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**Clinical Study Protocol**

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

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**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.**

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**Test product and dose**

N/A

**Sponsor signatory**

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## 1 STUDY SYNOPSIS

<b>Study title</b>	
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.	
<b>Study code</b>	<b>Planned study period</b>
SM22-03	Q1 2023 to Q2 2023
<b>Coordinating/Principal Investigator</b>	
(b) (6)	
(b) (4)	
<b>Study design</b>	
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.	
<b>Objectives</b>	
<u>Primary objective</u>	
<ul style="list-style-type: none"> <li>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.</li> </ul>	
<u>Secondary objectives</u>	
<ol style="list-style-type: none"> <li>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.</li> <li>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.</li> <li>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.</li> <li>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.</li> <li>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.</li> <li>To evaluate the safety and tolerability of nicotine pouches, tobacco-based snus, and combustible cigarettes in current users of these nicotine products.</li> </ol>	
<u>Exploratory objective</u>	
<ol style="list-style-type: none"> <li>To correlate the extracted amounts of nicotine, NNN, and NNK, multiplied by the used number of pouches, with plasma and urine concentrations of BoE.</li> </ol>	

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 $\alpha$ , 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  $\geq 25$  to  $\leq 45$  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  $\geq 1$  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  $\geq 1$  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  $\geq 1$  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:  $\geq 200$  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 (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). 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.

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### 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 <sub>4</sub>	Leukotriene E <sub>4</sub>
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)

### Coordinating /Principal Investigator

(b) (6)

Phone: (b) (6)

E-mail: (b) (6)

### Clinical conduct

(b) (4)

### 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 TSNA's (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 $\alpha$ , 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  $\geq 25$  to  $\leq 45$  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:  $\geq 200$  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, will collect (b) (4) (Samples A) and on (b) (4) (Samples B) and (b) (4) (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 (b) (4) 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  $\geq 25$  to  $\leq 45$  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  $\geq 1$  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  $\geq 200$  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  $\geq 1$  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  $\geq 200$  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  $\geq 1$  year, with a minimum daily consumption of 4 or more combustible cigarettes, prior to screening.
2. Urinary cotinine levels  $\geq 200$  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  $\leq 18$  and  $\geq 33$  kg/m<sup>2</sup>.
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 (b) (4) 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:

<b>Recovered/resolved</b>	The subject has recovered completely, and no symptoms remain.
<b>Recovering/resolving</b>	The subject's condition is improving, but symptoms still remain.
<b>Recovered/resolved with sequelae</b>	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).
<b>Not recovered/not resolved</b>	The subject's condition has not improved, and the symptoms are unchanged ( <i>e.g.</i> , an atrial fibrillation has become chronic).
<b>Fatal</b>	
<b>Unknown</b>	

#### 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)
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**Table 12.4-2 Estimated blood volumes, Group D**

(b) (4)
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## 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 TSNAs (NNAL, NNN, NAB, and NAT) in urine
- anatabine, anabasine, and 3-OH-BaP in urine
- eicosanoids (8-iso-PGF2 $\alpha$ , 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, (b) (4)

(b) (4)

### ***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.

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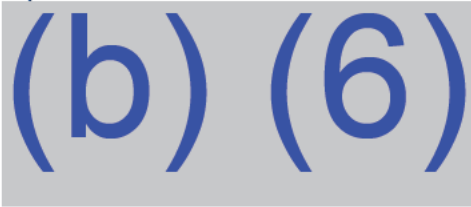


## 19 SIGNATURES

### 19.1 Principal Investigator statement

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

#### Principal Investigator

DocuSigned by:  
  
 \_\_\_\_\_  
 (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

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**Clinical Study Report**

Study code	SM22-03
Report version and date	Final version 2.0; 06DEC2023

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**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**

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**Study duration**

First subject screened: 12JAN2023  
First subject included: 30JAN2023  
Last subject last visit: 15MAR2023

**Sponsor signatory**

(b) (6)

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

**Principal/Coordinating Investigator**

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

**Clinical study conduct**

(b) (4)

**Clinical study management**

(b) (4)

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**This clinical study was conducted, and essential study documentation archived, in compliance with company standard operating procedures and applicable regulatory requirements.**

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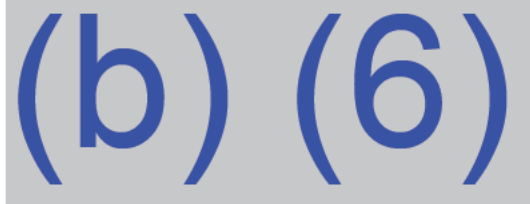
## DOCUMENT HISTORY

Version	Date	Summary of changes
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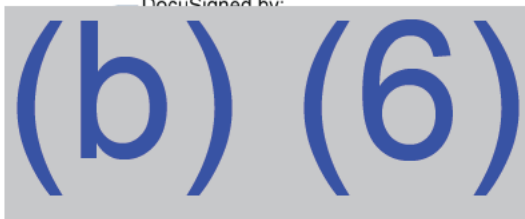
## 1. SIGNATURES

*I, the undersigned, have read this report and confirm that to the best of my knowledge it accurately describes the conduct and results of the study.*

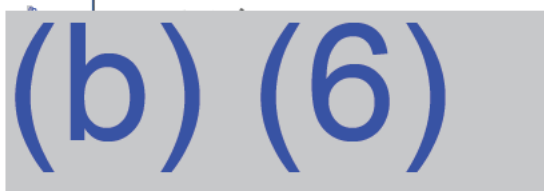
### 1.1 Principal Investigator

DocuSigned by:  
  
(b) (4)

### 1.2 Biostatistician

DocuSigned by:  
  
(b) (4)

### 1.3 Sponsor signatory

DocuSigned by:  
  
Swedish Match NE

## 2 STUDY SYNOPSIS

<b>Study title</b> 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.
<b>Study code</b> SM22-03
<b>Study period</b> Date of first subject screened: 12JAN2023 Date of first subject included: 30JAN2023 Date of last subject last visit: 15MAR2023
<b>Principal/Coordinating Investigator</b> (b) (4), (b) (6)
<b>Study centers</b> (b) (4)
<b>Publication (reference)</b> N/A
<b>Study design</b> This was a multi-center, 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 used their product of choice <i>ad libitum</i> throughout the 14-day study period.
<b>Objectives</b> <u>Primary objective</u> To compare 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, tobacco-based snus, or combustible cigarettes, and nonusers of tobacco/nicotine products. <u>Secondary objectives</u> <ol style="list-style-type: none"> <li>1. To compare urine concentrations of nicotine and its metabolites and tobacco-specific nitrosamines (TSNA) between users of nicotine pouches, tobacco-based snus, or combustible cigarettes, and nonusers of tobacco/nicotine products.</li> <li>2. To compare urine concentrations of anatabine, anabasine, and 3-hydroxybenzo(a)pyrene (3-OH-B[a]P) between users of nicotine pouches, tobacco-based snus, or combustible cigarettes, and nonusers of tobacco/nicotine products.</li> </ol>

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, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (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.

#### **Number of subjects**

The study included a total of (b) (4) subjects:

- (b) (4) subjects who were exclusive nicotine pouch users (group A),
- (b) (4) subjects who were exclusive tobacco-based snus users (group B),
- (b) (4) subjects who were exclusive users of combustible cigarettes (group C),
- and (b) (4) subjects who were nonusers of tobacco/nicotine products (group D).

The male/female ratio was (b) (4) in the nicotine pouch user group, (b) (4) in the tobacco-based snus group, (b) (4) in the combustible cigarette group, and (b) (4) in the nonuser of tobacco/nicotine products group.

#### **Diagnosis and main eligibility criteria**

Healthy male and female subjects aged  $\geq 25$  to  $\leq 45$  years meeting the criteria for each group, respectively, were considered to be eligible for participation in the study.

The criteria for the 4 groups were:

A) exclusive users of a Swedish Match brand nicotine pouch product, with a nicotine content between 3 and 16 mg per pouch, for  $\geq 1$  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  $\geq 1$  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  $\geq 1$  year, with a minimum daily consumption of 4 or more combustible cigarettes, prior to screening.

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) used different brand, type, flavor, and nicotine strength, only 1 type of product was to be used during the 14-day study period.

No exposure to passive smoking (from living with someone who smokes at home) was allowed in any of the user groups, except for the combustible cigarette users.

All subjects had to be willing to comply with study procedures and give written informed consent. Subjects who were pregnant, breastfeeding, or intend to become pregnant during the study, and/or subjects with a history or presence of diagnosed hypertension or cardiovascular disease (CVD) or

other medical condition that could interfere with the BoE or could put the subject at risk because of participation in the study, and/or intend to stop using nicotine-containing products, were excluded from the study.

## Methodology

All subjects provided informed consent prior to study procedures. The subjects reported 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) took place within 4 weeks prior to Visit 2 and included 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 were not allowed to eat within 1 hour prior to spirometry assessments, nor were subjects 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, were assessed by urinary cotinine strip test (cotinine cut off:  $\geq 200$  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) choose 1 product which they exclusively used during the study. This was the product brand that they had mostly used in the past month in case they were not exclusive users of 1 product brand and this also implied nicotine strength and flavor variation of the same brand. The brand, including nicotine strength and flavor, was documented in the electronic case report form (eCRF) during screening, at Visit 2, as well as at the end-of study visit (Visit 3).

All subjects (including the nonusers of tobacco/nicotine products) were informed how to collect the first morning urine void. Subjects in the nonusers of tobacco/nicotine products were provided with a urine sample collection container and a cooling bag for transportation to the study sites at the screening visit. The same material were provided to users of nicotine pouches, tobacco-based snus, and combustible cigarettes at Visit 2. Furthermore, during Visit 2, users of nicotine pouches and tobacco-based snus were provided with collection containers and an extra cooling bag for used pouches.

All subjects reported to the study sites for Visit 2. Blood was 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 exclusively used 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 was documented in the eCRF. Also, the users of nicotine pouches and tobacco-based snus collected (b) (4)

(Samples A) and on (b) (4) (Samples B) and (b) (4). For the nonusers of tobacco/nicotine products blood and urine for all analysis of BoE and BoPH were collected at 1 study visit (Visit 2). Thus, this group of subjects also brought 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 did 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 reported to the study sites for Visit 3. The subjects brought their morning urine void to the study sites, collected in the container, and placed in the cooling bag provided at Visit 2. The subjects were interviewed about experienced adverse events (AEs), used brand, nicotine strength, and flavor and there was a compliance check of the electronic diary. Also, (b) (4)

(b) (4). Blood was collected from all subjects (users of nicotine pouches, tobacco-based snus, and combustible cigarettes) for analysis of BoE and BoPH.



If the subjects forgot to bring the collected morning urine void, they informed the study sites, and a new appointment was made as soon as possible (preferably the next day). If the nicotine pouch and tobacco-based snus users forgot to bring their used pouches to the study site at Visit 3, they re-visited 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 purchased the applicable products used by the subjects in Group A and B for chemical characterization of unused reference pouches.

### Investigational products

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

- Subjects in Group A were required to exclusively use 1 brand of a Swedish Match nicotine pouch product (3-16 mg nicotine per pouch) throughout the study.
- Subjects in Group B were required to exclusively use 1 brand of a Swedish tobacco-based snus product (4-20 mg nicotine per pouch) throughout the study.
- Subjects in Group C were required to exclusively use 1 brand of a commercially manufactured combustible cigarette product throughout the study.
- Subjects in Group D were required to continue to *not use* tobacco/nicotine products from screening to Visit 2.

### Duration of treatment

Fourteen 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 remained abstaining from tobacco/nicotine products.

### Duration of subject's involvement in the study

Each subject (tobacco/nicotine products users) participated in the study for 14 days, not including the up to 28-day screening period. The nonusers of tobacco/nicotine products participated in the study for 1 day, not including the preceding screening period.

### Blood and urine sampling for analysis of biomarkers

- Analysis of baseline plasma concentrations of nicotine, cotinine, OH-cotinine, NNAL, and NNN (only in users of tobacco/nicotine products at Visit 2).
- Analysis of plasma and urine concentrations of BoE and BoPH for nonusers of tobacco/nicotine products (Visit 2).
- Analysis of plasma and urine concentrations of BoE and BoPH after 14 days of *ad libitum* usage of tobacco/nicotine products (Visit 3).

### Chemical analysis of study products

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

### Nicotine extraction assessment

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

### Safety assessments

AEs were collected from Visit 2 and up until Visit 3 (end-of study visit) for tobacco/nicotine users.

### Statistical methods

Descriptive statistics were 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) were calculated for metric parameters, additionally, graphical presentation of data where applicable. Categorical and ordinal parameters were summarized using the number and percentages of subjects in each group.

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

Individual subject data were listed by subject number, user group, and, where applicable, by assessment time.

All descriptive summaries and statistical analyses were performed using SAS Version 9.4 (SAS Institute, Inc., Cary, NC).

Baseline was defined as the last data collected prior to the start of the 14-day *ad libitum* usage period.

No adjustment for multiple comparisons was made and no imputation of missing data was performed.

## Summary of results

### Biomarkers in plasma (primary endpoint)

- There were no significant differences in nicotine plasma levels between users of nicotine pouches ((b) (4) ng/mL), tobacco-based snus ((b) (4) ng/mL), and combustible cigarettes ((b) (4) ng/mL) 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 ((b) (4) and ((b) (4) ng/mL, respectively) and tobacco-based snus users ((b) (4) and ((b) (4) ng/mL, respectively). In contrast, users of combustible cigarettes had significantly lower levels of these biomarkers ((b) (4) and ((b) (4) ng/mL, respectively) compared to both nicotine pouch users and tobacco-based snus users ((b) (4) ((b) (4) ).
- Nicotine pouch users had significantly lower plasma levels of NNAL and NNN ((b) (4) ((b) (4) ((b) (4) )) compared to tobacco-based snus users ((b) (4) ((b) (4) )) and combustible cigarette users ((b) (4) ((b) (4) ). Additionally, plasma levels of NNAL and NNN did not significantly differ between nicotine pouch users and nonusers of tobacco/nicotine products. Furthermore, there were no significant differences in plasma levels of NNAL and NNN between tobacco-based snus users ((b) (4) and ((b) (4) pg/mL, respectively) and combustible cigarette users ((b) (4) and ((b) (4) pg/mL, respectively).
- ((b) (4) .

### Biomarkers in urine (secondary endpoints)

*All analytes assessed in urine were normalized (divided) by urine creatinine concentrations to correct for variable dilution and all units are hence expressed by mg creatinine.*

- Mean urine levels of nicotine, cotinine, OH-cotinine, and nicotine equivalents were:
  - ((b) (4) ng/mg, ((b) (4) ng/mg, ((b) (4) ng/mg, and ((b) (4) μmol/mg, respectively, for nicotine pouch users
  - ((b) (4) ng/mg, ((b) (4) ng/mg, ((b) (4) ng/mg, and ((b) (4) μmol/mg, respectively, for tobacco-based snus users
  - ((b) (4) ng/mg, ((b) (4) ng/mg, ((b) (4) ng/mg, and ((b) (4) μmol/mg, respectively, for combustible cigarette users

There were no significant differences in urine levels of nicotine, cotinine, OH-cotinine, or nicotine equivalents between nicotine pouch users and tobacco-based snus users. Users of combustible cigarettes had significantly lower levels of all parameters compared to both

nicotine pouch users ((b) (4)) and tobacco-based snus users ((b) (4)).

- Mean urine levels of TSNA (NNAL, NNN, NAB, and NAT) were:
  - ((b) (4)) pg/mg, ((b) (4)) pg/mg, BLQ, and ((b) (4)) pg/mg, respectively, for nicotine pouch users
  - ((b) (4)) pg/mg, ((b) (4)) pg/mg, ((b) (4)) pg/mg, and ((b) (4)) pg/mg, respectively, for tobacco-based snus users
  - ((b) (4)) pg/mg, ((b) (4)) pg/mg, ((b) (4)) pg/mg, and ((b) (4)) pg/mg, respectively, for combustible cigarette users
- Mean urine levels of anatabine, anabasine, and 3-OH-B[a]P were:
  - ((b) (4)) ng/mg, ((b) (4)) ng/mg, and ((b) (4)) fg/mg, respectively, for nicotine pouch users
  - ((b) (4)) ng/mg, ((b) (4)) ng/mg, and ((b) (4)) fg/mg, respectively, for tobacco-based snus users
  - ((b) (4)) ng/mg, ((b) (4)) ng/mg, and ((b) (4)) fg/mg, respectively, for combustible cigarette users

Nicotine pouch users had significantly lower urine levels of TSNA (NNAL, NNN, NAB, and NAT), anatabine, and anabasine compared to tobacco-based snus users and combustible cigarette users ((b) (4)). In contrast, there were no significant differences between nicotine pouch users and nonusers of tobacco/nicotine products in relation to these biomarkers, except for NNAL.

There were no significant differences in urine levels of NNAL, NNN, and NAT between tobacco-based snus users and combustible cigarette users. Urine levels of NAB were significantly higher ((b) (4)), and urine levels of anatabine and anabasine were significantly lower ((b) (4)), in combustible cigarette users compared to tobacco-based snus users.

Urine levels of 3-OH-B[a]P were low and there were no significant differences between nicotine pouch users, nonusers of tobacco/nicotine products, and tobacco-based snus users. In contrast, combustible cigarette users had significantly higher urine levels of 3-OH-B[a]P compared to all other groups ((b) (4)).

- In general, nonusers of tobacco/nicotine products had urine levels BLQ for nicotine, cotinine, OH-cotinine, nicotine equivalents, TSNA, anatabine, and anabasine.
- Mean urine levels of eicosanoids (8-iso PGF<sub>2α</sub>, 11-dh-TXB<sub>2</sub>, 2,3-d-TXB<sub>2</sub>, and LTE<sub>4</sub>) were within the lower range for all user groups including nonusers of tobacco/nicotine products:
  - ((b) (4)), and ((b) (4)) ng/mg, respectively, for nicotine pouch users
  - ((b) (4)), and ((b) (4)) ng/mg, respectively, for tobacco-based snus users
  - ((b) (4)), and ((b) (4)) ng/mg, respectively, for combustible cigarette users

There were no significant differences in 8-iso PGF<sub>2α</sub> levels between nicotine pouch users, users of combustible cigarettes, and nonusers of tobacco/nicotine products. In contrast, tobacco-based snus users had significantly lower 8-iso PGF<sub>2α</sub> levels compared to both nicotine pouch users ((b) (4)) and combustible cigarette users ((b) (4)).

Nicotine pouch users and tobacco-based snus users had significantly lower urine levels of 11-dh-TXB<sub>2</sub>, 2,3-d-TXB<sub>2</sub>, and LTE<sub>4</sub> compared to combustible cigarette users ((b) (4)). However, while urine levels of LTE<sub>4</sub> in combustible cigarette users were significantly higher than in nonusers of tobacco/nicotine products ((b) (4)), there were no significant differences in 11-dh-TXB<sub>2</sub> or 2,3-d-TXB<sub>2</sub> urine levels in combustible cigarette users compared to nonusers of tobacco/nicotine products.

Plasma concentration of sICAM-1 and GDF-15 results (secondary endpoint)

- Mean plasma levels of sICAM-1 and GDF-15 were:
  - (b) (4) ng/mL and (b) (4) pg/mL, respectively, for nicotine pouch users
  - (b) (4) ng/mL and (b) (4) pg/mL, respectively, for tobacco-based snus users
  - (b) (4) ng/mL and (b) (4) pg/mL, respectively, for combustible cigarette users

There were no significant differences in plasma levels of sICAM-1 and GDF-15 between nicotine pouch users, tobacco-based snus users, and nonusers of tobacco/nicotine products. In contrast, combustible cigarette users had significantly higher plasma levels of sICAM-1 and GDF-15 compared to nicotine pouch users ((b) (4)), tobacco-based snus users ((b) (4)) and nonusers of tobacco/nicotine products ((b) (4)).

Extracted amounts and fractions results (secondary endpoint)

- Nicotine pouch users had a higher mean extracted amount ((b) (4) mg/unit) and fraction ((b) (4) of nicotine compared to tobacco-based snus-users ((b) (4) mg/unit and (b) (4), respectively). However, the mean total nicotine exposure over the 14-day study period was lower for nicotine pouch users ((b) (4) mg) compared to tobacco-based snus users ((b) (4) mg).
- (b) (4)
- For tobacco-based snus users, the mean total exposure to NNK and NNN was ((b) (4) µg and (b) (4) µg, respectively.

Safety results (secondary endpoint)

- (b) (4) in the study were of mild intensity and assessed as unlikely related to the tobacco/nicotine products used. There were no differences in reporting frequency between the nicotine user groups.

Exploratory endpoints

(b) (4)

## Conclusions

### Biomarkers of exposure - nicotine and nicotine metabolites in plasma and urine

- There were no significant differences in plasma levels of nicotine between nicotine pouch users, tobacco-based snus users, and combustible cigarette users, while the levels of the metabolites cotinine and OH-cotinine were significantly lower for combustible cigarette users after 14 days of *ad libitum* use of either product.
- There were no significant differences in urine levels of nicotine, cotinine, OH-cotinine, and nicotine equivalents between nicotine pouch users and tobacco-based snus users while users of combustible cigarettes had significantly lower levels of these biomarkers.

### Biomarkers of exposure - TSNA in plasma and urine

(b) (4)

### Biomarkers of potential harm – eicosanoids in urine

- The urine levels of eicosanoids (8-iso PGF<sub>2α</sub>, 11-dh-TXB<sub>2</sub>, 2,3-d-TXB<sub>2</sub>, and LTE<sub>4</sub>) were within the lower range for all user groups including nonusers of tobacco/nicotine products and statistical comparisons were ambiguous.

### Biomarkers of potential harm - sICAM-1 and GDF-15 in plasma

- There were no significant differences in plasma levels of sICAM-1 and GDF-15 between nicotine pouch users, tobacco-based snus users, and nonusers of tobacco/nicotine products. In contrast, combustible cigarette users had significantly higher levels of sICAM-1 and GDF-15 compared to all other user groups.

### Total exposure and extracted amounts and fractions of nicotine and TSNA from pouches used by nicotine pouch and tobacco-based snus users

(b) (4)

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## 4 LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

Abbreviation	Explanation
2,3-d-TXB <sub>2</sub>	2,3-dinor-thromboxane B <sub>2</sub>
3-OH-B[a]P	3-hydroxybenzo[a]pyrene
8-iso PGF <sub>2α</sub>	8-iso Prostaglandin F <sub>2α</sub>
11-dh-TXB <sub>2</sub>	11-dehydrothromboxane B <sub>2</sub>
AE	Adverse event
ANOVA	Analysis of variance
ATC	Anatomical therapeutic chemical
BLQ	Below the limit of quantification
BMI	Body mass index
BoE	Biomarkers of exposure
BoPH	Biomarkers of potential harm
CI	Confidence intervals
CS	Clinically significant
CSP	Clinical study protocol
(b) (4)	(b) (4)
CTCAE	Common terminology criteria for adverse events
CV	Coefficient of variation
CVD	Cardiovascular diseases
DBL	Database lock
DSCF	Dwass, Steel, Critchlow-Fligner
ECG	Electrocardiogram
eCRF	Electronic case report form
EDC	Electronic data capture
EEA	European Economic Area
EU	European Union
FAS	Full analysis set
GCP	Good clinical practice
GDF-15	Growth differentiation factor 15
GDPR	General data protection regulation
GM	Geometric mean
HIV	Human immunodeficiency virus
ICF	Informed consent form

ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
IQR	Interquartile range
LLOQ	Lower limit of quantification
LTE <sub>4</sub>	Leukotriene E <sub>4</sub>
Max	Maximum
MedDRA	Medical dictionary for regulatory activities
Min	Minimum
NA	Not available
NAB	N'-nitrosoanabasine
NAT	N'-nitrosoanatabine
NCS	Not clinically significant
NNAL	4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol
NNK	4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone
NNN	N-nitrosonornicotine
OH-cotinine	Trans-3'-hydroxycotinine
PR interval	(ECG) The time from the onset of the P wave to the start of the QRS complex
PT	Preferred term
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
SAP	Statistical analysis plan
SD	Standard deviation
SDV	Source data verification
SERA	Swedish Ethical Review Authority
sICAM-1	Soluble intercellular adhesion molecule-1
SOC	System organ class
TMF	Trial master file
TSNA	Tobacco-specific nitrosamines
US	United States (of America)
WHO	World Health Organization
WOCBP	Women of child-bearing potential

## 5 ETHICAL AND REGULATORY REQUIREMENTS

### 5.1 Independent ethics committee

The Principal Investigator was responsible for the submission of the clinical study protocol (CSP), the subject information, informed consent forms (ICFs) and any other written information to be provided to the subjects, as well as advertisements used for the recruitment of subjects, to the Swedish Ethical Review Authority (SERA) for approval.

The study and the CSP version 1.0 dated 09NOV2022 were approved in writing by SERA on 30NOV2022. Subject recruitment started after this approval.

There were no amendments made to the CSP during the conduct of the study. The CSP (version 1.0) is provided in Appendix 16.1.1.

### 5.2 Ethical conduct of the study

The study was conducted in accordance with ethical principles that have their origin in the Declaration of Helsinki [1] and are compliant with applicable section of the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) Good Clinical Practice (GCP) E6 (R2) guidance [2], and applicable sections of the European Union (EU) Clinical Trials Directive 2001/20/EC [3], and applicable local regulatory requirements. The study was registered in the international standard randomized controlled trial number (ISRCTN) clinical trial registry (ISRCTN38557348) [4].

### 5.3 Subject information and consent

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

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

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

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

The written subject information and ICFs are provided in the trial master file (TMF).

#### 5.4 Subject data protection

The ICFs included information that data were recorded, collected, and processed and could be transferred to European Economic Area (EEA) or non-EEA countries. In accordance with the European Union (EU) General Data Protection Regulation (GDPR), Regulation (EU) 2016/679 [5], the data did not allow for identification of any persons taking part in the study.

The potential study subject was informed that by signing the ICF he/she approved that the authorized representatives from the Sponsor, (b) (4) and the SERA had direct access to their medical records for verification of clinical study procedures. This agreement was substantiated in a separate document, according to local requirements.

The subject had the right to request access to their personal data and to request rectification of any data that were not correct and/or complete, in accordance with the EU GDPR Regulation (EU) 2016/679 [5]. Such requests were raised to the Principal Investigator.

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



## 6 INVESTIGATORS AND STUDY ADMINISTRATIVE STRUCTURE

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## 7 INTRODUCTION

### 7.1 Project 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 [6]. 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 1 preventable death with more than 8 million deaths globally each year [7]. 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 [8]. 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 United States (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 [9]. 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 EU [10]. In 2020, only 7 % of the adult population in Sweden used cigarettes, which is partly attributed to the growing prevalence of snus use as an alternative tobacco product [11]. 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 [12].

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 (TSNA) [13]. 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 [14].

### 7.2 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 TSNA like 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and N-nitrosonornicotine (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 [14,15,16,17].

Swedish snus shows low TSNA concentrations, partly due to the use of pasteurization as the primary tobacco-processing method [18]. Tobacco-leaf free, oral nicotine pouches have the potential to further reduce the risk from TSNA exposure as suggested from recent chemical characterization studies [13].

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 were assessed in plasma and urine. In addition, this study included the measurement of biomarkers of potential harm (BoPH) related to CVD and cancer. Such data could be helpful to categorize the products on the risk continuum scale of tobacco and nicotine use.

This study aimed 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.

## 8 STUDY OBJECTIVES AND ENDPOINTS

### 8.1 Primary, secondary, and exploratory objectives and endpoints

The study objectives and endpoints are summarized in [Table 8.1-1](#).

**Table 8.1-1 Summary of objectives, endpoints, and assessments**

Primary objective	Primary endpoint	Assessment	Reported in Section
To compare plasma concentrations of nicotine, cotinine, trans-3'-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.	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.	Assessment of biomarkers (Section <a href="#">9.4.3</a> ).	Section <a href="#">11.1</a>
Secondary objectives	Secondary endpoints	Assessment	Reported in Section
1. To compare urine concentrations of nicotine and its metabolites and TSNA between users of nicotine pouches, tobacco-based snus, or combustible cigarettes, and nonusers of tobacco/nicotine products.	1. Difference in urine concentrations of total nicotine equivalents and TSNA (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.	Assessment of biomarkers (Section <a href="#">9.4.3</a> ).	Urine concentrations of total nicotine equivalents and TSNA (Section <a href="#">11.2.1.1</a> ).
2. To compare urine concentrations of anatabine, anabasine, and 3-hydroxybenzo(a)pyrene (3-OH-B[a]P) 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-B[a]P between users of nicotine pouches, tobacco-based snus, or combustible cigarettes, and nonusers of tobacco/nicotine products.	Assessment of biomarkers (Section <a href="#">9.4.3</a> ).	Urine concentrations of anatabine, anabasine, and 3-OH-B[a]P (Section <a href="#">11.2.1.2</a> ).
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.	3. Difference in urine concentrations of eicosanoids (8-iso prostaglandin F2 $\alpha$ [8-iso PGF $_{2\alpha}$ ], 11-dehydrothromboxane B2 [11-dh-TXB $_2$ ], 2,3-dinor-thromboxane B2 [2,3-d-TXB $_2$ ], and leukotriene E4 [LTE $_4$ ]) between users of nicotine pouches, tobacco-based snus, or combustible cigarettes, and nonusers of tobacco/nicotine products.	Assessment of biomarkers (Section <a href="#">9.4.3</a> ).	Urine concentrations of eicosanoids (Section <a href="#">11.2.1.3</a> ).

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.	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.	Assessment of biomarkers (Section 9.4.3).	Plasma concentrations of sICAM-1 and GDF-15 (Section 11.2.1.4).
5. To compare the extracted amounts and fractions of nicotine, NNK, and NNN from nicotine pouches and tobacco-based snus.	5. Difference in the extracted amounts (mg/unit) and fractions (%) of nicotine, NNK, and NNN from nicotine pouches and tobacco-based snus.	Nicotine, NNK, and NNN extraction from pouches (Section 9.4.3.3).	Estimated total exposure, and average extracted amounts and fractions (Section 11.2.2).
6. To evaluate the safety and tolerability of nicotine pouches, tobacco-based snus, and combustible cigarettes in current users of these nicotine products.	6. Frequency, seriousness, and intensity of adverse events (AEs).	AEs (Section 9.6.5.4).	AEs (Section 12.2).
Exploratory objectives	Exploratory endpoints	Assessment	Reported in Section
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.	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.	Correlation analysis (Section 9.6.6.1).	Correlation analysis (Section 11.3.1)
2. To analyze the pattern of use between users of nicotine pouches, tobacco-based snus, and combustible cigarettes.	2. Difference in the pattern of use between users of nicotine pouches, tobacco-based snus, and combustible cigarettes.	Pattern of use (Section 9.6.6.2).	Analysis of pattern of use (Section 11.3.2).

## 9 INVESTIGATIONAL PLAN

### 9.1 Overall study design and schedule of events

This was 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 used their product of choice *ad libitum* throughout the 14-day study period.

Four separate user groups were recruited:

- A) exclusive users of a 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 provided informed consent prior to study procedures. The subjects reported 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) took place within 4 weeks prior to Visit 2 and included 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 were not allowed to eat within 1 hour prior to spirometry assessments, nor were subjects 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, were assessed by urinary cotinine strip test (cotinine cut off:  $\geq 200$  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) choose 1 product which they exclusively used during the study. This was the product brand that they had mostly used in the past month in case they were not exclusive users of 1 product brand and also implied nicotine strength and flavor variation of the same brand. The brand, including nicotine strength and flavor, was documented in the eCRF during screening, at Visit 2, as well as at the end-of study visit (Visit 3).

All subjects (including the nonusers of tobacco/nicotine products) were informed how to collect the first morning urine void. Subjects in the nonusers of tobacco/nicotine products were provided with a urine sample collection container and a cooling bag for transportation to the study sites at the screening visit. The same material were provided to users of nicotine pouches, tobacco-based snus, and combustible cigarettes at Visit 2. Furthermore, during Visit 2, users of nicotine pouches and tobacco-based snus were provided with collection containers and an extra cooling bag for used pouches.

All subjects reported to the study sites for Visit 2. Blood was 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 exclusively used 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 was documented in the eCRF. Also, the users of nicotine pouches and tobacco-based snus, collected (b) (4) (Samples A) and on (b) (4) (Samples B) and (b) (4)

For the nonusers of tobacco/nicotine products blood and urine for all analysis of BoE and BoPH were collected at 1 study visit (Visit 2, Table 9.1-1) Thus, this group of subjects also brought 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 did not report to the study sites for Visit 3.

After 14 days, the users of nicotine pouches, tobacco-based snus, and combustible cigarettes reported to the study sites for Visit 3. The subjects brought their morning urine void to the study sites, collected in the container, and placed in the cooling bag provided at Visit 2. The subjects were interviewed about experienced AEs, used brand, nicotine strength, and flavor and there was a compliance check of the electronic diary. Also, the users of nicotine pouches and tobacco-based snus brought their used and frozen pouches collected on 4 separate days (in a separate cooling bag to avoid cross contamination with the urine sample). Blood was collected from all subjects (users of nicotine pouches, tobacco-based snus, and combustible cigarettes) for analysis of BoE and BoPH.

If the subjects forgot to bring the collected morning urine void, they informed the study sites, and a new appointment was made as soon as possible (preferably the next day). If the nicotine pouch and tobacco-based snus users forgot to bring their used pouches to the study sites at Visit 3, they re-visited 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 purchased the applicable products used by the subjects in group A and B for chemical characterization of unused reference pouches.

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

The overall schedule of events is shown in Table 9.1-1. Study assessments are described in Section 9.4.



*Table 9.1-1 Schedule of events*

(b) (4)

(b) (4)

## 9.2 Selection of study population

Subjects were recruited from (b) (4) of volunteers and from advertising in media (including social media). The advertisement texts approved by the SERA were used to create the materials for recruitment.

### 9.2.1 Inclusion criteria

For inclusion in the study, the subjects had to 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  $\geq 25$  to  $\leq 45$  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) could occur in any of the study groups, except for the users of combustible cigarettes.
5. Women of child-bearing potential (WOCBP) had to be willing to use a sufficient contraceptive method for the duration of the study, this included 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 was allowed when this was the preferred and usual lifestyle of the subject.

#### 9.2.1.1 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  $\geq 1$  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  $\geq 200$  ng/mL on Visit 1.
4. Willingness to use only 1 specific Swedish Match brand nicotine pouch (type, flavor, and nicotine strength) product during the conduct of this study (total of 14 days).

#### 9.2.1.2 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  $\geq 1$  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  $\geq 200$  ng/mL on Visit 1.
4. Willingness to use only 1 specific tobacco-based snus product (brand, type, flavor, and nicotine strength) during the conduct of this study (total of 14 days).

### 9.2.1.3 *Additional inclusion criteria for Group C (Users of combustible cigarettes)*

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

### 9.2.1.4 *Additional inclusion criteria for Group D (Nonusers)*

1. Nonusers of tobacco/nicotine products who had 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.

### 9.2.2 *Exclusion criteria*

Subjects were excluded from the study if any of the following exclusion criteria were fulfilled:

1. History of any clinically significant disease or disorder which, in the opinion of the Investigator, could either put the subject at risk because of participation in the study, or influence the results or the subject's ability to participate in the study.
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, could interfere with the absorption, distribution, metabolism, or excretion of the nicotine products or could 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 were pregnant, breastfeeding, or intended 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 were expected given the subject's medical history and prescribed medications could 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.  $BMI \leq 18$  and  $\geq 33$  kg/m<sup>2</sup>.
10. Regular use of any medication, especially those which could 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] were permitted.

11. Subjects who intended 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 considered 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 studies were not to be excluded.

#### **9.2.2.1 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 1 or more days during the study.
3. Exposure to passive smoking in the household.

#### **9.2.2.2 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 1 or more days during the study.
3. Exposure to passive smoking in the household.

#### **9.2.2.3 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 1 or more days during the study.

#### **9.2.2.4 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.

### **9.2.3 Restrictions during the study**

Subjects had to be willing to comply with the restrictions as outlined in Section [9.2.3.1](#) below.

#### **9.2.3.1 General restrictions**

1. The subjects were 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 were expected to use contraceptive methods in accordance with inclusion criterion #5 or practice abstinence from heterosexual intercourse (only allowed when this was the preferred and usual lifestyle of the subject) during the study.

3. If the nicotine product user groups (nicotine pouches, tobacco-based snus, or combustible cigarettes) used different brands/products, only 1 should be used during the 14-day study period.
4. Drugs of abuse: Subjects should abstain from any drugs of abuse during the study, *i.e.*, from screening (Visit 1) to the last study visit (Visit 3).
5. Subjects were 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).

#### **9.2.4 Removal of subjects from therapy or assessment**

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

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

Reasons for discontinuation could include:

- Withdrawal of consent (subject decision).
- Severe non-compliance to CSP procedures, as judged by the Investigator and/or Sponsor.
- Subject was lost to follow-up. A subject was considered lost to follow-up if he/she failed to come for consecutive scheduled visits and if he/she was 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.
- Pregnancy.
- Death.
- Meeting of an exclusion criterion during the study, which, in the opinion of the Investigator, could pose a risk for the subject.

### **9.3 Investigational products**

#### **9.3.1 Product administration and identity of investigational products**

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

- Group A, nicotine pouch users: 1 Swedish Match nicotine pouch product of choice, containing 3-16 mg nicotine per pouch throughout the 14-day study.
- Group B, tobacco-based snus users: 1 Swedish tobacco-based snus product of choice, containing 4-20 mg nicotine per pouch throughout the 14-day study.
- Group C, combustible cigarette users: 1 commercially manufactured combustible cigarette product of choice throughout the 14-day study.
- Group D, nonusers of tobacco/nicotine products: No tobacco/nicotine product throughout the 14-day study.

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

### **9.3.2 Method of assigning subjects to user groups**

Not applicable.

### **9.3.3 Blinding**

Not applicable.

### **9.3.4 Prior and concomitant therapy**

There were no restrictions (except for as specified below) concerning concomitant medications or therapies, as long as the subject was on a stable course of medication from the screening visit to the last visit. Prescribed medications taken *pro re nata* could be a reason for exclusion as judged by the Investigator if they affected the subject's general condition and salivation. Usage of hormonal contraceptives (females) and non-prescription pain medication (paracetamol) were permitted.

#### Prohibited medications

Regular use of any medication which could 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 were prohibited within 14 days prior to screening and during the study.

### **9.3.5 Administration compliance**

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

## **9.4 Study assessments and variables**

### **9.4.1 Recording of data**

The Principal Investigator provided the Sponsor with all data produced during the study from the scheduled study assessments. The Principal Investigator ensured the accuracy, completeness, legibility, and timelines of the data reported to the Sponsor in the eCRF and in all required reports.

### **9.4.2 Demographics and other baseline characteristics**

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

The following demographic data were recorded in the eCRF: gender, age, ethnicity, and race. Weight and height were measured without shoes. BMI was calculated from the height and weight recorded. Medical/surgical history, prior medications and history of tobacco/nicotine

product usage were obtained by subject interview and registered in the eCRF. All concomitant medications were registered in the eCRF.

### **9.4.3 Assessments related to primary and secondary endpoints**

#### **9.4.3.1 Assessment of biomarkers**

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

#### **9.4.3.2 Analysis of biomarkers of exposure and biomarkers of potential harm**

The BoEs and BoPHs analyzed (at Visit 2 in the nonuser group, Group D, and at Visit 3 in the tobacco/nicotine user groups, Group A-C) are specified in [Table 9.4-1](#).

**Table 9.4-1 BoEs and BoPHs analyzed in plasma and urine**

(b) (4)



#### 9.4.3.3 *Nicotine, NNK, and NNN extraction from pouches*

Used pouches were 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 purchased the applicable products used by the subjects in Group A and B for chemical characterization of unused reference pouches at Swedish Match. The extracted amount and fraction of nicotine, NNK, and NNN were calculated by subtracting the average of the pouches used by the nicotine pouch and tobacco-based snus users from the average of (b) (4).

#### 9.4.4 *Additional analyses of selected biomarkers*

At Visit 2, plasma samples were collected from the tobacco/nicotine user groups (Group A-C) for selected BoEs, which were subsequently analyzed using validated bioanalytical methods. The following BoEs were included in the analysis: nicotine, cotinine, OH-cotinine, NNAL, and NNN.

#### 9.4.5 *Safety assessments*

##### 9.4.5.1 *Adverse Events*

AEs (including serious AEs [SAEs]) were collected from Visit 2 until the last visit (Visit 3). The grading of the severity/intensity (grade 1 to grade 5) of AEs followed the common terminology criteria for AEs (CTCAE) v5.0 [19]. AEs were assessed as unlikely, possibly, or probably related to tobacco/nicotine products.

For definitions of AEs, details regarding grading, causality, and outcome assessments, as well as reporting procedures for SAEs refer to the CSP in Appendix 16.1.1.

##### 9.4.5.2 *Vital signs*

Systolic and diastolic blood pressure and pulse were measured in supine position after 10 minutes of rest. Any vital signs outside of normal ranges were specified and documented as clinically significant or not clinically significant.

##### 9.4.5.3 *Lung function measured by spirometry*

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

##### 9.4.5.4 *12-lead electrocardiogram*

Single 12-lead ECGs were recorded in supine position after 10 minutes of rest using an ECG machine. The resting heart rate and PQ/PR, QRS, QT, and QTcF intervals were recorded. Safety ECGs were reviewed and interpreted on-site by the Investigator. Any abnormalities were specified and documented as clinically significant or not clinically significant.

#### 9.4.5.5 *Laboratory assessments*

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

Plasma samples for analysis of HIV, serum hepatitis B surface antigen, hepatitis C antibody were taken at the screening visit.

Blood samples for the analysis of clinical chemistry and hematology were collected through venipuncture or an indwelling venous catheter and sent to Synlab Sverige AB and analyzed by routine analytical methods.

The laboratory parameters are defined in [Table 9.4-2](#) and were assessed at the screening visit.

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

**Table 9.4-2 Laboratory parameters**

(b)	(4)
-----	-----

#### 9.4.5.6 *Physical examination*

A brief physical examination included assessments of the head, nose, throat, skin, neurological, lungs, cardiovascular, abdomen (liver and spleen), and extremities. Any abnormalities were specified and documented as clinically significant or not clinically significant.

#### **9.4.6 Assessments related to exploratory endpoints**

Correlation analyses were conducted using total nicotine exposure, as well as NNN and NNK exposure, in relation to plasma and urine concentrations of BoE. The extraction procedures are outlined in Section 9.4.3.3 while the correlation analysis is described in Section 9.6.6.1.

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

#### **9.4.7 Appropriateness of measurements**

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

### **9.5 Data quality assurance**

The study was performed in compliance with GCP, applicable regulations, and (b) (4) Standard Operating Procedures.

Before inclusion of the first subject into the study, a study initiation visit was performed by (b) (4) at each site in order to inform and train relevant study staff. The Investigator was thereafter responsible for providing appropriate study related training to new staff and to forward any new information of relevance to the performance of this study to the staff involved.

An eCRF was completed for each subject included. A sample of the eCRF is included as Appendix 16.1.2.

The study sites were periodically visited by a Monitor from (b) (4). The Monitor had direct access to medical records and original data for source data verification according to the risk-based monitoring plan.

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

Data management was performed by (b) (4) in accordance with the data management plan. When all data had been cleaned, coded, validated, signed, and locked, clean file was declared, and the database was locked on 22MAY2023.

No audits were performed during this study.

### **9.6 Statistical methods planned in the CSP and determination of sample size**

#### **9.6.1 General**

Descriptive statistics are 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) have been calculated for metric parameters, additionally, graphical presentation of data where applicable. Categorical and ordinal parameters are summarized using the number and percentages of subjects in each group.

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

Individual subject data were listed by subject number, user group, and, where applicable, by assessment time.

All descriptive summaries and statistical analyses were performed using SAS Version 9.4 (SAS Institute, Inc., Cary, NC).

Baseline was defined as the last data collected prior to the start of the 14-day *ad libitum* usage period. No adjustment for multiple comparisons was made and no imputation of missing data was performed.

### **9.6.2      *Determination of sample size***

The primary endpoint in this study was 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 was performed as available data for this study design was lacking. Based on previous experiences with other user groups, (b) (4) subjects ((b) (4) subjects per user group) were considered to generate sufficient data for the purpose of this study, also with an estimated dropout rate of 10 % per user group.

### **9.6.3      *Analysis data sets***

The Full Analysis Set (FAS) consisted of all subjects who were included and who had at least 1 post-baseline data point.

### **9.6.4      *Description of study population***

#### **9.6.4.1      *Demographics and baseline characteristics***

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

#### **9.6.4.2      *Medical/surgical history and prior/concomitant medications***

Medical/surgical history are presented by system-organ-class (SOC) and preferred term (PT). Prior/concomitant medications are presented by anatomical therapeutic chemical (ATC) level 4 and 5.

All data were listed by subject number.

#### **9.6.4.3      *History of tobacco/nicotine product use***

The history of nicotine use in terms of tobacco/nicotine product use, 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) are presented.

#### **9.6.4.4      *Treatment compliance***

The number of subjects in each group, and their individual usage were listed.

## 9.6.5 Analysis of primary and secondary endpoints

### 9.6.5.1 Analysis of biomarkers

The difference in plasma and urine concentrations of various biomarkers among users of nicotine pouches, tobacco-based snus, combustible cigarettes, and nonusers of tobacco/nicotine products were assessed using analysis of variance (ANOVA). Pairwise comparisons between these groups were conducted, and their corresponding p-values were calculated.

In all biomarker formal statistical analyses, the last collected biomarker data were used, *i.e.*, Visit 2 for nonusers and Visit 3 for users of tobacco/nicotine products.

#### 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 were assessed using ANOVA. Pairwise comparisons between these groups were conducted, and their corresponding p-values were calculated.

#### Analysis of secondary endpoints

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

- total nicotine equivalents and TSNA (NNAL, NNN, NAB, and NAT) in urine,
- anatabine, anabasine, and 3-OH-B[a]P in urine,
- eicosanoids (8-iso-PGF<sub>2α</sub>, 2,3-dinor-TXB<sub>2</sub>, 11-dehydro-TXB<sub>2</sub>, and LTE<sub>4</sub>) in urine,
- sICAM-1 and GDF-15 in plasma.

Pairwise comparisons between these groups were conducted, and their corresponding p-values were calculated.

### 9.6.5.2 Post-hoc analysis of biomarkers

A number of comparisons could not be performed using the original parametric ANOVA analysis due to too many values below the limit of quantification (BLQ) for several parameters in several user groups (Table 9.6-1 and Table 9.6-2). Therefore, the Kruskal-Wallis test, a non-parametric counterpart to ANOVA, was used to analyze the difference between groups. Also, non-parametric pairwise comparisons between groups were calculated with their corresponding p-values using the Dwass, Steel, Critchlow-Fligner (DSCF) approach, which is based on pairwise two-sample Wilcoxon comparisons.

***Table 9.6-1 User groups excluded from pairwise comparisons based on ANOVA due to >50% of observations below the limit of quantification: primary endpoints biomarkers in plasma***

(b) (4)

**Table 9.6-2** *User groups excluded from pairwise comparisons based on ANOVA due to >50% of observations below the limit of quantification: secondary endpoints biomarkers in urine and plasma*

(b) (4)

(b) (4)



(b) (4)

(b) (4)

### 9.6.5.3 *Analysis of extracted amount of nicotine, NNK, and NNN*

Nicotine, NNK, and NNN were 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) and used pouches was used to calculate the *in vivo* extraction. The calculated extracted amounts of nicotine, NNK, and NNN per pouch were averaged per subject and multiplied with the consumption (number of pouches) reported in the electronic diary over the 14-day *ad libitum* usage period, to receive the total exposure.

Total exposure to nicotine, NNK, and NNN was summarized descriptively.

Average extracted amounts (mg/unit) and fractions (%) of nicotine, NNK, and NNN were summarized descriptively.

### 9.6.5.4 *Adverse events*

An overview of all AEs, including SAEs, intensity, relationship to use, and deaths are presented. The incidence of AEs and SAEs were summarized by SOC and PT by group and overall.

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

### 9.6.5.5 *Vital signs, ECG, laboratory analysis, and physical examinations*

Vital signs (systolic/diastolic blood pressure, pulse rate, and lung function) are summarized by group.

All ECGs were categorized as "normal", "abnormal, not clinically significant", or "abnormal, clinically significant" (as judged by the Investigator) and summarized by group.

Safety laboratory data were summarized by group. Clinically significant and non-clinically significant abnormal findings were specified and presented by subject number and summarized by group. All data were listed by subject number.

## 9.6.6 *Analysis of exploratory endpoints*

### 9.6.6.1 *Correlation analysis*

Total exposure to nicotine, NNK, and NNN was calculated. The intake was 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, as shown in [Table 9.6-3](#).

**Table 9.6-3 Correlation analysis models**

(b)	(4)
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To all of the models above, user group (A or B) was fitted to the models as dummy variables together with the interaction effect total exposure to nicotine/NNK/NNN\*user group to produce different regression slopes for Group A and Group B. The regression result was presented in a graph together with 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.

#### 9.6.6.2 *Pattern of use*

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

### 9.7 **Changes in the conduct of the study or planned analyses**

There were no amendments to the final CSP (version 1.0 dated 09NOV2022).

#### 9.7.1 *Changes in the planned statistical analyses*

Changes to the planned analyses and the timing of these are summarized in [Table 9.7-1](#).

**Table 9.7-1 Changes in the planned statistical analyses**

(b) (4)

## 10 STUDY SUBJECTS

### 10.1 Disposition of subjects

The subject disposition is summarized in [Table 10.1-1](#). Screening failures are detailed in [Table 10.1-2](#).

In total, (b) (4) were screened, and (b) (4) were included in the study ([Table 10.1-1](#)). (b) (4)

In total, (b) (4) subjects were withdrawn prior to Visit 2. Of these, (b) (4) were screening failures ([Table 10.1-2](#)). (b) (4)

(presented as “Other” in [Table 10.1-1](#)).

The subjects were recruited as follows:

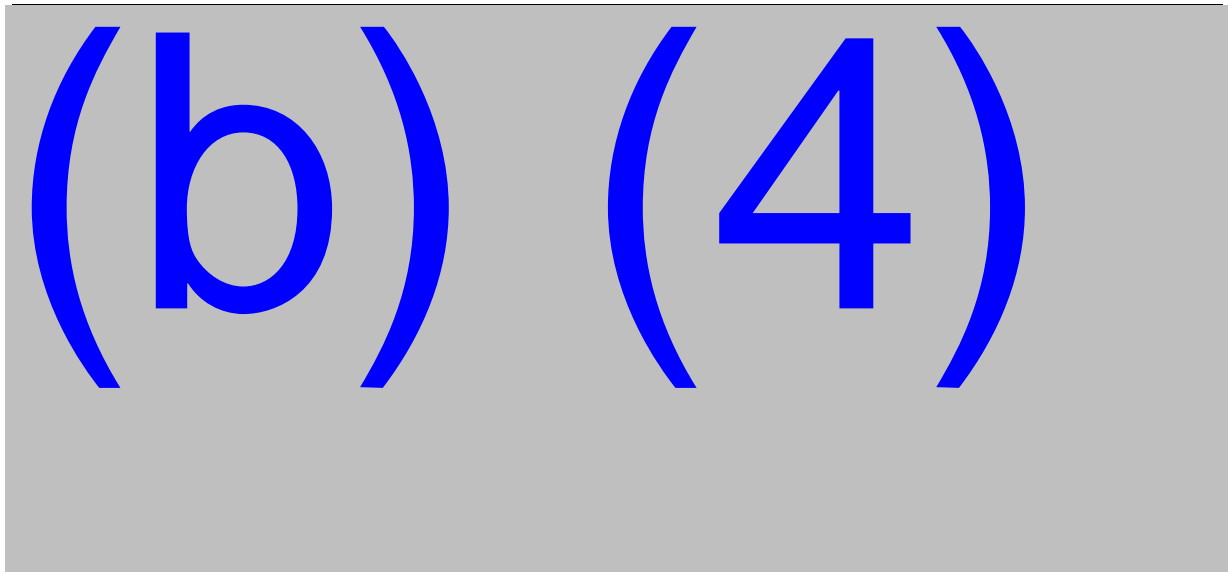
- (b) (4)  
Group A: (b) (4), Group B: (b) (4), Group C: (b) (4), Group D: (b) (4)  
○ (b) (4)
- (b) (4)  
Group A: (b) (4), Group B: (b) (4), Group C: (b) (4), Group D: (b) (4)  
○ (b) (4)

(b) (4)

Individual data are provided in Appendix 16.2.1.

***Table 10.1-1 Subject disposition (all subjects)***

(b) (4)



*Table 10.1-2 Reason for screening failures*

The table area is redacted with a solid grey background. In the upper left portion of this redacted area, the text "(b)" is written in a large, bold, blue font. To its right, the text "(4)" is also written in a large, bold, blue font. The rest of the table content is obscured by the grey redaction box.

### 10.1.1 Study discontinuations

Of the (b) (4), (b) (4) in the nicotine pouch group (Group A) was lost to follow-up after Visit 2 (Appendix 16.2.1).

## 10.2 Protocol deviations

A total of 30 protocol deviations were reported by (b) (4) of the study subjects (including (b) (4) subjects who were not included in the study). All protocol deviations are listed in Appendix 16.2.2.

### 10.2.1 Major protocol deviations

There were 17 major protocol deviations affecting (b) (4) subjects recorded during the study. All deviations were related to signing or dating of the ICF (study procedures) at the screening visit:

- The ICF was signed by the subject, but the Investigator mistakenly dated it, instead of the subject, resulting in 14 major protocol deviations.
- The Investigator changed the date format on the ICF, leading to 2 major protocol deviations.
- The subject binder was signed only by the Investigator and not by the subject, resulting in 1 major protocol deviation.

In all cases, the dating and signing was corrected by the subject on the next study visit or confirmed by the subject via telephone. Of the 17 subjects affected by these deviations, 2 subjects were screening failures and were not included in the study. The remaining 15 subjects were all included in the FAS.

### 10.2.2 Minor protocol deviations

There were 13 minor protocol deviations affecting 13 subjects recorded during the study.

Nine minor protocol deviations were related to study procedures:

- 2 deviations related to missing laboratory values.
- 2 deviations related to centrifugation of plasma samples outside allowed time window.
- 3 deviations related to missing PR value on the ECG.
- 1 deviation related to missed marking of pouch number.
- 1 deviation related to too few collected pouches on the first day of the *ad libitum* usage period. All subjects were included in the FAS.

Three minor protocol deviations were reported as “Other”:

- (b) (4)
- (b) (4)



- (b) (4)

(b) (4)

The subject was included in the FAS.

### 10.3 Data sets analyzed

One analysis set was defined for the study. The FAS consisted of all subjects who had been included in the study and this population was also used for the analysis of safety. (b) (4)

1.

Individual subject data are provided in Appendix 16.2.3.

### 10.4 Demographics and other baseline characteristics

Individual listings for subject demographic and baseline data for all included subjects are presented in Appendix 16.2.4.

#### 10.4.1 Demographics

The demographics and baseline characteristics for subjects included in the study are summarized in Table 10.4-1. (b) (4)

(b) (4)

(b) (4)

*Table 10.4-1 Baseline characteristics and demographics (Full analysis set)*

(b) (4)	(b) (4)
---------	---------

(b) (4)

#### 10.4.2 Medical history

In total, (b) (4) medical history events were reported by (b) (4). These events are summarized by SOC and PT in [Table 14.1-1](#) in Section 14.1.1.

(b) (4)

Individual data are listed in Appendix 16.2.4.

### 10.4.3 Prior and concomitant medication

Prior and concomitant use of medications are summarized in Table 14.1-2 and Table 14.1-3 in Section 14.1.2.

Prior medications were reported by (b) (4). The most commonly reported prior medication was paracetamol, taken by (b) (4), and ibuprofen, taken by (b) (4)s.

Concomitant medications were reported by (b) (4). The most commonly used medication during the study were (b) (4), followed by paracetamol, taken by (b) (4).

All other usage of concomitant medications were reported by < 5 % of the subjects in the study.

(b) (4)

None of the subjects frequently took medication which could interfere with the cyclooxygenase pathway or drugs known to be strong inducers/inhibitors of CYP450 enzymes (refer to exclusion criteria no 10).

### 10.4.4 History of nicotine use

History of nicotine use is summarized in Table 14.1-4 in Section 14.1.3.

- Group A: The nicotine pouch user group had consumed in average 12.18 pouches per day for the last 30 days (range between 4 and 26 pouches per day), with an average duration of use of 3.24 years (range between 1 and 10 years).
- Group B: The tobacco-based snus group had consumed in average 14.58 pouches per day for the last 30 days (range between 5 and 30 pouches per day) with an average duration of use of 11.10 years (range between 1 and 28 years).
- Group C: The combustible cigarette group had consumed in average 12.34 cigarettes per day for the last 30 days (range between 4 and 30 cigarettes per day), with an average of use of 14.48 years (range between 3 and 27 years).

## 10.5 Measurements of treatment compliance

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

## 11 BIOMARKER EVALUATION

### 11.1 Evaluation of primary endpoints

#### 11.1.1 Plasma concentrations of nicotine, cotinine, OH-cotinine, NNAL, and NNN

##### Nicotine, cotinine, and OH-cotinine

The plasma concentrations of nicotine, cotinine, OH-cotinine, NNAL, and NNN in the different user groups are presented in [Table 11.1-1](#), while statistical analyses are shown in [Table 11.1-2](#).

Mean nicotine plasma concentrations were (b) (4), and (b) (4) ng/mL for nicotine pouch users, tobacco-based snus users, and combustible cigarette users, respectively ([Table 11.1-1](#)). There were no significant differences in mean nicotine plasma concentrations between the groups ([Table 11.1-2](#)).

Mean levels of cotinine and OH-cotinine in plasma were (b) (4) and (b) (4) ng/mL for nicotine pouch users, (b) (4) and (b) (4) ng/mL for tobacco-based snus users, and (b) (4) and (b) (4) ng/mL for combustible cigarette users, see [Table 11.1-1](#). There were no significant differences in cotinine and OH-cotinine plasma levels between nicotine pouch users and tobacco-based snus users. Users of combustible cigarettes had significantly lower plasma levels of cotinine and OH-cotinine compared to both nicotine pouch users ((b) (4) for both comparisons, ANOVA) and tobacco-based snus users ((b) (4) for both comparisons, ANOVA), see [Table 11.1-2](#).

The post-hoc non-parametric test (Wilcoxon rank sum test) showed that plasma concentrations of nicotine, cotinine, and OH-cotinine were significantly lower in the nonusers of tobacco/nicotine compared to the other groups ((b) (4) for all comparisons and parameters), see [Table 11.1-2](#).

##### NNAL and NNN

NNAL and NNN were BLQ in plasma for all subjects in the nicotine pouch user group, and the originally planned pairwise comparisons (ANOVA) with the other groups could therefore not be performed ([Table 11.1-1](#)). For tobacco-based snus users, the mean NNAL and NNN plasma concentrations were (b) (4) and (b) (4) pg/mL, respectively, while combustible cigarette users had mean NNAL and NNN plasma concentrations of (b) (4) and (b) (4) pg/mL, respectively ([Table 11.1-1](#)). There were no significant differences in the mean NNAL and NNN concentrations between tobacco-based snus-users and combustible cigarette users ([Table 11.1-2](#)).

Nicotine was BLQ in (b) (4) out of (b) (4) nonusers of tobacco/nicotine products, while cotinine, OH-cotinine, NNAL, and NNN were BLQ in plasma of all nonuser subjects ([Table 11.1-1](#)). The originally planned pairwise comparisons (ANOVA) to the other groups could therefore not be performed for any of these parameters.

The post-hoc non-parametric test (Wilcoxon rank sum test) showed that NNAL and NNN plasma concentrations in nicotine pouch users were significantly lower compared to tobacco-based snus users ((b) (4) for both parameters) and combustible cigarette users ((b) (4) for both parameters) while there were no significant differences between nicotine pouch users and nonusers of tobacco/nicotine products in terms of either NNAL or NNN, see [Table 11.1-2](#).

***Table 11.1-1 Descriptive summary of primary endpoint biomarkers in plasma (Full analysis set)***

(b) (4)

(b) (4)

***Table 11.1-2 Statistical analyses of primary endpoint biomarkers in plasma (Full analysis set)***

(b) (4)

(b) (4)



(b) (4)

## 11.2 Evaluation of secondary endpoints

### 11.2.1 Biomarkers of exposure and biomarkers of potential harm

A descriptive summary of secondary endpoint biomarkers is presented in [Table 11.2-1](#) while statistical analyses are shown in [Table 11.2-2](#).

Three subjects in the nicotine pouch group had biomarker values that deviated from the values of the other subjects in the group. These subjects were included in the analysis of biomarkers, which may have impact on the mean biomarker concentrations for the nicotine pouch group. For details, see Section [11.6.3.1](#).

#### 11.2.1.1 Urine concentrations of total nicotine equivalents and TSNA

All analytes assessed in urine were normalized (divided) by urine creatinine concentrations to correct for variable dilution and all units are hence expressed by mg creatinine.

#### Urine nicotine, cotinine, OH-cotinine, and nicotine equivalents

Mean nicotine levels in urine were (b) (4) and (b) (4) ng/mg for nicotine pouch users, tobacco-based snus users, and combustible cigarette users, respectively. Mean cotinine levels in urine were (b) (4), and (b) (4) ng/mg for the corresponding user groups while mean OH-cotinine levels were (b) (4), and (b) (4) ng/mg and mean nicotine equivalent levels were (b) (4), and (b) (4) µmol/mg ([Table 11.2-1](#)).

There were no significant differences in urine levels of nicotine, cotinine, OH-cotinine, or nicotine equivalents between nicotine pouch users and tobacco-based snus users. Users of combustible cigarettes had significantly lower urine levels of nicotine, cotinine, OH-cotinine, and nicotine equivalents compared to both nicotine pouch users (b) (4) for all comparisons, ANOVA) and tobacco-based snus users (b) (4) for all comparisons, ANOVA), see [Table 11.2-2](#).

For nonusers of tobacco/nicotine products, nicotine and cotinine were BLQ in urine in all subjects. Similarly, the vast majority of OH-cotinine concentrations (b) (4) subjects) were BLQ in urine of nonusers of tobacco/nicotine products. The originally planned pairwise comparisons (ANOVA) to the other groups could therefore not be performed for any of these parameters ([Table 11.2-1](#) and [Table 11.2-2](#)).

The post-hoc non-parametric test (Wilcoxon rank sum test) showed that urine concentrations of nicotine, cotinine, OH-cotinine, and nicotine equivalents were significantly lower in the nonusers of tobacco/nicotine compared to the other groups (b) (4) for all comparisons and parameters), see [Table 11.2-1](#) and [Table 11.2-2](#).

#### NNAL and NNN

Mean NNAL levels in urine were (b) (4), and (b) (4) pg/mg for nicotine pouch users, tobacco-based snus users, combustible cigarette users, and non-users of tobacco/nicotine products, respectively, while mean NNN levels were (b) (4) and (b) (4) pg/mg for the corresponding user groups ([Table 11.2-1](#)). There were no significant differences in urine NNAL or NNN levels between tobacco-based snus users and combustible cigarette users. Users of nicotine pouches had significantly lower urine levels of NNAL compared to both tobacco-based snus users and combustible cigarette users (b) (4) for both comparisons, ANOVA), see [Table 11.2-2](#).

NNN was BLQ in (b) (4) out of (b) (4) subjects in the nicotine pouch user group and the originally planned pairwise comparisons (ANOVA) to the other groups could therefore not be performed for this parameter (Table 11.2-1 and Table 11.2-2).

Similarly, for nonusers of tobacco/nicotine products, most NNN (43/49 subjects) concentrations were BLQ and the originally planned pairwise comparisons (ANOVA) to the other groups could therefore not be performed for this parameter (Table 11.2-1 and Table 11.2-2).

The post-hoc non-parametric test (Wilcoxon rank sum test) showed that urine concentrations of NNN were significantly lower in nicotine pouch users compared to both tobacco-based snus users and combustible cigarette users ( $p < 0.0001$  for both comparisons) and that there was no significant difference compared to nonusers of tobacco/nicotine products (Table 11.2-2).

## NAB and NAT

Mean NAB levels in urine were (b) (4) and (b) (4) pg/mg for tobacco-based snus users and combustible cigarette users, respectively, while mean NAT levels in urine were (b) (4), and (b) (4) pg/mg for nicotine pouch users, tobacco-based snus users, and combustible cigarette users (Table 11.2-1). Users of combustible cigarettes had significantly higher urine levels of NAB compared to tobacco-based snus users (b) (4), ANOVA), while there were no significant difference in urine NAT levels between combustible cigarette users and tobacco-based snus users, see Table 11.2-2.

NAB was BLQ in urine in all subjects in the nicotine pouch user group and NAT concentrations were BLQ in (b) (4) out of (b) (4) subjects in this group (Table 11.2-1). The originally planned pairwise comparisons (ANOVA) to the other groups could therefore not be performed for these parameters (Table 11.2-2).

Similarly, for nonusers of tobacco/nicotine products, most NAB ((b) (4) subjects) and NAT ((b) (4) subjects) concentrations were BLQ and the originally planned pairwise comparisons (ANOVA) to the other groups could therefore not be performed for these parameters (Table 11.2-1 and Table 11.2-2).

The post-hoc non-parametric test (Wilcoxon rank sum test) showed that urine concentrations of NAB and NAT were significantly lower in nicotine pouch users compared to both tobacco-based snus users and combustible cigarette users ((b) (4) for both parameters and comparisons) and that there was no significant difference between nicotine pouch users and nonusers of tobacco/nicotine products in terms of either NAB or NAT.

### 11.2.1.2 Urine concentrations of anatabine, anabasine, and 3-OH-B[a]P

#### Anatabine and anabasine

Mean anatabine levels in urine were (b) (4), and (b) (4) ng/mg and mean anabasine levels were (b) (4), and (b) (4) ng/mg for nicotine pouch users, tobacco-based snus users, and combustible cigarette users, respectively (Table 11.2-1).

Users of tobacco-based snus had significantly higher urine levels of both anatabine and anabasine compared to combustible cigarette users ((b) (4) for both comparisons, ANOVA), see Table 11.2-2.

Anatabine concentrations were BLQ in (b) (4) out of (b) (4) subjects in the nicotine pouch user group, while anabasine were BLQ in (b) (4) out of (b) (4) subjects, see Table 11.2-1. Similarly, for nonusers of tobacco/nicotine products, a vast majority of anatabine concentrations (b) (4) subjects) and a majority of anabasine concentrations (b) (4) subjects) were BLQ (Table 11.2-1). The

originally planned pairwise comparisons (ANOVA) to the other groups could therefore not be performed for these parameters (Table 11.2-2).

The post-hoc non-parametric test (Wilcoxon rank sum test) showed that urine concentrations of anatabine and anabasine were significantly lower in nicotine pouch users compared to both tobacco-based snus users and combustible cigarette users (b) (4) for both parameters and comparisons) and that there was no significant difference between nicotine pouch users and nonusers of tobacco/nicotine products in terms of either anatabine or anabasine (Table 11.2-2).

### 3-OH-B[a]P

Mean 3-OH-B[a]P levels in urine were (b) (4) and (b) (4) fg/mg for nicotine pouch users, tobacco-based snus users, and combustible cigarette users, respectively (Table 11.2-1).

A majority of 3-OH-B[a]P concentrations were BLQ in nicotine pouch users (b) (4) subjects), tobacco-based snus users (b) (4) subjects) and nonusers of tobacco/nicotine products ((b) (4) subjects) (Table 11.2-1). Consequently, none of the originally planned pairwise comparisons (ANOVA) could be performed for this parameter (Table 11.2-2).

The post-hoc non-parametric test (Wilcoxon rank sum test) showed no significant differences in 3-OH-B[a]P levels in urine among nicotine pouch users, tobacco-based snus users, and nonusers of tobacco/nicotine products. However, combustible cigarette users had significantly higher urine levels of 3-OH-B[a]P compared to all other groups ((b) (4) (b) (4) (Table 11.2-2).

#### 11.2.1.3 Urine concentrations of eicosanoids

### 8-iso PGF<sub>2α</sub>

Mean 8-iso PGF<sub>2α</sub> levels in urine were (b) (4), and (b) (4) ng/mg for nicotine pouch users, tobacco-based snus users, combustible cigarette users, and nonusers of tobacco/nicotine products, respectively (Table 11.2-1).

There were no significant differences in 8-iso PGF<sub>2α</sub> levels between nicotine pouch users, combustible cigarette users, and nonusers of tobacco/nicotine products, see Table 11.2-2. However, tobacco-based snus users had significantly lower 8-iso PGF<sub>2α</sub> levels compared to both nicotine pouch users (b) (4), ANOVA) and combustible cigarette users (b) (4), ANOVA), while there was no significant difference compared to nonusers of tobacco/nicotine products (Table 11.2-2).

Corresponding results were obtained using the post-hoc non-parametric test (Wilcoxon rank sum test), see Table 11.2-2.

### 11-dh-TXB<sub>2</sub>

Mean 11-dh-TXB<sub>2</sub> levels in urine were (b) (4), and (b) (4) ng/mg for nicotine pouch users, tobacco-based snus users, combustible cigarette users, and nonusers of tobacco/nicotine products, respectively (Table 11.2-1).

There were no significant differences in 11-dh-TXB<sub>2</sub> levels between nicotine pouch users and tobacco-based snus users. Combustible cigarette users had significantly higher urine levels of 11-dh-TXB<sub>2</sub> compared to both nicotine pouch users (b) (4), ANOVA) and tobacco-based snus users (b) (4), ANOVA), but there was no significant difference between combustible cigarette users and nonusers of tobacco/nicotine products (Table 11.2-2).

Corresponding results were obtained using the post-hoc non-parametric test (Wilcoxon rank sum test), see Table 11.2-2.

## 2,3-d-TXB<sub>2</sub>

Mean 2,3-d-TXB<sub>2</sub> levels in urine were (b) (4), and (b) (4) ng/mg for nicotine pouch users, tobacco-based snus users, combustible cigarette users, and nonusers of tobacco/nicotine products, respectively (Table 11.2-1).

Combustible cigarette users had significantly higher urine levels of 2,3-d-TXB<sub>2</sub> compared to tobacco-based snus users ((b) (4), ANOVA), but there was no significant difference between combustible cigarette users and nonusers of tobacco/nicotine products.

2,3-d-TXB<sub>2</sub> levels in urine were BLQ for the majority of nicotine pouch users ((b) (4) subjects). Consequently, the originally planned pairwise comparisons using ANOVA with the other groups could not be conducted for this parameter (Table 11.2-1 and Table 11.2-2).

The post-hoc non-parametric test (Wilcoxon rank sum test) showed that urine concentrations of 2,3-d-TXB<sub>2</sub> were significantly lower in nicotine pouch users compared to combustible cigarette users ((b) (4)), while there were no significant differences compared to either tobacco-based snus users or nonusers of tobacco/nicotine products (Table 11.2-2).

## LTE<sub>4</sub>

Mean LTE<sub>4</sub> levels in urine were (b) (4), and (b) (4) ng/mg for nicotine pouch users, tobacco-based snus users, combustible cigarette users, and nonusers of tobacco/nicotine products, respectively (Table 11.2-1).

Nicotine pouch users had significantly lower levels of LTE<sub>4</sub> compared to both tobacco-based snus users ((b) (4), ANOVA) and combustible cigarette users ((b) (4) 1, ANOVA), see (Table 11.2-2). Tobacco-based snus users had significantly lower levels of LTE<sub>4</sub> compared to combustible cigarette users ((b) (4)).

There were no significant differences between nonusers of tobacco/nicotine products and users of either of nicotine pouches or tobacco-based snus. However, combustible cigarette users had significantly higher levels of LTE<sub>4</sub> compared to nonusers of tobacco/nicotine products ((b) (4), ANOVA), see (Table 11.2-2).

Corresponding results were obtained using the post-hoc non-parametric test (Wilcoxon rank sum test) except that there was no significant difference between nicotine pouch users and tobacco-based snus users in the non-parametric test, see Table 11.2-2

### 11.2.1.4 Plasma concentrations of sICAM-1 and GDF-15

Mean sICAM-1 plasma levels were (b) (4) and (b) (4) ng/mL and mean GDF-15 plasma levels were (b) (4), and (b) (4) pg/mL for nicotine pouch users, tobacco-based snus users, combustible cigarette users, and nonusers of tobacco/nicotine-products, respectively (Table 11.2-1).

There were no significant differences in sICAM-1 and GDF-15 plasma levels between nicotine pouch users, tobacco-based snus users, and nonusers of tobacco/nicotine products.

Combustible cigarette users had significantly higher sICAM-1 and GDF-15 plasma levels compared to nicotine pouch users ((b) (4) ANOVA), tobacco-based snus users ((b) (4) for both comparisons, ANOVA), and nonusers of tobacco/nicotine products ((b) (4) for both comparisons, ANOVA), see Table 11.2-2.

Corresponding results were obtained using the post-hoc non-parametric test (Wilcoxon rank sum test) except that there was no significant difference between tobacco-based snus users and combustible cigarette users in the non-parametric test of GDF-15, see Table 11.2-2

***Table 11.2-1 Descriptive summary of secondary endpoints biomarkers (Full analysis set)***

(b) (4)

(b) (4)

(b) (4)



(b) (4)

(b) (4)

***Table 11.2-2 Statistical analyses of secondary endpoints biomarkers in urine and plasma (Full analysis set)***

(b) (4)

(b) (4)

(b) (4)

(b) (4)

(b) (4)

(b) (4)



(b) (4)

### 11.2.2 *Estimated total exposure and average extracted amounts and fractions*

Difference in total exposure, average extracted amounts and fractions of NNK, NNN, and nicotine between nicotine pouch users and tobacco-based snus users are presented in Table 11.2-3.

Table 14.2-2 in Section 14.2.2 presents an overall summary of nicotine, NNK, and NNN content in the reference pouches for each user group. Furthermore, Table 14.2-3 and Table 14.2-4 in Section 14.2.2 provide detailed information of the nicotine, NNK, and NNN content in the reference pouches for each individual product within each group. A summary of average residual nicotine, NNK, and NNN in used nicotine pouches and tobacco-based snus pouches is shown in Table 14.2-5 in Section 14.2.2.

For nicotine pouch users, the mean extracted amount of nicotine was (b) (4) mg/unit, the mean extracted fraction of nicotine was (b) (4) and the mean total exposure to nicotine was (b) (4) mg (Table 11.2-3). The mean nicotine content in reference pouches was (b) (4) mg/unit (Table 14.2-2).

For tobacco-based snus users, the mean extracted amount of nicotine was (b) (4) mg/unit, the mean extracted fraction of nicotine was (b) (4) and the mean total exposure to nicotine was (b) (4) mg (Table 11.2-3). The mean nicotine content in reference pouches was (b) (4) mg/unit (Table 14.2-2).

The levels of NNK and NNN in nicotine pouches, both used and reference pouches, were BLQ. As a result, nicotine pouch users were not exposed to quantifiable levels of NNK and NNN (Table 11.2-3 and Table 14.2-2).

For tobacco-based snus users the mean extracted amount of NNK was (b) (4) µg/unit, the mean extracted fraction of NNK was (b) (4) and the mean total exposure to NNK was (b) (4) µg. The mean extracted amount of NNN was (b) (4) µg/unit, the mean extracted fraction of NNN was (b) (4) and the mean total exposure to NNN was (b) (4) µg (Table 11.2-3). In reference pouches, the mean NNK content was (b) (4) µg/unit and the mean NNN content was (b) (4) µg/unit (Table 14.2-2).

**Table 11.2-3 Summary of estimated total exposure, average extracted amounts, and fractions of nicotine, NNK, and NNN (Full analysis set)**

(b) (4)

(b) (4)

(b) (4)

### 11.2.3 *By subject displays*

Individual subject data are listed in Appendix 16.2.6.

## 11.3 **Evaluation of exploratory endpoints**

### 11.3.1 *Correlation analysis and total exposure versus biomarkers in plasma and urine*

#### 11.3.1.1 *Exposure to nicotine and biomarkers in plasma and urine*

Correlation analysis of exposure to nicotine and biomarkers in plasma and urine for nicotine pouch users and tobacco-based snus users are presented in [Figure 14.2-1](#) to [Figure 14.2-13](#) in Section 14.2.3.1. The total exposure to nicotine versus biomarkers in plasma and urine are presented in [Figure 14.2-24](#) to [Figure 14.2-34](#) in Section 14.2.3.5.

For both nicotine pouch users and tobacco-based snus users, the nicotine, cotinine, and OH-cotinine levels in urine normalized for creatinine showed a positive correlation with total exposure to nicotine. There were no significant differences between groups ([Figure 14.2-1](#), [Figure 14.2-2](#) and [Figure 14.2-3](#) in Section 14.2.3.1). The correlation analyses of these biomarkers in plasma showed similar results ([Figure 14.2-8](#), [Figure 14.2-9](#) and [Figure 14.2-10](#) in Section 14.2.3.1).

In terms of NNAL in urine, there was a positive correlation with total nicotine exposure for both groups ([Figure 14.2-4](#) in Section 14.2.3.1). Regarding NNAL in plasma, tobacco-based snus users showed a positive correlation with nicotine exposure, while the nicotine pouch users were excluded from the analysis due to a large number of values BLQ ([Figure 14.2-11](#) in Section 14.2.3.1).

As for NNN in both urine and plasma, tobacco-based snus users exhibited a correlation with nicotine exposure, while the nicotine pouch user group was excluded from the analysis due to too many values BLQ ([Figure 14.2-5](#) and [Figure 14.2-12](#) in Section 14.2.3.1). The correlation between nicotine and the sum of NNAL and NNN in both urine and plasma yielded similar results ([Figure 14.2-7](#) and [Figure 14.2-13](#) in Section 14.2.3.1).

In terms of nicotine equivalents, both the nicotine pouch users and the tobacco-based snus users showed a positive correlation with total exposure to nicotine ([Figure 14.2-6](#) in Section 14.2.3.1).

#### 11.3.1.2 *Exposure of NNK and NNN and biomarkers in plasma and urine*

Correlation analysis of exposure to NNK and biomarkers in plasma and urine are presented in [Figure 14.2-14](#) to [Figure 14.2-17](#) in Section 14.2.3.2. The total exposure to NNK versus biomarkers in plasma and urine are presented in [Figure 14.2-35](#) to [Figure 14.2-38](#) in Section 14.2.3.6.

Correlation analysis of NNN and biomarkers in plasma and urine are presented in [Figure 14.2-18](#) to [Figure 14.2-21](#) in Section 14.2.3.3. Total exposure to NNN versus biomarkers in plasma and urine are presented in [Figure 14.2-39](#) to [Figure 14.2-42](#) in Section 14.2.3.7.

Correlation analysis of exposure to the sum of NNK and NNN and biomarkers in plasma and urine are presented in [Figure 14.2-22](#) and [Figure 14.2-23](#) in Section 14.2.3.4. The total exposure to the sum of NNK and NNN versus biomarkers in urine and plasma is presented in [Figure 14.2-43](#) to [Figure 14.2-46](#) in Section 14.2.3.8.

The nicotine pouch user group was excluded from the correlation analysis of NNK and NNN levels in both urine and plasma since all values of NNK and NNN were BLQ.

For tobacco-based snus users, a positive correlation was observed between normalized NNAL levels in both urine and plasma and total exposure to NNK ([Figure 14.2-14](#) and [Figure 14.2-16](#) in Section 14.2.3.2). Similarly, a positive correlation was found between normalized NNAL levels in urine and plasma and exposure to NNN in the tobacco-based snus group ([Figure 14.2-18](#) in Section 14.2.3.3 and [Figure 14.2-20](#) in Section 14.2.3.3). Additionally, the sum of NNAL and NNN in urine showed a positive correlation with the sum of NNK and NNN ([Figure 14.2-23](#) in Section 14.2.3.4).

In the case of the tobacco-based snus group, no correlation was observed between normalized NNN levels in urine and total exposure to NNK ([Figure 14.2-15](#) in Section 14.2.3.2), while a positive correlation was found between NNN levels in plasma and total exposure to NNK ([Figure 14.2-17](#) in Section 14.2.3.2).

Furthermore, normalized NNN levels in both urine and plasma showed a positive correlation with total exposure to NNN in the tobacco-based snus group ([Figure 14.2-19](#) in Section 14.2.3.3 and [Figure 14.2-21](#) in Section 14.2.3.3). Finally, the sum of NNAL and NNN in urine demonstrated a positive correlation with the sum of NNK and NNN ([Figure 14.2-22](#) in Section 14.2.3.4).

### 11.3.2 Analysis of pattern of use

A summary of pattern of use for the different nicotine user groups are presented in [Table 14.2-6](#) in Section 14.2.3.9.

The mean number of tobacco/nicotine products used was as follows:

- Nicotine pouch users: (b) (4) pouches per day (range between (b) (4) pouches),
- Tobacco-based snus users: (b) (4) pouches per day (range between (b) (4) pouches),
- Combustible cigarette users: (b) (4) cigarettes per day (range between (b) (4) cigarettes).

## 11.4 Additional analyses

For nicotine pouch users, tobacco-based snus users, and combustible cigarette users, selected biomarker data were collected both at Visit 2 (Day 1) and at Visit 3 (Day 14). The results from Visit 3 are used in descriptive summaries and statistical analyses throughout this report but plasma concentrations of nicotine, cotinine, OH-cotinine, NNAL, and NNN at Visit 2 are shown together with the results at Visit 3 in [Table 14.2-1](#) in Section 14.2.1.

Mean plasma concentrations of all biomarkers were comparable at both Visit 2 and Visit 3 ([Table 14.2-1](#) in Section 14.2.1).

## 11.5 Summary of results

### 11.5.1 Primary endpoint

- There were no significant differences in nicotine plasma levels between users of nicotine pouches ((b) (4) ng/mL), tobacco-based snus ((b) (4) ng/mL), and combustible cigarettes ((b) (4) ng/mL) 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 ((b) (4) and ((b) (4) ng/mL, respectively) and tobacco-based snus users ((b) (4) and ((b) (4) ng/mL, respectively). In contrast, users of combustible cigarettes had significantly lower levels of these biomarkers ((b) (4) and ((b) (4) ng/mL, respectively) compared to both nicotine pouch users and tobacco-based snus users ((b) (4) ).
- Nicotine pouch users had significantly lower plasma levels of NNAL and NNN (all values were BLQ) compared to ((b) (4) ) and combustible cigarette users ((b) (4) ). Additionally, plasma levels of NNAL and NNN did not significantly differ between nicotine pouch users and nonusers of tobacco/nicotine products. ((b) (4) ) and ((b) (4) ) and ((b) (4) pg/mL, respectively) ((b) (4) ) and ((b) (4) pg/mL, respectively).
- Nonusers of tobacco/nicotine products had plasma concentrations BLQ for nicotine, cotinine, OH-cotinine, NNAL, and NNN.

### 11.5.2 Secondary endpoints

#### 11.5.2.1 Biomarkers in urine

All analytes assessed in urine were normalized (divided) by urine creatinine concentrations to correct for variable dilution and all units are hence expressed by mg creatinine.

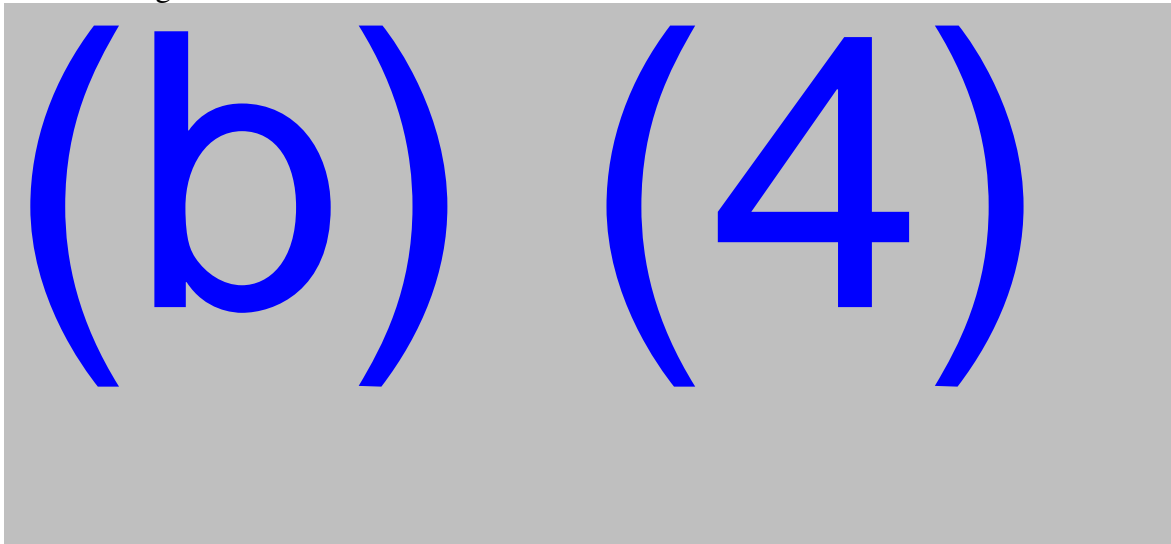
- Mean urine levels of nicotine, cotinine, OH-cotinine, and nicotine equivalents were:
  - ((b) (4) ng/mg, ((b) (4) ng/mg, ((b) (4) ng/mg, and ((b) (4) μmol/mg, respectively, for nicotine pouch users
  - ((b) (4) ng/mg, ((b) (4) ng/mg, ((b) (4) ng/mg, and ((b) (4) μmol/mg, respectively, for tobacco-based snus users
  - ((b) (4) ng/mg, ((b) (4) ng/mg, ((b) (4) ng/mg, and ((b) (4) μmol/mg, respectively, for combustible cigarette users

((b) (4)

- Mean urine levels of TSNA (NNAL, NNN, NAB, and NAT) were:
  - (b) (4) pg/mg, (b) (4) pg/mg, (b) (4), and (b) (4) pg/mg, respectively, for nicotine pouch users
  - (b) (4) pg/mg, (b) (4) pg/mg, (b) (4) pg/mg, and (b) (4) pg/mg, respectively, for tobacco-based snus users
  - (b) (4) pg/mg, (b) (4) pg/mg, (b) (4) pg/mg, and (b) (4) pg/mg, respectively, for combustible cigarette users

Mean urine levels of anatabine, anabasine, and 3-OH-B[a]P were:

- (b) (4) ng/mg, (b) (4) ng/mg, and (b) (4) fg/mg, respectively, for nicotine pouch users
- (b) (4) ng/mg, (b) (4) ng/mg, and (b) (4) fg/mg, respectively, for tobacco-based snus users
- (b) (4) ng/mg, (b) (4) ng/mg, and (b) (4) fg/mg, respectively, for combustible cigarette users



- In general, nonusers of tobacco/nicotine products had urine levels BLQ for nicotine, cotinine, OH-cotinine, nicotine equivalents, TSNA, anatabine and anabasine.
- Mean urine levels of eicosanoids (8-iso PGF<sub>2α</sub>, 11-dh-TXB<sub>2</sub>, 2,3-d-TXB<sub>2</sub>, and LTE<sub>4</sub>) were within the lower range for all user groups including nonusers of tobacco/nicotine products:
  - (b) (4), and (b) (4) ng/mg, respectively, for nicotine pouch users
  - (b) (4), and (b) (4) ng/mg, respectively, for tobacco-based snus users
  - (b) (4), and (b) (4) ng/mg, respectively, for combustible cigarette users

There were no significant differences in 8-iso PGF<sub>2α</sub> levels between nicotine pouch users, users of combustible cigarettes, and nonusers of tobacco/nicotine products. In contrast, tobacco-based snus users had significantly lower 8-iso PGF<sub>2α</sub> levels compared to both nicotine pouch users (p<0.01) and combustible cigarette users (p<0.001).

Nicotine pouch users and tobacco-based snus users had significantly lower urine levels of 11-dh-TXB<sub>2</sub>, 2,3-d-TXB<sub>2</sub>, and LTE<sub>4</sub> compared to combustible cigarette users

( $p < 0.01$  for all comparisons). However, while urine levels of  $\text{LTE}_4$  in combustible cigarette users were significantly higher than in nonusers of tobacco/nicotine products ( $p < 0.001$ ), there were no significant differences in 11-dh-TXB<sub>2</sub> or 2,3-d-TXB<sub>2</sub> urine levels in combustible cigarette users compared to nonusers of tobacco/nicotine products.

#### 11.5.2.2 Biomarkers in plasma

- Mean plasma levels of sICAM-1 and GDF-15 were:
  - (b) (4) ng/mL and (b) (4) pg/mL, respectively, for nicotine pouch users
  - (b) (4) ng/mL and (b) (4) pg/mL, respectively, for tobacco-based snus users
  - (b) (4) ng/mL and (b) (4) pg/mL, respectively, for combustible cigarette users

There were no significant differences in plasma levels of sICAM-1 and GDF-15 between nicotine pouch users, tobacco-based snus users, and nonusers of tobacco/nicotine products. In contrast, combustible cigarette users had significantly higher plasma levels of sICAM-1 and GDF-15 compared to nicotine pouch users ((b) (4)), tobacco-based snus users ((b) (4) comparisons) and nonusers of tobacco/nicotine products ((b) (4)).

#### 11.5.2.3 Total exposure and average extracted amounts and fractions

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

#### 11.5.3 Exploratory endpoints

- There was a positive correlation between total exposure to nicotine and urine levels of normalized nicotine, cotinine, OH-cotinine, NNAL, and nicotine equivalents for both nicotine pouch and tobacco-based snus users.
- NNAL levels in plasma and NNN levels in both plasma and urine, were BLQ for the nicotine pouch user group. However, there was a positive correlation between NNAL in plasma and NNN in both plasma and urine and exposure to nicotine for the tobacco-based snus group.
- The analysis of correlation to NNK and NNN in both urine and plasma excluded the nicotine pouch user group because all NNK and NNN values were BLQ. There was a positive correlation between NNAL and total exposure to both NNK and NNN for the tobacco-based snus group.
- During the 14-day study period, the nicotine pouch users used an average of ((b) (4)), tobacco-based snus users used an average of ((b) (4)) ((b) (4)), and combustible cigarette users used an average of ((b) (4)).



## 11.6 Statistical/analytical issues

### 11.6.1 Significance level

All hypothesis testing used a 5 % significance level ( $\alpha=0.05$ ).

### 11.6.2 Adjustments for covariates

Not applicable. No adjustments for covariates were performed.

### 11.6.3 Handling of dropouts or missing data

Outliers were included in summary tables and listings and were not handled separately in any analyses. In general, no imputation of data was performed. However, when calculating statistics for plasma and urine concentrations, concentrations under the lower limit of quantification (LLOQ) were replaced with LLOQ/2.

In case of missing start and stop times of AEs that could not be investigated further, missing data were imputed according to a worst-case scenario.

One subject in the nonusers of tobacco/nicotine products group ((b) (4)) was excluded from all biomarker analyses since the subject was found to have used nicotine products, see Section 10.3.

#### 11.6.3.1 Outliers included in the analysis of biomarkers

Three subjects in the nicotine pouch user group, (b) (4), and (b) (4), had biomarker values that deviated from the values of the other subjects in the group.

(b) (4)

In addition, (b) (4), had high levels of creatinine-normalized NNN ((b) (4) pg/mg, (b) (4) pg/mg and (b) (4) pg/mg, respectively) in urine, which may have been formed endogenously or during storage at low urine pH.

(b) (4)

All these subjects were included in the analysis despite their unusual biomarker values, which was considered to be a conservative approach.

### 11.6.4 Interim analyses and data monitoring

Not applicable.

### **11.6.5    *Multi-center studies***

Two clinical sites were used in this study: 1 site in Uppsala, Sweden and 1 site in Stockholm, Sweden. No calculations were performed to adjust for potential differences between sites.

### **11.6.6    *Multiple comparison/multiplicity***

No adjustments for multiple comparison/multiplicity were performed in the ANOVA model. All significant findings were reviewed for medical relevance. However, the p-values of the Wilcoxon rank sum test for pairwise comparisons (post-hoc analysis) were adjusted for multiple comparisons according to the approach of Dwass, Steel and Critchlow-Fligner (DSCF).

Furthermore, pairwise comparisons between user groups extracted from ANOVA models were only interpreted if the overall F-test p-value for the ANOVA was significant. Similarly, the p-values from the non-parametric pairwise comparisons were only interpreted if the overall p-value from the Kruskal-Wallis test was significant (post-hoc analysis). Likewise, p-values for individual effects extracted from linear regression models were only interpreted providing the overall model F-test statistics was significant.

## **11.7        *Tabulation of individual response data***

Individual subject data are listed in Appendix 16.2.6.

## 12 SAFETY EVALUATION

### 12.1 Extent of exposure

A summary of the number of the used nicotine products per day is shown in [Table 14.2-6](#) in [Section 14.2.3](#).

Individual compliance data are listed by subject in [Appendix 16.2.5](#).

### 12.2 Adverse events

#### 12.2.1 Brief summary of adverse events

An overview of AE reporting frequency, causality, and severity by user group is provided in [Table 12.2-1](#). Individual AE data per subject are listed in [Appendix 16.2.7](#).

In total, (b) (4) reported (b) (4) AEs during the study. All AEs were mild in intensity and assessed as unlikely to be related to the usage of nicotine products. There were no differences in AE reporting frequency between the tobacco/nicotine user groups. No AEs were collected from the nonuser group; therefore, the reporting frequency in this group was zero ([Table 12.2-1](#)).

**Table 12.2-1 Overview of adverse events (Full analysis set)**

(b) (4)

(b) (4)

### 12.2.2 *Display and analysis of adverse events*

An overview of AEs by SOC and PT is presented in [Table 14.3-1](#) in Section 14.3.1.

The most commonly reported AEs during the study were nasopharyngitis, reported on 1 occasion each by (b) (4) and headache, reported on 1 occasion each by (b) (4). All other AEs were single occurring events. All AEs were of mild intensity with a duration of (b) (4). There were no differences in reporting frequency between the tobacco/nicotine user groups.

### 12.2.3 *Listing of adverse events by subject*

All AEs are listed by subject in Appendix 16.2.7.

## 12.3 **Deaths, other serious adverse events, and other significant adverse events**

There were no deaths, other SAEs, or other significant AEs during the study.

## 12.4 **Vital signs and spirometry evaluation (screening only)**

Vital signs measurements and vital signs interpretations are summarized in [Table 14.3-2](#) and [Table 14.3-3](#), respectively, in Section 14.3.2.

There were no clinically significant differences in vital signs measurements (systolic blood pressure, diastolic blood pressure, and pulse rate) between user groups at screening ([Table 14.3-2](#)).

In general, for all spirometry assessments (PEF, FVC, FEV1, and FEV<sub>25-75%</sub>) users of combustible cigarettes displayed slightly lower values compared to the other groups at screening ([Table 14.3-2](#)).

All vital signs and spirometry assessments were assessed as normal or abnormal “non-clinically significant” ([Table 14.3-3](#) and Appendix 16.2.9).

## 12.5 **Electrocardiogram evaluation (screening only)**

ECG measurements and ECG interpretations are summarized in [Table 14.3-4](#) and [Table 14.3-5](#), respectively, in Section 14.3.3.

There were no clinically significant differences in any of the ECG parameters (heart rate, PR, QRS, QT, and QTcF) between user groups at screening ([Table 14.3-4](#)).

All ECG measurements were assessed as normal or abnormal “non-clinically significant” ([Table 14.3-5](#)).

## 12.6 Clinical laboratory evaluation (screening only)

Clinical chemistry and hematology measurements are summarized in [Table 14.3-6](#) and [Table 14.3-7](#), respectively, in Section [14.3.4](#).

In general, there were no clinically significant differences in any of the clinical chemistry parameters between groups at screening ([Table 14.3-6](#)).

(b) (4)

### 12.6.1 Listing of individual laboratory measurements by subject and each abnormal laboratory value

Individual subject listings are shown in Appendix 16.2.8.

## 12.7 Physical examination (screening only)

Physical examinations are summarized in [Table 14.3-8](#) in Section [14.3.5](#).

All physical examinations were assessed as normal or abnormal “non-clinically significant”.

## 12.8 Safety summary and conclusions

There were no deaths, other SAEs, or withdrawals due to AEs during the study. (b) (4) (b) (4) in the study were of mild intensity and assessed as unlikely related to the tobacco/nicotine products used. There were no differences in reporting frequency between the nicotine user groups.

## 13 DISCUSSION AND OVERALL CONCLUSIONS

### 13.1 Discussion

This was a multi-center, cross-sectional, 4-group, non-randomized study, designed to assess BoE and BoPH related to CVD and cancer in plasma and urine, in current daily users of nicotine pouches, tobacco-based snus, or combustible cigarettes, or nonusers of tobacco/nicotine products. Additionally, the study aimed to assess the extracted amount and fraction of nicotine and TSNA from pouches used by nicotine pouch and tobacco-based snus users. The subjects in the 3 nicotine user groups used their product of choice *ad libitum* throughout the 14-day study period while the nonuser group remained abstaining from tobacco/nicotine products.

(b) (4)

The primary objective of the study was to compare plasma concentrations of nicotine and its metabolites cotinine, OH-cotinine, as well as TSNA (NNAL and NNN) between user groups. There were no significant differences in nicotine plasma concentrations between nicotine pouch users, tobacco-based snus users, and combustible cigarette users. For cotinine and OH-cotinine plasma levels, there were no significant differences between nicotine pouch users and tobacco-based snus users, while combustible cigarette users displayed significantly lower levels.

There were no significant differences in creatinine-normalized levels of nicotine, cotinine, OH-cotinine, and nicotine equivalents in urine between nicotine pouch users and tobacco-based snus users. In contrast, users of combustible cigarettes had significantly lower urine levels of these parameters.

Nicotine pouch users had significantly lower levels of TSNA (NNAL and NNN) plasma concentrations compared to both tobacco-based snus users, and combustible cigarette users. All plasma NNAL and NNN concentrations in nicotine pouch users were BLQ.

Similarly, nicotine pouch users had significantly lower urine levels of creatinine-normalized TSNA (NNAL, NNN, NAB, and NAT), anatabine and anabasine levels compared to both tobacco-based snus users and combustible cigarette users. Urine NNN, NAB, NAT, anatabine, and anabasine levels were BLQ in most subjects in the nicotine pouch user group. As expected, nonusers of tobacco/nicotine products displayed insignificant levels of these biomarkers.

Nicotine pouch users, tobacco-based snus users, and nonusers of tobacco/nicotine products had low urine levels of creatinine-normalized 3-OH-B[a]P, whereas combustible cigarette users had significantly higher levels compared to all other groups.

For all user groups, including nonusers of tobacco/nicotine products, the urine levels of creatinine-normalized eicosanoids (8-iso PGF<sub>2α</sub>, 11-dh-TXB<sub>2</sub>, 2,3-d-TXB<sub>2</sub>, and LTE<sub>4</sub>) were low. Nicotine pouch users and tobacco-based snus users had significantly lower levels of 11-dh-TXB<sub>2</sub>, 2,3-d-TXB<sub>2</sub>, and LTE<sub>4</sub> compared to combustible cigarette users. However, while urine levels of LTE<sub>4</sub> in combustible cigarette users were significantly higher than in nonusers of tobacco/nicotine products, there were no significant differences in 11-dh-TXB<sub>2</sub> or 2,3-d-

TXB<sub>2</sub> urine levels in combustible cigarette users compared to nonusers of tobacco/nicotine products, *i.e.*, the data were ambiguous and difficult to interpret.

For both nicotine pouch users and tobacco-based snus users, the nicotine, cotinine, OH-cotinine, NNAL, and nicotine equivalents levels in urine showed a positive correlation with total exposure to nicotine. For nicotine pouch users, NNAL in plasma and NNN in both plasma and urine were BLQ. A positive correlation for NNAL in plasma and NNN in plasma and urine was shown with exposure to nicotine for the tobacco-based snus group.

For the analysis of correlation between NNK and NNN in both urine and plasma, the nicotine pouch user group was excluded due to all NNK and NNN values being BLQ. A positive correlation was observed between NNAL and total exposure with both NNK and NNN in the tobacco-based snus group. In comparison to tobacco-based snus users, nicotine pouch users exhibited lower total exposure to nicotine. Additionally, no NNK or NNN could be extracted from used or reference pouches for the nicotine pouch users.

For the inflammatory markers sICAM-1 and GDF-15, the plasma concentrations were significantly higher for combustible cigarette users as compared to the other 3 study groups. No significant differences in sICAM-1 and GDF-15 levels were observed between nicotine pouch users, tobacco-based snus users, and nonusers of nicotine products.

Safety was assessed by AE reporting. There were no deaths, other SAEs, or withdrawals due to AEs in the study. All AEs ((b) (4)) were of mild intensity and assessed as unlikely related to nicotine products. There were no differences in reporting frequency between the nicotine user groups.

## 13.2 Overall conclusions

### Biomarkers of exposure - nicotine and nicotine metabolites in plasma and urine

- There were no significant differences in plasma levels of nicotine between nicotine pouch users, tobacco-based snus users, and combustible cigarette users, while the levels of the metabolites cotinine and OH-cotinine were significantly lower for combustible cigarette users after 14 days of *ad libitum* use of either product.
- There were no significant differences in urine levels of nicotine, cotinine, OH-cotinine, and nicotine equivalents between nicotine pouch users and tobacco-based snus users, while users of combustible cigarettes had significantly lower levels of these biomarkers.

### Biomarkers of exposure - TSNA in plasma and urine

- Nicotine pouch users had significantly lower plasma and urine levels of carcinogenic TSNA (NNAL and NNN in plasma and NNAL, NNN, NAB, and NAT in urine) and TSNA precursors (anatabine and anabasine in urine) compared to both tobacco-based snus users and combustible cigarette users.
- Nicotine pouch users had significantly lower urine levels of 3-OH-B[a]P compared to combustible cigarette users while there were no significant differences compared to tobacco-based snus users and nonusers of tobacco/nicotine.

### Biomarkers of potential harm – eicosanoids in urine

- The urine levels of eicosanoids (8-iso PGF<sub>2α</sub>, 11-dh-TXB<sub>2</sub>, 2,3-d-TXB<sub>2</sub>, and LTE<sub>4</sub>) were within the lower range for all user groups including nonusers of tobacco/nicotine products and statistical comparisons were ambiguous.

### Biomarkers of potential harm - sICAM-1 and GDF-15 in plasma

- There were no significant differences in plasma levels of sICAM-1 and GDF-15 between nicotine pouch users, tobacco-based snus users, and nonusers of tobacco/nicotine products. In contrast, combustible cigarette users had significantly higher levels of sICAM-1 and GDF-15 compared to all other user groups.

### Total exposure and extracted amounts and fractions of nicotine and TSNA from pouches used by nicotine pouch and tobacco-based snus users

- Nicotine pouch users had a higher mean extracted amount ((b) (4) mg/unit) and fraction ((b) (4) %) of nicotine compared to tobacco-based snus-users ((b) (4) mg/unit and (b) (4) %, respectively). However, the mean total nicotine exposure over the 14-day study period was lower for nicotine pouch users compared to tobacco-based snus users.
- The levels of NNK and NNN in nicotine pouches, both used and reference products, were BLQ. For tobacco-based snus users, the mean total exposure to NNK and NNN was (b) (4) µg and (b) (4) µg, respectively, over the 14-day study period.



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

### 14.1 Demographic data

#### 14.1.1 Medical history events

*Table 14.1-1 Medical history events by system organ class and preferred term (Full analysis set)*

(b) (4)

(b) (4)

(b) (4)

(b) (4)

(b) (4)

**14.1.2 Prior and concomitant medications**

**Table 14.1-2 Prior medications by ATC levels 4 and 5 (Full analysis set)**

(b) (4)

(b) (4)

*Table 14.1-3 Concomitant medications by ATC levels 4 and 5 (Full analysis set)*

(b) (4)



(b) (4)

(b) (4)

(b) (4)

(b) (4)

### 14.1.3 History of nicotine use

**Table 14.1-4 History of nicotine use - years of use and average consumption (Full analysis set)**

(b) (4)

## 14.2 Data from primary/secondary/exploratory endpoints

### 14.2.1 Primary endpoints

**Table 14.2-1 Descriptive summary of selected plasma biomarkers at Visit 2 and Visit 3 (Full analysis set)**

(b) (4)

(b) (4)

(b) (4)

#### 14.2.2 Secondary endpoints

**Table 14.2-2 Overall summary of reference pouches (Full analysis set)**

(b)	(4)
-----	-----

**Table 14.2-3 Summary of reference pouches for nicotine pouch users (Full analysis set)**

(b)	(4)
-----	-----



(b) (4)

(b) (4)

(b) (4)

(b) (4)

(b) (4)

(b) (4)

(b) (4)

(b) (4)



(b) (4)

(b) (4)

(b) (4)

(b) (4)

(b) (4)

*Table 14.2-5 Summary of average residual nicotine, NNK, and NNN in used nicotine/tobacco snus (Full analysis set)*

(b) (4)

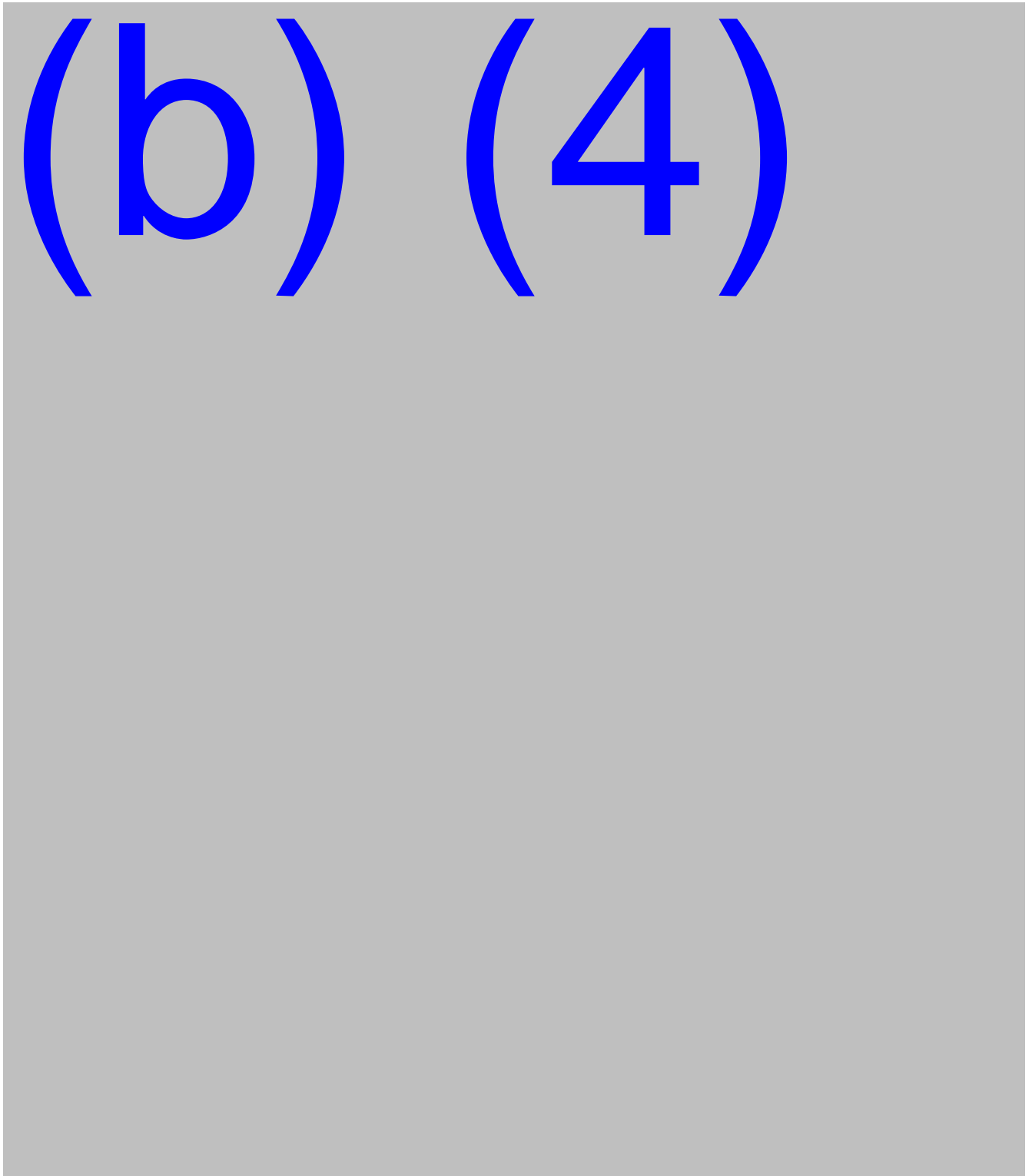
### 14.2.3 *Exploratory endpoints*

#### 14.2.3.1 *Correlation analysis: exposure to nicotine and biomarkers in plasma and urine*

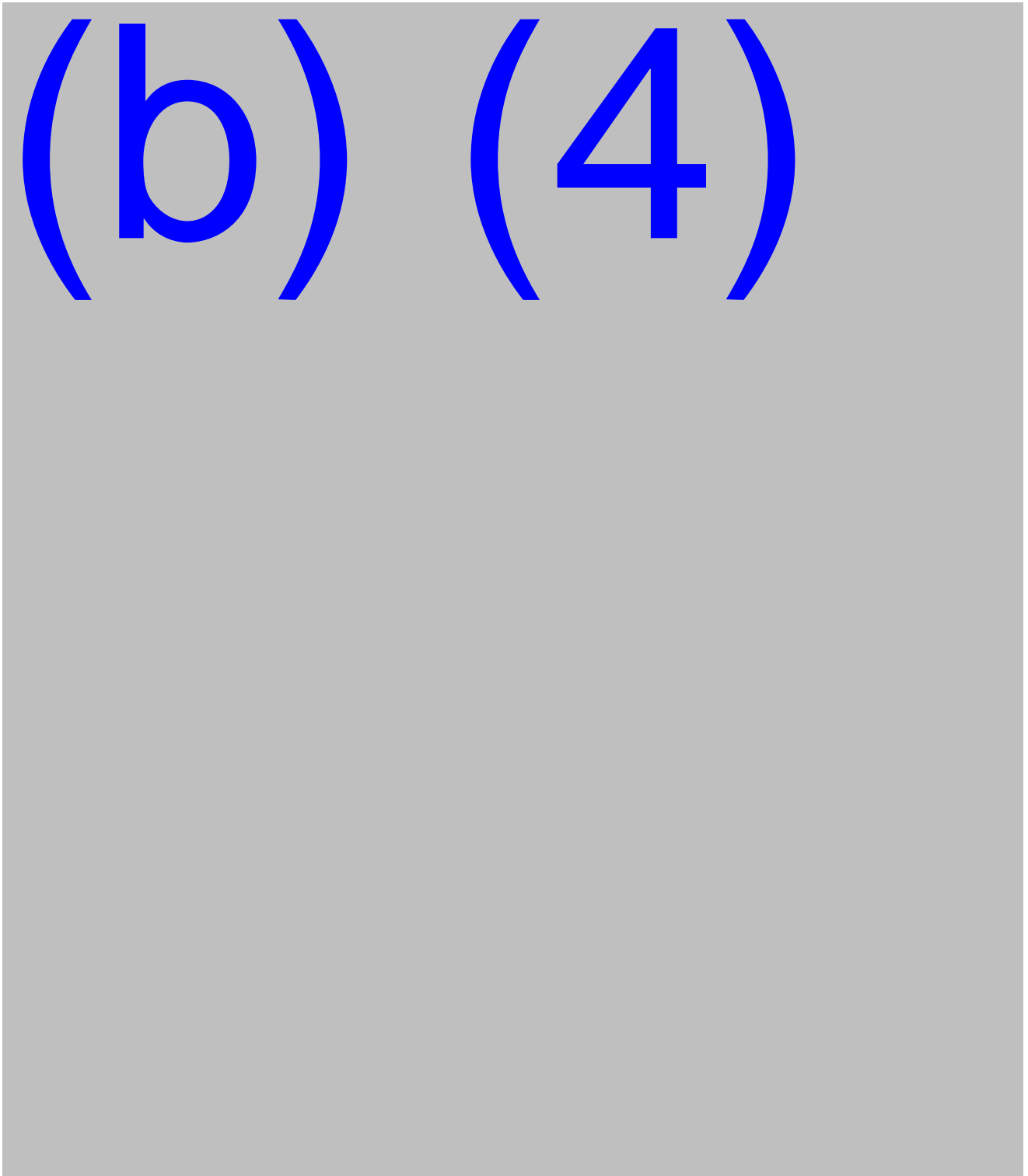
**Figure 14.2-1** (b) (4)

(b) (4)

**Figure 14.2-2** (b) (4)



**Figure 14.2-3** (b) (4)





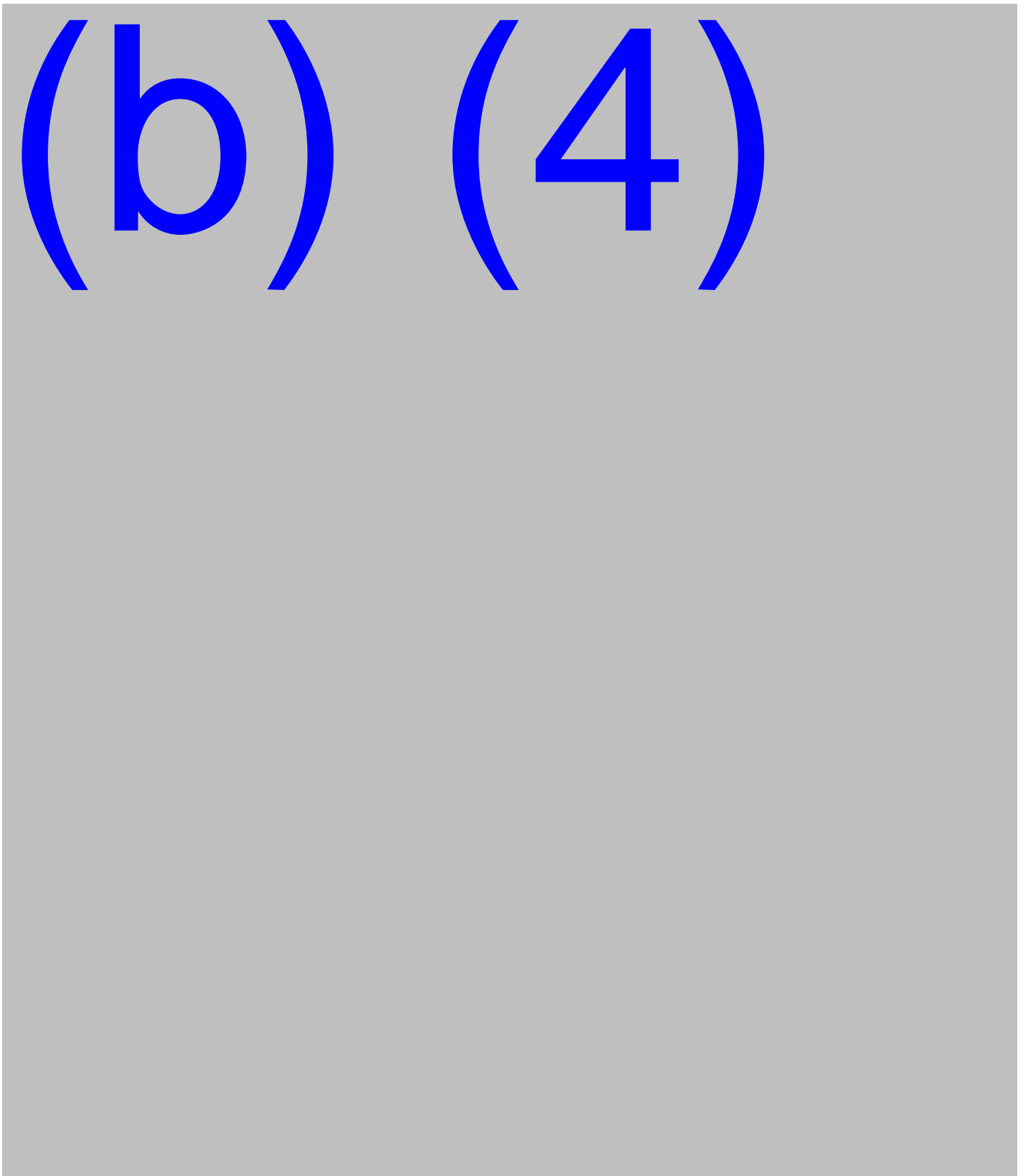
**Figure 14.2-4** (b) (4)

(b) (4)

**Figure 14.2-5** (b) (4)

(b) (4)

**Figure 14.2-6** (b) (4)



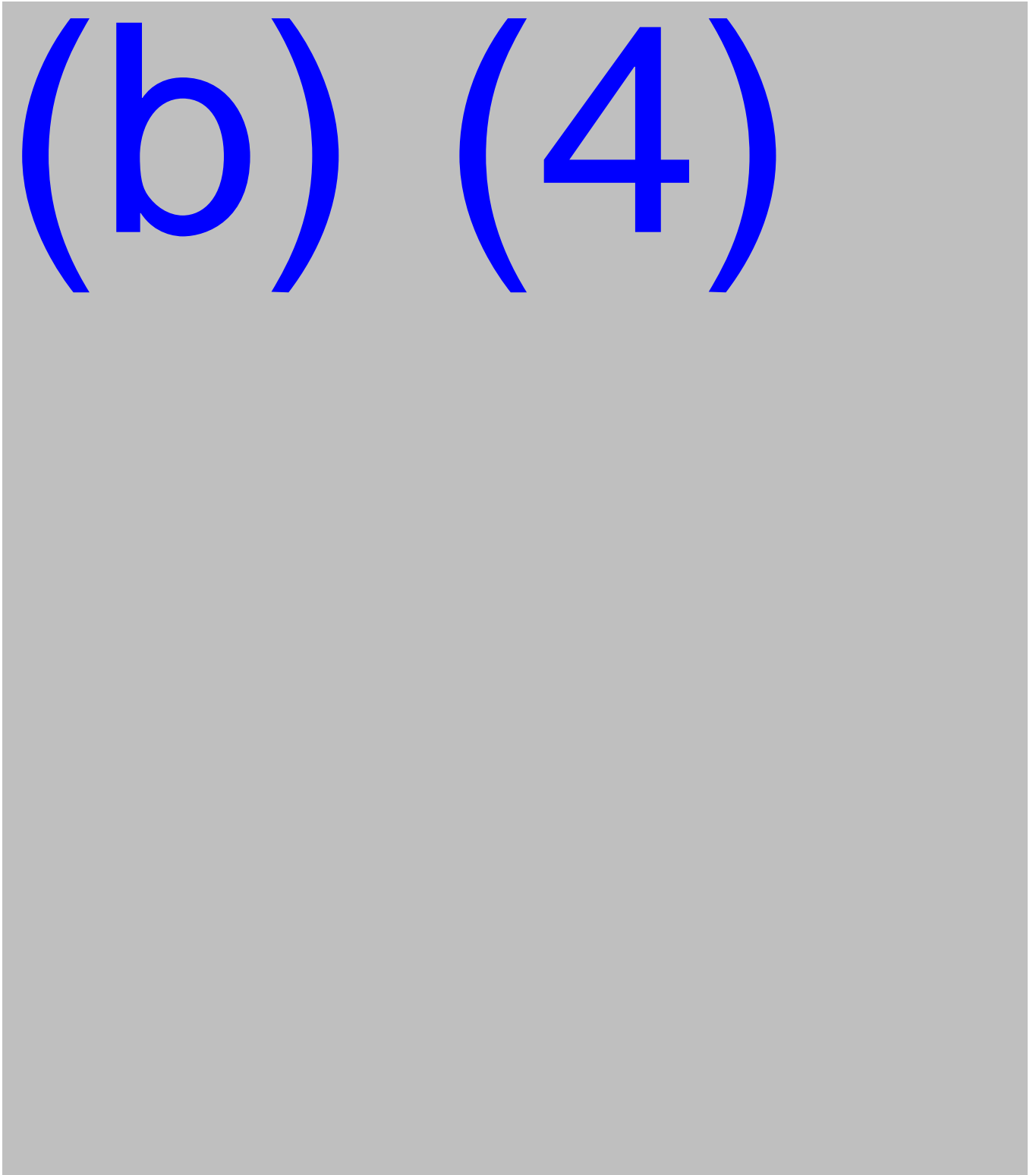
**Figure 14.2-7** (b) (4)

(b) (4)

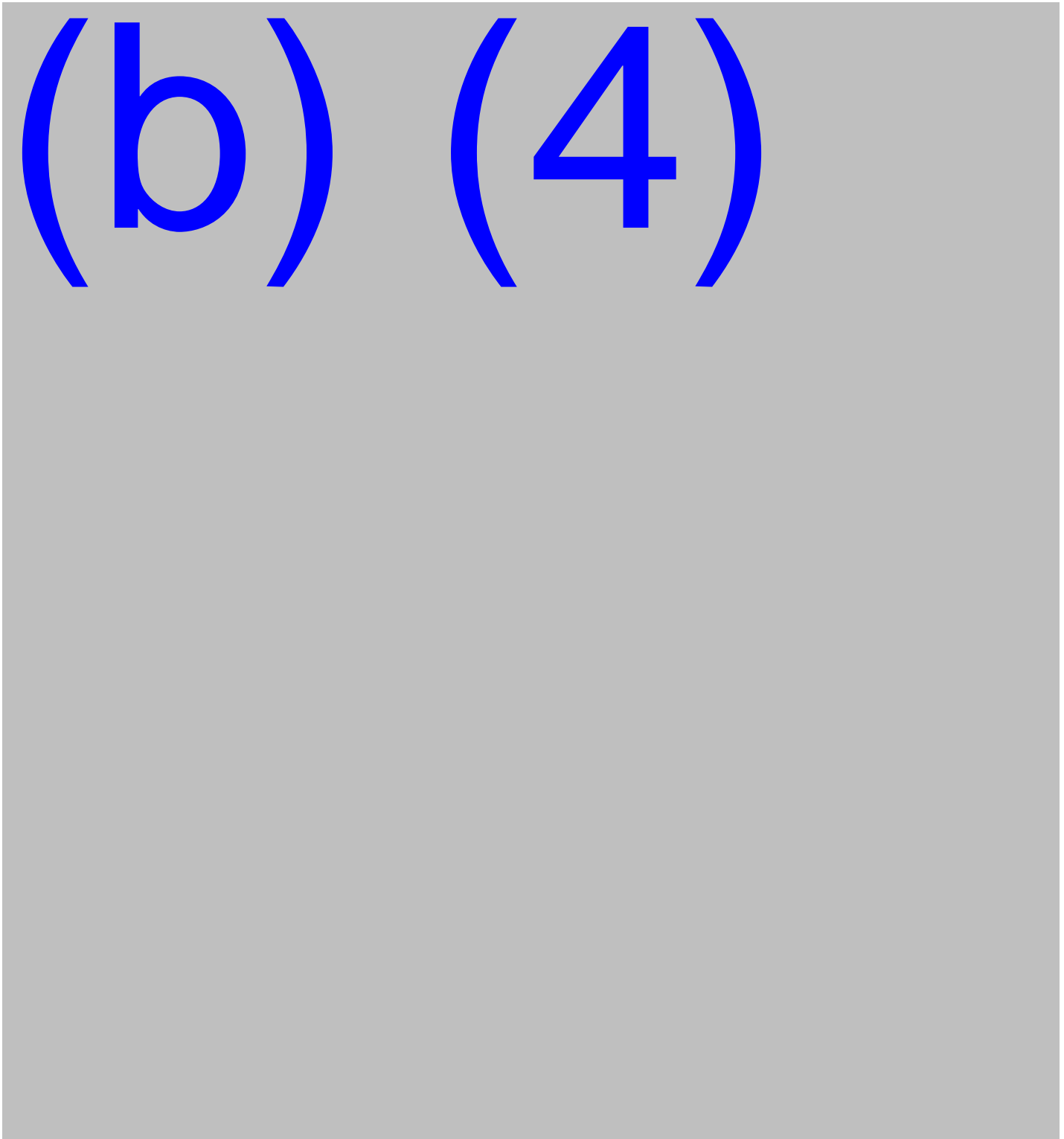
**Figure 14.2-8** (b) (4)

(b) (4)

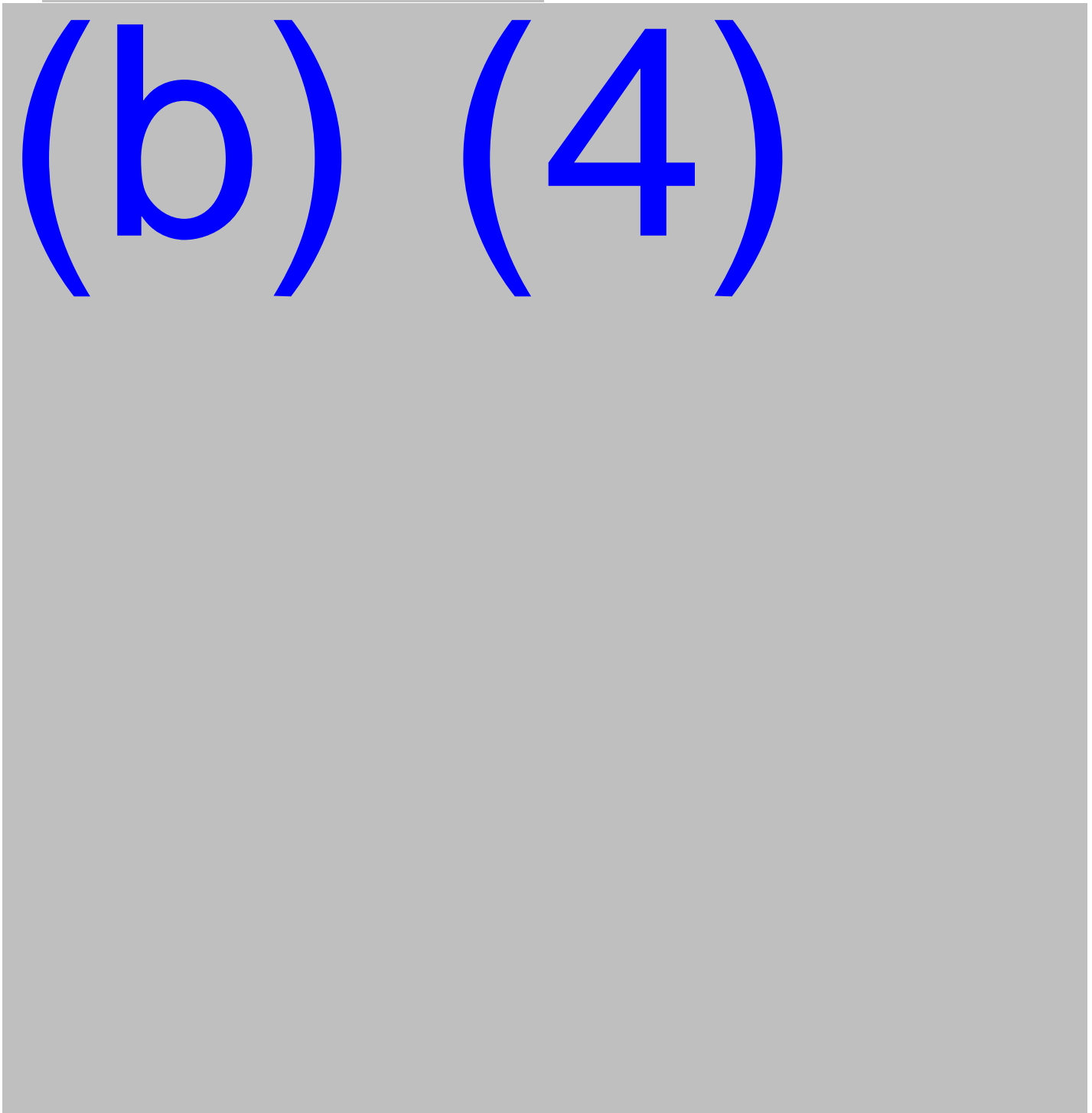
**Figure 14.2-9** (b) (4)



**Figure 14.2-10** (b) (4)



**Figure 14.2-11**(b) (4)

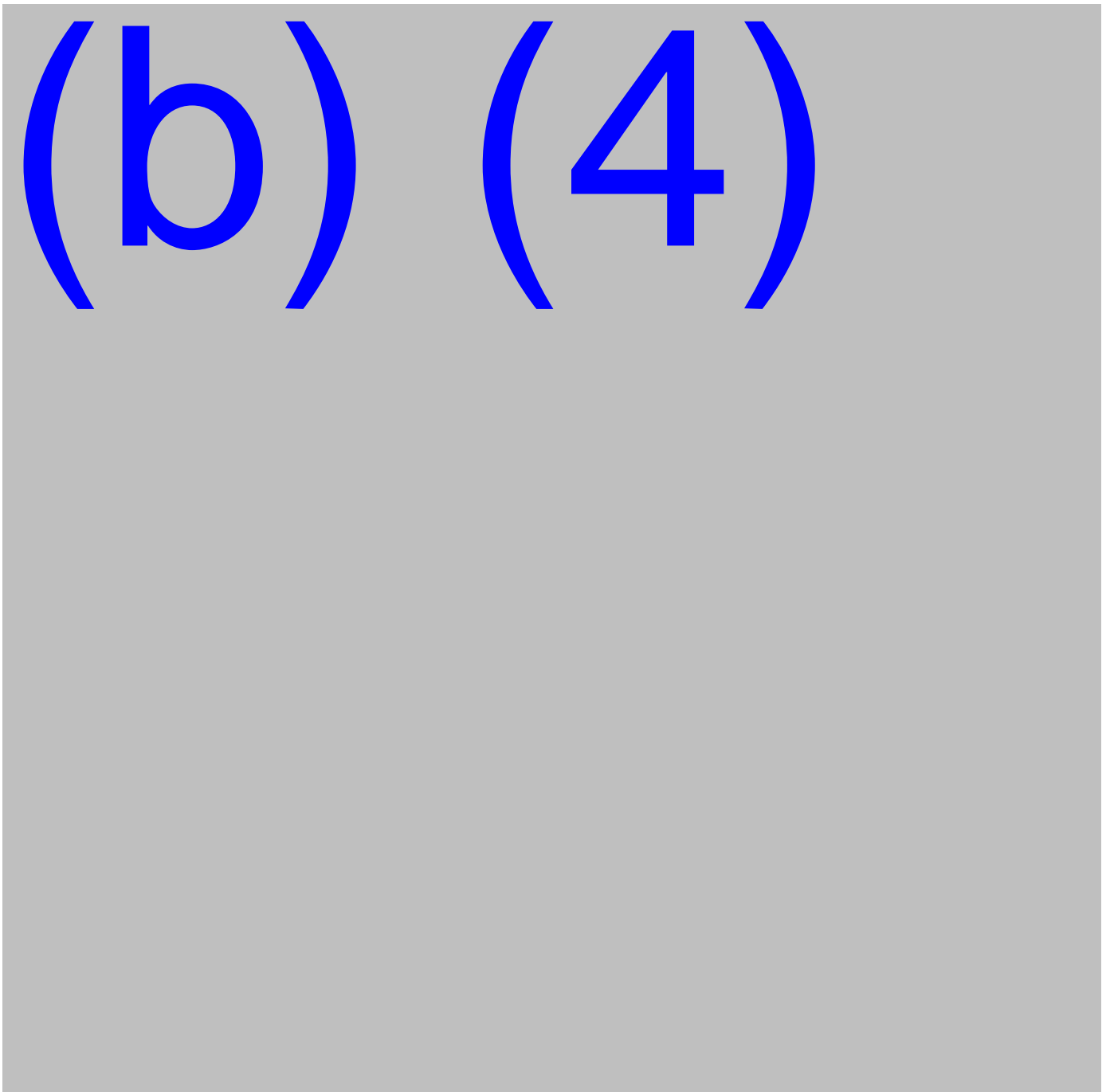




**Figure 14.2-12** (b) (4)

(b) (4)

**Figure 14.2-13** (b) (4)



14.2.3.2 Correlation analysis: exposure to NNK and biomarkers in plasma and urine

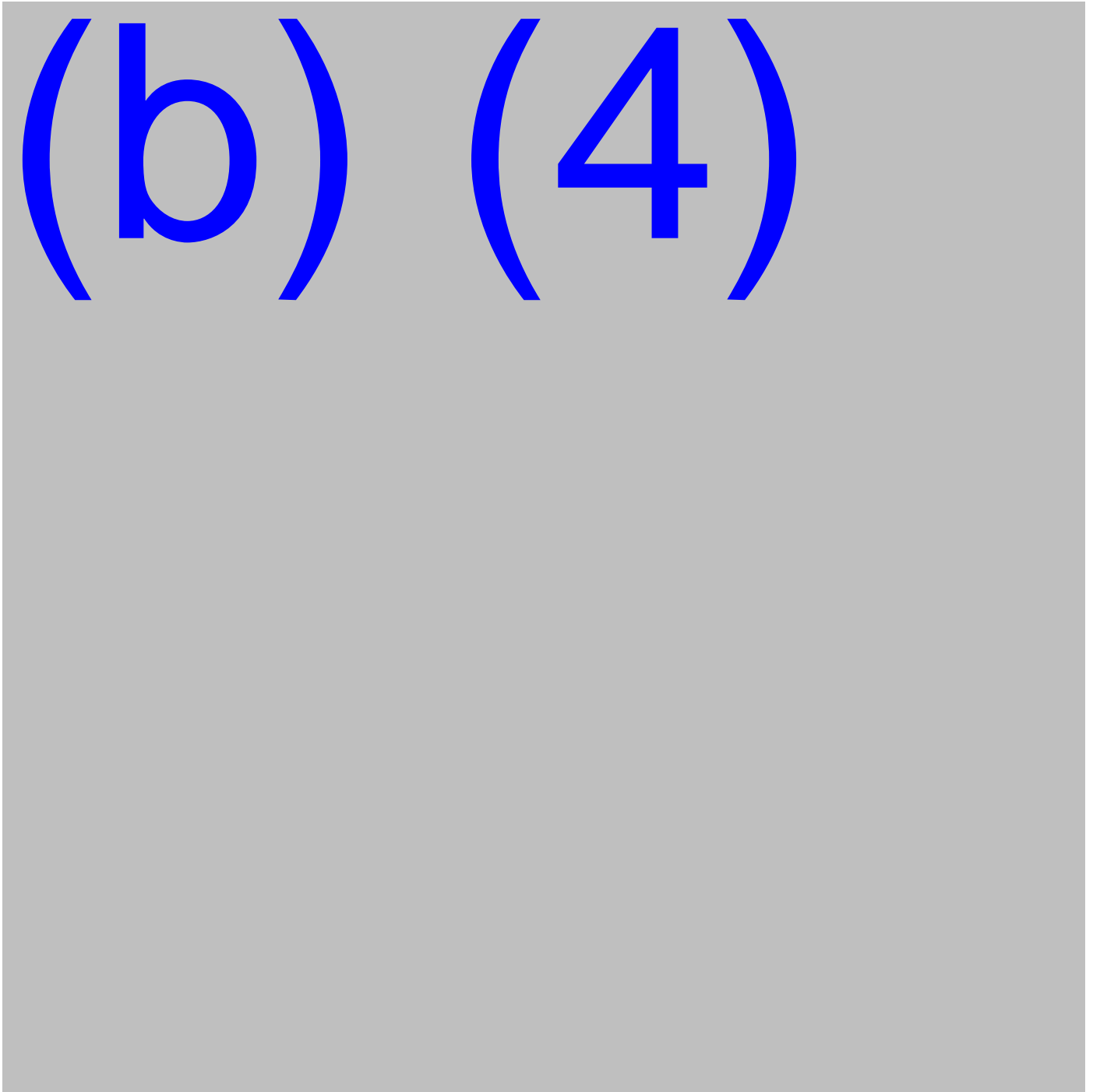
**Figure 14.2-14** (b) (4)

(b) (4)

**Figure 14.2-15** (b) (4)

(b) (4)

**Figure 14.2-16** (b) (4)



**Figure 14.2-17** (b) (4)

(b) (4)

14.2.3.3 Correlation analysis: exposure to NNN and biomarkers in plasma and urine

Figure 14.2-18 (b) (4)

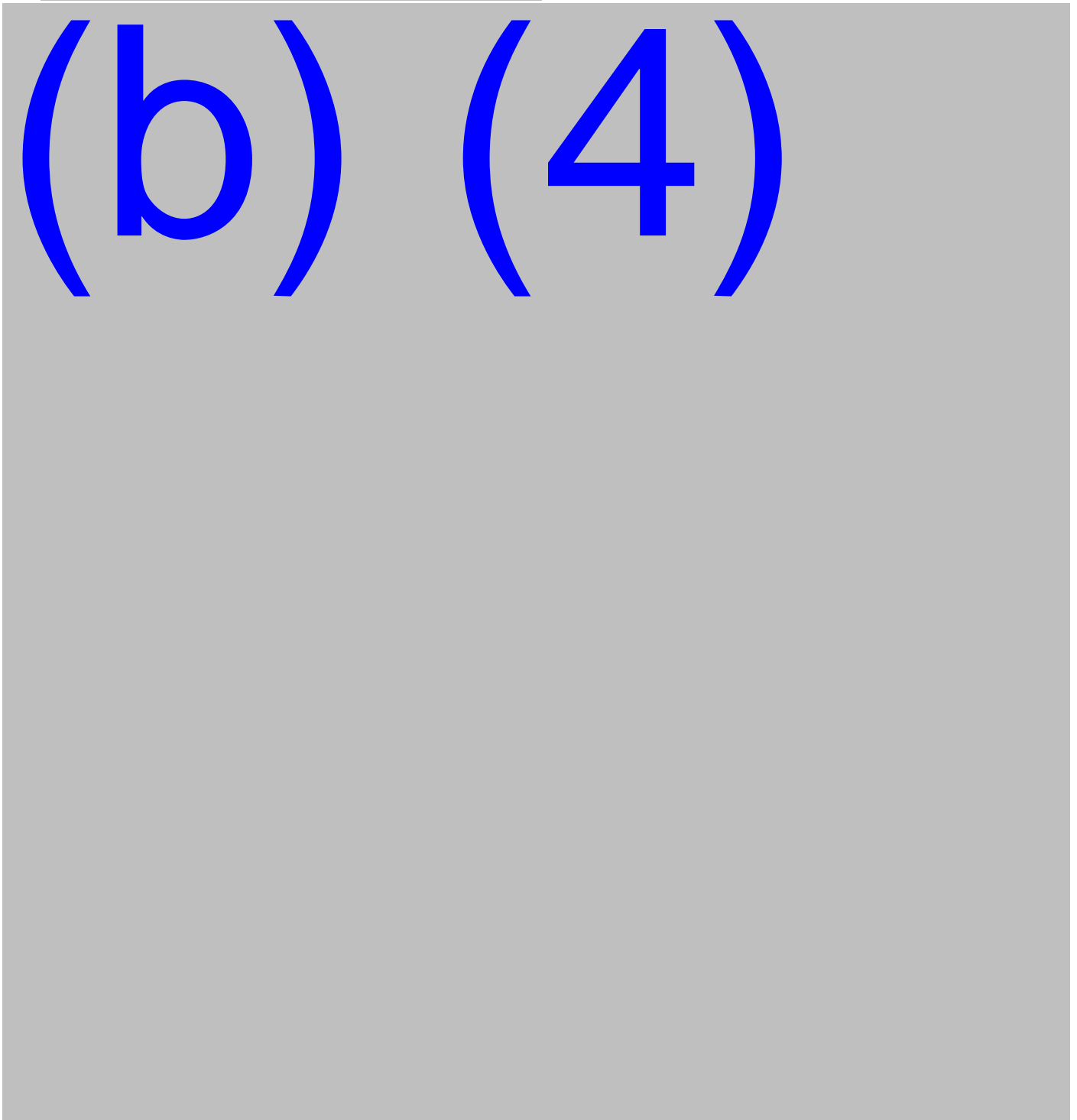
(b) (4)

**Figure 14.2-19** (b) (4)

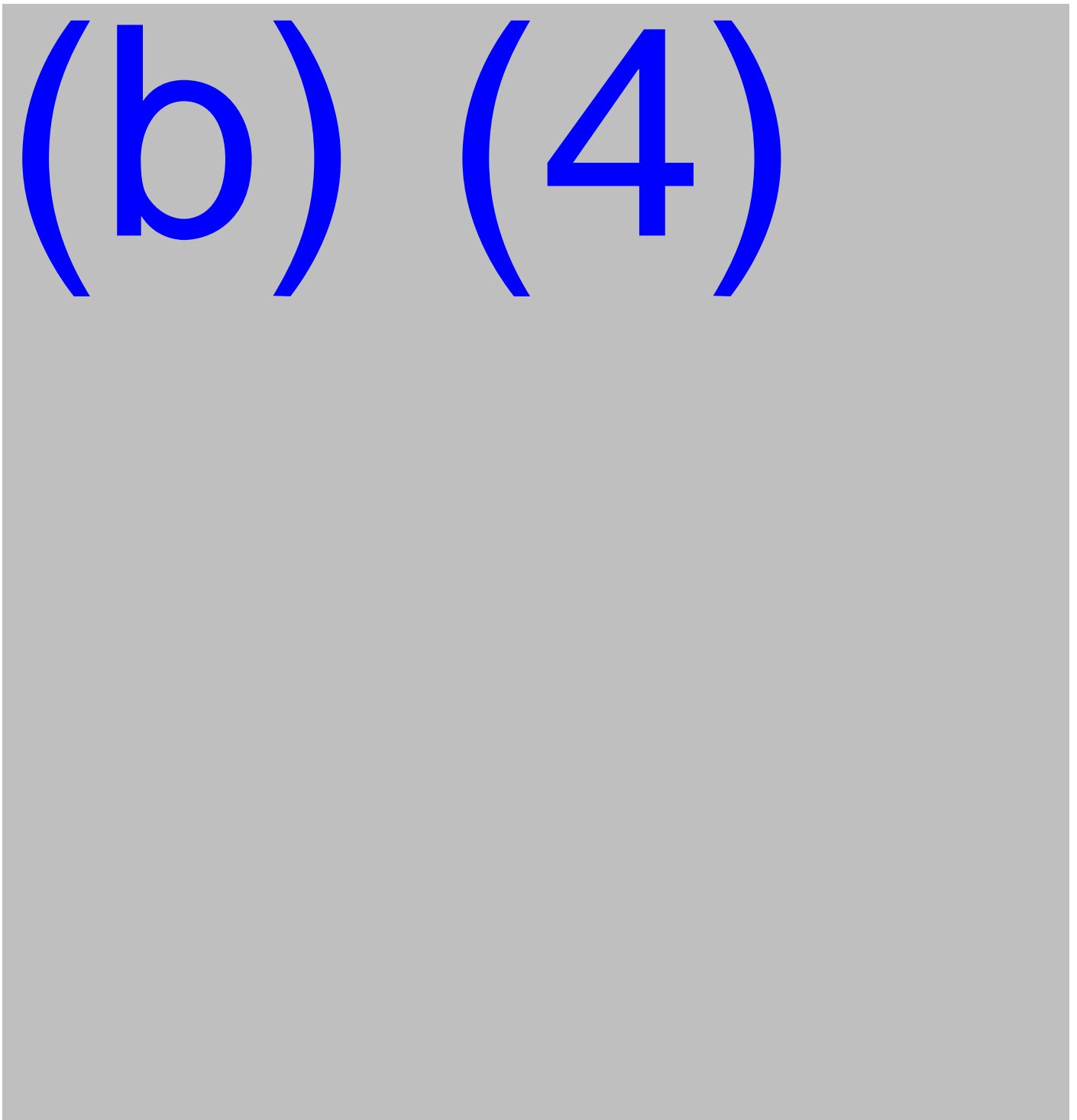
(b) (4)



**Figure 14.2-20** (b) (4)



**Figure 14.2-21** (b) (4)



14.2.3.4 Correlation analysis: exposure to the sum of NNK and NNN and biomarkers in plasma and urine

Figure 14.2-22 (b) (4)

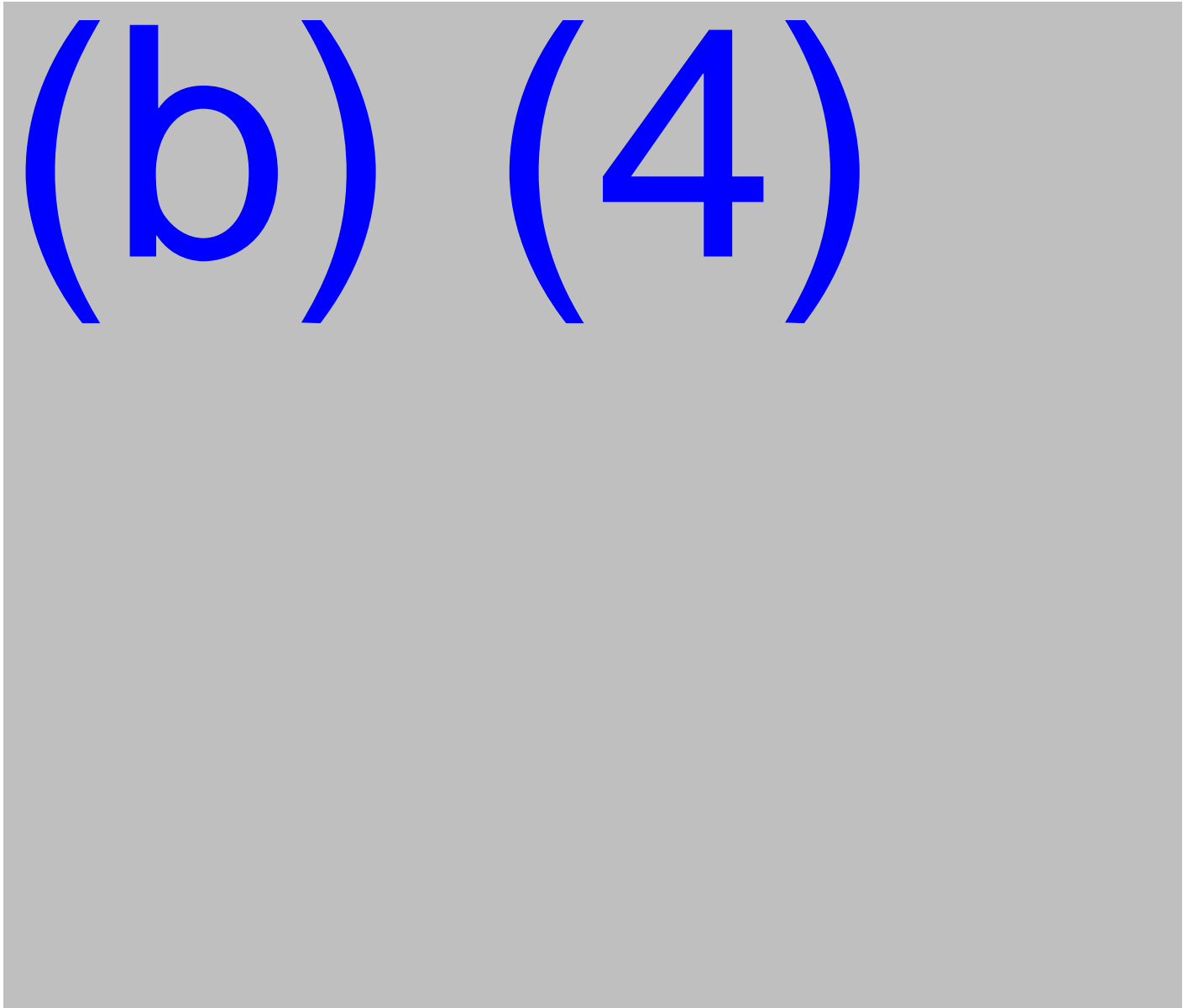
(b) (4)

**Figure 14.2-23** (b) (4)

(b) (4)

14.2.3.5 *Total exposure to nicotine versus biomarkers in plasma and urine*

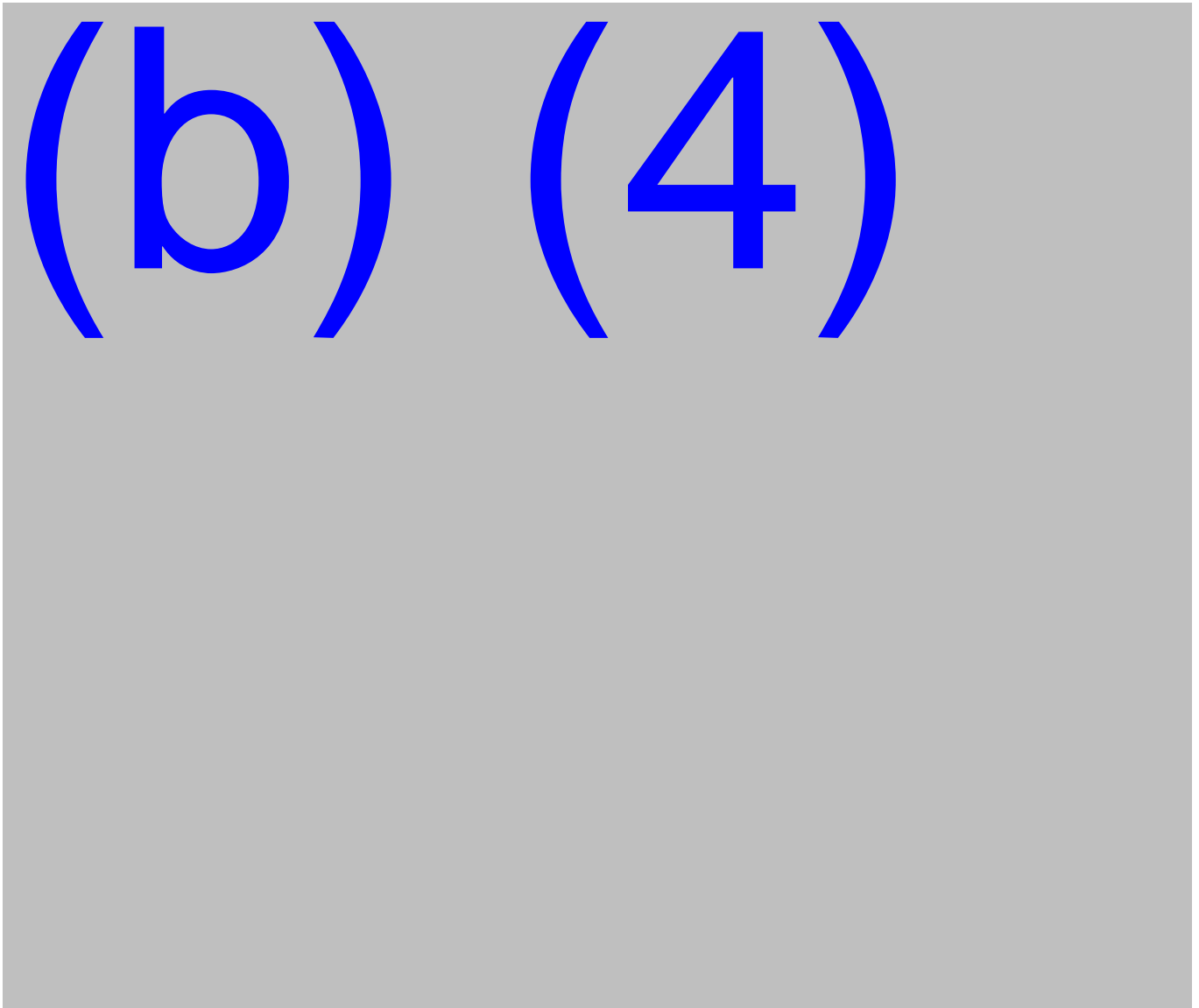
**Figure 14.2-24** (b) (4)



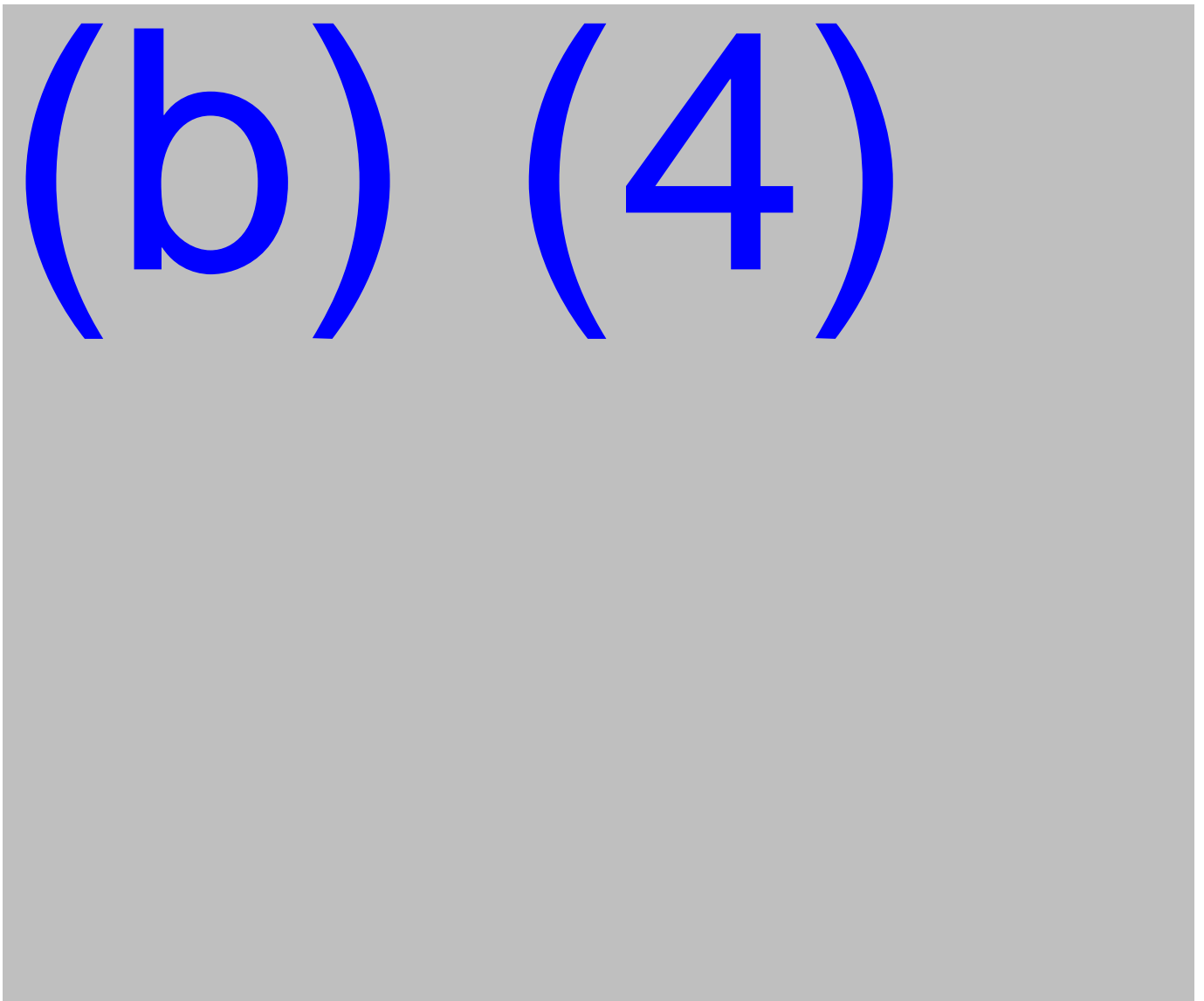
**Figure 14.2-25** (b) (4)

(b) (4)

**Figure 14.2-26** (b) (4)

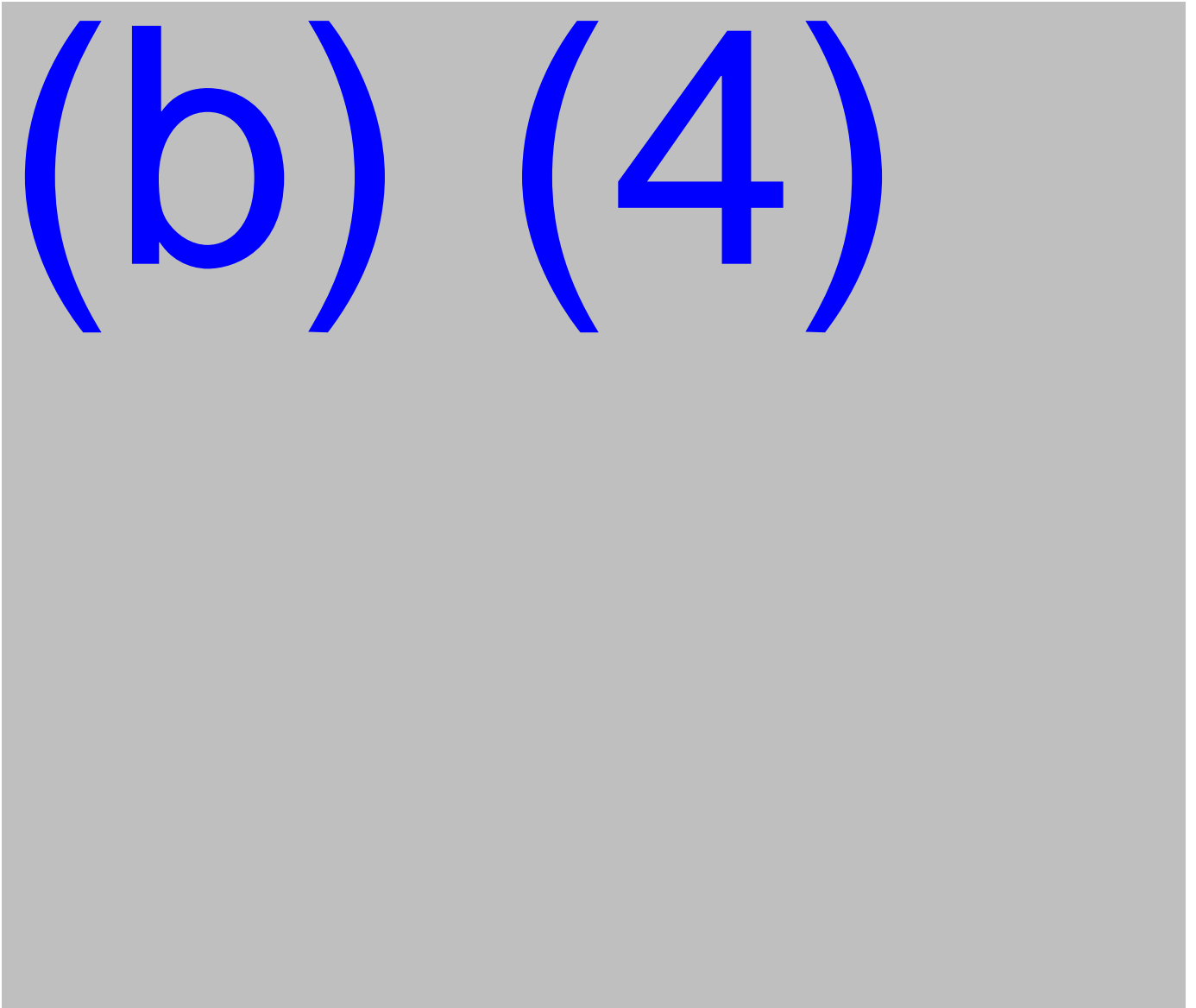


**Figure 14.2-27** (b) (4)





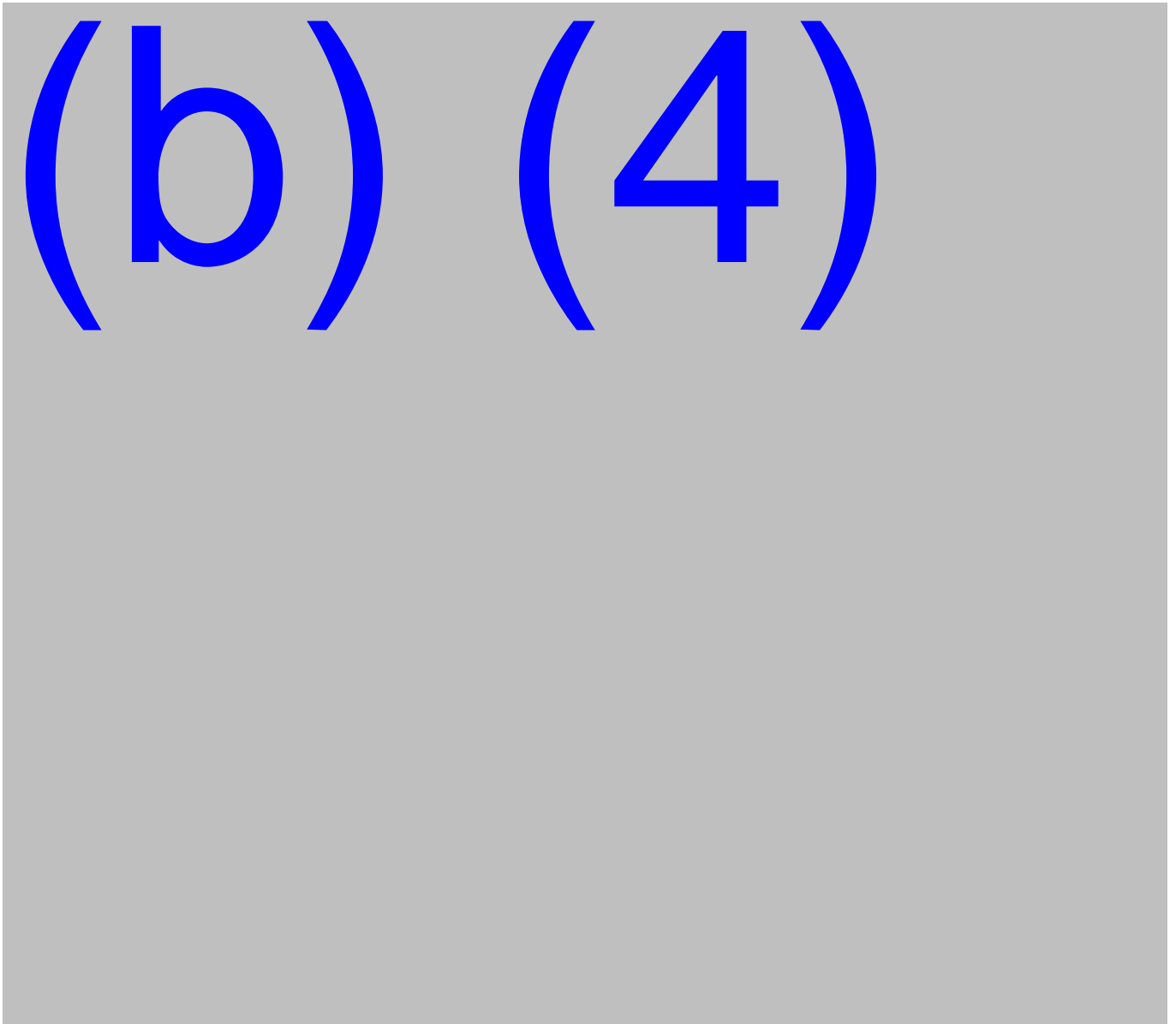
**Figure 14.2-28** (b) (4)



**Figure 14.2-29** (b) (4)

(b) (4)

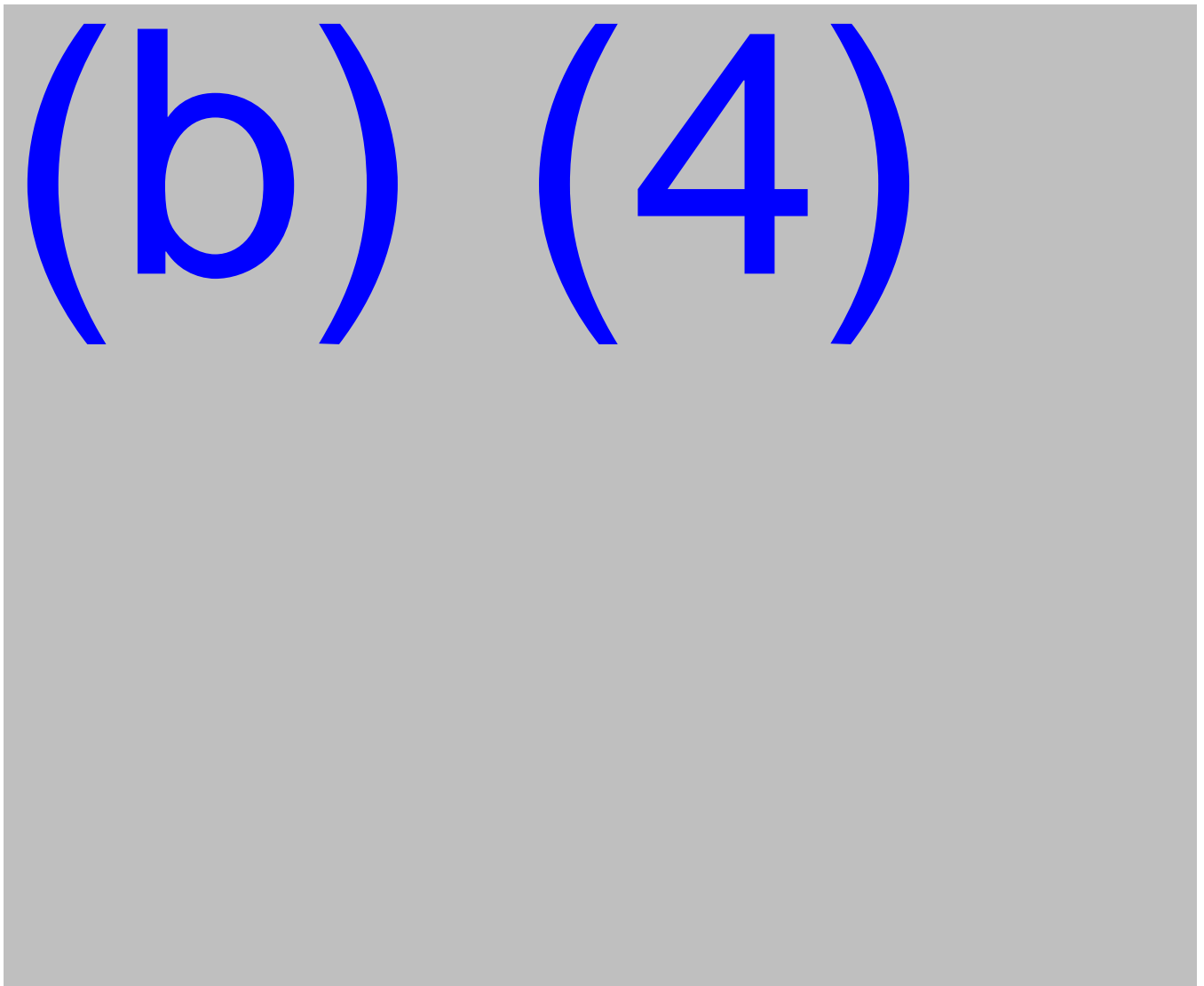
**Figure 14.2-30** (b) (4)



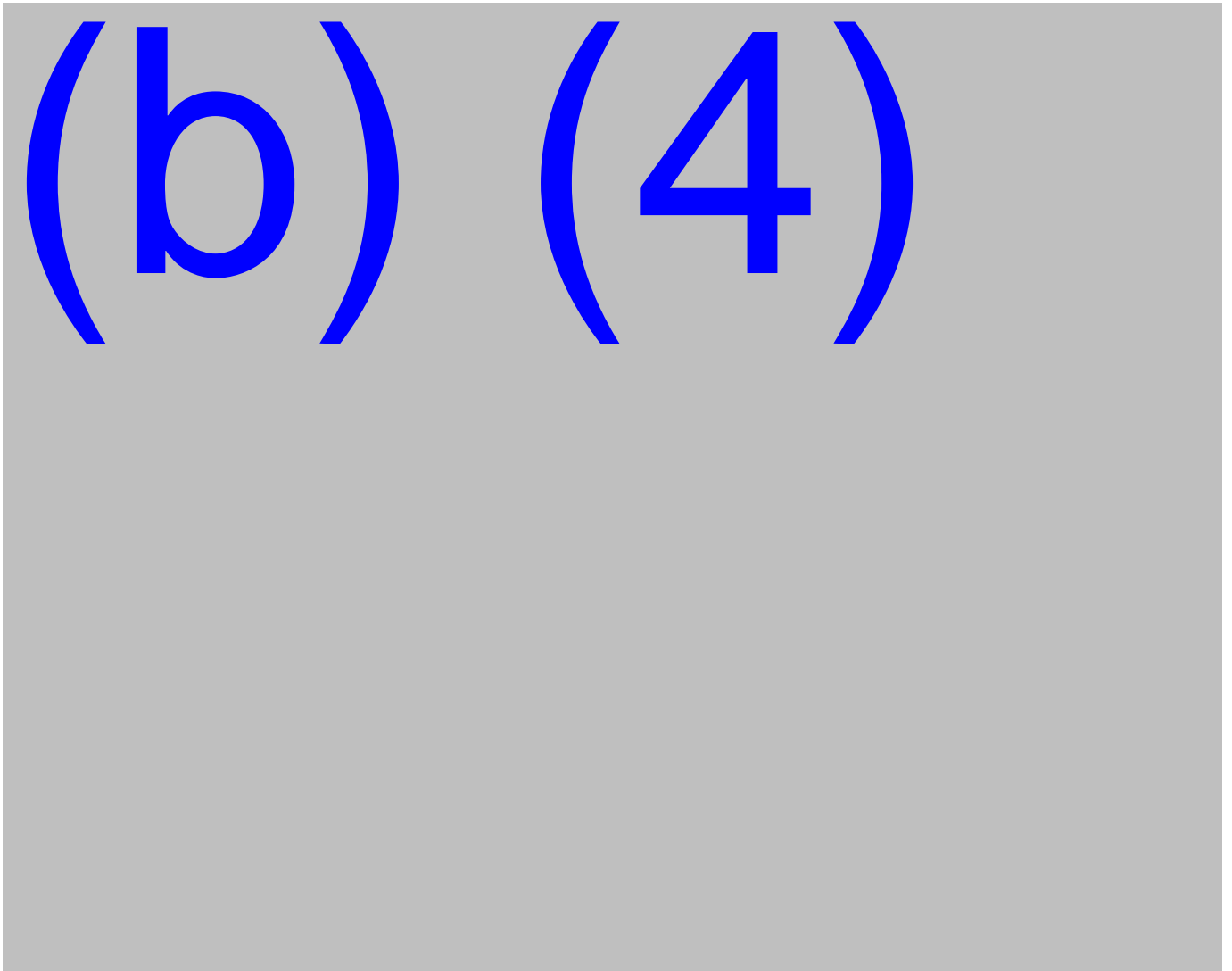
**Figure 14.2-31** (b) (4)

(b) (4)

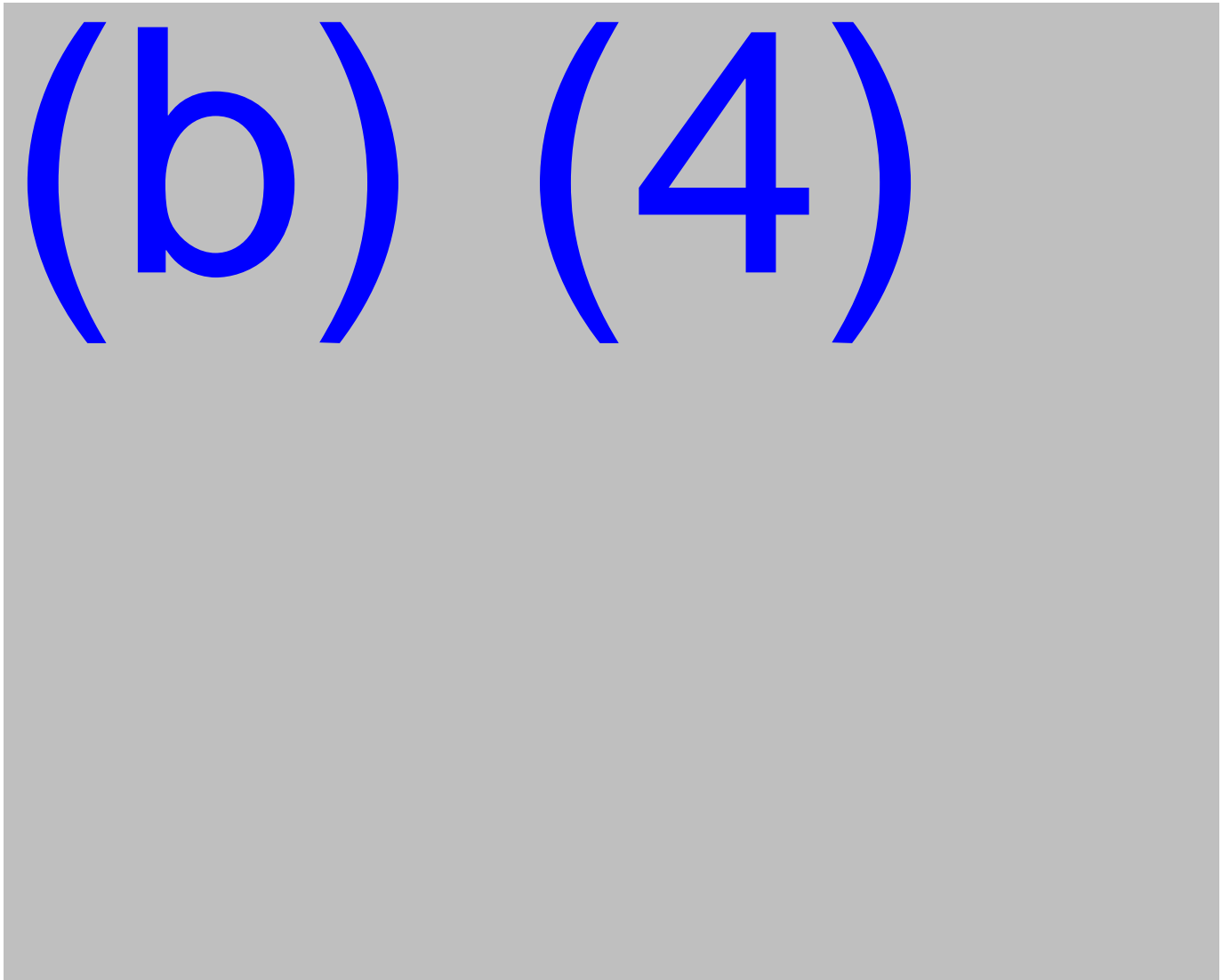
**Figure 14.2-32** (b) (4)



**Figure 14.2-33** (b) (4)

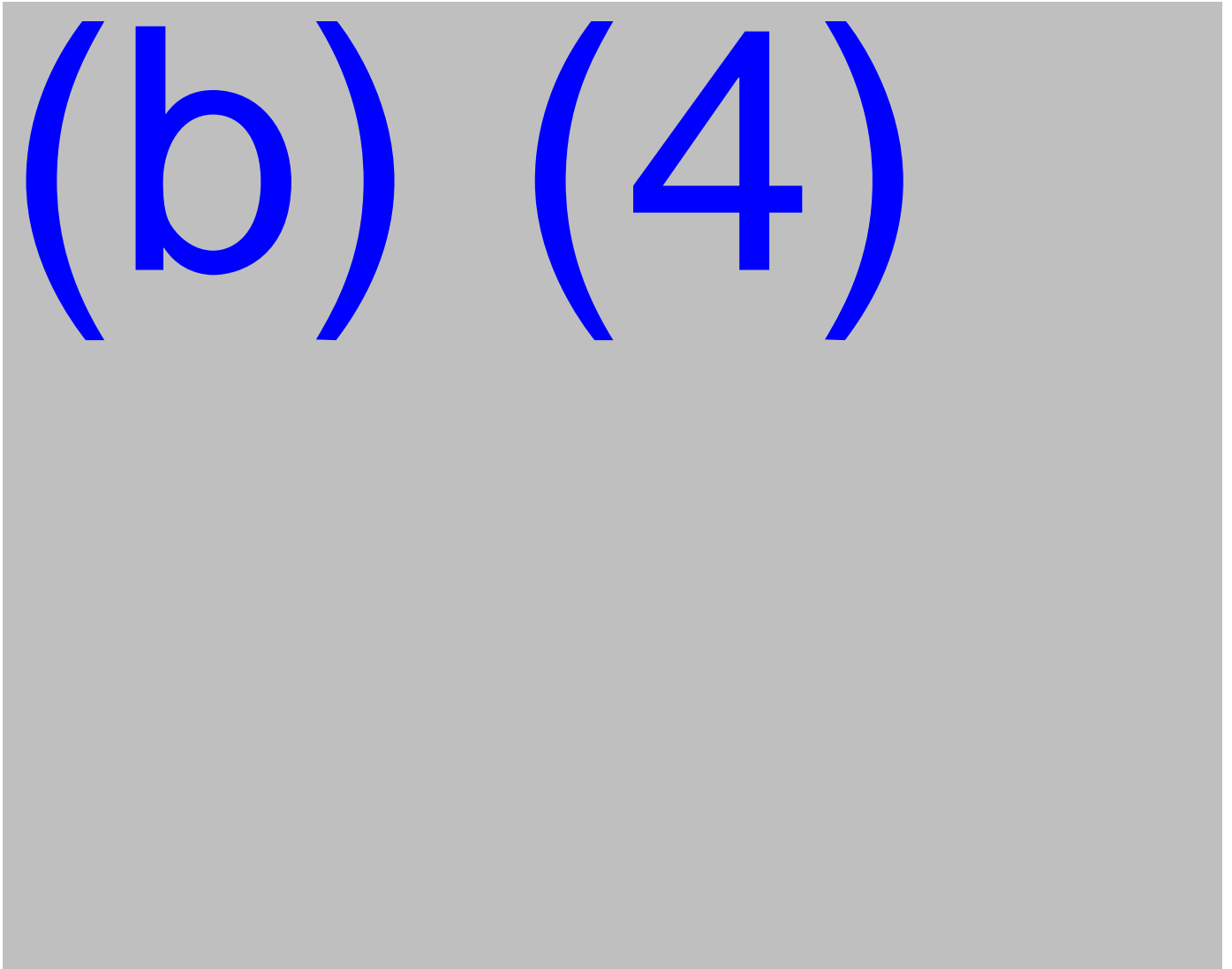


**Figure 14.2-34** (b) (4)



14.2.3.6 *Total exposure to NNK versus biomarkers in plasma and urine*

**Figure 14.2-35** (b) (4)

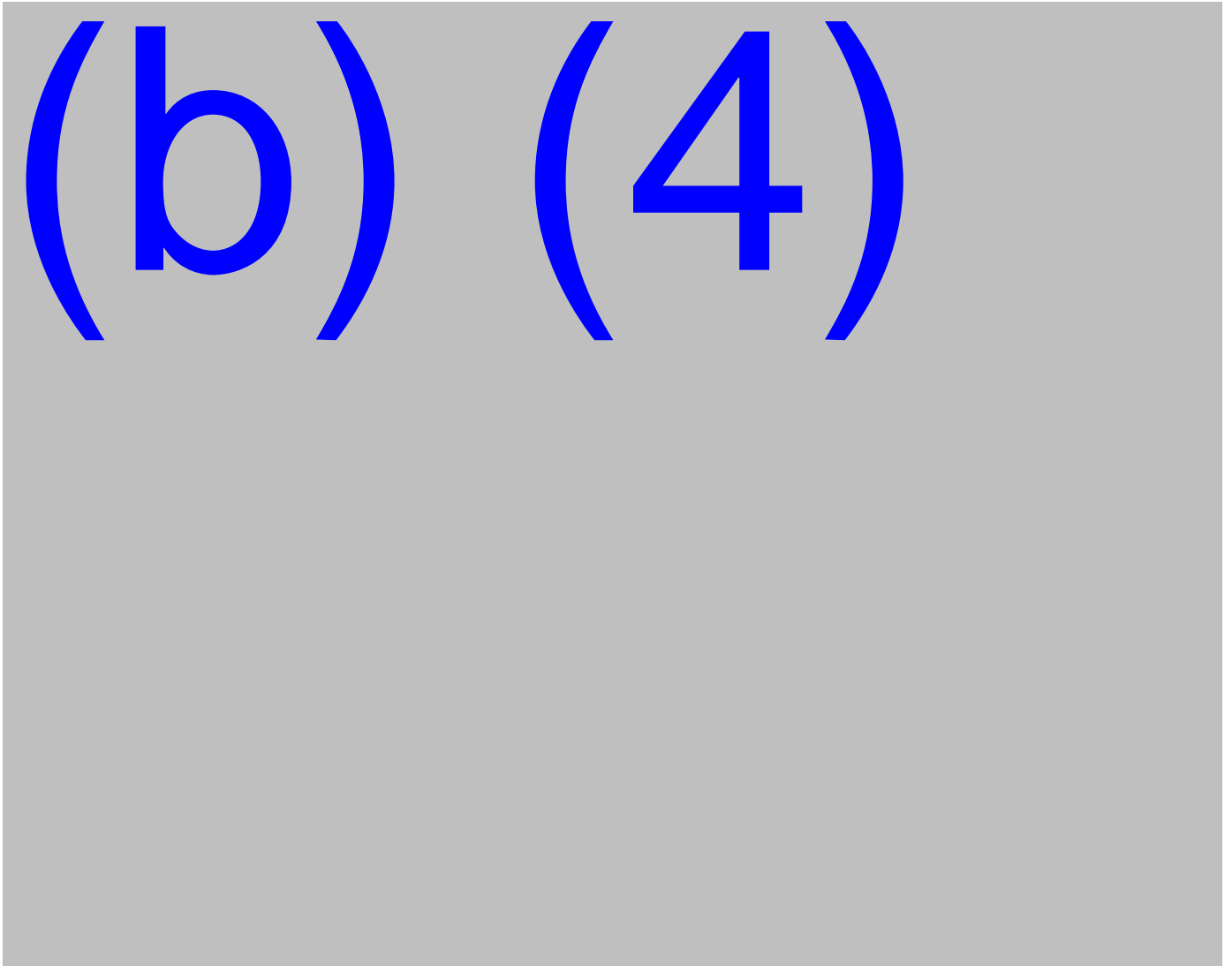




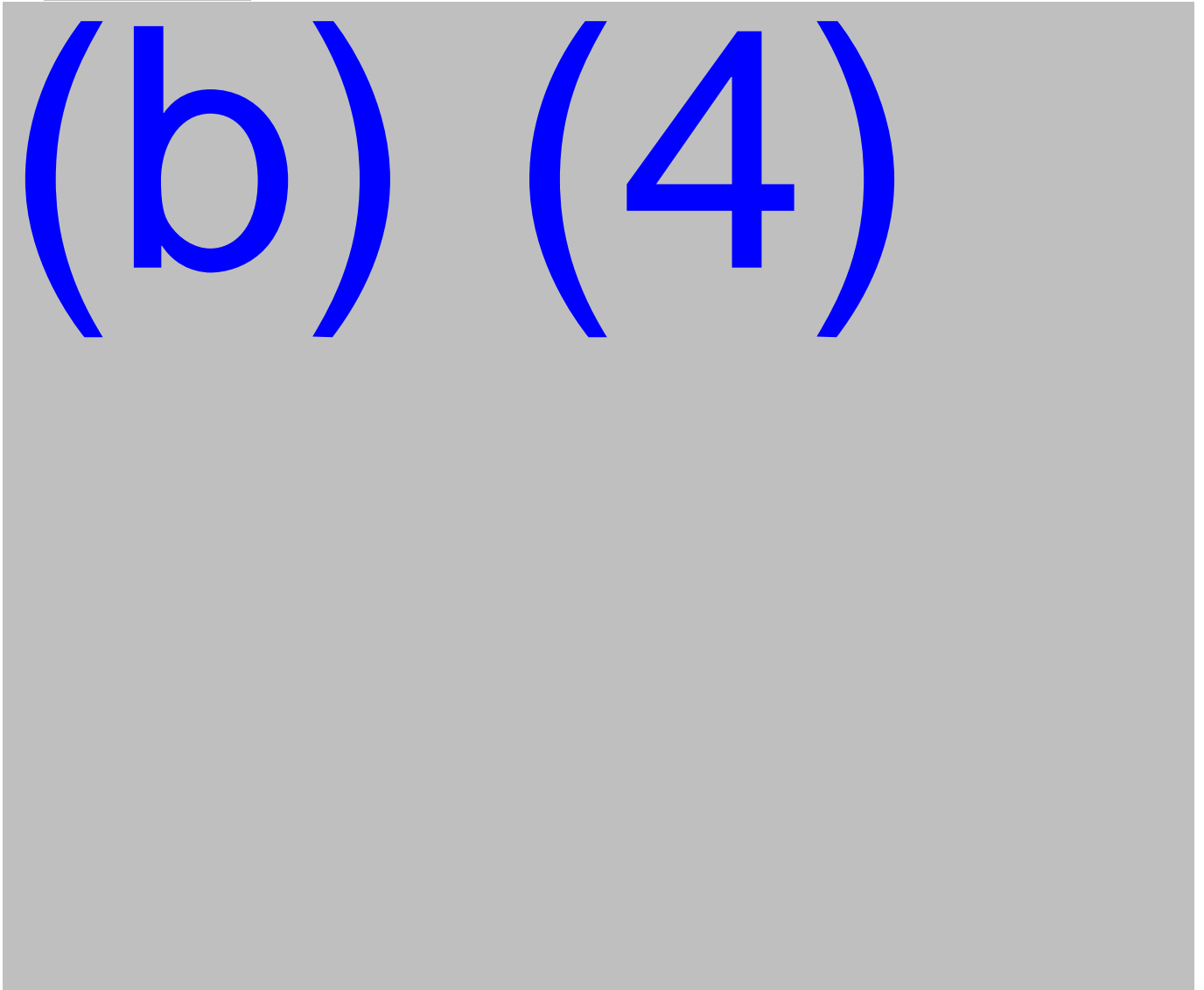
**Figure 14.2-36** (b) (4)

(b) (4)

**Figure 14.2-37** (b) (4)

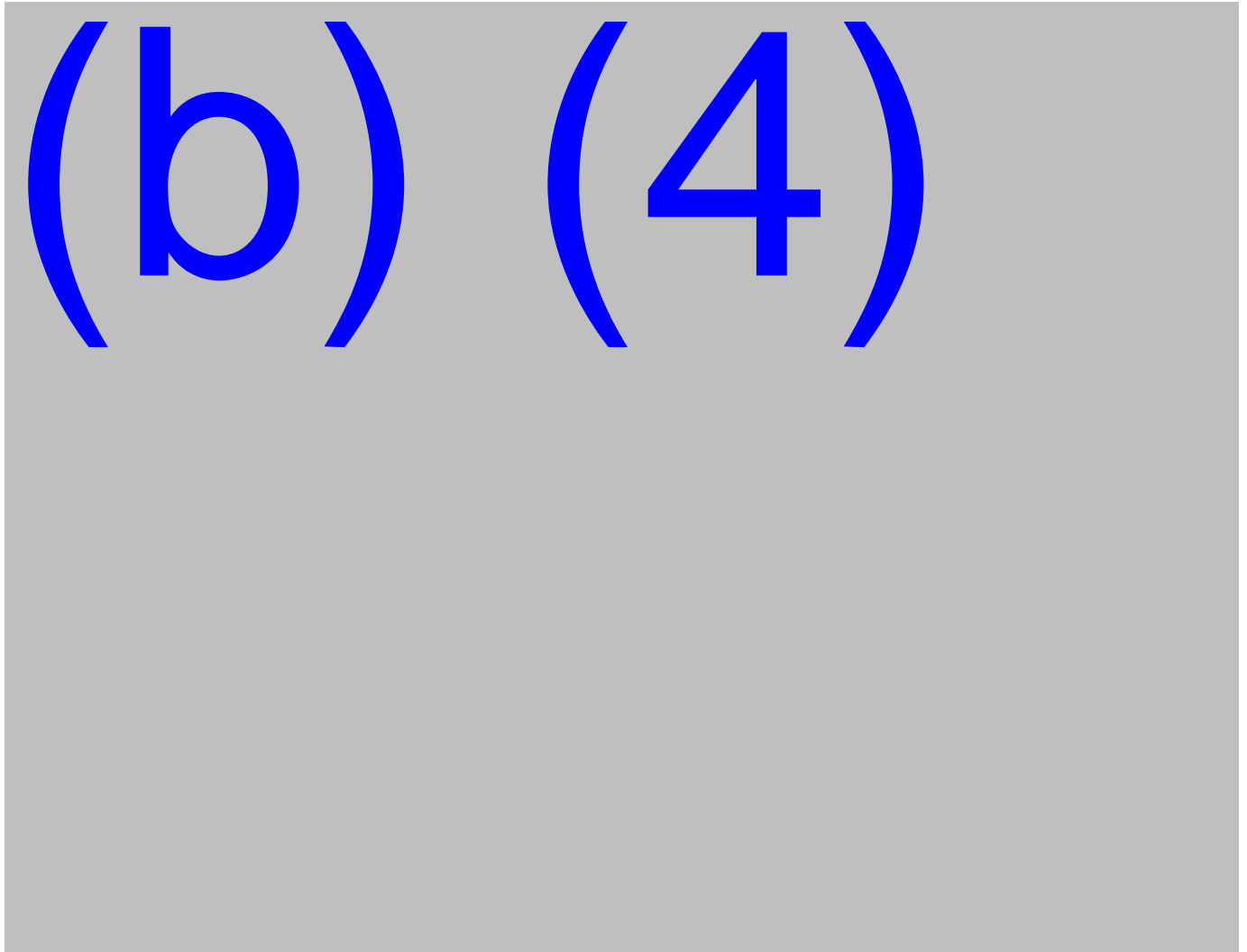


**Figure 14.2-38** (b) (4)

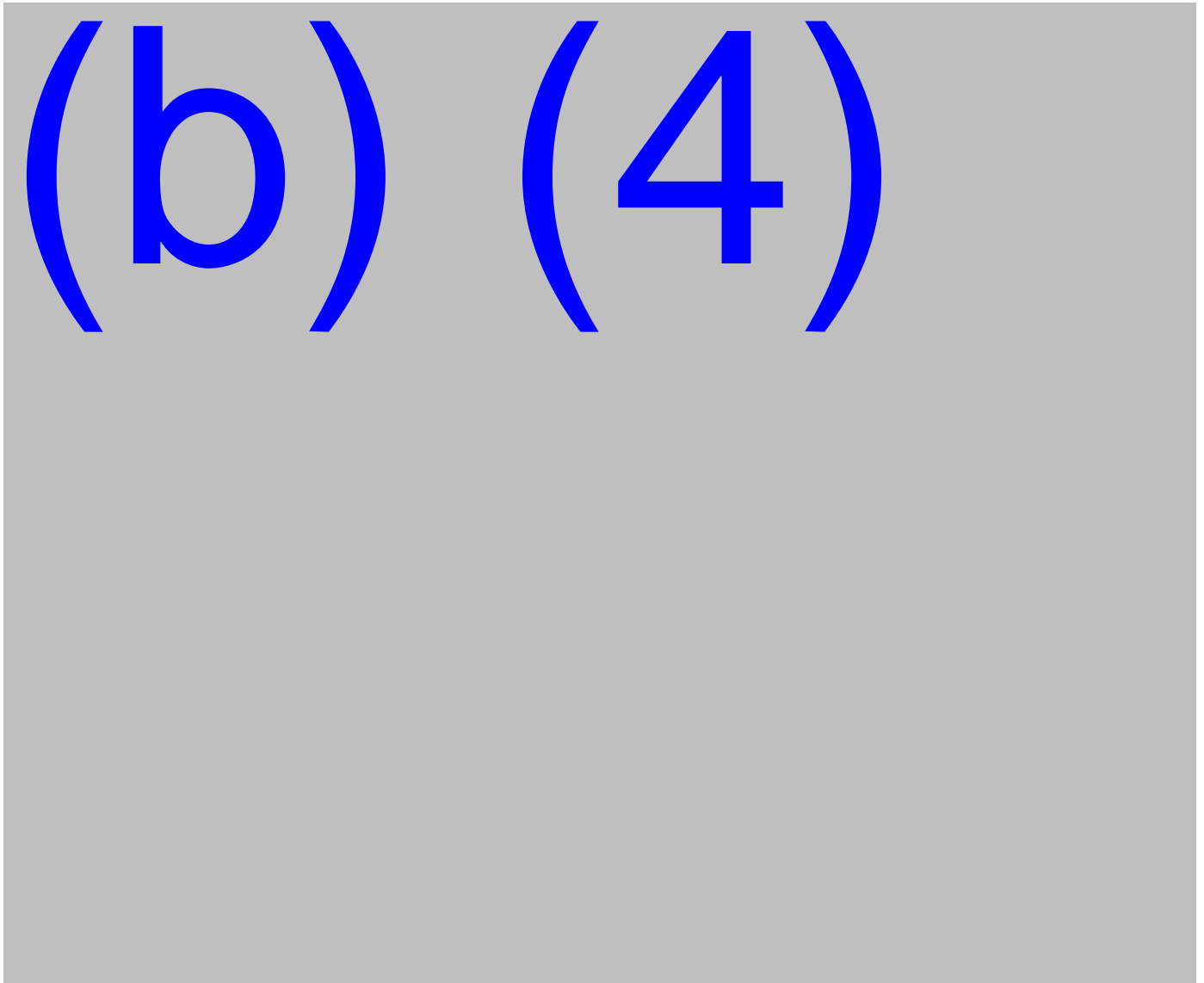


14.2.3.7 *Total exposure to NNN versus biomarkers in plasma and urine*

**Figure 14.2-39** (b) (4)



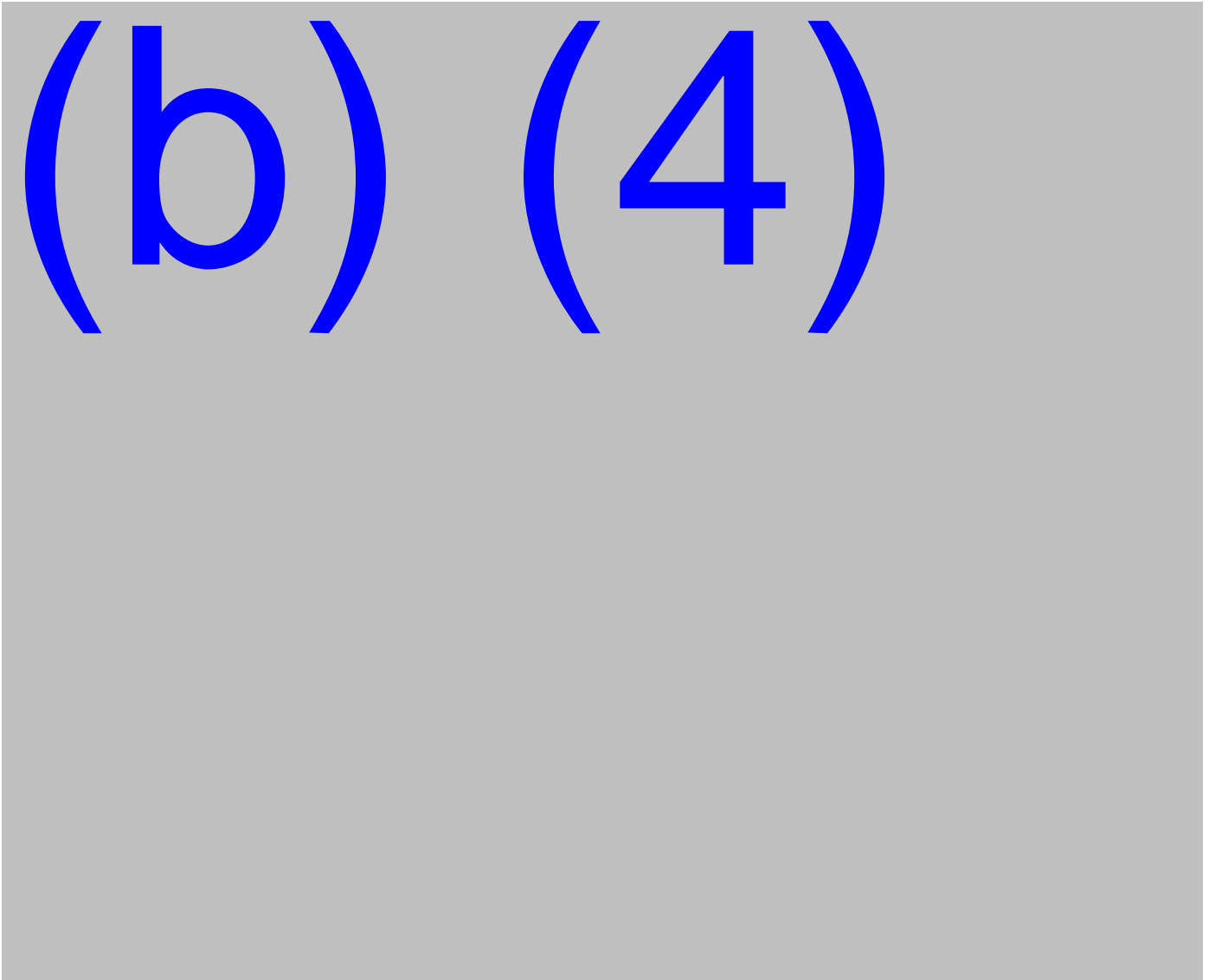
**Figure 14.2-40** (b) (4)



**Figure 14.2-41**(b) (4)

(b) (4)

**Figure 14.2-42** (b) (4)



14.2.3.8 *Total exposure to the sum of NNK and NNN versus biomarkers in plasma and urine*

**Figure 14.2-43** (b) (4)

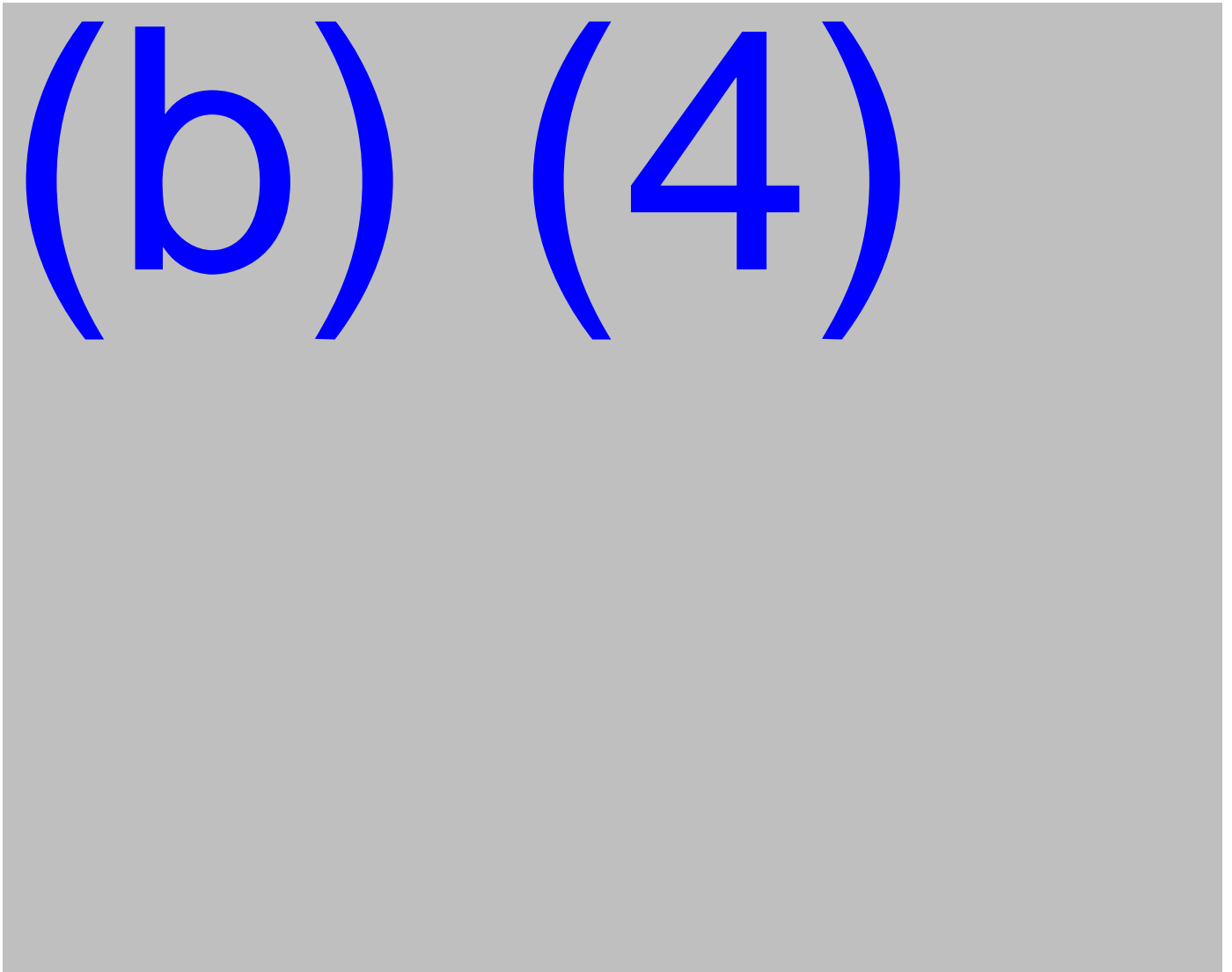
(b) (4)



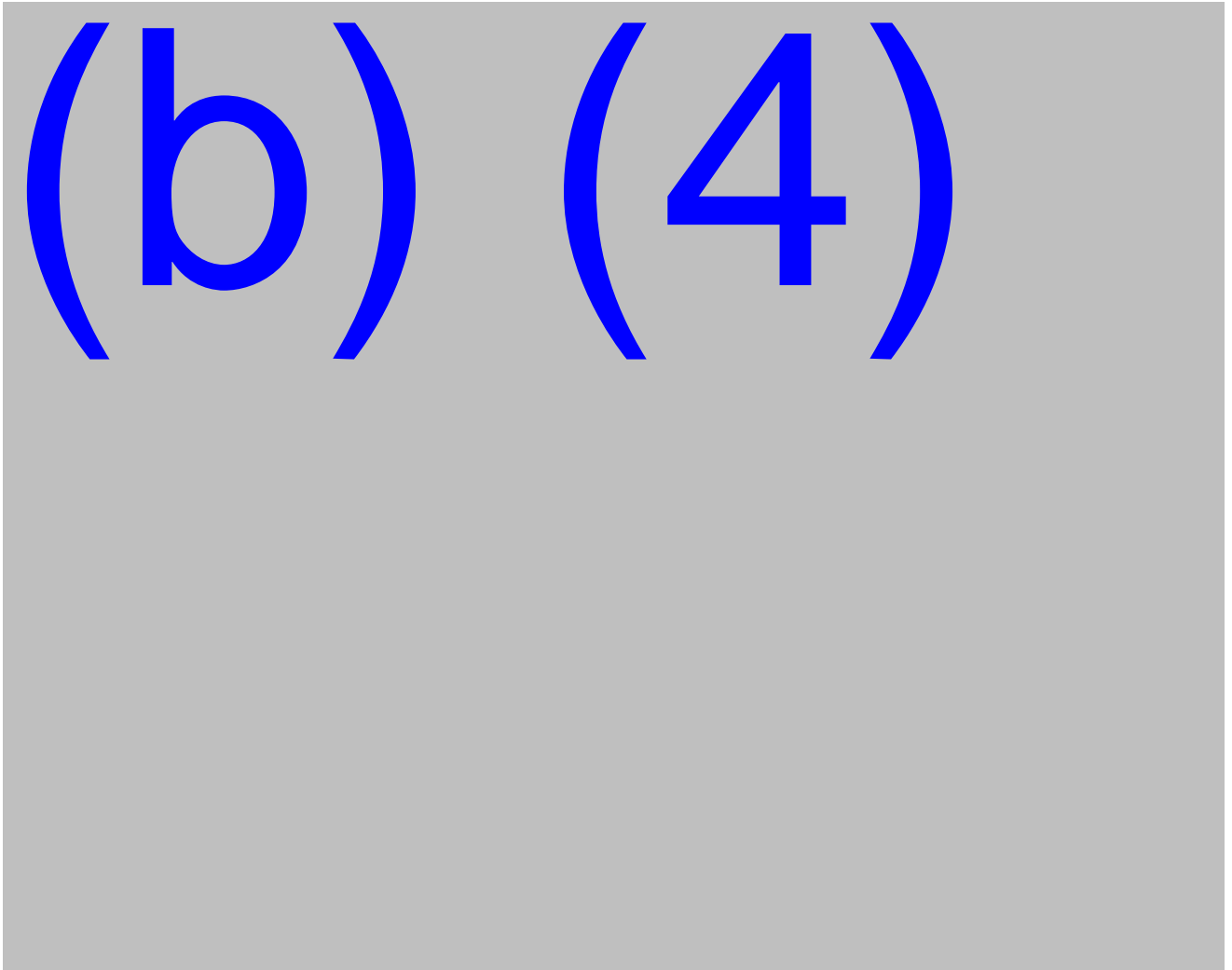
**Figure 14.2-44** (b) (4)

(b) (4)

**Figure 14.2-45** (b) (4)



**Figure 14.2-46** (b) (4)



14.2.3.9 Pattern of use

**Table 14.2-6 Summary of pattern use (Full analysis set)**

(b) (4)

(b) (4)

### 14.3 Safety data

#### 14.3.1 Adverse events

##### 14.3.1.1 Displays of adverse events

**Table 14.3-1 Adverse events by system organ class and preferred term (Full analysis set)**

(b) (4)

(b) (4)

**14.3.2 Vital signs**

***Table 14.3-2 Vital signs measurements (Full analysis set)***

(b) (4)

(b) (4)

***Table 14.3-3 Vital signs interpretations (Full analysis set)***

(b) (4)



### 14.3.3 *Electrocardiogram*

**Table 14.3-4 ECG measurements (Full analysis set)**

(b) (4)

**Table 14.3-5 ECG interpretations (Full analysis set)**

(b) (4)

**14.3.4 Clinical laboratory**

***Table 14.3-6 Safety laboratory measurements - clinical chemistry (Full analysis set)***

(b) (4)

(b) (4)

***Table 14.3-7 Safety laboratory measurements - hematology (Full analysis set)***

(b) (4)

#### 14.3.5 Physical examination

**Table 14.3-8 Physical examinations (Full analysis set)**

(b)	(4)
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## 16 APPENDICES

### 16.1 Study Information

- 16.1.1 Protocol and Protocol Amendments
- 16.1.2 Sample of CRF (Unique Pages Only)
- 16.1.3 List of IECs or IRBs (Plus the Name of the Committee Chair if Required by the Regulatory Authority) – Representative Written Information for Patient and Sample Consent Forms. *Provided in the TMF.*
- 16.1.4 List and Description of Investigators and Other Important Participants in the Study, Including Brief (1 page) CVs or Equivalent Summaries of Training and Experience Relevant to the Performance of the Clinical Study. *The CVs are provided in the TMF.*
- 16.1.5 Signatures of Principal or Coordinating Investigator(s) or Sponsor's Responsible *The signatures are provided in Section 0 in this CSR.*
- 16.1.6 Listing of Subjects Receiving Investigational Products From Specific Batches, Where More Than One Batch Was Used. *Not applicable.*
- 16.1.7 Randomization Scheme and Codes (Subject Identification and Treatment Assigned). *Not applicable.*
- 16.1.8 Audit Certificates (If Available). *Not applicable.*
- 16.1.9 Documentation Of Statistical Methods.
- 16.1.10 Documentation of Inter-Laboratory Standardization Methods and Laboratory Quality Assurance Procedures if Used.
- 16.1.11 Publications Based On The Study. *Not applicable.*
- 16.1.12 Important Publications Referenced in the Report. *Available on request.*

### 16.2 Subject Data Listings

- 16.2.1 Discontinued Subjects, Disposition
- 16.2.2 Protocol Deviations
- 16.2.3 Population definitions
- 16.2.4 Demographic Data
- 16.2.5 Compliance Data
- 16.2.6 Individual Efficacy Response Data
- 16.2.7 Adverse Event Listings
- 16.2.8 Listing of Individual Laboratory Measurements by Subject.
- 16.2.9 Listings of Vital Signs, ECG, Physical Examination and Lung Function Data



**16.3 Case report forms**

- 16.3.1 CRFs of Deaths, Other Serious Adverse Events and Withdrawals for AE.
- 16.3.2 Other CRFs Submitted.

Appendix 16.1 to 16.3 are provided separately to the CSR.