemical characterization of tobacco-free "modern" oral nicotine pouches and their position on the toxicant and risk continuums. *Drug Chem Toxicol* 2021, 1-9. DOI: 10.1080/01480545.2021.1925691 From NLM.
10. Hecht, S. S.; Hatsukami, D. K. Smokeless tobacco and cigarette smoking: chemical mechanisms and cancer prevention. *Nature reviews. Cancer* 2022, 22 (3), 143-155. DOI: 10.1038/s41568-021-00423-4 From NLM Medline.
11. Jablonski, J.; Cheetham, A.; Martin, A. Market Survey of Modern Oral Nicotine Products: Determination of Select HPHCs and Comparison to Traditional Smokeless Tobacco Products. *Separations* 2022, 9(3), 65; <https://doi.org/10.3390/separations9030065>.
12. International Agency for Research on Cancer (IARC). Smokeless tobacco and some tobacco-specific N-Nitrosamines; IARC Press, 2007.
13. Rutqvist, L. E.; Curvall, M.; Hassler, T.; Ringberger, T.; Wahlberg, I. Swedish snus and the GothiaTek® standard. *Harm Reduct J* 2011, 8, 11. DOI: 10.1186/1477-7517-8-11 From NLM.
14. European Medicines Agency. Points to consider on implication of Coronavirus disease (Covid-19) on methodological aspects of ongoing clinical trials. June 26, 2020. EMA/158330/2020 Rev. 1. Published on [www.ema.europa.eu](https://www.ema.europa.eu/en/documents/scientific-guideline/points-consider-implications-coronavirus-disease-covid-19-methodological-aspects-ongoing-clinical_en-0.pdf). https://www.ema.europa.eu/en/documents/scientific-guideline/points-consider-implications-coronavirus-disease-covid-19-methodological-aspects-ongoing-clinical_en-0.pdf (last accessed 12OCT2022).
15. European Medicines Agency. Guidance on the management of clinical trials during the Covid-19 (coronavirus) pandemic. Version 4. April 2, 2021. Published on [ec.europa.eu](https://ec.europa.eu/health/sites/health/files/files/eudralex/vol-10/guidanceclinicaltrials_covid19_en.pdf). https://ec.europa.eu/health/sites/health/files/files/eudralex/vol-10/guidanceclinicaltrials_covid19_en.pdf (last accessed 12OCT2022).
16. European Medicines Agency. ICH E2A Clinical safety data management: definitions and standards for expedited reporting. June 1995. Published on [ema.europa.eu](https://www.ema.europa.eu).

https://www.ema.europa.eu/en/documents/scientific-guideline/international-conference-harmonisation-technical-requirements-registration-pharmaceuticals-human-use_en-15.pdf (last accessed 12OCT2022).

17. National Cancer Institute Division of Cancer Treatment and Diagnosis, Cancer Therapy Evaluation Program. Common terminology criteria for adverse events, CTCAE v5.0. November 27, 2017. Published on ctep.cancer.gov.
https://ctep.cancer.gov/protocoldevelopment/electronic_applications/ctc.htm (last accessed 12OCT2022).
18. US Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Biologics Evaluation and Research (CBER). Guidance for Industry, Drug-Induced Liver Injury: Premarketing Clinical Evaluation. July 2009. Published on fda.org. <https://www.fda.gov/media/116737/download> (last accessed 12OCT2022).
19. European Medicines Agency. ICH E6(R2) Guideline for Good Clinical Practice. July 1, 2002. Last updated December 15, 2016. EMA/CHMP/ICH/135/1995. Published on ema.europa.eu. <https://www.ema.europa.eu/en/ich-e6-r2-good-clinical-practice> (last accessed).
20. European Commission. Clinical Trials – Directive 2001/20/EC. April 4, 2001. Published on ec.europa.eu. https://ec.europa.eu/health/human-use/clinical-trials/directive_en (last accessed).
21. European Commission. Regulation (EU) 2016/679 of the European Parliament and of the Council of 27 April 2016 on the protection of natural persons with regard to the processing of personal data and on the free movement of such data, and repealing Directive 95/46/EC (General Data Protection Regulation). 2016. Published on eur-lex-europa.eu. <https://eur-lex.europa.eu/eli/reg/2016/679/oj> (last accessed 12OCT2022).
22. European Medicines Agency. ICH E3 Structure and content of clinical study reports. July 1, 1996. CPMP/ICH/137/95. Published on ema.europa.eu. <https://www.ema.europa.eu/en/ich-e3-structure-content-clinical-study-reports> (last accessed 12OCT2022).
23. World Health Organization. Blood Donor Selection: Guidelines on Assessing Donor Suitability for Blood Donation. Geneva. 2012. Chapter 4, General donor assessment. Available from <https://www.ncbi.nlm.nih.gov/books/NBK138219/> (last accessed 12OCT2022).

19 SIGNATURES

19.1 Principal Investigator statement

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

Principal Investigator

DocuSigned by:

(b) (6)

(b) (4)

19.2 Approval of the clinical study protocol

I, the undersigned, approve this CSP.

Sponsor signatory

DocuSigned by:

(b) (6)

Swedish Match AB