ISRCTN38557348 https://doi.org/10.1186/ISRCTN38557348

Levels of biomarkers of exposure in users of tobacco/nicotine products.

Submission date 25/11/2022	**Recruitment status** No longer recruiting	[X] Prospectively registered [X] Protocol [_] Statistical analysis plan [_] Results [_] Individual participant data [_] Record updated in last year
Registration date 05/12/2022	**Overall study status** Completed	
Last Edited 11/03/2025	**Condition category** Other	

Plain English summary of protocol

Background and study aim(s):
Cigarette smoking causes many diseases, such as cancer, pulmonary disease, and cardiovascular disease (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 tobacco-specific nitrosamines (TSNAs). So, although the health effects are substantially smaller for tobacco-based snus compared to cigarette smoking, some adverse effects cannot be ruled out.
Oral nicotine pouches constitute a substitute to both combusted and non-combusted tobacco-containing inhalation products (e.g., conventional cigarettes, heated tobacco vaporizer, and electronic cigarettes) and to oral tobacco products (e.g., tobacco-based snus and moist snuff) and nicotine pouches has gained large popularity in recent years. In contrast to tobacco-based snus, these products contain no tobacco and as such generally have a low burden (if any) from tobacco-derived toxicants such as TSNAs. Hence, these products have the potential to further reduce tobacco-related harm.
To assess the health risks attributed to usage of 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) and biomarkers of potential harm (BoPH).
Herein, applicable BoE for nicotine and its metabolites and TSNA exposure in plasma and urine will be assessed in users of nicotine pouches related to Swedish snus users as well as users of combustible cigarettes and nonusers of tobacco/nicotine products. In addition, this study includes the measurement of BoPH in order to investigate the potentially reduced risk of these products with respect to CVD and cancer. Such data are needed to categorize the products on the risk continuum scale of tobacco and nicotine use. Hence, 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 TSNAs from pouches used by nicotine pouch and tobacco-based snus users.

Who can participate?
Healthy male and female user of nicotine pouches, tobacco-based snus, or combustible cigarettes, or nonusers of tobacco/nicotine products aged ≥25 to ≤45 years. The subjects in the three nicotine user groups (group A-C) are required to have been daily users of these products for at least 1 year, with a minimum daily consumption of four or more pouches/cigarettes, to be eligible for participation in this study.

What does the study involve?
The subjects in the three nicotine user groups (group A-C) will use their product of choice ad libitum throughout the 14-day study period, following their regular pattern of use. The subjects will report to the study sites for a screening visit (Visit 1), followed by 1 (nonusers, group D) or 2 (group A-C) study visits (Visit 2 and Visit 3). Screening (Visit 1) will take place within 4 weeks prior to Visit 2. For subjects in group A-C, the brand, including nicotine strength and flavor, will be documented in the electronic case report form (eCRF).
All subjects will report to the study sites for Visit 2. For nonusers of tobacco/nicotine products, blood and urine (morning urine void collected by the subjects and brought to the clinic) will be collected for all analysis of BoE and BoPH; hence, these subjects will not need to report to the study site for Visit 3. Blood will be collected from subjects in the three nicotine user groups (group A-C) for the analysis of plasma concentrations of nicotine, cotinine, OH-cotinine, NNAL, and NNN and from this visit, the subjects will document their consumption via an electronic diary during the 14-day study period (once per day). Also, users of nicotine pouches and tobacco-based snus (group A-B) will collect 8 used pouches per week.
After 14 days, the subjects in the three nicotine user groups (group A-C) report to the study sites for Visit 3. The subjects will bring their morning urine void to the study sites and the subjects will be interviewed about experienced AEs. Also, the users of nicotine pouches and tobacco-based snus (group A-B) will bring their used and frozen pouches. Blood will be collected from the subjects for analysis of BoE and BoPH.

What are the possible benefits and risks of participating?
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.
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 three nicotine user groups (group A-C) are required to have been daily users of these products for at least one 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. 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. The nonusers of tobacco/nicotine products will remain abstaining from tobacco/nicotine products and the above-mentioned risks do thus not apply.
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 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 nicotine pouches, tobacco-based snus, and combustible cigarette users to some key biomarkers which may impact their health.

Where is the study run from?
CTC Clinical Trial Consultants AB: CTC Oscar in Uppsala and CTC Karolinska in Stockholm, Sweden.

When is the study starting and how long is it expected to run for?
November 2022 to March 2023

Who is funding the study?
Swedish Match North Europe (Sweden)

Who is the main contact?
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Contact information

Type(s)
Scientific

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Additional identifiers

Protocol serial number
SM22-03

Study information

Scientific 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.

Acronym
SM22-03

Study objectives
Users of nicotine pouches are exposed to lower levels of tobacco-specific nitrosamines than users of tobacco-based snus and combustible cigarettes.

Ethics approval required
Old ethics approval format

Ethics approval(s)
Approved 30/11/2022, Swedish Ethical Review Authority (Etikprövningsmyndigheten, Box 2110, 750 02, Uppsala, Sweden; +46 10-475 08 00; registrator@etikprovning.se), ref: 2022-06076-01

Study design
Multicenter cross-sectional 4-group non-randomized study

Primary study design
Other

Study type(s)
Other

Health condition(s) or problem(s) studied
Nicotine use and non-nicotine use

Interventions
There will be no investigational product provided in this study. Four groups of subjects will be included:
• Subjects in group A will be required to use exclusively one brand of Swedish Match nicotine pouch product throughout the study.
• Subjects in group B will be required to use exclusively one brand of tobacco-based snus product 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.

The subjects in the three nicotine user groups (group A-C) will use their product of choice ad libitum throughout the 14-day study period, following their regular pattern of use; the brand, including nicotine strength and flavor, will be documented in the electronic case report form (eCRF). The subjects will report to the study sites for a screening visit (Visit 1), followed by 1 (nonusers, group D) or 2 (group A-C) study visits (Visit 2 and Visit 3). Screening (Visit 1) will take place within 4 weeks prior to Visit 2. Blood and urine will be collected for analysis of biomarkers of exposure (BoE) and biomarkers of potential harm (BoPH).

Intervention Type
Other

Primary outcome(s)
Plasma concentrations of nicotine, cotinine, 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.

Plasma analysis will be assessed using validated bioanalytical methods (liquid chromatography-tandem mass spectrometry (LC-MS/MS)).

Key secondary outcome(s))

1. 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. Urine analysis will be assessed using validated bioanalytical methods (LC-MS/MS). Creatinine will also be assessed for urine normalization (colorimetric assay).
2. 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. Urine analysis will be assessed using validated bioanalytical methods (LC-MS/MS). Creatinine will also be assessed for urine normalization (colorimetric assay).
3. Urine concentrations of eicosanoids (8-iso prostaglandin F2α, 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. Urine analysis will be assessed using validated bioanalytical methods (LC-MS/MS). Creatinine will also be assessed for urine normalization (colorimetric assay).
4. 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. Plasma analysis will be assessed using commercially available ELISA-kits (colorimetric assays).
5. The extracted amounts (mg/unit) and fractions (%) of nicotine, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), and NNN from nicotine pouches and tobacco-based snus. The in vivo extraction of nicotine, NNK, and NNN will be calculated by subtracting the residual amount after use from the mean of 10 unused pouches. Used pouches will be frozen and analyzed using gas chromatography-mass spectrometry (GC-MS).
6. Frequency, seriousness, and intensity of adverse events (AEs). AEs will be collected by subject interview at the last visit (Visit 3) for users of tobacco/nicotine products (group A-C).

Completion date

15/03/2023

Eligibility

Key inclusion 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 anticonception 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.

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).

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).

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).

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.

Participant type(s)
Healthy volunteer

Healthy volunteers allowed
No

Age group
Adult

Sex
All

Total final enrolment
198

Key exclusion criteria
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 cardiovascular disease (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/m^2.
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 nonprescription 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.

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 for one or more days during the study.
3. Exposure to passive smoking in the household.

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 for one or more days during the study.
3. Exposure to passive smoking in the household.

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 for one or more days during the study.

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.

Date of first enrolment
13/01/2023

Date of final enrolment
21/02/2023

Locations

Countries of recruitment
Sweden

Study participating centre
CTC Clinical Trial Consultants AB
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Sponsor information

Organisation
Swedish Match North Europe

Funder(s)

Funder type

Industry

Funder Name
Swedish Match North Europe

Results and Publications

Individual participant data (IPD) sharing plan
The data-sharing plans for the current study are unknown and will be made available at a later date

IPD sharing plan summary
Data sharing statement to be made available at a later date

Study outputs

Output type	Details	Date created	Date added	Peer reviewed?	Patient-facing?
Protocol file	version 1.0	09/11/2022	05/12/2022	No	No