

Module 5.2 New Clinical Cross-sectional Biomarker Study SM 22-03

Assessing biomarkers of exposure in plasma and urine in current, daily users of nicotine pouches, tobacco-based snus, or combustible cigarettes, or non-users of tobacco/nicotine products

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1. STUDY INTRODUCTION

SM 22-03 was a multi-center, cross-sectional, four-group, non-randomized study completed in Sweden, designed to assess BoEs and BoPHs in current, daily users of nicotine pouches, tobacco-based snus, or combusted cigarettes, relative to non-users of tobacco or nicotine products (TNPs). Subjects in the nicotine user groups used their product of choice *ad libitum* throughout the 14-day study period. The overarching aim of the study was to determine the risk profile of nicotine pouches, in terms of BoEs, compared to cigarettes or snus. While the *proposed MRTPs* were not used exclusively in this study, all nicotine pouches used in the study were manufactured by Swedish Match, according to the same standards. Similarly, although not all snus products used by the subjects were the *authorized MRTPs*, all were Swedish snus and adhered to similar manufacturing procedures, meeting the GOTHIA TEK® standard. The average nicotine content of the nicotine pouch products in the study was higher ((b) (4) mg/pouch) than in the *proposed MRTPs* (3 mg or 6 mg/pouch), and the product components and product manufacturing standards were similar for all products. Therefore, the majority of nicotine pouch products used in the studies that were not the *proposed MRTPs*, were likely to expose users to slightly higher if not comparable levels of nicotine, but comparable levels of other constituents. Similar reasoning can be used for the snus products used in the studies and the *authorized products*. Therefore, the study results are representative of the *proposed MRTPs* and *authorized MRTPs*.

1.1. Primary objective

- To compare the plasma concentrations of nicotine, cotinine, trans-3'-hydroxycotinine (OH-cotinine), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), and N'-nitrosonornicotine (NNN) between users of nicotine pouches, snus, and combusted cigarettes, and non-users of TNPs

1.2. Secondary objectives

- To compare urine concentrations of nicotine and its metabolites and TSNA between users of nicotine pouches, snus, or combusted cigarettes, and non-users of TNPs.
- To compare urine concentrations of anatabine, anabasine, and 3-hydroxybenzo[a]pyrene (3-OH-B[a]P) between users of nicotine pouches, snus, or combusted cigarettes, and non-users of TNPs.
- To compare urine concentrations of eicosanoids in urine between users of nicotine pouches, snus, or combusted cigarettes, and non-users of TNPs.
- To compare plasma concentrations of soluble intercellular adhesion molecule-1 (sICAM-1) and growth differentiation factor 15 (GDF-15) between users of nicotine pouches, snus, or combusted cigarettes, and TNPs.
- To compare the extracted amounts and fractions of nicotine, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), and NNN from nicotine pouches and snus.
- To evaluate the safety and tolerability of nicotine pouches, snus, and combusted cigarettes in current users of these nicotine products.

1.3. Exploratory objectives

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

2. STUDY PROCEDURES

Subjects were recruited from the (b) (4) database of healthy adult volunteers and media advertisements. The study included (b) (4) subjects, divided into four groups. All subjects in the three nicotine user groups were exclusive users who had used the product type for ≥ 1 year, with a minimum daily consumption of four or more units (Table 1). The non-users of TNPs had to have used < 100 units of TNPs during their lifetime, with no usage during last 1 year. No exposure to passive smoking (from living with someone who smokes at home) was allowed in any of the nicotine user groups, except for the combusted cigarette users. Subjects had to be willing and able to give written informed consent for participation in the study, (b) (4).

Table 1. Biomarker Study SM 22-03 Study Groups and Products category

Study group	Product category
Nicotine pouch users	One Swedish Match brand nicotine pouch product (usual brand) containing (b) (4) mg nicotine per pouch
Tobacco-based snus users	One Swedish pouch tobacco-based snus product (usual brand) containing (b) (4) mg nicotine per pouch
Combusted cigarette users	One commercially manufactured combusted cigarette (usual brand)
Non-users of tobacco/nicotine products	None

Eligible subjects in the nicotine user groups participated in the study for 14 days, while non-users of TNPs participated for 1 day, excluding the up to 28-day screening period. Following a screening visit (Visit 1), all subjects attended Visit 2 at the study sites. Non-users of TNPs provided blood and morning urine samples for the analysis of BoEs and BoPHs during this visit and were not required to return for Visit 3. During Visit 2, blood was collected for the analysis of selected BoEs for subjects in the three nicotine user groups. Subsequently, they exclusively used their product of choice *ad libitum* for 14 days, following their regular usage pattern and documenting consumption via an electronic diary once per day. Nicotine pouch users and snus users also collected four used pouches on two separate days per week and brought these to the clinic for Visit 3. During Visit 3, the three nicotine user groups also provided blood and morning urine samples for the analysis of BoEs and BoPHs and were interviewed about experienced adverse events (AEs).

3. STUDY RESULTS

(b) (4) subjects were screened, and (b) (4) subjects were included in this study; specifically, (b) (4) nicotine pouch users, (b) (4) snus users, (b) (4) combusted cigarette users, and (b) (4) non-users of TNPs. The study population consisted of (b) (4) females (b) (4) and (b) (4) males (b) (4) with a mean age of (b) (4) and a mean body mass index of (b) (4) kg/m². One subject was withdrawn before study completion as the subject was lost to follow-up after Visit 2.

3.1. BoEs – nicotine and nicotine metabolites in plasma and urine

Mean nicotine plasma concentrations did not significantly differ for nicotine pouch users (b) (4) ng/mL, snus users (b) (4) ng/mL, and combusted cigarette users (b) (4) ng/mL; see Figure 1). There were no significant differences in cotinine and OH-cotinine plasma levels between nicotine pouch users (b) (4) ng/mL and (b) (4) ng/mL, respectively) and snus users (b) (4) ng/mL and (b) (4) ng/mL, respectively). Users of combusted cigarettes (b) (4) ng/mL and (b) (4) ng/mL, respectively) had significantly lower plasma levels of cotinine and OH-cotinine compared to both nicotine pouch users and snus users

((b) (4)). Nicotine, cotinine, and OH-cotinine levels were below the limit of quantification (BLQ) in non-users of TNPs and were significantly lower compared to the nicotine user groups ((b) (4)).



Figure 1. Comparisons of nicotine (A), cotinine (B), and OH-cotinine (C) levels in plasma across cohorts.

NP = nicotine pouch, CC = combusted cigarette, ns = not significant. Asterisks denote statistically significant differences between cohorts.

All analytes assessed in urine were normalized by urine creatinine concentrations to correct for variable dilution, so quantities are expressed per mg creatinine. There were no significant differences in urine levels of nicotine, cotinine, OH-cotinine, and nicotine equivalents between nicotine pouch users ((b) (4) ng/mg, ((b) (4) ng/mg, ((b) (4) ng/mg, and ((b) (4) μmol/mg, respectively) and snus users ((b) (4) ng/mg, ((b) (4) ng/mg, ((b) (4) ng/mg, and ((b) (4) μmol/mg, respectively). In contrast, users of combusted cigarettes ((b) (4) ng/mg, ((b) (4) ng/mg, ((b) (4) ng/mg, and ((b) (4) μmol/mg, respectively) had significantly lower urine levels of all these analytes compared to both nicotine pouch users ((b) (4) ((b) (4)) and snus users ((b) (4)). Urine concentrations of nicotine, cotinine, OH-cotinine, and nicotine equivalents were significantly lower in the non-users of TNPs compared to the other groups ((b) (4)).

3.2. BoEs – TSNA_s in plasma and urine

NNAL and NNN were ((b) (4) in plasma for all subjects in the nicotine pouch user and non-user groups and these two groups did not significantly differ from each other. However, both snus users ((b) (4) pg/mL NNAL and ((b) (4) pg/mL NNN) and combusted cigarette users ((b) (4) pg/mL NNAL and ((b) (4) pg/mL NNN) had significantly higher levels compared to both nicotine pouch user and non-user groups ((b) (4)).

There were no significant differences in urine NNAL or NNN levels between nicotine pouch users ((b) (4) pg/mg NNAL and ((b) (4) pg/mg NNN) and non-users ((b) (4) pg/mg NNAL and ((b) (4) pg/mg NNN), ((b) (4) pg/mg NNAL and ((b) (4) pg/mg NNN) and combusted cigarette users ((b) (4) pg/mg NNAL and ((b) (4) pg/mg NNN). However, nicotine pouch users and non-users had significantly lower urine levels of NNAL and NNN compared to both snus users and combusted cigarette users ((b) (4)).

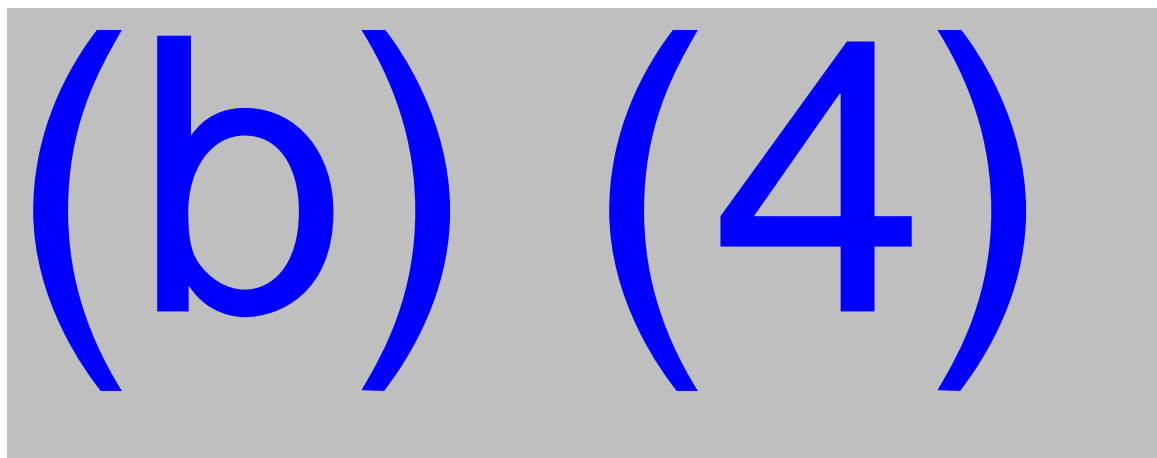


Figure 2. Comparisons of NNAL (A) and NNN (B) levels in plasma across cohorts.

NP = nicotine pouch, CC = combusted cigarette, ns = not significant. Asterisks denote statistically significant differences between cohorts.

N-nitrosoanabasine (NAB) and N'-nitrosoanatabine (NAT) urine levels (b) (4) in the nicotine pouch user and non-user groups and did not significantly differ between these two groups. However, users of combusted cigarettes (b) (4) pg/mg NAB and (b) (4) pg/mg NAT) had significantly higher urine levels of NAB compared to snus users (b) (4) pg/mg NAB and (b) (4) pg/mg NAT) ((b) (4)), while there was no significant difference in urine NAT levels between these two groups. Additionally, nicotine pouch users and non-users had significantly lower urine concentrations of both NAB and NAT compared to both snus users and combusted cigarette users ((b) (4)).

Urine levels of TSNA precursors anatabine and anabasine (b) (4) in both the nicotine pouch user and non-user groups and did not significantly differ between these two user groups. However, both nicotine pouch users and non-users had significantly lower urine levels of these analytes compared to both snus users (b) (4) ng/mg (b) (4) and (b) (4) ng/mg (b) (4)) and combusted cigarette users (b) (4) ng/mg (b) (4) and (b) (4) ng/mg (b) (4)) ((b) (4)).

3.3. BoEs – 3-OH-B[a]P

Urine levels of 3-OH-B[a]P (b) (4) in the nicotine pouch user, snus user, and non-user groups and these groups did not significantly differ from each other. However, combusted cigarette users (b) (4) fg/mg) had significantly higher urine levels of 3-OH-B[a]P compared to nicotine pouch users (b) (4) fg/mg), snus users (b) (4) fg/mg), and non-users of TNPs ((b) (4) (b) (4)).

3.4. BoPHs

Urine levels of eicosanoids (8-iso PGF2 α , 11-dh-TXB2, 2,3-d-TXB2, and LTE4) were within the lower range for all user groups, including non-users of TNPs, and statistical comparisons were ambiguous. Additionally, sICAM-1 and GDF-15 plasma levels did not significantly differ between nicotine pouch users, snus users, and non-users of TNPs. However, combusted cigarette users ((b) (4) and (b) (4)) had significantly higher levels of these two analytes compared to nicotine pouch users ((b) (4) and (b) (4)), snus users ((b) (4) (b) (4) and (b) (4)), and non-users of TNPs ((b) (4) and (b) (4)).

3.5. *Exposure to nicotine and TSNAs*

The mean number of TNPs used per day was (b) (4) pouches for nicotine pouch users, (b) (4) pouches for snus users, and (b) (4) for combusted cigarette users. Nicotine pouch users had a higher mean extracted amount and fraction of nicotine (b) (4) mg/unit and (b) (4), respectively) compared to snus users (b) (4) mg/unit and (b) (4), respectively). However, the mean total exposure over the 14-day study period was lower for nicotine pouch users (b) (4) mg) compared to snus users (b) (4) mg). The levels of NNK and NNN in unused, reference, nicotine pouches were BLQ. Hence, nicotine pouch users were not exposed to quantifiable levels of NNK and NNN; in contrast, for snus users, the mean total exposure to NNK and NNN was (b) (4) µg and (b) (4) µg, respectively.

3.6. *Safety Evaluation*

(b) (4). All AEs were mild in intensity and assessed as unlikely to be related to the usage of nicotine products. Additionally, there were no differences in AE reporting frequency between the tobacco/nicotine user groups. Notably, no AEs were collected from the non-user group, resulting in a reporting frequency of zero in this group.

4. CONCLUSIONS

- There were no significant differences in nicotine plasma levels between users of nicotine pouches, snus, and combusted cigarettes after 14 days of ad libitum use of either product.
- There were no significant differences in cotinine and OH-cotinine plasma levels between nicotine pouch users and snus users, but the levels were significantly higher compared to users of combusted cigarettes.
- Plasma levels of NNAL and NNN did not significantly differ between nicotine pouch users and non-users but were significantly lower compared to snus users and combusted cigarette users. (b) (4).
- There were no significant differences in urine levels of nicotine, cotinine, OH-cotinine, or nicotine equivalents between nicotine pouch users and snus users, but both groups had significantly higher levels of these analytes compared to users of combusted cigarettes.
- Nicotine pouch users had significantly lower urine levels of TSNAs (NNAL, NNN, NAB, and NAT), anatabine, and anabasine compared to snus users and combusted cigarette users. In contrast, there were no significant differences between nicotine pouch users and non-users of TNPs in relation to these biomarkers, except for NNAL.
- (b) (4). Urine levels of NAB were significantly higher, and urine levels of anatabine and anabasine were significantly lower, in combusted cigarette users compared to snus users.
- Urine levels of 3-OH-B[a]P were low and there were no significant differences between nicotine pouch users, non-users of TNPs, and snus users. In contrast, combusted cigarette users had significantly higher urine levels of 3-OH-B[a]P compared to all other groups.
- There were no significant differences in plasma levels of sICAM-1 and GDF-15 between nicotine pouch users, snus users, and non-users of TNPs. In contrast, combusted cigarette users had significantly higher plasma levels of sICAM-1 and GDF-15 compared to all other groups.
- Nicotine pouch users tended on average to use few pouches per day (b) (4) pouches) compared to snus users (b) (4) pouches) and combusted cigarette users (b) (4) cigarettes). Therefore, the mean total exposure over the 14-day study period was lower for nicotine pouch users compared

to snus users, despite having a higher mean extracted amount and fraction of nicotine compared to snus-users

- (b) (4)
- All reported AEs (b) (4) AEs) were of mild intensity and assessed as unlikely related to nicotine products, and there were no differences in reporting frequency between the nicotine user groups.

5. DISCUSSION

The study demonstrates the users of nicotine pouches manufactured according to Swedish Match standards are exposed to significantly lower levels of TSNA compared to both users of cigarettes and tobacco-based snus when using their usual brand *ad libitum*, in a real-life setting. Further, the data shows nicotine pouch users are not exposed to higher levels of nicotine compared to users of snus and 3-OH-B[a]P levels were significantly higher in cigarette smokers compared to both nicotine pouch users and snus users. Collectively, the study demonstrates users of Swedish Match nicotine pouches are exposed to lower levels of potentially harmful constituents than both cigarette smokers and snus users and therefore, the use of the *proposed MRTPs* does not put the user at higher risk of mouth cancer, heart disease, lung cancer, stroke, emphysema, and chronic bronchitis than the *authorized MRTPs*.

As the *authorized MRTPs* have already been demonstrated to put users at lower risk of developing these serious health conditions compared to cigarettes, as evidenced by FDA granting authorization to use the proposed claim with the *authorized MRTPs*, it is evident the *proposed MRTPs* would similarly put users at lower risk of developing these serious health conditions compared to cigarettes.