A Randomized, Controlled, Open-label Short-term Study to Evaluate Changes in Exposure to Harmful and Potentially Harmful Constituents in Adult Smokers Who Completely Switch to on!® Nicotine Pouches in a Clinical Setting

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Protocol

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Information described herein is confidential and may be disclosed only with the express written permission of the sponsor.

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SPONSOR APPROVAL

I have read the protocol and approve it:

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INVESTIGATOR AGREEMENT

I have read the protocol and agree to conduct the study as described herein.

Name, Qualifications Principal Investigator	Date

STUDY IDENTIFICATION

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SYNOPSIS

Study Title

A Randomized, Controlled, Open-label Short-term Study to Evaluate Changes in Exposure to Harmful and Potentially Harmful Constituents in Adult Smokers Who Completely Switch to on!® Nicotine Pouches in a Clinical Setting

Objectives

The primary objective of the study is:

- To compare 24-hour urinary total 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) in subjects using nicotine pouches (NP) for 7 days versus subjects who continue to smoke cigarettes for 7 days.

The secondary objectives of the study are:

- To compare biomarkers of exposure (BOEs)[1] (except total NNAL) in subjects using NP for 7 days versus subjects who continue to smoke cigarettes for 7 days
- To compare BOEs in subjects using NP for 7 days versus subjects who stopped using any tobacco products for 7 days
- To characterize product use behaviors (such as: number of cigarettes smoked per day, number of NP use per day, number of NPs per use, average duration of each NP use).

Study Design

This is an open-label, randomized, 5 parallel-group clinical study evaluating changes in exposure to selected harmful and potentially harmful constituents (HPHCs) and product use behavior in adult smokers who are randomly assigned to 1 of the 5 groups; continue smoking (Group 1), completely switch to 2 mg (Group 2), 4 mg (Group 3), or 8 mg (Group 4) on!® NP, or stop using any tobacco products (Group 5) for 7 days. Potential subjects will be screened to assess their eligibility to enter the study within 28 days prior to check-in (Day -3).

[1] BOEs : Urine: total 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), nicotine equivalents (NE), 2-aminonaphthalene (2-AN), 4-aminobiphenyl (4-ABP), 2-hydroxyethyl mercapturic acid (HEMA), 2-cyanoethylmercapturic acid (CEMA), S-phenyl mercapturic acid (S-PMA), 3-hydroxy-1-methylpropylmercapturic acid (3-HMPMA), 3-hydroxypropylmercapturic acid (3-HPMA), 2-hydroxypropylmercapturic acid (2-HPMA), N-acetyl-S-(2-carbamoylethyl) cysteine (AAMA), N-acetyl-S-(2-hydroxy-2-carbamoylethyl)cysteine (GAMA), 2-hydroxybutenylmercapturic acid (2-MHBMA), 2-OH-Fluorene (2-OHFle), 2-Naphthol,1-OH-Phenanthrene (1-OHPhe), 3-hydroxybenzo-a-pyrene (3-HB[a]P), urine mutagenicity, 1-hydroxypyrene (1-OHP). Blood: carboxyhemoglobin (COHb)

Subjects will be admitted into the study site on Day -3 and be confined to the study site until discharge on Day 8.

Study Groups

	Product Code	Product Description
Group 1	A	Subject's Usual Brand Cigarette
Group 2	B	Mint on!® 2 mg NP
Group 3	C	Mint on!® 4 mg NP
Group 4	D	Mint on!® 8 mg NP
Group 5	-	Tobacco Cessation Group

Number of Subjects

Approximately 150 subjects will be enrolled in the study.

Diagnosis and Main Criteria for Inclusion

Healthy males and females (no more than 60% of either gender), 21-45 years of age, self-affirmed either menthol or non-menthol combustible cigarette smokers (average daily consumption between 10 and 30 factory-manufactured combustible cigarettes for at least 12 months prior to screening).

Study Products

- Product A (reference): subject's usual brand cigarette, ad libitum, from Day 1 to Day 7
- Product B (test): 2 mg NP marketed as Mint on!® 2 mg nicotine pouches, at least 3 pouches/day, from Day 1 to Day 7
- Product C (test): 4 mg NP marketed as Mint on!® 4 mg nicotine pouches, at least 3 pouches/day, from Day 1 to Day 7
- Product D (test): 8 mg NP marketed as Mint on!® 8 mg nicotine pouches, at least 3 pouches/day, from Day 1 to Day 7

Duration of Subject Participation in the Study

Planned screening duration: up to 28 days

Planned study duration (screening to follow-up): approximately 5 weeks.

Endpoints

Primary endpoint:

- 24-hour total urinary NNAL (mg/24 hours) excreted on Day 7

Secondary endpoints:

- 24-hour urinary NE, 2-AN, 4-ABP, HEMA, CEMA, S-PMA, 3 HMPMA, 3-HPMA, , 2-HPMA, AAMA, GAMA, 2-MHBMA, 2 OHFle, 2-Naphthol, 1 OHPhe, 3-HB[a]P, mutagenicity, and 1-OHP excreted on Day 7
- COHb on Day 7
- Product use behavior daily from Day 1 to Day 7 (ie, number of cigarettes smoked per day[CPD], number of NP per day [NPPD], number of NP per use [NPPU], and average duration of each NP use)

Safety:

Adverse events, clinical laboratory evaluations (hematology, clinical chemistry, urinalysis), 12-lead electrocardiograms, vital signs measurements, and physical examinations.

Statistical Methods

Linear mixed models for analysis of covariance will be used to compare the Day 7 biomarker values between groups as described in the study objectives. In the statistical models, the outcome variable will be included as a dependent variable; group and gender will be included as fixed effects; and baseline values of corresponding biomarker will be included as covariates. For the NP groups compared to the continue cigarette smoking group, Dunnett's method will be used for the adjustment of multiple comparisons. The SAS procedure Proc Mixed will be used. The least-squares means (LSM) difference and 95% confidence interval for the LSM difference between the test and reference groups and p-values will be provided.

Product use behaviors and safety parameters will be analyzed using descriptive statistics.

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LIST OF ABBREVIATIONS

Abbreviation	Definition
AAMA	N-acetyl-S-(2-carbamoylethyl) cysteine
4-ABP	4-aminobiphenyl
AE	adverse event
ALCS	Altria Client Services
2-AN	2-aminonaphthalene
BOE	biomarker of exposure
CC	continue smoking cigarettes
CEMA	2-cyanoethylmercapturic acid
CFR	Code of Federal Regulations
CLIA	Clinical Laboratory Improvement Amendments
COHb	carboxyhemoglobin
COVID	coronavirus disease
CPD	number of cigarettes smoked per day
CRO	contract research organization
DMP	Data Management Plan
ECG	electrocardiogram
eCRF	electronic case report form
EDC	electronic data capture
EOS	end of study
FDA	Food and Drug Administration
FSH	follicle-stimulating hormone
GAMA	N-acetyl-S-(2-hydroxy-2-carbamoylethyl)cysteine
GCP	good clinical practice
3-HB[a]P	3-hydroxybenzo-a-pyrene
HEMA	2-hydroxyethyl mercapturic acid
HPHC	harmful and potentially harmful constituents
3-HMPMA	3-hydroxy-1-methylpropylmercapturic acid
2-HPMA	2-hydroxypropylmercapturic acid
3-HPMA	3-hydroxypropylmercapturic acid
ICF	informed consent form
ICH	International Council for/Conference on Harmonization
IRB	institutional review board
LSM	least-squares means
MedDRA	Medical Dictionary for Regulatory Activities
2-MHBMA	2-hydroxybutenylmercapturic acid

MST	moist smokeless tobacco
NE	nicotine equivalents
NNAL	4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol
NP	nicotine pouch
NP2	2 mg nicotine pouch
NP4	4 mg nicotine pouch
NP8	8 mg nicotine pouch
NPPD	number of nicotine pouches used per day
NPPU	number of nicotine pouch per use
NT	no tobacco
1-OHPhe	1-OH-Phenanthrene
2-OHFle	2-OH-Fluorene
1-OHP	1-hydroxypyrene
OTDN	Oral Tobacco-Derived Nicotine
PK	pharmacokinetic
QTcF	QT interval corrected for heart rate using Fridericia's method
SAE	serious adverse event
SoA	Schedule of Assessments
S-PMA	S-phenyl mercapturic acid
UBC	usual brand cigarette

1. INTRODUCTION

1.1. Background

The harm caused by tobacco product use is primarily attributable to cigarette smoking. Smoking is the primary causal factor for at least 30% of all cancer deaths, for nearly 80% of deaths from chronic obstructive pulmonary disease, and early cardiovascular disease and deaths. The scientific evidence has clearly established that smoking cessation leads to a significant reduction in smoking-related morbidity and mortality. [1,2,3,4] While cessation is the most desirable outcome for reducing the harm from smoking-related diseases, many adult smokers are unable or unwilling to quit. Public health authorities, including the FDA, have acknowledged a continuum of risk among tobacco products, with combustible cigarettes at the highest end and non-combustible products on the lower end of that spectrum.[5,6]

The Oral Tobacco-Derived Nicotine (OTDN) products are non-combustible and intended for oral consumption. The OTDN category is one of the fastest growing tobacco product segments in the United States.

The on!® nicotine pouches (NPs) are one such portfolio of products that belong to the OTDN category. The on!® NP are innovative oral tobacco products that do not contain cut, ground, powdered, or leaf tobacco – a point of differentiation compared to smokeless tobacco products in the United States. The on!® NP contain tobacco-derived nicotine and non-tobacco ingredients used in foods. The nicotine in the on!® products is extracted from tobacco plants and crystalized into nicotine salt. The on!® products do not contain any of the major food allergens (milk, eggs, fish, shellfish, tree nuts, peanuts, wheat, and soy).

on!® product contains tobacco-derived nicotine, which is addictive. Nicotine can harm a baby for a female who is pregnant or nursing. Nicotine can increase heart rate, blood pressure and aggravate diabetes. Nicotine can cause dizziness, nausea and stomach pain. A burning sensation may be experienced while using oral nicotine products. on!® product packages have the following warning: “WARNING: This product contains nicotine. Nicotine is an addictive chemical.”

These products have been available since 2016. The on!® NP are well tolerated and minimal consumer complaints have been reported. As an example of this, we received only 17 consumer reports in a 6-month timeframe, July through December 2019, during which time period 2,026,251 cans had been purchased.

Two pharmacokinetic (PK) studies were conducted with on!® NP.

The first study (ALCS-REG-19-12-OTDN) included 6 flavor variants (Berry, Cinnamon, Citrus, Coffee, Original, and Wintergreen) of the 4 mg on! ® NP and the subject’s usual brand cigarette (UBC) as a reference product. The purpose of this PK study was to characterize the nicotine plasma PK profile from single use (1 pouch use for 30 minutes or 10 inhalations from 1 cigarette). Prior to randomization, subjects had a Product Trial period to use the NP at home for at least 3 days. In addition, subjects were allowed to use each of the NP ad libitum for 4 hours following randomization. A total of 42 (25 males, mean age 36.3 years) healthy adult cigarette smokers were enrolled and 41 completed the study. Starting from the first

product use in the Product Trial until the time of randomization, a total of 9 mild adverse events (AEs) were reported by 6 (14%) subjects. The most frequent events were hiccups and throat irritation, experienced by 2 (5%) subjects each. The investigator considered 3 AEs (dyspepsia, pharyngeal paresthesia, and throat irritation) to be definitely related to study product, 2 AEs (hiccups and throat irritation) to be likely related, 2 AEs (hiccups and productive cough) to be possibly related, and the remaining 2 AEs (headache and toothache) not related. Of note, the investigator was unable to determine whether the AEs were related to NP or cigarette since both products were allowed ad libitum during the Product Trial. From randomization on Day -1 through study completion on Day 7, a total of 25 mild AEs were reported by 18 (43%) subjects, with more subjects experiencing AEs following cigarettes (8 [19%]) compared to NP (≤ 3 [7%] subjects across flavors). Pain in extremity (due to venipunctures/blood draws) was the most frequently reported event, experienced by 4 (10%) subjects. All remaining AEs were reported by 3 or fewer (≤ 7%) subjects each. The investigator considered 3 AEs (dizziness [cigarette], gingival bleeding [Cinnamon NP], and hyperhidrosis [cigarette]) to be likely related to study product, 3 AEs (1 dyspepsia [Citrus NP] and 2 headaches [cigarette]) to be possibly related, and the remaining events unlikely related or not related. There were no serious AEs (SAEs) reported in this study and no subjects were discontinued due to AEs.

The second study (ALCS-REG-19-13-OTDN) included 5 levels (1.5, 2. 3.5, 4, and 8 mg) of Mint on! ® NP. A total of 30 (29 males, mean age 34.9 years) healthy adult dual tobacco users (those who smoke cigarette and also use moist smokeless tobacco [MST]) were enrolled and completed the study with the similar design as the first study. During the Product Trial period, during which subjects were allowed to use ad libitum the supplied Mint on! ® NP, and their usual brands of combustible cigarettes and loose MST, a total of 9 mild AEs were reported by 6 (20%) subjects. The most frequent event was headache, experienced by 4 (13%) subjects. The investigator considered all events unlikely or not related to study product. From the time of randomization on Day -1 until the end of study on Day 7, a total of 61 AEs were reported by 20 (67%) subjects across study products. The majority of the events (59) were mild in severity and 2 (headache [cigarette] and nausea [8 mg NP]) were moderate. Headache was the most frequently reported event, experienced by 8 (27%) subjects, followed by nausea, experienced by 6 (20%) subjects. All remaining events were reported by 4 or fewer (≤ 13%) subjects each. The investigator considered 7 events to be likely related to study product and 13 events possibly related. The likely/possibly related events occurred across study products and included, but were not limited to, nausea, vomiting, dizziness, and headache. The remaining 41 events were considered unlikely/not related to study product. There were no SAEs reported in this study and no subjects were discontinued due to AEs.

1.2. Study Rationale

A dose-response relationship has been demonstrated between cigarette smoking and cancer of the lung, larynx, oral cavity, and urinary bladder (2004 SGR Report – The Health Consequences of Smoking[7]). Therefore, sustained and prolonged reduction in exposure to the harmful and potentially harmful constituents (HPHCs) from cigarette smoke will result in reduction in smoking-related disease risks. The exposure can be assessed by measuring the levels of constituents or metabolites in urine or blood (biomarkers of exposure - BOEs).

The purpose of this study is to generate evidence regarding the extent of reduction in exposure to selected HPHCs in adult smokers switching to on![®] NPs. The study intends to determine changes in exposure to selected HPHCs by measuring biomarkers in adult smokers who completely switch from smoking to use of on![®] NP compared to those who continue smoking cigarettes (CC) or stop using all tobacco products (NT).

2. OBJECTIVES AND ENDPOINTS

2.1. Objectives

The primary objective of the study is:

- To compare 24-hour urinary total 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) in subjects using NP for 7 days versus subjects who continue to smoke cigarettes for 7 days.

The secondary objectives of the study are:

- To compare BOEs (except total NNAL) in subjects using NP for 7 days versus subjects who continue to smoke cigarettes for 7 days
- To compare BOEs in subjects using NP for 7 days versus subjects who stopped using any tobacco products for 7 days
- To characterize product use behaviors (such as: number of cigarettes smoked per day [CPD], number of NPs used per day [NPPD], number of NPs per use [NPPU], average duration of each NP use).

2.2. Endpoints

2.2.1. Primary Endpoints

The primary endpoint is presented in Table 1.

Table 1: Primary Endpoint

Biomarker	Abbreviation	Associated Toxicant	Matrix
Total 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol	NNAL	4-[Methyl(nitroso)amino]-1-(3-pyridinyl)-1-butanone	Urine (24-hour)

2.2.2. Secondary Endpoints

The secondary BOE endpoints are presented in Table 2.

Table 2: Secondary Endpoints – Biomarkers of Exposure

Biomarker of Exposure	Abbreviation	Associated Toxicant	Matrix
Nicotine equivalents (nicotine, cotinine, 3-hydroxycotinine and their glucuronide conjugates)	NE	Nicotine	Urine (24 hours)

Biomarker of Exposure	Abbreviation	Associated Toxicant	Matrix
3-hydroxypropylmercapturic acid	3-HPMA	Acrolein	Urine (24 hours)
3-hydroxy-1-methylpropylmercapturic acid	3-HMPMA	Crotonaldehyde	Urine (24 hours)
2-hydroxypropylmercapturic acid	2-HPMA	Propylene oxide	Urine (24 hours)
S-phenylmercapturic acid	S-PMA	Benzene	Urine (24 hours)
2-cyanoethylmercapturic acid	CEMA	Acrylonitrile	Urine (24 hours)
1-hydroxypyrene	1-OHP	Pyrene	Urine (24 hours)
2-hydroxyethylmercapturic acid	HEMA	Ethylene oxide	Urine (24 hours)
2-hydroxybutenylmercapturic acid (2-MHBMA)	2-MHBMA	1,3 butadiene	Urine (24 hours)
2-Naphthol	-	Naphthalene	Urine (24 hours)
4-aminobiphenyl	4-ABP	4-aminobiphenyl	Urine (24 hours)
2-aminonaphthalene	2-AN	2-aminonaphthalene	Urine (24 hours)
N-acetyl-S-(2-carbamoylethyl) cysteine	AAMA	Acrylamide	Urine (24 hours)
N-acetyl-S-(2-hydroxy-2-carbamoylethyl)cysteine	GAMA	Acrylamide	Urine (24 hours)
2-OH-Fluorene	2-OHFle	Fluorene	Urine (24 hours)
Naphthol,1-OH-Phenanthrene	1-OHPhe	Benzo-a-pyrene	Urine (24 hours)
3-hydroxybenzo-a-pyrene	3-HB[a]P	Benzo-a-pyrene	Urine (24 hours)
Urine mutagenicity	-	-	Urine (24 hours)
Carboxyhemoglobin	COHb	Carbon monoxide	Blood

Additional endpoint is:

- Product use behavior daily from Day 1 to Day 7 (ie, CPD, NPPD, NPPU, and the average duration of each NP use).

3. INVESTIGATIONAL PLAN

3.1. Overall Study Design and Plan

This will be an open-label, randomized, 5 parallel-groups clinical study evaluating changes in exposure to selected HPHCs and product use behavior in adult smokers.

Study population will be randomized into 5 groups:

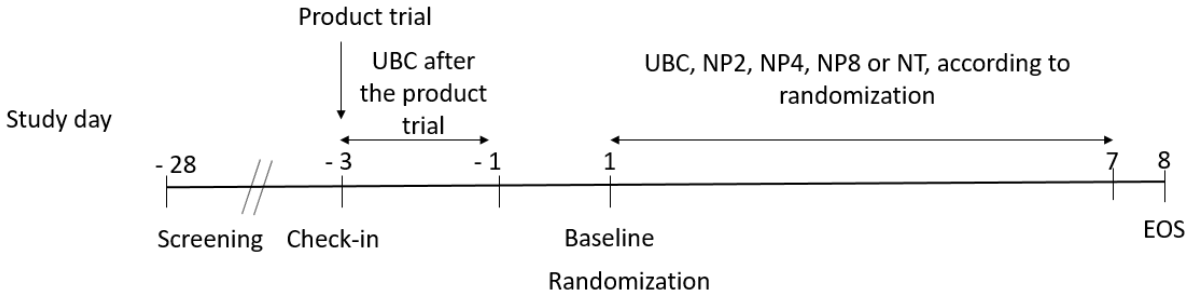
- Group 1 (n = 30): CC; subjects will be asked to continue smoking their UBCs ad libitum for 7 days.
- Group 2 (n = 30): 2 mg NP (NP2); subjects will exclusively use 2 mg NP, using at least 3 pouches per day for 7 days.
- Group 3 (n = 30): 4 mg NP (NP4); subjects will exclusively use 4 mg NP, using at least 3 pouches per day for 7 days.
- Group 4 (n = 30): 8 mg NP (NP8); subjects will exclusively use 8 mg NP, using at least 3 pouches per day for 7 days.

- Group 5 (n = 30): No Tobacco (NT); subjects will completely stop all tobacco product usage for 7 days.

The goal is to recruit approximately 150 subjects (not more than 60% of either gender) with the aim of at least 100 subjects completing the study (20 subjects, minimum per group).

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

Figure 1: Study Schematic



Abbreviations: EOS = end of the study; NP2 = 2 mg nicotine pouches; NP4 = 4 mg nicotine pouches; NP8 = 8 mg nicotine pouches; NT = no tobacco; UBC = usual brand cigarette.

Potential subjects will be screened to assess their eligibility to enter the study within 28 days prior to the first product administration. Subjects will be admitted into the study site on Day -3 and be confined to the study site until discharge on Day 8.

The total duration of study participation for each subject (from screening through last visit) is anticipated to be approximately 5 weeks.

The start of the study is defined as the date the first subject signs an informed consent form (ICF). The point of enrollment occurs at the time of subject check-in visit. The study completion is defined as the date of the last subject's last assessment (scheduled or unscheduled).

A Schedule of Assessments (SoA) is presented in Appendix 5.

3.2. Discussion of Study Design

This is an open-label, randomized, 5 parallel-group clinical study evaluating changes in exposure to selected HPHCs and product use behavior in adult smokers.

Study population will be divided into 5 groups (see Section 3.1 for details). Group 1 (subjects continuing to smoke their UBC) and Group 5 (NT) will serve as references, indicating maximum and minimum levels of BOEs.

Collecting CPD, NPPD, NPPU, and the average duration of each NP use will help to define use behaviors.

This study follows the recommendations of the Modified Risk Tobacco Product Applications draft FDA guidance.[8]

3.3. Selection of Study Products

on!® Nicotine pouches are available in 5 levels (1.5, 2, 3.5, 4, and 8 mg) of nicotine. The 2-, 4-, and 8-mg levels will be assessed in this study as they represent the range of nicotine levels across the portfolio. The Mint flavor was chosen as it is the most widely used flavor variant. Subjects will be required to use NP at least 3 times per day, to ensure a minimum use of the product.

4. SELECTION OF STUDY POPULATION

Self-affirmed adult smokers will be screened to enroll approximately 150 subjects (not more than 60% of either gender). Study population will be divided into 5 groups:

- Group 1 (n = 30): CC; subjects will be asked to continue smoking their UBCs ad libitum for 7 days.
- Group 2 (n = 30): 2 mg NP (NP2); subjects will exclusively use 2 mg NP, using at least 3 pouches per day for 7 days.
- Group 3 (n = 30): 4 mg NP (NP4); subjects will exclusively use 4 mg NP, using at least 3 pouches per day for 7 days.
- Group 4 (n = 30): 8 mg NP (NP8); subjects will exclusively use 8 mg NP, using at least 3 pouches per day for 7 days.
- Group 5 (n = 30): NT; subjects will completely stop all tobacco product usage for 7 days.

4.1. Inclusion Criteria

Subjects must satisfy all of the following criteria at the screening visit unless otherwise stated:

1. Voluntary consent to participate in this study documented on the signed ICF
2. Healthy adult males and females 21 to 45 years of age, inclusive, at screening
3. Smoking history (self-reported at screening) of an average of at least 10 but no more than 30 factory-manufactured combustible cigarettes (either menthol or non-menthol) daily for at least 12 months prior to screening. Brief periods (ie, up to 7 consecutive days) of non-smoking during the 3 months prior to screening (eg, due to illness or participation in a study where smoking was prohibited) will be permitted
4. Positive urine cotinine (≥ 500 ng/mL) at screening
5. Female subjects (see Appendix 3) who are heterosexually active and of childbearing potential (eg, neither surgically sterile at least 6 months prior to check-in nor postmenopausal with amenorrhea for at least 12 months prior to check-in with follicle-stimulating hormone [FSH] levels consistent with postmenopausal status) must have been using one of the following forms of contraception for the time period indicated and agree to continue using it through completion of the study:

- Hormonal (eg, oral, vaginal ring, transdermal patch, implant, injection) consistently for at least 3 months prior to check-in, double barrier (ie, condom with spermicide or diaphragm with spermicide) consistently for at least 4 weeks prior to check-in, an intrauterine device for at least 4 months prior to check-in
- Exclusive partner who has been vasectomized for at least 6 months (inclusive) prior to check-in

Female subjects of childbearing potential who are not currently engaging in heterosexual intercourse must agree to use one of the above methods of birth control through completion of study, in the event that they have heterosexual intercourse during the course of the study.

6. Female subjects who are of nonchildbearing potential must have undergone one of the following sterilization procedures at least 6 months prior to check-in:
   - Hysteroscopic sterilization (including Essure® or similar nonsurgical sterilization procedures); bilateral tubal ligation or bilateral salpingectomy; hysterectomy; bilateral oophorectomy

   Or be postmenopausal with amenorrhea for at least 12 months prior to check-in and have FSH levels consistent with postmenopausal status
7. Willing to comply with the requirements of the study
8. Willing to use all 3 on!® NP after the Product Trial at check-in
9. Willing and able to abstain from cigarettes from Day 1 through the end of the study (EOS).

4.2. Exclusion Criteria

Subjects will be excluded from the study if there is evidence of any of the following criteria at screening, check-in, or prior to randomization, as appropriate.

1. Use of any type of tobacco- or nicotine-containing products other than manufactured cigarettes (eg, e-vapor products, roll-your-own cigarettes, bidis, snuff, nicotine inhaler, pipe, cigar, chewing tobacco, nicotine patch, nicotine spray, nicotine lozenge, or nicotine gum) in the 7 days prior to check-in
2. Self-reported puffers (ie, adult smokers who draw smoke from the cigarette into the mouth and throat but do not inhale)
3. Planning to quit smoking in the next 30 days (from screening visit)
4. History or presence of clinically significant gastrointestinal, renal, hepatic, neurologic, hematologic, endocrine, oncologic, urologic, existing respiratory diseases, immunologic, psychiatric, lymphatic, or cardiovascular disease, or any other condition that, in the opinion of the investigator, would jeopardize the safety of the subject or impact the validity of the study results

5. Clinically significant abnormal findings on the vital signs, physical examination, medical history, electrocardiogram (ECG), or clinical laboratory results, in the opinion of the investigator
6. Positive test for human immunodeficiency virus, hepatitis B surface antigen, or hepatitis C virus at screening
7. History or presence of any type of malignant tumors
8. Current evidence or any history of congestive heart failure
9. Diabetes mellitus (fasting glucose ≥126 mg/L [7 mmol/L]) that is not controlled by diet/exercise alone, in the opinion of the investigator
10. An acute illness (eg, upper respiratory infection, viral infection) requiring treatment with prescribed medicines within 2 weeks prior to check-in
11. Dentition that prevents subjects from using on!® NP products
12. Allergic to or cannot tolerate mint flavoring agents or phenylalanine
13. Any planned surgery from the time of screening through EOS
14. History of drug or alcohol abuse within 24 months prior to check-in
15. Fever (ie, body temperature >100.5°F) at screening or check-in. One recheck may be performed at the investigator's discretion
16. Body mass index greater than 40.0 kg/m^2 or less than 18.0 kg/m^2 at screening
17. Systolic blood pressure >150 mmHg and/or diastolic blood pressure > 90 mmHg at screening or check-in, measured after being seated for at least 10 minutes. One recheck may be performed at the investigator's discretion
18. Estimated creatinine clearance (by Cockcroft-Gault equation) < 80 mL/minute at screening
19. Serum alanine aminotransferase ≥1.5 times the upper limit of normal and/or aspartate aminotransferase ≥1.5 times the upper limit of normal at screening
20. Positive screen for alcohol (breath) or any of the following drugs of abuse (urine), regardless of the reason of use: amphetamines, methamphetamines, opiates, cannabinoids, or cocaine at screening or check-in
21. Female subjects who are pregnant (positive serum pregnancy test at screening or urine pregnancy test at check-in), lactating, or intend to become pregnant from screening through EOS
22. Use of prescription or over-the-counter bronchodilator medication (eg, inhaled or oral ß-agonists) within 12 months prior to check-in
23. Use of medications or foods known or are suspected to interact with cytochrome P450 2A6 (including, but not limited to, amiodarone, amlodipine, amobarbital, buprenorphine, clofibrate, clotrimazole, desipramine, disulfiram, entacapone, fenofibrate, isoniazid, grapefruit, ketoconazole, letrozole, methimazole, methoxsalen, metyrapone, miconazole, modafinil, orphenadrine, pentobarbital, phenobarbital, pilocarpine, primidone, propoxyphene, quinidine, rifampicin, rifampin, secobarbital,

selegiline, sulconazole, tioconazole, tranylcypromine) within 14 days or 5 half-lives of the drug, whichever is longer, prior to check-in or during the study

24. Use of antibiotic treatment within 2 weeks prior to check-in
25. Plasma donation within 7 days prior to check-in
26. Donation of blood or blood products (with the exception of plasma as noted above), had significant blood loss, or received whole blood or a blood product transfusion within 56 days prior to check-in
27. Participation in a previous clinical study for an investigational drug, device, biologic, or a tobacco product within 30 days prior to check-in
28. Participation in 2 or more Altria Client Services (ALCS) studies within the past 12-month period prior to check-in
29. Subject or a first-degree relative (ie, parent, sibling, child, spouse/partner) is a current or former employee of the tobacco industry or a named party or class representative in litigation with any tobacco company
30. Subject or a first-degree relative (ie, parent, sibling, child, spouse/partner) is a current employee of the study site
31. Positive result for coronavirus disease (COVID)-19 test at screening or check-in
32. One or more “yes” answers to any of the questions on the COVID-19 screening questionnaire (provided by the study site) at screening or check-in.

4.3. Subject Number and Identification

Subjects will be assigned a unique subject identification number upon signing informed consent.

Subjects will be identified by subject number on all study documentation.

4.4. Subject Withdrawal and Replacement

A subject is free to withdraw from the study at any time. In addition, a subject will be withdrawn from the study if any of the following criteria are met:

- Change in compliance with any inclusion/exclusion criterion that is clinically relevant and affects subject safety as determined by the investigator (or designee)
- Noncompliance with the study restrictions that might affect subject safety or study assessments/objectives, as considered applicable by the investigator (or designee)
- Any clinically relevant sign or symptom that, in the opinion of the investigator (or designee), warrants subject withdrawal.

If a subject is withdrawn from product use, the sponsor will be notified and the date and reason(s) for the withdrawal will be documented in the subject’s electronic case report form (eCRF). If a subject is withdrawn from the study, efforts will be made to perform all EOS assessments, if possible (Appendix 5). Other procedures may be performed at the

investigator's (or designee's) and/or sponsor's discretion. If the subject is in-house, these procedures should be performed before the subject is discharged from the clinic. The investigator (or designee) may also request that the subject return for an additional follow-up visit. All withdrawn subjects will be followed until resolution of all their AEs or until the unresolved AEs are judged by the investigator (or designee) to have stabilized.

Subjects withdrawing from the study may be replaced at the discretion of the sponsor. Subjects enrolled in the Product Trial but who failed at check-in or dropped prior to randomization may be replaced without consultation of the sponsor. Subjects completing, withdrawing, or who are removed from this study cannot re-enter.

4.5. Study Termination

The study may be discontinued at the discretion of the investigator (or designee), sponsor, or sponsor's medical monitor if any of the following criteria are met:

- Adverse events unknown to date (ie, not previously reported in any similar study on the study products with respect to their nature, severity, and/or duration)
- Increased frequency, severity, and/or duration of known, anticipated, or previously reported AEs (this may also apply to AEs defined at check-in as baseline signs and symptoms)
- Medical or ethical reasons affecting the continued performance of the study
- Difficulties in the recruitment of subjects
- Other administrative reasons.

5. STUDY PRODUCTS

5.1. Description, Storage, Packaging, and Labeling

Study products will be supplied by the sponsor (or designee), along with the batch/lot numbers and certificates of analysis.

Details on study products can be found in Table 3.

Table 3: Study Products

Study Product	Product Code	Format	Product	Nicotine Yield/Content
1	B	Pouch	Mint on!® 2 mg nicotine pouches	2 mg/pouch
2	C	Pouch	Mint on!® 4 mg nicotine pouches	4 mg/pouch
3	D	Pouch	Mint on!® 8 mg nicotine pouches	8 mg/pouch

The study products will be packaged in the original commercial packs in sealed pre-packaging. Each product pack contains 20 pouches. To open the packages, the sealed pre-packaging will have to be broken and the lid opened.

Study products will be stored at the study site in a location that is locked with restricted access.

The study product containers will be labeled in accordance with applicable laws and regulations.

5.2. Study Product Administration

5.2.1. Product Trial

At Day -3, subjects will engage in a brief Product Trial using 1 Mint on!® 8 mg NP for 10 minutes to allow them to become accustomed to the product and to confirm their ability to tolerate the study product. Subjects will be instructed to place the pouch between the upper lip and gum, on either side of the mouth, per subject's choice. Subjects will be informed that these products are spit-free.

5.2.2. Product Use from Day 1

Subjects will begin using the assigned study products or completely stop using tobacco products on the morning of Day 1 and continue through Day 7 according to the randomization:

- Subjects in Group 1 will be allowed to smoke their UBCs ad libitum (ie, no restriction on the duration of use or the number of CPD) from 07:00 through 23:00. Subjects will be allowed to smoke upon request to the clinic staff but will only be allowed 1 cigarette at a time.
- Subjects in Groups 2, 3, and 4 will use the assigned NPs ad libitum (ie, no restrictions on the NPPU, the NPPD, or the duration of use) except for 3 specific NP use opportunities at approximately 11:00, 15:00, and 19:00 each day during which subjects will be asked to keep the assigned NP in their mouth for at least 10 minutes. Subjects will be allowed to use the NPs upon request.
- Subjects in Group 5 will not be allowed to smoke or use NPs.

Study product use will not be permitted from 23:00 to 07:00 each day during the study from check-in (Day -3) until Day 7. Study products and cigarettes will not be allowed on Day 8 (discharge day).

5.3. Randomization

On Day 1, the subjects will be randomized to 1 of the following 5 groups in a 1:1:1:1:1 ratio, with not more than 60% of either gender. Subjects will be stratified by gender and screening visit self-reported CPD (≤16; >16).

Table 4: Group Allocation

Group number	Intervention	Number of subjects
Group 1	Continue Smoking (CC)	30
Group 2	2 mg NP (NP2)	30
Group 3	4 mg NP (NP4)	30
Group 4	8 mg NP (NP8)	30
Group 5	No Tobacco (NT)	30

5.4. Blinding

This is an open-label study.

5.5. Study Product Compliance

For study integrity, all smoked cigarette butts and all used NPs will be collected throughout the study (from Day -3 to Day 7).

The following measures will be employed to ensure study product use compliance:

- Subjects from Group 1 will request a cigarette from the clinic staff, each time they want to smoke a cigarette. They will be instructed to return each cigarette butt upon completion. Only 1 cigarette will be dispensed for use at a time and subjects will be instructed to return the cigarette butt before being allowed to obtain another UBC
- Subjects from Groups 2, 3, and 4 will request NPs from the clinic staff, each time they want to use a NP. At approximately 11:00, 15:00, and 19:00, they will use their assigned NP in the presence of a clinical staff member. Subjects will be instructed to return each used NP upon completion.

5.6. Study Product Accountability, Storage, and Preparation

All study products will be provided by the sponsor, except UBCs. The study staff at the site will coordinate shipping of the study products from the sponsor. The study staff will document the date each shipment was received and record it in the inventory records. The study staff will document and reconcile the total number of products shipped to the site, the total number of study products used during the study, and the total number of unused study products remaining at the EOS. The site pharmacy will retain and store 2 cans/packages of each study product at the site until finalization of the final study report.

All subjects will be required to provide a sufficient supply of their UBC to the study site for use from check-in through Day 7 (10 days) in case they are randomized to continue smoking their UBC. This supply will be calculated from the number of CPD reported at screening plus an additional 20% rounded up to the next pack. For example, a subject reporting to smoke 15 CPD would bring 9 packs (15 CPD x 10 days = 150 cigarettes + an additional 30 cigarettes = 180 cigarettes total [9 packs]). The clinical site will purchase additional cigarettes if subjects run out of cigarettes during the study.

All study products will be stored in a locked, limited-access area at the study site and kept at controlled room temperature (defined as 15°C – 25°C [59°F – 77°F], with excursions permitted to 30°C [86°F]). A sufficient supply for each subject may be transferred and kept in a secure area in the clinic (eg, locked drawer or cupboard) each day as necessary, with appropriate documentation of transfer noted as above.

Any unused packs of cigarettes will be returned to the study subjects upon their completion or withdrawal from the study.

6. CONCOMITANT THERAPIES AND OTHER RESTRICTIONS

6.1. Concomitant Therapies

Any medications, and the reason for its use, taken from 30 days prior to check-in through check-in will be recorded as prior medications.

Any concomitant medications, and the reason for its use, taken from check-in through the EOS (or upon early termination) will be recorded as concomitant medication.

Prohibited medications are included in the exclusion criteria (Section 4.2).

Stable doses (ie, no dosage adjustments within 30 days prior to check-in) of prescription or over-the-counter medications required to treat an investigator-approved disease or condition are permitted at the discretion of the investigator. Hormonal contraceptives (eg, oral, vaginal ring, transdermal patch, implant, injection) and hormonal replacement therapy are permitted. Use of over-the-counter analgesics (eg, acetaminophen, ibuprofen), milk-of-magnesia, antihistamines, and nasal decongestants are permitted as needed to treat AEs experienced by subjects, at the discretion of the investigator. Note that some decongestants might cause a positive urine/saliva drug screen result and therefore their use should be discouraged within 5 to 7 days of those tests.

The administration of any other concomitant medications during the study is prohibited without prior approval of the investigator (or designee), unless its use is deemed necessary for the treatment of an AE.

6.2. Diet

While confined at the study site, subjects will receive a standardized diet at scheduled times that do not conflict with other study-related activities.

No foods or beverages containing alcohol will be allowed for 48 hours prior to screening, check-in, and during confinement at the study site.

Consumption of broiled or pan-fried meat, pre-cooked meats (eg, tuna, ham, corned beef, smoked lunchmeats), bacon, or sausage will not be allowed for 48 hours prior to check-in and during the confinement at the study site.

Caffeinated beverages (up to 1 cup per meal) may be served while subjects are confined at the study site.

Subjects will be fasted overnight (at least 8 hours) before collection of blood samples for clinical laboratory evaluations.

6.3. Smoking

Following Product Trial on Day -3, subjects will continue to smoke their UBC through 23:00 on Day -3 and from 07:00 to 23:00 on Days -2 and -1.

Subjects from Groups 2, 3, 4, and 5 will not be allowed to smoke from Day 1 to the EOS

Smoking will be limited to a designated area of the clinic. Subjects permitted to smoke (Group 1) will be housed separately from the subjects from Groups 2, 3, 4, and 5.

Any illicit use of any tobacco- or nicotine-containing products or sharing of study products will be strictly prohibited and will be grounds for immediate termination from the study at the discretion of the investigator.

6.4. Exercise

Strenuous exercise will be forbidden for 48 hours prior to check-in and while the subject is confined at the study site.

7. STUDY ASSESSMENTS AND PROCEDURES

Every effort will be made to schedule and perform the procedures as closely as possible to the nominal time, giving considerations to appropriate posture conditions, practical restrictions, and the other procedures to be performed at the same timepoint.

The highest priority procedures will be performed closest to the nominal time. The order of priority for scheduling procedures around a timepoint is (in descending order of priority):

- 24-hour urine sampling
- Vital signs
- ECG
- Physical examination including an oral exam

- Blood samples.

7.1. Harmful and Potentially Harmful Constituent Assessments

7.1.1. Carboxyhemoglobin Assessments

7.1.1.1. Blood Sample Collection and Processing

Blood samples will be collected by venipuncture or cannulation at the times indicated in the SoA in Appendix 5.

Procedures for collection, processing, and shipping of blood samples will be detailed in a separate document.

The maximum blood volume for the entire study, including samples taken for safety evaluation, will not exceed 450 mL.

7.1.1.2. Analytical Methodology

Blood concentrations of COHb will be determined using a validated analytical procedure. Specifics of the analytical method will be provided in a separate document.

7.1.2. Biomarkers of Exposure (other than COHb) Assessments

Biomarkers of exposure other than COHb (see list in Table 1 and Table 2) will be assessed in 24-hour urine.

7.1.2.1. Urine Sample Collection and Processing

A 24-hour urine collection will be performed at the times indicated in the SoA in Appendix 5.

All urine voids over a 24-hour period (24-hour urine) will be collected for BOE analysis. The 24-hour urine collection begins on each scheduled day after the first morning void and any void prior to 07:00, and finishes the following morning with the last void collected at approximately 07:00 (including first morning void).

Procedures for collection, processing, and shipping of urine samples will be detailed in a separate document.

7.1.2.2. Analytical Methodology

Urine concentrations of BOEs will be determined using validated analytical procedures. Specifics of the analytical methods will be provided in a separate document.

Urine creatinine will be measured in each 24-hour collection and may be used to adjust the concentration values of urine BOEs.

7.2. Product Use Behavior Assessments

The following parameters will be collected to assess product use behavior:

- Number of CPD: number of cigarettes smoked from 07:00 to 23:00, each day
- NPPD: number of NP used from 07:00 to 23:00, each day
- NPPU: number of NPs that could be used at once will not be limited
- Average duration of each NP use: time of NP placement in the subject's mouth and time it was removed from the subject's mouth.

7.3. Safety Assessments

7.3.1. Adverse Events

Adverse event definitions, assignment of severity and causality, and procedures for reporting SAEs are detailed in Appendix 1.

The condition of each subject will be monitored from the time of signing the ICF to final discharge from the study.

Subjects will be observed for any signs or symptoms and asked about their condition by open questioning, such as "How have you been feeling since you were last asked?", at least once each day while resident at the study site and at each study visit. Subjects will also be encouraged to spontaneously report AEs occurring at any other time during the study.

All nonserious AEs, whether reported by the subject voluntarily or upon questioning, or noted on physical examination, will be recorded from initiation of study product until study completion. Serious AEs will be recorded from the time the subject signs the ICF until study completion. The nature, time of onset, duration, and severity will be documented, together with an investigator's (or designee's) opinion of the relationship to the use of the study product.

Adverse events recorded during the course of the study will be followed up, where possible, to resolution or until the unresolved AEs are judged by the investigator (or designee) to have stabilized. This will be completed at the investigator's (or designee's) discretion.

7.3.2. Clinical Laboratory Evaluations

Blood and urine samples will be collected for clinical laboratory evaluations at the times indicated in the SoA in Appendix 5. Clinical laboratory evaluations are listed in Appendix 2.

Subjects will be asked to provide urine samples for drugs of abuse screen and cotinine test and will undergo an alcohol breath test at the times indicated in the SoA in Appendix 5. For female subjects, a pregnancy test will be performed at the times indicated in the SoA in Appendix 5.

All clinical laboratory tests will be conducted by a laboratory accredited by Centers for Medicare and Medicaid Services (Clinical Laboratory Improvement Amendments [CLIA] of 1988) or at the clinical study site using CLIA-waived kits or procedures.

An investigator (or designee) will perform a clinical assessment of all clinical laboratory data.

Values for the laboratory parameters are to be within the laboratory normal ranges or deemed not clinically significant in the opinion of the investigator (or designee) or otherwise meet the specified values (ranges) in the protocol. One recheck may be performed at the investigator's discretion for all clinical laboratory tests except for the urine/saliva drug screen, urine/breath alcohol screen, and urine cotinine test.

7.3.3. Vital Signs

Seated blood pressure, seated pulse rate, and oral body temperature will be assessed at the times indicated in the SoA in Appendix 5. Vital signs may also be performed at other times if judged to be clinically appropriate.

All measurements will be performed singly and repeated once if outside the relevant clinical reference range.

Subjects must be seated for at least 5 minutes before blood pressure and pulse rate measurements.

Product use is to be stopped 15 minutes prior to vital sign measurement.

7.3.4. 12-Lead Electrocardiogram

Resting 12-lead ECGs will be recorded after the subject has been supine and at rest for at least 5 minutes at the times indicated in the SoA in Appendix 5. Single 12-lead ECGs will be repeated twice, and an average taken of the 3 readings, if either of the following criteria apply:

- QT interval corrected for heart rate using Fridericia's method (QTcF) is >500 ms
- QTcF change from the baseline (pre study product exposure) is >60 ms.

Additional 12-lead ECGs may be performed at other times if judged to be clinically appropriate. The investigator (or designee) will perform a clinical assessment of each 12-lead ECG.

7.3.5. Physical Examination

A full physical examination will be performed at screening. Symptom-directed physical examination will be performed at the other timepoints specified in the SoA (Appendix 5).

7.3.6. COVID-19 Screening

All subjects will be tested for COVID-19 at the times indicated in the SoA (Appendix 5). Details of the testing will be provided separately.

Throughout confinement, daily temperature will also be measured prior to any product use as part of the site's COVID-19 measures/mitigation and will be captured separately from safety vital signs assessment.

7.3.7. Tobacco Cessation Information

The investigator (or designee), at screening and at the EOS or upon early termination, will advise all adult tobacco product users that to reduce the health effects of tobacco, the best thing to do is to quit. The investigator (or designee) will refer all adult tobacco product users to the Quit Assist® website (using information cards, subject handouts, etc.), which contains citations to a number of third-party information sources, including websites, telephone resources, and other organizations with additional information.

8. DATA MANAGEMENT

Data management activities will be detailed in the Data Management Plan (DMP). All data for this study will be captured in the Medrio system, supplied by ALCS or designee. Medrio is 21 Code of Federal Regulations (CFR) Part 11 compliant. Electronic case report forms (eCRF) will be developed according to the study protocol specifications and will follow ALCS data standards. Analytical data will be collected externally to the database.

Data will be captured on paper source and will be entered into the electronic data capture (EDC) system by the site. All data captured will have an audit trail.

Programmed edits checks will be used to ensure the accuracy and integrity of the database. Edit checks will be programmed within the system to check for errors and discrepancies, such as missing data, data inconsistencies, and inappropriate date ranges. Corrections will be made by the site as necessary prior to database lock. Database lock will occur after all reviews are completed, all queries are resolved, and there are no outstanding issues. Any changes to the data following database lock will be documented and approved by the sponsor prior to unlocking the database to make the required changes.

Adverse events will be coded using the most current version of the Medical Dictionary for Regulatory Activities (MedDRA). Concomitant medications will be coded using the most current version of World Health Organization Drug Dictionary. The versions will remain the same throughout the study. Coding will be completed by ALCS Data Management and will be reviewed by the medical monitor at ALCS and Covance.

All casebooks (eCRFs) will be signed by the investigator prior to database lock. Submission casebooks will be extracted after database lock and provided to the site.

9. MONITORING OF THE STUDY

The responsible study monitor will contact and visit the investigator as necessary, and he/she will be allowed, upon request, to inspect and verify all records of the study (eg, source document, ICFs, eCRFs, regulatory documents) in a manner consistent with good clinical practice (GCP) and all other applicable state and federal law.

It will be the study monitor’s responsibility to inspect the source documents to verify the adherence to the protocol and the completeness, consistency, and accuracy of the data being entered in the eCRF. The monitor will verify that each subject has consented in writing prior to any study procedures being performed. Where the terms of the ICF, GCP, and all other

applicable state and federal law permit, the monitor should have access to laboratory test reports and other subject records needed to verify the entries on the eCRF. The investigator (or designee) agrees to cooperate with the monitor to ensure that any issues detected in the course of these monitoring visits are resolved.

In addition, the sponsor's internal auditors (or designee), institutional review board (IRB) reviewers, and government inspectors may evaluate the study and must be allowed access to eCRFs, source documents, and other study files.

The investigator must notify the sponsor (or designee) promptly of any inspections of the study or activities related to the study conducted by regulatory authorities, allow the sponsor (or designee) to be present during the inspection, and promptly forward copies of inspection reports to the sponsor (or designee).

10. SAMPLE SIZE AND DATA ANALYSIS

Full details of the statistical analysis methods for the study will be specified in the Statistical Analysis Plan.

10.1. Determination of Sample Size

Up to 150 subjects will be enrolled in order that 20 subjects per group complete the study.

This study is being conducted to assess the differences in BOE values after adult smokers switched to on!® NP with the total NNAL (ng/24 h, measured at Day 7) as the primary endpoint. The sample size estimation is based on total NNAL data, assuming a two-sided test, 85% power and α =0.017 Type I error rate to account for the multiplicity adjustment for the comparisons (each of the 3 NP groups versus CC group), a sample size of approximately 20 subjects per group is needed to detect a statistically significant difference between the NP groups and CC group. Thirty subjects will be randomized to make sure 20 subjects per group complete the study. The sample size calculation used to derive the sample size for this study was based on summary statistics data of total NNAL from a previous study with a similar study design,[9] where the mean ± standard deviation of total NNAL at Day 7 were 476.1 ± 296.58 ng/24 h for the CC group and 167.3 ± 100.17 ng/24 h and 176.9 ± 135.17 ng/24 h for the groups that used a chewable OTDN product with 2 flavors, respectively.

10.2. Analysis Populations

10.2.1. Biomarker of Exposure Population

The BOE population will include all subjects from Groups 1, 2, 3, and 4 who used at least 1 of the assigned study products and all Group 5 subjects. To be included in the BOE population, subjects must have baseline (Day 1) and at least 1 postbaseline evaluable BOE data.

10.2.2. Safety Population

The safety population will include all subjects from Groups 1, 2, 3, and 4 who used at least 1 of the assigned study product and Group 5 subjects.

10.3. Biomarker of Exposure Analyses

Urinary biomarkers:

Linear mixed models for analysis of covariance will be used to compare the Day 7 biomarker values between groups as described in the study objectives. In the statistical models, the outcome variable will be included as a dependent variable; group and gender will be included as fixed effects; and baseline values of corresponding biomarker will be included as covariates. For the NP groups compared to the CC group, Dunnett's method will be used for the adjustment of multiple comparisons. The SAS procedure Proc Mixed will be used. The least-squares means (LSM) difference and 95% confidence interval for the LSM difference between the test and reference groups and p-values will be provided.

10.4. Product Use Behavior

The number of each product used per day (cigarettes or NPs), NPPU, and the duration of each NP used during each product use period will be listed and summarized by study product using descriptive statistics, as appropriate.

10.5. Safety Analysis

All AEs will be listed and summarized using descriptive methodology. Each AE will be coded using MedDRA. Frequency counts of AEs will be provided by body system, preferred term, and study product. Frequency counts of AEs will also be summarized by severity and relationship to study product.

Observed values for clinical laboratory test data, 12-lead ECGs, vital signs, and physical examination findings will be listed.

10.6. Interim Analysis

No interim analyses are planned for this study.

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12. APPENDICES

Appendix 1: Adverse Event Reporting

Definitions

The following is the definition for an **AE**:

Any unfavorable and unintended sign (including an abnormal laboratory finding[10]), symptom, or disease[10] temporally associated with the use of a study product, **whether or not** related to the study product.[10, 11]

All AEs occurring during this study after the subject has signed the ICF and after the first use of the study product during the Product Trial and through Day 8 or EOS/Early Termination must be recorded in the eCRF, including the date and time of onset and outcome of each event. Events occurring between signing of the ICF and prior to the first use of study product during the Product Trial will be documented as medical history.

Surgical procedures themselves are not AEs; they are therapeutic measures for conditions that require surgery. The condition for which the surgery is required may be an AE. Planned surgery permitted by the clinical study protocol and the condition(s) leading to this surgery are not AEs.

No causal relationship with the study products or with the clinical study itself is implied by the use of the term "adverse event."

Assessment of Severity

The investigator (or designee) will review each event and rate each reported sign or symptom on a 3-point severity scale. The following definitions for **rating severity**[10] will be used:

- Mild: The AE is easily tolerated and does not interfere with daily activity
- Moderate: The AE interferes with daily activity, but the subject is still able to function
- Severe: The AE is incapacitating and requires medical intervention. *Note: This is not the same as "serious," which is based on the outcome or action criteria usually associated with events that pose a threat to life or functioning.*

Relationship to Study Product

Each AE will also be assessed by the investigator (or designee) for **relationship to study product (causality)** using the following grades of certainty[12, 13] (the strength of a causal association may be revised as more information becomes available):

Not related: Clearly and definitely due to extraneous cause (eg, disease, environment)

Unlikely:

a. Does not follow a probable temporal (ie, time) sequence from the use of study product.

b. Does not follow a known pattern of response to the study product.

c. Could plausibly have been produced by the subject's clinical state/underlying disease or other drugs or chemicals the subject received.

d. Does not reappear or worsen when the study product is re-administered.

Possible:

a. Follows a reasonable temporal (ie, time) sequence from the use of study product.

b. Follows a known pattern of response to the study product.

c. Could also have been produced by the subject's clinical state/concurrent disease or other drugs or chemicals the subject received.

Likely:

a. Follows a reasonable temporal (ie, time) sequence from the use of study product.

b. Follows a known pattern of response to the study product.

c. Could not readily have been produced by the subject's clinical state/concurrent disease or other drugs or chemicals.

d. Follows a clinically reasonable response on withdrawal (dechallenge), ie, disappears or decreases when the study product is stopped or reduced.

e. Rechallenge information is **not** required to fulfill this definition.

Definitely:

a. Follows a reasonable temporal (ie, time) sequence from the use of study product.

b. Follows a known pattern of response to the study product.

c. Cannot be explained by the subject's clinical state/concurrent disease or other drugs or chemicals.

d. Follows a clinically reasonable response on withdrawal (dechallenge), ie, disappears or decreases when the study product is stopped or reduced.

e. Recurs with re-exposure to study product (rechallenge). *NOTE: Re-exposure of the subject is NOT required, but the "definitely related" category may only be used when recurrence is observed.*

Follow-up of Adverse Events

Every reasonable effort will be made to follow up with subjects who have ongoing AEs at the EOS visit. Any subject who has an ongoing AE that is possibly related or related to the study product or study procedures at the EOS visit will be followed up, where possible, until resolution or until the unresolved AE is judged by the investigator (or designee) to have stabilized. This will be completed at the investigator's (or designee's) discretion. Any subject who has an ongoing AE that is not related or unlikely related to the study product or study procedures at the EOS visit can be closed out as ongoing at the investigator's discretion.

Serious Adverse Events

An SAE is defined as any untoward medical occurrence that either:

- Results in death
- Is life-threatening
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity (disability is defined as a substantial disruption of a person's ability to conduct normal life functions)
- Results in a congenital anomaly/birth defect
- Results in an important medical event (see below).

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered SAEs when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

Instances of death or congenital abnormality, if brought to the attention of the investigator at any time after cessation of the study product and considered by the investigator to be possibly related to the study product, will be reported to the sponsor.

Definition of Life-threatening

An AE is life-threatening if the subject was at immediate risk of death from the event as it occurred (ie, does not include a reaction that might have caused death if it had occurred in a more serious form). For instance, drug-induced hepatitis that resolved without evidence of hepatic failure would not be considered life-threatening even though drug-induced hepatitis can be fatal.

Definition of Hospitalization

Adverse events requiring hospitalization should be considered serious. In general, hospitalization signifies that the subject has been detained (usually involving an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate at the study site. When in doubt as to whether hospitalization occurred or was necessary, the AE should be considered as serious.

Hospitalization for elective surgery or routine clinical procedures, which are not the result of an AE, need not be considered AEs and should be recorded on a clinical assessment form and added to the eCRF. If anything untoward is reported during the procedure, this must be reported as an AE and either 'serious' or 'nonserious' attributed according to the usual criteria.

Serious Adverse Event Reporting

AEs that are associated with the use of the study product and are serious and unexpected will be reported by the study site to the sponsor, medical monitor assigned by the sponsor, and the responsible IRB.

The sponsor and medical monitor will be notified in writing (eg, facsimile) within 24 hours of when a serious, unexpected AE that is associated with use of the study product associated is first recognized or reported.

Subsequently, a written confirmation or summary of the AE (using FDA Form 3500A or equivalent) will be sent to the sponsor within 3 working days of the original notification.

The IRB will be notified of any serious, unexpected AE that is associated with the use of the study product in accordance with the IRB's procedures.

Pregnancy

A positive pregnancy test prior to enrollment will be documented as a screen failure. Pregnancy occurring in a female study subject (after check-in through EOS/Early Termination) will be documented in a pregnancy form (provided separately) and as a protocol deviation to the IRB.

Pregnancy itself is not an AE. The investigator or designee will discontinue the pregnant subject from the study and will advise her to seek prenatal care and counseling from her primary care provider. The investigator (or designee) will refer her to the Quit Assist® website, which contains citations to a number of third-party information sources, including websites, telephone resources, and other organizations with additional information. Advice given will be documented in the subject's source document.

All pregnancies must be reported by telephone and by fax or email to the sponsor and the medical monitor within 24 hours of the site's learning of the pregnancy or, at the latest, on the following workday.

The study site staff will request the pregnant subject to notify the site of the outcome of the pregnancy (ie, birth, loss, or termination). To help ensure this, the study site staff will follow up with the subject until the end of pregnancy, if in compliance with the site's standard operating procedures and with the subject's consent. This request and the subject's response will be documented in the subject's source document. A final report of pregnancy outcome will be sent to the medical monitor.

Appendix 2: Clinical Laboratory Evaluations

Clinical chemistry:	Hematology:	Urinalysis:
Alanine aminotransferase Albumin Alkaline phosphatase Aspartate aminotransferase Bicarbonate Blood urea nitrogen Chloride Creatinine[c] Glucose Potassium Sodium Total bilirubin[a] Total protein Uric acid	Hematocrit Hemoglobin Platelet count Red blood cell (RBC) count White blood cell (WBC) count WBC differential: Basophils Eosinophils Lymphocytes Monocytes Neutrophils	Bilirubin Blood Color and appearance Glucose Ketones Leukocyte esterase Nitrite pH Protein Specific gravity Urobilinogen Microscopic examination[d] (RBCs, WBCs, casts, and bacteria)
Serology:	**Drug screen:**	**Hormone panel - females only:**
Hepatitis B surface antigen Hepatitis C antibody Human immunodeficiency virus (HIV-1 and HIV-2) antibodies	Including but not limited to: Amphetamines/methamphetamines Cocaine (metabolite) Opiates Tetrahydrocannabinol/cannabinoids Alcohol Cotinine test[e]	Follicle-stimulating hormone[f] (postmenopausal females only) Serum pregnancy test[g] (human chorionic gonadotropin) Urine pregnancy test[b]

[a] Direct and indirect bilirubin will be analyzed if total bilirubin is elevated.
[b] Performed for all females at check-in. A positive urine pregnancy test will be confirmed with a serum pregnancy test.
[c] At screening, estimated glomerular filtration rate will be calculated.
[d] Microscopic examination will be conducted if protein, leukocyte esterase, nitrite, and/or blood are abnormal.
[e] A positive qualitative test (≥ 500 ng/mL) will be required for participation in the study.
[f] To confirm postmenopausal status.
[g] Performed for all females at screening.

In addition, COVID-19 testing will be performed at screening and check-in.

Appendix 3: Contraception Guidance

Definitions

Women of Childbearing Potential: premenopausal females who are anatomically and physiologically capable of becoming pregnant following menarche.

Women of Nonchildbearing Potential:

1. **Surgically sterile:** females who are permanently sterile via hysterectomy, bilateral salpingectomy, and/or bilateral oophorectomy by reported medical history and/or medical records. Surgical sterilization to have occurred a minimum of 6 months, or at the investigator's discretion, prior to screening.
2. **Postmenopausal:** females at least 45 years of age with amenorrhea for 12 months without an alternative medical reason with confirmatory FSH levels of ≥40 mIU/mL. The amenorrhea should not be induced by a medical condition such as anorexia nervosa, hypothyroid disease, or polycystic ovarian disease or by extreme exercise. It should not be due to concomitant medications that may have induced the amenorrhea such as oral contraceptives, hormones, gonadotropin-releasing hormones, anti-estrogens, or selective estrogen receptor modulators.

Contraception Guidance

Female Subjects

Female subjects who are heterosexually active and of childbearing potential (eg, not surgically sterile at least 6 months prior to check-in nor postmenopausal with amenorrhea for at least 12 months prior to check-in and FSH levels consistent with postmenopausal status) must have been using one of the following forms of contraception and agree to continue using it through completion of the study:

- Hormonal (eg, oral, vaginal ring, transdermal patch, implant, injection) consistently for at least 3 months prior to check-in
- Double barrier (eg, condom with spermicide or diaphragm with spermicide) consistently for at least 4 weeks prior to check-in
- Intrauterine device for at least 4 months prior to check-in
- Exclusive partner who has been vasectomized for at least 6 months (inclusive) prior to check-in.

Female subjects of childbearing potential who are not currently engaging in heterosexual intercourse must agree to use one of the above methods of birth control through completion of study, in the event that they have heterosexual intercourse during the course of the study.

Appendix 4: Regulatory, Ethical, and Study Oversight Considerations

Regulatory and Ethical Considerations

This study will be conducted in accordance with the protocol and with the following:

- Consensus ethical principles derived from international guidelines including the Declaration of Helsinki and Council for International Organizations of Medical Sciences International Ethical Guidelines.
- Applicable International Conference on Harmonization (ICH) GCP Guidelines.
- Applicable laws and regulations.

The protocol, protocol amendments, ICF, and other relevant documents must be submitted to an IRB by the investigator and reviewed and approved by the IRB before the study is initiated.

Any amendments to the protocol will require IRB approval before implementation of changes made to the study design, except for changes necessary to eliminate an immediate hazard to study subjects.

The investigator will be responsible for the following:

- Providing written summaries of the status of the study to the IRB annually or more frequently in accordance with the requirements, policies, and procedures established by the IRB.
- Notifying the IRB of SAEs or other significant safety findings in accordance with the IRB's procedures.
- Providing oversight of the conduct of the study at the site and adherence to requirements of ICH guidelines and all other applicable regulations.

Finances and Insurance

Financing and insurance will be addressed in a separate agreement.

Informed Consent

Prior to starting participation in the study, each subject will be provided with a study-specific ICF giving details of the study products, procedures, and potential risks of the study. Subjects will be instructed that they are free to obtain further information from the investigator (or designee) and that their participation is voluntary and they are free to withdraw from the study at any time. Subjects will be given an opportunity to ask questions about the study prior to providing consent for participation.

Following discussion of the study with study site personnel, subjects will sign the ICF in the presence of a suitably trained member of staff to indicate that they are freely giving their informed consent. A copy of the ICF will be given to the subjects.

If the ICF is amended at any time after subjects have started participating in the study, those subjects must be re-consented to the most current version of the ICF(s) during their participation in the study.

Subject Data Protection

Subjects will be assigned a unique identifier and will not be identified by name in eCRFs, study-related forms, study reports, or any related publications. Subject and investigator personal data will be treated in compliance with all applicable laws and regulations. In the event the study protocol, study report, or study data are included in a public registry, all identifiable information from individual subjects or investigators will be redacted according to applicable laws and regulations.

The subject must be informed that his/her personal study-related data will be used by the sponsor in accordance with local data protection law. The level of disclosure must also be explained to the subject. The subject must also be informed that his/her study-related data may be examined by sponsor or contract research organization (CRO) auditors or other authorized personnel appointed by the sponsor, by appropriate IRB members, and by inspectors from regulatory authorities.

Disclosure

All information provided regarding the study, as well as all information collected and/or documented during the course of the study, will be regarded as confidential. The investigator (or designee) agrees not to disclose such information in any way without prior written permission from the sponsor.

Data Quality Assurance

The following data quality steps will be implemented:

- All relevant subject data relating to the study will be recorded on eCRFs unless directly transmitted to the sponsor or designee electronically (eg, laboratory data). The investigator is responsible for verifying that data entries are accurate and correct by electronically signing the eCRF.
- The investigator must maintain accurate documentation (source data) that supports the information entered in the eCRF.
- The investigator must permit study-related monitoring, audits, IRB review, and regulatory agency inspections and provide direct access to source data documents.
- The sponsor or designee is responsible for the data management of this study including quality checking of the data. Predefined agreed risks, monitoring thresholds, quality tolerance thresholds, controls, and mitigation plans will be documented in a risk management register. Additional details of quality checking to be performed on the data may be included in a DMP.
- A study monitor will perform ongoing source data verification to confirm that data entered into the eCRF by authorized site personnel are accurate, complete, and

verifiable from source documents; that the safety and rights of subjects are being protected; and that the study is being conducted in accordance with the currently approved protocol and any other study agreements, ICH GCP, and all applicable regulatory requirements.

- Records and documents, including signed ICFs, pertaining to the conduct of this study must be retained by the investigator for a minimum of 20 years after the completion or termination of the study. No records may be destroyed during the retention period without the written approval of the sponsor. No records may be transferred to another location or party without written notification to the sponsor.

Investigator Documentation Responsibilities

All individual, subject-specific study data will also be entered into a 21 CFR Part 11-compliant EDC system on an eCRF in a timely fashion.

All data generated from external sources (eg, laboratory and bioanalytical data), and transmitted to the sponsor or designee electronically, will be integrated with the subject's eCRF data in accordance with the DMP.

An eCRF must be completed for each enrolled subject who undergoes any screening procedures, according to the eCRF completion instructions. The sponsor, or CRO, will review the supporting source documentation against the data entered into the eCRFs to verify the accuracy of the electronic data. The investigator will ensure that corrections are made to the eCRFs and that data queries are resolved in a timely fashion by the study staff.

The investigator will sign and date the eCRF via the EDC system's electronic signature procedure. These signatures will indicate that the investigator reviewed and approved the data on the eCRF, data queries, and site notifications.

Appendix 5: Schedule of Assessments

Study Procedures	**Screening**	**Days -3 to -1[f]**	**Days 1 (Baseline) to 7**	**EOS (Day 8) or Early Termination**
Informed consent	X			
Inclusion/exclusion criteria	X	X (Day -3)		
Demographic data	X			
Medical history	X	X (Day -3)[a]		
Tobacco use history[b]	X			
Fagerström Test for Cigarette Dependence (Appendix 6)			X (Day 1, only)	
Urinary drug screen	X	X (Day -3)		
Cotinine test	X			
Alcohol breath test	X	X (Day -3)		
Serology[c]	X			
Pregnancy test[d]	X	X (Day -3)		
FSH[e]	X			
Height, body weight, and BMI	X			
COVID-19 test	X	X (Day -3)		
Study residency:				
Check-in		X (Day -3)		
Check-out				X (after last Day 7 urine collection)
Study product administration[g]:				
Product trial		X[h] (Day -3, only)		
Randomization[i]			Day 1	
2 mg NP (Group 2)			X[j] (ad libitum[k])	
4 mg NP (Group 3)			X[j] (ad libitum[k])	
8 mg NP (Group 4)			X[j] (ad libitum[k])	
UBC (Group 1)			X[l] (ad libitum[m])	
No Tobacco (Group 5)			X	
Pharmacokinetics:				
Blood sampling[n]		X (Day -1, only)	X (Day 7, only)	

Study Procedures	Screening	Days -3 to -1[f]	Days 1 (Baseline) to 7	EOS (Day 8) or Early Termination
24-hour urine sampling[o]		X (Day -1, only)	X (Day 7, only)	
Urine creatinine[p]		X (Day -1, only)	X (Day 7, only)	
Safety:				
Adverse event recording		X	Ongoing	X
Prior/concomitant medication monitoring	X	X	Ongoing	X
Clinical laboratory evaluations[q]	X			
Vital signs[r]	X	X	X	X
12-lead ECG	X			
Physical examination[s]	X	X (Day -3)		X

Abbreviations: BMI = body mass index; BOE = biomarker of exposure; COHb = carboxyhemoglobin; COVID = coronavirus disease; CPD = cigarettes smoked per day; ECG = electrocardiogram; EOS = end of the study; FSH = follicle-stimulating hormone; HBsAg = hepatitis B surface antigen; HCV = hepatitis C virus; HIV= human immunodeficiency virus; NP = nicotine pouch; UBC = usual brand cigarettes.

[a] Interim medical history.

[b] The following characteristics of the subject's usual brand will be documented: brand, brand style, and flavor. The number of uses per day (single number, not a range) will also be documented. Subjects will bring with them a new pack of their most commonly used cigarettes. The pack will be color photocopied and the copy will be placed in the source documents at screening and at any time during the study in which the subject changes cigarette brand.

[c] HIV, HBsAg, HCV tests.

[d] Serum pregnancy test for all females at screening; urine pregnancy test for all females at check-in. A positive urine pregnancy test will be confirmed with a serum pregnancy test.

[e] To confirm postmenopausal status in self-reported postmenopausal females only.

[f] Following Product Trial on Day -3, subjects will continue to smoke their UBCs through 23:00 on Day -3 and from 07:00 to 23:00 on Days -2 and -1.

[g] Product use will not be permitted from 23:00 to 07:00 each day during the study from check-in (Day -3) until Day 7. Product use and cigarette smoking will not be allowed at Day 8 (EOS).

[h] Subjects will engage in a brief Product Trial using one 8 mg NP for 10 minutes to allow subjects to become accustomed to the products.

[i] Subjects will be randomized into each group on Day 1 based on gender and CPD.

[j] Subjects will be allowed to use the NPs upon request and they will be instructed to return each used NP upon completion.

[k] No restrictions on the number of NP used at once, the number of NP used per day, or the duration of use except for 3 specific NP use opportunities at approximately 11:00, 15:00, and 19:00 each day during which subjects will be asked to keep the assigned NP in their mouth for at least 10 minutes.

[l] Subjects will be allowed to smoke upon request to the clinic staff but will only be allowed 1 cigarette at a time and will be instructed to return each cigarette butt upon completion.

[m] No restriction on the duration of use or the number of CPD.

[n] At approximately 21:30. The sample will be used for COHb analysis, and banked for biomarker of potential harm analysis.

[o] All urine voids over a 24-hour period (24-hour urine) will be collected for BOE analysis. The 24-hour urine collection begins on each scheduled day after the first morning void and any void prior to 07:00, and finishes the following morning with the last void collected at approximately 07:00 (including first morning void).

[p] Urine creatinine will be measured in each 24-hour collection and may be used to adjust the concentration values of urine BOEs.

[q] Hematology, clinical chemistry, routine urinalysis (see Appendix 2) at screening.

[r] Blood pressure, pulse rate, oral temperature. All vital signs assessed at screening, Day -3 and EOS. From Day -2 to Day 7: daily oral temperature only.
[s] Full physical examination at screening; symptom-directed physical examinations at other timepoints.

Appendix 6: Fagerström Test for Cigarette Dependence[14] Questionnaire

1. How soon after you wake up do you smoke your first cigarette?
   - ☐ Within 5 minutes (3)
   - ☐ 6-30 minutes (2)
   - ☐ 31-60 minutes (1)
   - ☐ After 60 minutes (0)

2. Do you find it difficult to refrain from smoking in places where it is forbidden?
   - ☐ Yes (1)
   - ☐ No (0)

3. Which cigarette would you hate most to give up?
   - ☐ The first one in the morning (1)
   - ☐ All others (0)

4. How many cigarettes/day do you smoke?
   - ☐ 10 or less (0)
   - ☐ 11-20 (1)
   - ☐ 21-30 (2)
   - ☐ 31 or more (3)

5. Do you smoke more frequently during the first hours after waking than during the rest of the day?
   - ☐ Yes (1)
   - ☐ No (0)

6. Do you smoke if you are so ill that you are in bed most of the day?
   - ☐ Yes (1)
   - ☐ No (0)