Document Coversheet

Study Title:

A randomized, parallel-group open-label trial of ENDS users switching from flavors of potentially high-toxicity profile to flavors of potentially low-toxicity profile (CRoFT_3.2)

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A Randomized, Parallel-group Open-label Trial of ENDS Users Switching from Flavors of Potentially High-toxicity Profile to Flavors of Potentially Low-toxicity Profile (CRoFT_3.2)

PROTOCOL NUMBER:

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PRINCIPAL INVESTIGATOR:

Maciej Goniewicz. PhD, PharmD
Roswell Park Comprehensive Cancer Center
Elm and Carlton Street
Buffalo, New York 14263
716-845-8541
Maciej.Goniewicz@Roswellpark.org

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1 OBJECTIVES

There is a critical knowledge gap in the potential impact of flavored electronic nicotine delivery systems (ENDS) aerosols on pulmonary inflammation and respiratory health in ENDS users. Recently, New York State prohibited the sale of flavored e-cigarettes which took effect mid May 2020. Shortly thereafter, a newly emerging class of 'tobacco free' nicotine pouches began to gain popularity in the United Sates. This study will determine for the first time if a transition from NYS banned flavorings to non-prohibited flavors, such as tobacco, and/or use of 'tobacco free' nicotine pouches can improve to the respiratory health in established ENDS users.

We will conduct a randomized, parallel-group open-label trial to evaluate respiratory symptoms in ENDS users switching from banned flavors to a non-banned flavor (tobacco) or 'tobacco free' nicotine pouches. Participants will be randomized into 1 of 3 Arms: (Arm1) subjects will be asked to continue using their usual flavor (Arm2) subjects will be asked to switch to tobacco flavor (Arm3) subjects in this arm are asked to refrain from using their ENDS and switch to using 'tobacco free' nicotine pouches.

Recent data obtained from an ongoing observational study demonstrates that out of a cohort of 126 study subjects, about 80% use flavored e-cigarettes/e-liquid and there was no change in use patterns after the ban went into effect. From monitoring the study subjects we have learned that they are able to obtain the flavored products from places that the policy does not cover including Native Territories, out of state locations (Pennsylvania), and online stores.

1.1 Primary Objectives

- To evaluate respiratory symptoms in 1) ENDS users switching from banned flavors of potentially high-toxicity profile to non-banned flavors of potentially low-toxicity profile and,
- To evaluate respiratory symptoms in ENDS users who stop vaping and start using 'tobacco free' nicotine pouches.

1.2 Secondary Objectives

- To determine the compliance of ENDS users ability to switch from using banned flavors to either non-banned flavors and/or 'tobacco free' nicotine pouches.
- Evaluate the amount, type, and frequency of flavored product use.

2 BACKGROUND

Capitalizing on the 2009 US Food and Drug Administration (FDA) ban on flavorings in cigarettes (with the exception of menthol), manufacturers' inclusion of a variety of flavorings in emerging tobacco products is considered a significant selling point that tends to draw cigarette smokers towards these products. Many of the flavorings chemicals used in tobacco products are included on the U.S. Food and Drug Administration's (FDA) Generally Recognized as Safe (GRAS) list, which is comprised of additives shown to be "generally recognized as safe" under conditions of intended use; however this designation applies to consumption use, and the GRAS criteria do not include an examination of inhalation risks. Additionally, a number of flavorings have not been tested adequately for safety when heated and inhaled in aerosolized form. At present, there are no restrictions on the composition of flavors in tobacco products, and no regulatory program to assess

the toxicity and health hazards of the flavorings in tobacco products. There is no proposed regulation of the composition of flavorings based on respiratory health effects, in part due to the lack of available research on the toxic and adverse health effects of these flavorings.

Given the uncertainty surrounding adverse respiratory health effects from long-term inhalation of flavoring ingredients, data are urgently needed to clearly define the potential respiratory health effects of flavored aerosols.

The overarching theme of the WNY-based **Center for Research on Flavored Tobacco (CRoFT)** is the effects of flavors in tobacco, with an end goal of developing a scientific framework for evaluating the potential effects of flavors on tobacco product users. Common flavorings used in tobacco products have potential respiratory effects and have been shown to further enhance inhalation toxicity of tobacco smoke. The most familiar and intensely-studied flavoring in tobacco products is menthol, a biologically active compound that has effects by itself and in conjunction with nicotine to enhance cytotoxicity of cigarettes (1). Concerns have been raised about the potential respiratory health risks of ENDS use, including effects of inhaled flavorings. For example, high levels of diacetyl were detected in ENDS products, where it is likely added to impart a buttery or creamy taste (4,5). The National Institute for Occupational Safety and Health (NIOSH), after investigating microwave popcorn and flavoring production facilities, has suggested that high diacetyl exposures may contribute to or cause severe respiratory disorders, including *bronchiolitis obliterans* (sometimes referred to as constrictive bronchiolitis or "popcorn lung") (6). The other flavor in ENDS of potential concern is cinnamon. Behar et al. tested cytotoxicity of cinnamon-flavored ENDS and found that cytotoxic effects correlated with the amount of cinnamaldehyde in the products (7). Our group has studied benzaldehyde, a key ingredient in natural fruit-flavored products, which has been shown to cause irritation of respiratory airways in occupational exposure studies. Of 145 flavored ENDS products, we found that the highest levels of benzaldehyde in cherry-flavored products (8). Sousssy et al. reported high levels of furfural and 5-hydroxyfurfural in sweet-flavored ENDS liquids (9). Furfural has been shown to cause irritation to the upper respiratory tract in humans, and both detected compounds show tumorigenic activity in mice (10).

While ENDS seem to be a promising harm reduction tool for smokers of combusted tobacco, evidence citied above indicate that using these products could result in repeated inhalation of respiratory toxicants, irritants, and sensitizers. ENDS users often report respiratory symptoms include persistent cough, muco-purulent secretion from the respiratory tract, wheezing, and dyspnea/breathlessness. Findings of cytotoxicity in cinnamon-flavored refill fluids reported by Behar et al. (7) were consistent with reports made by ENDS users: cinnamon-flavored products have caused throat, mouth, and lung irritations and for some users, the irritation ceased after switching to a different flavor. Short-term use of flavored ENDS results in changes in respiratory function in healthy volunteers and *in vitro* studies have found that flavored ENDS aerosol induced release of cytokines and pro-inflammatory mediators in exposed lung epithelial cells (11). Human studies have similarly suggested that ENDS may impair immunity and increase peripheral airway resistance (12). Our group showed that unvaporized liquids were oxidative in a manner dependent on flavor additives, while sweet or fruit flavors were stronger oxidizers than tobacco flavors (13). Our supporting data are robust, as similar levels of inflammatory mediators were seen in smokers and to a lesser extent in ENDS users. McConnell et al. (14) analyzed the relationship between ENDS use and respiratory symptoms in the Southern California Children's Health Study and found that ENDS use was associated with increased odds of bronchitis symptoms that increased with

frequency of ENDS usage over the prior 30 days. These findings echo results from a cross-sectional study of Chinese adolescents, which reported an association between respiratory symptoms and use of flavored ENDS (15).

Despite the increasing popularity of ENDS little is known about users' preferences, selection and switching between various flavors. Farsalinos et al. (16) conducted an online survey of over 4,000 ENDS users and found that flavors, and particularly the variety of flavors, were an important factor in the maintenance of ENDS use by current and former smokers. The results also indicated that smokers tended to start with tobacco-flavored products, and then would switch to multiple flavors as they transitioned from dual use to complete (or almost complete) substitution of ENDS for their usual cigarettes. Berg (17) recruited 1,567 adults, aged 18–34 years, through Facebook ads targeting tobacco users and non-users. Among smokers and non-smokers, fruity ENDS flavors were the most preferred. These results are mirrored by the larger, nationally representative PATH study data, where nearly 60% of adult ENDS users reported a fruit flavor. For conventional tobacco cigarette, the substantially greater preference for menthol cigarettes that has been reported among African American smokers relative to smokers of European ancestry has given rise to speculation that menthol may in some way account for the elevated incidence and severity of some smoking-related diseases among African Americans (1). Some studies suggest that menthol may impact societal disease burdens by influencing the rates of smoking initiation and cessation (1).

In mid-May 2020, the state of New York prohibited the sale of flavored e-cigarettes. Since then, retailers are now only allowed to sell e-cigarette products that are tobacco, menthol, mint, wintergreen, or unflavored. According to the National Conference of State Legislatures (NCSL), five states have put laws into place that ban flavored e-cigarettes. These states include California, Massachusetts, New Jersey, New York, and Rhode Island. However, even with the ban in place restricting the sale of certain flavors, ENDS users have been still able to obtain their desired flavors by buying on the internet or purchasing the products on local Native Territories where the flavor ban does not take effect. ENDS users in Western New York Region are particularly able to obtain their flavors because of the two local Native Territories in close proximity as well as the Pennsylvania boarder close by. In addition, a new product category emerged as several large tobacco companies started selling 'tobacco free' nicotine pouches.

These products are typically sold as pouches with a white nicotine-containing powder and do not contain tobacco leaf. Users are meant to place the pouch between their lip and gum and spitting is not required (unlike traditional smokeless tobacco products). Oral nicotine pouches may have potential as reduced risk products because they are not combusted and do not contain tobacco leaf, and they seem to contain low levels of tobacco-specific nitrosamines, such as the oral carcinogen NNN (49).

Embedded within this health effects study, we will gather data on product usage patterns that may influence health effects, including total consumption, puffing topography, and flavor preference.

3 INCLUSION AND EXCLUSION CRITERIA

Screening: Upon calling, potential participants will be given a brief explanation of the study and their eligibility to participant will be determined (**Appendix D**). This study will only enroll healthy volunteers and not cancer patients cared for at Roswell Park. In addition, we will inform all potential participants that their current flavors are included in the NYS flavor ban and give a brief

summary of the policy over the phone. Individuals who meet eligibility criteria and come in for study sessions will be provided a written educational document (Appendix AA). All participants who meet the inclusion and exclusion criteria summarized in Section 3.1 and Section 3.2 will be treated on an outpatient basis.

3.1 Inclusion Criteria

To be included in this study, participants must meet the following criteria:

1. Age ≥ 21 years and <55 years.
2. ENDS users as determined by: (a) using banned flavored ENDS with nicotine such as fruit, candy, dessert flavors, and/or any product that indicates such flavors (b) using ENDS daily, regularly for the past 6 months (self-reported).
3. No smoking tobacco or using smokeless tobacco for the past 6 months.
4. Subjects should be free of acute respiratory illness within the proceeding 30 days prior to recruitment (self-reported).
5. Participant must understand the investigational nature of this study and sign an Independent Ethics Committee/Institutional Review Board approved written informed consent form prior to receiving any study related procedure.
6. After receiving information regarding the NYS flavor ban individuals must not want to quit vaping or stop using their flavored product for the next 90 days.

Refer to **Appendix** A **A** for the Investigator Study Eligibility Verification Form: Inclusion Criteria.

3.2 Exclusion Criteria

1. Individuals with health conditions and therapies that may affect immune responses and levels of inflammatory markers, including allergic rhinitis, aspirin/NSAID therapy, asthma, any use of inhaled corticosteroid or injectable steroid, immunodeficiency (HIV or other), Guillain-Barre Syndrome, COPD, or fever/respiratory illness within 30 days prior to entry into study (self-reported).
2. Pregnant or nursing female participants (self-reported on telephone screener, pregnancy test on Visit#1).
3. Unable to communicate in English.
4. Unable or unwilling to follow protocol requirements.
5. Self-report having active, untreated medical/psychiatric conditions.
6. History of serious side effects from nicotine or from any nicotine replacement therapies.
7. Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.
8. Vulnerable populations, such as cognitively impaired adults, individuals who are not yet adults, pregnant women, and prisoners.

9. Any condition which in the Investigator's opinion deems the participant an unsuitable candidate to receive study drug.
10. After receiving information about the NYS flavor ban, participants who report that they are thinking about quitting or stop using their flavor within the next 90 days.

Refer to **Appendix** B **B** for the Investigator Study Eligibility Verification Form: Exclusion Criteria.

3.3 Special Populations

Special populations that will be excluded from participating in the study are:

- Cognitively impaired adults/adults with impaired decision-making capacity
- Individuals who are not yet adults (infants, children, teenagers)
- Pregnant women
- Prisoners

3.4 Inclusion of Women and Minorities

Both men and women and members of all races and ethnic groups are eligible for this study.

4 LOCAL AND STUDY-WIDE NUMBER OF SUBJECTS

A maximum of 216 evaluable participants at Roswell Park will be enrolled. Accrual is expected to take up to 3 years.

5 LOCAL AND STUDY-WIDE RECRUITMENT METHODS

Participants will be recruited from advertisements in local newspapers/magazines, posters, community centers, churches, several websites (Roswell websites, Facebook, Instagram, Craigslist, etc.), local tobacco control networks, and lists of prior study participants. An example of the participant recruitment material can be found in **Appendix C** . Applicants will be invited to call a local number. On calling, applicants will be given a brief explanation of the study and their eligibility to participate will be assessed (**Appendix D**). Those who meet the eligibility criteria will be invited to participate in the study sessions.

Those who meet eligibility criteria be randomized to one of three Arms and attend 4 visits over 90 days. We anticipate that each visit will take 45-60 minutes. **All participants** will be paid **$400** for completing all 4 visits. Cash will be given after each session is complete as follows:

Table 1: Cash Compensation given to all Participants

ARM	Visit 1	Visit 2	Visit 3	Visit 4	Follow-Up
1, 2 & 3	**$50**	**$75**	**$125**	**$150**	**$20**

Subjects in **Arm 1** will be asked to continue using their usual flavor and not to change flavors during 90 days. Subjects in **Arm 1** will simply be observed throughout the 90 days. No product will be provided to subjects randomized to Arm 1. Subjects randomized to **Arm 2** will be asked to

use new assigned tobacco flavor regularly for 90 days and not use any alternative or additional flavors during this time. Study subjects will be provided with tobacco flavored products similar to what they normally use. Participants using MOD devices will be provided free-based tobacco refill solution 4mg nicotine. Study subjects using POD devices will be receiving Juul devices including Virginia Tobacco refill pods 5% nicotine. Study subjects using disposable devices will be provided with the Hyde device Gold flavor 5% nicotine. Subjects in **Arm 3** will be asked to refrain from using any ENDS for 90 days and instead only use the provided 'tobacco free' nicotine pouches.

Subjects in Arms 2 and 3 will receive refill solutions for free (this is on top of the scheduled cash allotted in Table 1), and we will implement strict product dispensation: weekly supply of solutions between visits 1 and 2, monthly supply between visits 2, 3 and 4 will be provided to subjects during study visits or by mail (with delivery confirmation). Subjects will be asked to return all used and unused refill bottles, pods, devices, pouches and will be asked to document any deviations.

All subjects will be encouraged to adhere to their allocated product, but they will be allowed to remain in the study if they report non-compliance with the assigned product.

6 MULTI-SITE RESEARCH

All local site investigators will conduct the study in accordance with applicable federal regulations and local laws. All study visits will be conducted by Roswell Park Comprehensive Cancer Center employees and resources.

Urine, plasma, exhaled breath condensate (EBC), and nasal epithelium samples will be de-identified and sent to the Biomarkers, Genomics & Epigenomics (BGE) Core located at the University of Rochester Medical Center in Rochester, New York for analysis purposes only. Researchers at the University of Rochester will analyze EBC samples using a commercial enzyme immunoassay kit (Cayman) along with analysis on selected samples by GC-MS will be used to measure the levels of F_2-Isoprostanes, lipid peroxidation (MDA & 4-HNE), 8-hydroxyl-2-deoxyguanosine (8-OHdG), TBARS, GSH/GSSG, and myeloperoxidase (MPO) activity. Nasal epithelial cells will undergo transcriptomic analysis using the nCounter Inflammation v2 expression panel from NanoString (NanoString). The University of Rochester will also assess the expression of 255 genes inflammation-related targets. Additionally, UR will also use Human v3 miRNA Assays for profiling miRNAs. NanoString data will be normalized and processed using NanoString data analysis software (nSolver). Analysis of plasma at UR will include 1) oxidative/carbonyl stress; 2) inflammatory mediators (IL-1, IL-6, PGE2, C-reactive protein-CRP, fibrinogen); 3) anti-inflammatory lipid mediators [e.g., resolvin D1], and 4) microparticles/exosomes. UR researchers will analyze for cotinine and total nicotine equivalents (TNE), which are both markers of smoking or vaping and of environmental tobacco smoke exposure.

Researchers at UR will also provide biostatistical expertise and will be responsible for developing the database for the study and analyzing data collected during the study. At no point during the study will researchers at Rochester have access to any personal data of study participants. Additionally, UR will partake in writing publications arising from the study and will be listed as co-authors on published manuscripts.

Due to federal funding, associated with this study, Single IRB is being pursued with the University of Rochester. The Roswell Park Cancer Institute IRB will act as the sIRB for Rochester. Roswell Park will ensure that a signed, formal, written authorization/reliance agreement is in place, and

copies of this agreement and other necessary documentation are maintained. The authorization/reliance agreement will be in place prior to the sites obtaining IRB approval and engaging in research.

7 STUDY TIMELINES

A maximum of 216 participants at Roswell Park will be enrolled. Accrual is expected to take 3 years, with a follow-up phone call 1 month after the final visit. Upon completion of the follow-up phone call, participants will be taken off study.

Participants will come in for 4 total in-person study session followed by a phone call 1 month after their 4th study session. The 4 study sessions will go as follows: Day 1 (Visit #1-beginning of the trial), Day 7 (Visit#2-after one week ± 2 days), Day 30 (Visit#3-after 1 month ±7 days, Day 90 (Visit#4-after 3 months ± 14 days), respectively. Each study visit is expected to take 45-60 minutes.

Study subjects that are not able to come in for their scheduled sessions within the given time period listed above will be terminated from the study. Those terminated from the study due to not coming in for their scheduled sessions will be replaced.

8 STUDY ENDPOINTS

8.1 Primary Endpoints

- Endpoints include clinically relevant risk biomarkers of inflammation and oxidative stress, changes in respiratory function, subjective respiratory symptoms and side-effects, susceptibility to respiratory infections, and expression of immune and inflammatory response genes in nasal epithelial cells.

We anticipate one of the following outcomes:

- There will be statistically significant differences between Arms 1 and 2. This will demonstrate for the first time that potential health effects from inhaling banned flavorings among ENDS users may be mitigated by substituting potentially toxic flavorings with less toxic or non-toxic flavorings.
- An alternative scenario could be that there will be no statistically significant differences between Arms 1 and 2. This would suggest similar health risk from inhaling flavors of different toxicological profiles as predicted in animal and in vitro models.
- There will be statistically significant differences between Arm 3 and Arms 1&2. This will demonstrate for the first time that quitting use of flavored ENDS and substituting with ‘tobacco free’ nicotine pouches, leads to significant improvement in respiratory health.

8.2 Secondary Endpoints

- Assess the compliance of individuals randomized to Arm 2 or Arm 3. Usage of provided products outside of the lab will be closely monitored by study staff contacting participants between study sessions.
- Evaluate the amount, type, and frequency of recent flavored product use using a comprehensive set of questionnaires included in the PhenX Toolkit (see below) as well as

a combination of questions from the Population Assessment of Tobacco and Health (PATH) and the 2015 International Tobacco Control (ITC) 4-Country Survey instruments. (See Appendices E-N for questionnaires)

PhenX Toolkit (https://www.phenxtoolkit.org/index.php?pageLink=about)

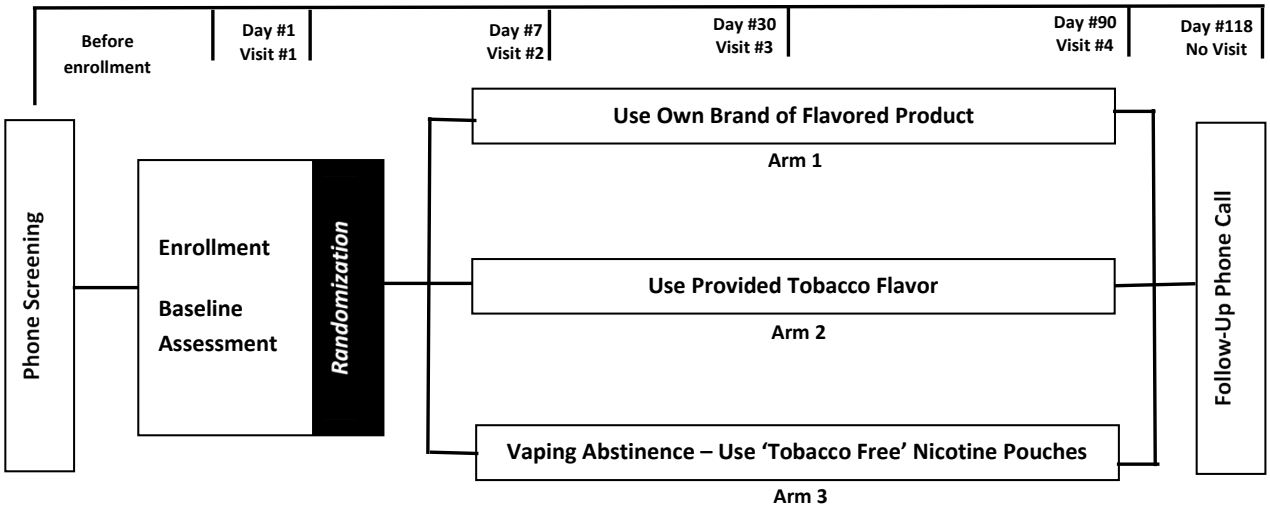
PhenX was established in September 2007 by a cooperative agreement from the National Human Genome Research Institute (NHGRI) of the National Institutes of Health (NIH), with Dr. Erin Ramos as NHGRI Project Scientist. Supplemental funding was provided by the NIH Office of Behavioral and Social Sciences Research (OBSSR). The goal of the PhenX project was to provide the scientific community with recommended, standard high-priority measures of phenotypes and exposures for use in Genome-wide Association Studies (GWAS) and more generally, epidemiological and biomedical research.

The PhenX Toolkit offers well-established, broadly validated measures of phenotypes and exposures relevant to investigators in human genomics, epidemiology, and biomedical research. The measures in the Toolkit are selected by Working Groups of domain experts using a consensus process. The Toolkit provides detailed protocols, information about the measures, and tools to help investigators incorporate PhenX measures into their studies. Inclusion of PhenX measures facilitates cross-study analysis downstream, thus increasing the scientific impact of each individual study.

9 DESIGN

This is a randomized, parallel-group open-label trial to evaluate respiratory symptoms in ENDS users switching from banned flavors to a non-banned flavor (tobacco) or 'tobacco free' nicotine pouches (see Figure 1: Study Design).

Figure 1 Design of Study



10 TREATMENT

Subjects randomized to **Arm 1** will be asked to continue using their usual flavor and not to change flavors during 90 days. Subjects in this arm will be monitored for the next 90 days. No products will be provided to these subjects.

Subjects randomized to **Arm 2** will be asked to use new assigned tobacco flavor regularly for 90 days and not use any alternative or additional flavors during this time. Study subjects will be provided with tobacco flavored products similar to what they normally use.

Subjects in **Arm 3** will be asked to refrain from using any ENDS for 90 days and instead only use the provided 'tobacco free' nicotine pouches. We will explain that this new study will let us test whether the restricted flavors show similar results when compared to non-restricted flavors in actual vapers who use them. The results will not prove whether or not a particular flavored product overall is toxic, but it will add to the evidence on differences between different flavors that can ultimately lead to determination of whether some products are less toxic than others.

STUDY PRODUCTS

Intervention will be administered on an outpatient basis. Reported adverse events (AEs) and potential risks are described in Section 13 and Section 17.

ENDS

Electronic nicotine delivery systems (ENDS) usually look similar to regular cigarettes, cigars, pipes, or pens, but do not contain tobacco.

Participants randomized to **Arm 2** will be provided with a device and liquid similar to what they are accustomed to using. Participants using MOD devices will be provided free-based tobacco refill solution 4mg nicotine. Study subjects using POD devices will be receiving Juul device including Virginia Tobacco refill pods 5% nicotine. Study subjects using disposable devices will be provided Hyde device Gold flavor 5% nicotine.

For this project, the pod device given will be the Juul, which is commercially available (Figure 2). The Juul is a pod-style ENDS, with each pod pre-filled with 0.7mL of a nicotine solution. Nicotine solutions will be Virginia Tobacco flavored and contain 5.0% nicotine (~50 mg/mL), primarily in the form of nicotine salts.

Figure 2 Juul

The liquid provided to MOD users will be free-based e-liquid solution tobacco flavor containing 4 mg of nicotine. The liquid will be purchased from a local vapor shop in Buffalo, NY (Yeti Vape).

Subjects that report using disposable devices will be provided a Hyde device with a pre-filled 1.6 mL tank built in. The solution in the tank contains 5.0% nicotine (~50 mg/mL) and is Gold tobacco flavor (Figure 3).

Figure 3 Hyde

ROGUE--Oral Nicotine Products

Oral nicotine pouches are similar to snus, a smokeless tobacco, in that they are portioned pouches designed to be placed between the lip and gum. These pouches do not produce any emissions and are 100% tobacco leaf-free.

For this study, participants randomized to **Arm 3** will be asked to refrain from using any ENDS for 90 days and instead only use the provided 'tobacco free' ROGUE nicotine pouches. The 3 mg pouches in Mango flavor will be used (Figure 4).

Figure 4 ROGUE

10.1 Dosing and Administration

Subjects randomized to **Arm 2** will be asked to use new assigned tobacco flavor regularly for 90 days and not use any alternative or additional flavors during this time. Study subjects will be provided with tobacco flavored products similar to what they normally use. Participants using MOD devices will be provided free-based tobacco refill solution 4 mg nicotine. Study subjects using POD devices will be receiving Juul device including Virginia Tobacco refill pods 5% nicotine. Study subjects using disposable devices will be provided Hyde device Gold flavor 5% nicotine.

Subjects in **Arm 3** will be asked to refrain from using any ENDS for 90 days and instead only use the provided 'tobacco free' nicotine pouches. The oral nicotine pouch, ROGUE, will contain 3 mg

of nicotine. We will implement strict product dispensation: weekly supply of solutions between visits 1 and 2, monthly supply between visits 2, 3 and 4 will be provided to subjects during study visits or by mail (with delivery confirmation). Subjects will be asked to return all used and unused refill bottles, pods, devices, pouches and will be asked to document any deviations.

10.2 Duration of Treatment

Participants who meet eligibility criteria will be randomized to one of three Arms and attend 4 visits over 90 days. We anticipate that each visit will take 45-60 minutes. There will be a follow-up phone call 1 month after the final visit.

Participants that wish to discontinue their participation in the study are able to do so at any point.

10.3 Compliance

Study procedures that take place in the lab will be under supervision and lab staff will monitor participant's engagement with study procedures. Usage of provided products outside of the lab will be closely monitored by study staff contacting participants between study sessions. Participants will be asked to self-report any non-compliance to the provided product given and to immediately report any adverse reaction to the study product. The registered nurse on study will provide all study subjects with a phone line that will be available for them to call 24/7 while on study. Participants that do not show for their scheduled sessions will be terminated from the study and replaced.

11 PROCEDURES INVOLVED

11.1 Participant Randomization and Registration

Informed consent **MUST** be completed prior to receiving any study related procedures.

Informed consent will be obtained, and eligibility will be confirmed prior to randomizing participants to condition order. Following informed consent, participants will be registered to Clinical Research Services. This study is a randomized, parallel-group open-trial. After enrollment, subjects will be randomized to 1 of 3 arms. For each arm, subjects are asked to attend 4 study sessions at Roswell with a follow-up phone call 1 month following the final study session (visit#4). After completion of the follow-up phone call the participant will be taken off study.

All recruited subjects will be asked to attend four study visits that will take place during Day 1 (beginning of the trial), Day 7 (after one week), Day 30 (after 1 month), and Day 90 (after 3 months), respectively. During study visits, we will collect questionnaire data, perform respiratory tests and collect blood, EBC, and urine samples as described below.

The first set of samples will be collected prior to randomization (Visit 1) to assess the baseline levels of biomarkers.

The remaining samples will be collected during Visits 2-4, respectively.

Subjects will be asked to refrain from smoking tobacco and marijuana, use of smokeless tobacco and drugs of abuse during whole study and refrain from eating or drinking 1hr before each visit.

Procedures and expected time to complete each procedure for every visit are as follows:

1. **Respiratory Rate and Pulse Oximetry (Rest):** A trained technician will place a sensor on a subject's fingertip to monitor the oxygenation of hemoglobin. Technician will count the number of complete respiratory cycles (one inspiration and one expiration) a subject breathes in 60 seconds. (2min)
2. **Expired Breath:** ***Expired Carbon Monoxide (expCO):*** Expired CO will be measured to confirm current cigarette smoking status (Smokerlyzer®). Those subjects with a reading ≥10ppm will be classified as current smokers and be excluded from the study. (2min) (See **Appendix Y**).
3. **Comprehensive set of questionnaires using iPad with REDCap software:** (15min) (See Appendices E-N).
    a. Demographics, Medical History and Drug Use (***only administered at the first visit and final visit***): We will use questions from the Population Assessment of Tobacco and Health (PATH) survey (PhenX). Questionnaire will determine demographics (age, gender, SES), drug (including marijuana smoking) and medication use (particularly use of any medical inhalators; ever and past 30 day), and diet. Women will be asked about their pregnancy status and screened for pregnancy using urine pregnancy test.
    b. History of Tobacco Use: *Current and Past Use of Tobacco Products*: We will administer questions from the Wave 1 PATH Survey instrument (PhenX) to measure a respondent's use and regularity of use of the following tobacco products: cigarettes, ENDS, cigars, pipe tobacco, hookah and shisha tobacco, smokeless tobacco, and dissolvable tobacco. Subjects will be asked to list all tobacco products they have ever used and have used in past 30 days. An analysis of the results will allow subjects to be categorized as: ever user, current user, everyday user, some-day user, 30-day user, experimental current user, experimental former user, former user, former user 12-month, former user 12-month plus. Subjects also will be asked if they smoked at least 100 cigarettes in their entire life, how old were they when they first started smoking cigarettes/vaping, and how old were they the first time they smoked part or all of a cigarette or vape one cartridge or one bottle of e-liquid.
    c. History of Switching from Tobacco to ENDS (***only administered at first visit***):We anticipate that majority of subjects in our study will be ex-smokers (as per inclusion criteria) who quit tobacco cigarettes and switched to ENDS products. We will modify the interviewer-administered National Health Interview Survey (NHIS) protocol (PhenX) that consists of questions used on respondents 18 years or older to determine number of times a smoker has gone a day without smoking, use tobacco and ENDS products at the same time (dual use), and when a respondent switched completely to ENDS products.
    d. Use of Flavored Product: *Amount, Type, and Frequency of Recent Flavored Product Use*: The TLFB (Timeline FollowBack) protocol (PhenX) will be used to track use of ENDS products–it traditionally tracks tobacco use for 30 days and has also been used for marijuana, alcohol and other drugs, and involves asking subjects to retrospectively estimate their products use 7 days to 30 days (modified) prior to

the interview date. Subjects will be asked to list all products they have used in past 30 days. Subjects will be asked to estimate the volume of refill solutions used per day.

e. Flavor Preference: We will use a 6-item adult questionnaire (PhenX) to determine flavor preference when using ENDS. Specifically, subjects will be asked about: 1) flavor used when they first started using ENDS; 2) flavors used since they started regular ENDS use; 3) flavor used in a past 30 days; 4) new flavors used in past 30 days; 5) flavors in the regular brand of liquid; and 6) flavors in the last brand used. Subjects will be asked to assign the following flavor category to their products: tobacco, menthol/mint, clove/spice, fruit, chocolate, alcoholic drinks, candy or other sweets. If multiple flavors are mixed together, subjects will be asked to choose all that apply.

f. Use of a Regular Brand of Flavored ENDS Products: Using questions from the Wave 1 Adult PATH Survey (PhenX), we will determine if a subject has a regular brand of flavored refill solution.

g. Personal History of Respiratory Symptoms/Diseases (past 30-days and past 24 hours): *At each study visit*, we will collect information about the personal history of respiratory symptoms and illnesses (PhenX) that subjects may have experienced since their last visit. Duration and intensity of respiratory symptoms, including allergic reactions, will also be assessed. To assess the negative impact of acute upper respiratory infection, presumed viral (the common cold), we will use the validated Wisconsin Upper Respiratory Symptom Survey (WURSS-21)(18).

h. Quality of Life as Affected by Respiratory Condition (past 30-days): We will use self-administered St. George's Respiratory Questionnaire (SGRQ) recommended by ATS (PhenX). SGRQ is a disease-specific instrument designed to measure impact on overall health, daily life, and perceived well-being (19-21). It contains 50 items and 76 weighted responses divided into three components: Symptoms (frequency and severity); Activity (activities that cause or are limited by breathlessness); and Impacts (social functioning, psychological disturbances resulting from airways conditions).

i. Secondhand Exposure: *Exposure to Secondhand Tobacco Smoke*: Although subjects should not smoke tobacco cigarette, they may be involuntary exposed to secondhand smoke. In order to control for potential secondhand smoke exposure, we will ask questions from the National Adult Tobacco Survey (NATS) and the PATH Survey. Subjects will assess smoking prevalence inside their homes and vehicles during the past 7 days, policies on tobacco use in the place where they work, and exposure to tobacco smoke from someone else who was smoking in an indoor or outdoor public places.

j. House Rules about Vaping and Smoking: We will use a protocol (PhenX) that includes questions from the Wave 1 Adult PATH Survey instrument adapted to vaping scenario. These specific questions ask about ENDS use in the home. These specific questions ask about ENDS use in the homes.

k. Oral Health: We will ask participants about their oral healthcare routine including visits to a dental care provider along with any changes in their oral care routine they may encounter monthly.

l. COVID-19 Questionnaires: Each month, we will be asking participants screening questions about their possible exposure to COVID-19 or any symptoms. In addition, we will be asking participants about their feelings regarding the virus and if they have received the vaccination against the virus. (See **Appendix W** and **Appendix X**)

m. Marijuana/Cannabis Use: While participants are encouraged to not use any tobacco products and or other drugs while on study, we would like to monitor marijuana/cannabis use with a few questions from PATH project 3 Wave 1 survey. (**Appendix Z)**

n. Subjective Gastrointestinal Symptoms: At every visit we will give participants the GERD Health-Related Quality of Life Questionnaire in order to monitor any gastrointestinal side effects that might be taking place. (**Appendix BB**)

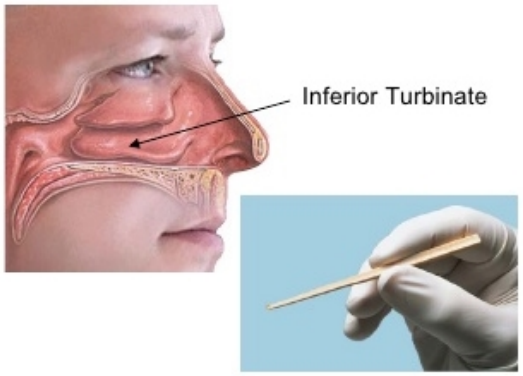
4. **Collection of Exhaled Breath Condensate (EBC):** We will use the RTube™ (Respiratory Research), a non-invasive method of collecting EBC samples. Typical condensate fluid yield is 100-250 µL/minute for an adult at normal tidal breathing effort and 2 mins of normal breathing will yield sufficient fluid quantity for planned laboratory tests. (5min) (See **Appendix O**).

5. **Blood Collection:** A blood sample will be collected via butterfly needles into: 1x8mL red top serum tube that will only be filled half way, (3x10mL) purple top EDTA tubes during each study visit along with 1x3mL EDTA purple top blood collection tube, and 2x3 mL DNA/RNA Shield green top blood collection tubes (43mL total blood at each visit). Blood will be taken from each volunteer in the sitting position. The participant will be asked to hyperextend his/ her arm and the registered nurse or phlebotomist will examine the antecubital area of the arm and choose a prominent vein. The median cubital vein, located

in the antecubital fossa, is generally the vein of choice; however the hand will also be used as a draw site if needed. After selecting the venipuncture site by palpating the area of arm vein location, the participant's arm will be wiped using an alcohol wipe. The butterfly needle will be inserted and secured with patches. Blood will be withdrawn with vacuum systems by pressing forward on the tube to puncture the cap and allowing the evacuated collection tube to fill up to capacity. Each tube will be gently removed from the holder/adapter keeping the needle positioned within the vein. The next tube will be inserted at designated time. When the last tube to be drawn is filling, the butterfly needle will be removed. The gauze will be pressed down on when the needle is out of the arm, applying adequate pressure to avoid formation of a hematoma. If the participant is experiencing excessive blood clotting, the butterfly needle will be removed and a new butterfly needle will be inserted to continue with blood collection (the number of new butterfly needle insertions will never exceed three per study visit). Blood collection tubes are supplied by the Goniewicz lab. (5min)

6. **Spirometry:** A spirometry test will be done using the MicroLoop system (CareFusion), a hand-held spirometer that completely complies with ATS/ERS 2005 standards (22). The primary measures will be forced vital capacity (FVC) and the forced expiratory volume in 1 second (FEV1). (5min) (See **Appendix P**).

7. **Nasal Epithelium Brushing Biopsies:** Trained study staff will perform the following procedure: the right nostril of the subjects is examined and the inferior turbinate is located. Subjects are then asked to blow their nose and attempt to remove any mucous from the nose. The sterile brush will be opened in the view of the participant. Opening the nostril, the lateral area underneath the inferior turbinate is then brushed for 3 seconds and the brush is placed in 2mL screw-cap Eppendorf tube containing 1.5mL of RNAProtect® Cell solution. The brush is then cut into the tube using a wire cutter cleaned with RNAse Zap® and alcohol. A second brush is collected in the same manner and placed in the same tube. (5min) (See **Appendix Q**).



Nasal epithelia sampled from inferior turbinate at 2 hours post dose: 2 scrapes (one per nostril) pooled yielding 1-10mg of tissue

8. **Fractional Exhaled Nitric Oxide** ***(FeNO):*** FeNO is an established biomarker for airway inflammation and it will be measured in subjects' breath using a FDA-cleared monitor NIOX VERO® (Aerocrine) according to ATS guidelines (23). (2min) (See **Appendix R**).

9. **Strength of Respiratory Muscles**: Maximal inspiratory and expiratory pressure (MIP/MEP) at the mouth and sniff nasal inspiratory pressure (SNIP) will be noninvasively measured using the MicroRPM respiratory pressure meter (CareFusion) following the supplier's standardized protocol. (5min) (See **Appendix S**).

10. **Urine Sample Collection:** Each subject will be asked to urinate in a sterile 90-mL urine specimen container. The urine specimen will be aliquoted and stored in a freezer immediately after collection. (5min)
11. **Collection of Product Sample:** All subjects will be asked to bring the products they currently use to visit #1. Subjects randomized to **Arm 1** will be asked to keep all bottles, cartridges, pods and/or devices used in between study visits and bring them in on their scheduled session. Subjects randomized to **Arm 2** or **Arm3** will be asked to collect and bring all used or unused bottles, pods, devices and/or pouch tins to each study session. .We will take photos and record product type, brand, nicotine content, flavor and battery power. We will also collect 1 to 2 drops (0.25 mL) of the participant's current e-liquid in an Eppendorf tube. If participants use devices with disposable pods or cartridges, we will evacuate approximately 0.25 mL of liquid from the cartridge or pod with a sterile syringe. If the pod or cartridge is nearly empty with little to no liquid remaining, we will ask if the participant if we may take the pod or cartridge with the residual liquid remaining. All samples will be labeled with the subject's ID number, protocol numbers, collection time, product, and date. Samples will then be transported to Dr. Goniewicz's laboratory by study staff to be stored. Samples will be analyzed by Dr. Goniewicz's laboratory when the study is complete. (2min)
12. **Puffing Topography:** Subjects will be provided with CressMicro™ monitor with a connector for ENDS products (Borgwaldt). At the end of each study session, subjects will be asked to puff *ad lib* on their device refilled with flavored solutions they currently use. The topography monitor will be calibrated before each puffing session using a smoking machine and a square-shaped puff profile (24). Measurement variables of puffing topography will include number of puffs, puff volume, intervals between puffs, and puff flow rate. (5min)
13. **Saliva Sampling:** Saliva samples will be collected from each participant at every visit using a Salivette® specially designed to determine important analytes from saliva. Participants will chew on the provided Salivette® swab for 60 seconds to simulate saliva production. The swab will be returned back to the tube and it will be centrifuged for 2 minutes before storage. (3min) (See **Appendix T**).
14. **Oral Rinse Specimen:** Oral cell samples will be collected by a non-invasive oral rinse specimen from each participant at every visit. Participants will provide an oral rinse using 10mL of Scope® mouthwash. A 30-second oral rinse is broken into three 5-second swish and 5-second gargle sessions. After each rinse session, participants expectorate the mouthwash rinse into a sterile collection cup. (5min) (See **Appendix V**).

12 WITHDRAWAL OF SUBJECTS

Subjects will be given phone numbers for research associates, study RN and supervising physicians to call if there are adverse effects or other study-related problems. In addition, the registered nurse will provide all study subjects with a phone line to call at any time, day or night, while on study. Subjects will be advised that if they have any adverse effects and/ or wish to stop participating in the study for any reason, they are free to do so. Subjects who are injured as a result of being in the study will have treatment available. The costs of such treatment may be covered by Roswell, depending on a number of factors.

Any participant who discontinues due to an adverse event or adverse experience (AE) must be followed until the event resolves or stabilizes. Appropriate medical care should be provided until signs and symptoms have abated, stabilized, or until abnormal laboratory findings have returned to acceptable or pre-study limits. The final status of AE will be reported in the participant's study records.

Reasons for intervention discontinuation/ withdrawal should be classified as follows:

- Death
- Toxicity; related or unrelated to intervention
- Investigator judgment
    - The Investigator may discontinue a participant if, in his/her judgment, it is in the best interest of the participant to do so.
- Noncompliance (defined as missing scheduled visit by more than 2 weeks without contact)
- Participant voluntary withdrawal
    - A participant may withdraw from the study at any time, for any reason. If a participant withdraws from the study, an attempt should be made to obtain information regarding the reason for withdrawal.

13 RISKS TO SUBJECTS

The potential risks to participants, and their likelihood and seriousness, are described below. Participants can choose, as an alternative, to not enroll in this study. Overall, there is minimal risk for serious adverse reactions as a consequence of enrolling in this study.

Nicotine overdose: Some people who use ENDS may experience symptoms of nicotine overdose such as nausea, sleep disturbance, headache, and vomiting; however, these symptoms are usually mild and temporary. Since all participant recruited for the studies will be regular daily ENDS users, we do not anticipate sever nicotine overdose symptoms. The PI and Co-I's have considerable experience with conducting outpatient studies on novel nicotine delivery devices and their effects on subjects and will be able to provide appropriate safety monitoring.

- The PK studies using nicotine products in participants will be performed in a clinical setting accustomed to monitoring for adverse events. The PI and Co-I's have considerable experience with conducting outpatient studies on novel nicotine delivery devices and their effects on participants, and will be able to provide appropriate safety monitoring.
- Participants will be instructed that the investigational products are not to be ingested. If a product is ingested by mistake, the participants will be instructed to call the study physician (716-845-5412). If they are experiencing serious symptoms of nicotine overdose, the participant should contact Poison Control or Emergency Medical Services.

Nicotine withdrawal: Many individuals who temporarily stop using ENDS products exhibit a pattern of symptoms related to withdrawal from tobacco use. These symptoms include sadness and anxiety, irritability, anger, difficulty concentrating, appetite change and weight gain, insomnia, and decreased heart rate. Because subjects in the studies will be asked to refrain from using ENDS for short periods of the study, it is likely that they will experience some of these symptoms. Consent forms will include sections describing the possibility of this occurrence. Study personnel will be trained to recognize these symptoms and educate the participants about them.

Respiratory irritation: Some people who use flavored ENDS may experience irritation of the mucosal membranes of the upper respiratory tract, but as they are already regular ENDS users we do not think that this is likely. We will exclude subjects or postpone procedures if they demonstrate chronic or acute respiratory problems.

Mouth irritation: Some participants who use oral nicotine products may experience irritation of the mucosal membranes of the mouth (inner lip, gum). We will exclude participants or postpose procedures if they demonstrate chronic or acute oral problems.

Inconvenience: It is quite probable that subjects will experience *inconvenience* due to the multiple study visits required.

Venipuncture is required for this study and this could lead to local pain, swelling, bruising or infection. Significant complications (beyond typical local pain and mild bruising) are expected to be uncommon. The volume of blood to be withdrawn will never exceed 45 mL on any planned visit. This amount of blood is safe for the subjects and will not produce any injury to a healthy individual.

Emotional distress: Subjects may experience psychological discomfort during assessments when discussing feelings and attitudes about using ENDS, or from learning about potential risks of inhaling flavorings. However, all subjects will be established ENDS users and we do not expect this type of reaction to be likely. Study personnel will be alerted to expect this from a small number of subjects and will be trained to make referrals for mental health services as needed. Personnel will be trained to query for adverse emotional reactions during assessments and will be trained to deal with such reactions and to provide additional referrals if needed. In addition, if assessments indicate psychiatric concerns, referrals to appropriate psychological services will be provided.

Reproductive Risks: Women will be advised to notify the study staff if they become or intend to become pregnant during the study period. Because nicotine and ENDS vapor safety for an unborn baby is unknown, participants will be told that they should not become pregnant while on this study nor should they nurse a baby. If a woman is pregnant or breast feeding, she may not participate in this study, and if she becomes pregnant during the study, she will be removed from the study.

As general measures to minimize the likelihood of participants experiencing these side effects we will:

1. Employ a stringent list of exclusionary criteria to limit the chance of side effects listed above occurring. Potential participants will be screened for medical illnesses that would preclude the ENDS use and/or increase the risk of adverse events or side effects (e.g., respiratory diseases, cardiovascular conditions).
2. Administer products of high quality.
3. Monitor self-reported side effects at each assessment time-point. Participants will be informed about the potential for adverse events or side effects, including upper respiratory tract mucosa irritation, cough, phlegm, headache, nausea, and emotional distress from learning about the adverse health consequences of smoking. Subjects will be told that they should proceed with caution until they are certain that the study products do not affect their performance.

4. The study physician will be alerted to any severe side effects or any reported adverse events. The study physician will review the information provided by the research staff and if applicable, will contact the study participant within 24 hours to gather more information and determine the appropriate course of action for the subject. Ultimately, the study physician will decide if the adverse event is related to the study intervention and whether the subject should discontinue its use.

Participants will be told that they should proceed with caution when using study products and to report any adverse symptoms or side effects to study personnel immediately.

14 POTENTIAL BENEFITS TO SUBJECTS

There is no direct benefit for those who decide to participate and enroll in this study.

Participants who enroll in this study will benefit from the knowledge that they are contributing in an important way to potentially furthering scientific knowledge concerning ways to improve safety of ENDS products and to inform FDA regulatory mandate. ENDS users may decide to quit vaping as a result of being in the study and, this will be permitted.

15 DATA AND SPECIMEN BANKING

Data Banking

For the purposes of this research, we will collect the following identifiable information: Participant name (to verify identification), date of birth (to verify age), telephone number and email address (to facilitate contact for scheduling). Identifiable information will be retained in a separate Excel file from the analytic dataset. The only link to identifiable information will be via a unique ID number.

All questionnaires are administered via REDCap, housed at RPCI. All data files will be electronic, including only the unique subject ID. All datafiles will be stored on the Cancer Prevention server (\\CancerPrev\CancerPrev$\HealthBehavior). Access to the folder is restricted to authorized users. Standard protocols for data encryption (via Credant) when using laptops and USB drives will be followed.

Biospecimen Banking

All samples will be immediately transported by study staff to the Health Behavior Department Laboratory (Roswell Park Comprehensive Cancer Center); the blood samples will be centrifuged to separate the plasma from whole blood. The plasma will be aliquoted into cryovials that are labeled with the participant's initials, participant's study number, clinical study number, protocol time point, product, and protocol day. Saliva samples will also be centrifuged for 2 minutes prior to storage. All samples will immediately be frozen at -70ºC or below in Dr. Goniewicz's Laboratory until analyzed (unless otherwise noted above). Female urine samples will be tested for pregnancy. Plasma will be tested for nicotine using UPLC-MS/MS method (Xu et al., 1996). All sample results are recorded in logs and kept in:

Dr. Goniewicz's Laboratory
Gratwick Basic Science Building (GBSB)
Health Behavior Department Laboratory
Laboratory location: 4th Floor, Room 4942
Laboratory phone: 716-845-8603

Dr. Goniewicz's office number: 716-845-8541

If there are remaining samples after all analysis has been done and the participant checks yes on the consent document to having any extra samples stored for future studies, then the samples will be kept in a -70°C or below freezer in Dr. Maciej Goniewicz's Laboratory listed above. If there are remaining samples and the participant checks no on the consent document to having any extra samples stored, then the extra samples of that participant will be immediately destroyed once they are taken off study or completed.

Any future research on the remaining samples will only be done under IRB approved protocols.

Analysis of Exhaled Breath Condensate (EBC):

RTubes with collected EBC will be labeled with the subject's ID number and protocol numbers, collection time, product, date and then transported to Dr. Goniewicz's laboratory and stored in -80°C. Samples will be sent to Biomarkers, Genomics & Epigenomics (BGE) Core and a commercial enzyme immunoassay kit (Cayman) along with analysis on selected samples by GC-MS will be used to measure the levels of F_2-Isoprostanes, lipid peroxidation (MDA & 4-HNE), 8-hydroxyl-2-deoxyguanosine (8-OHdG), TBARS, GSH/GSSG, and myeloperoxidase (MPO) activity.

Serum Analysis:

The blood sample from the one red-top collection tube will be centrifuged in the lab. The fresh serum (25 µL) will be aliquoted for a COVID-19 IgM IgG rapid test. The rapid test will allow us to determine if participants test positive or negative for antibodies of the virus in 15 minutes. The remaining, unused serum will be disposed of.

Plasma Analysis:

The 3 EDTA 10 mL blood collection tubes will be centrifuged in the lab. Plasma will be separated and aliquoted into cryovials, labeled with the subject's ID number and protocol numbers, collection time, product, date and then transported to Dr. Goniewicz's laboratory and stored in -80°C. The 3 mL EDTA tube and the 2 DNA/RNA Shield (3 mL) blood collection tubes will be frozen immediately. Samples will be sent to Biomarkers, Genomics & Epigenomics (BGE) Core for analysis of 1) oxidative/carbonyl stress; 2) inflammatory mediators (IL-1, IL-6, PGE2, C-reactive protein-CRP, fibrinogen); 3) anti-inflammatory lipid mediators [e.g., resolvin D1], and 4) microparticles/exosomes: ***Oxidative/carbonyl stress:*** We have established methodologies for determination of oxidative/carbonyl stress and inflammation (25,26-31) in plasma using ELISA (R&D Systems). Plasma samples will be used for determination of oxidative stress including lipid peroxidation products (malondialdehyde, MDA and 4-hydroxy-2-nonenal, 4-HNE), protein carbonylation by the OxyBlot™ kit, advanced glycation end-products (AGEs) by ELISA/ELISPOT kits (Cell Biolabs, Inc), 8-hydroxy-2-deoxyguanosine [8-OHdG] assays (Cell Biolabs/Enzo), and levels of reduced and oxidized GSH by enzymatic recycling method (29). The levels of pro-resolving lipid mediators by lipidomics facility and anti-inflammatory mediators (resolvin D1, resolvin E1, and IL-10) will be measured using high performance liquid chromatography-tandem electrospray ionization mass spectrometry (HPLC-ESI-MS-MS) (32,33). ***Inflammatory responses*** include cyclooxygenase 2 (COX-2), PGE2 (EIA/ELISA kits, Cayman, R&D/Enzo Life), NF-κB-dependent pro-inflammatory cytokines by the Luminex™ 100 (25,26)] as well as IgG and IgE measured by EIA. ***Anti-inflammatory lipid mediators:*** Lipid mediator concentrations will be determined by mass spectrometry as described previously (32,33) or by EIA

(PGE2, PGD2, and TxB2) according to the manufacturer's protocol. ***Microparticles and exosome assays:*** Currently, no information is available on the biological effects of ENDS on functional characteristics of exosomes in humans. We will determine the functional characteristics of exosomes and their cargos in the above recruited subjects. We will isolate and characterize MPs and exosomes from human plasma from users of flavored ENDS and correlate them with other toxicity biomarkers and lung function. The following methods will be used to isolate MPs and exosomes from human plasma samples from ENDS users: 1) Ultracentrifugation and 2) ExoQuick™ plasma precipitation kit (34-36). We will use nanoparticle tracking analysis for size and concentration analysis of the isolated exosomes using NanoSight NS300. Additionally, we will also perform electron microscopy analysis to confirm their size, morphology, and other minute structures on their surface. Furthermore, we will also perform phenotypic characterization of exosomes using specific protein markers by FACS analysis (36-39). Plasma will be also analyzed for ***nicotine and cotinine*** using UPLC-MS/MS (40).

Nasal Epithelium Brushing Biopsies:

The tubes will be labeled with the subject's ID number and protocol numbers, collection time, product, date and then transported to Dr. Goniewicz's laboratory and stored in -80°C. Samples will be sent to Biomarkers, Genomics & Epigenomics (BGE) Core for analysis for transcriptomic analysis using the nCounter® Inflammation v2 expression panel from NanoString (NanoString). We will assess the expression of 255 genes inflammation-related targets. Additionally, we will also use Human v3 miRNA Assays for profiling miRNAs. NanoString data will be normalized and processed using NanoString data analysis software (nSolver™).

Urine Analysis:

Each subject will be asked to urinate in a sterile 90 mL urine specimen container. The urine specimen will be aliquoted, labeled with the subject's ID number and protocol numbers, collection time, product, date and then transported to Dr. Goniewicz's laboratory and stored in -80°C. Samples will be sent to Biomarkers, Genomics & Epigenomics (BGE) Core for analysis of **Cotinine and Total Nicotine Equivalents (TNE)** are markers of either smoking or vaping and of environmental tobacco smoke exposure (PhenX). Cotinine in urine samples will be measured by gas chromatography mass spectrometry (GC-MS)(41). ***NNAL*** (PhenX) is tobacco-specific biomarker and will be used to verify tobacco use self-reported status reported by study subjects. NNAL will be measured by using liquid chromatography linked to tandem mass spectrometry (UPLC-MS/MS) (42). A commercial enzyme immunoassay kit (Cayman) along with analysis on selected samples by GC-MS will be used to measure the levels of ***F2α-isoprostanes*** (43). Urinary 3-hydroxyproply mercapturic acid, ***3-HPMA*** will be assayed to determine total acrolein exposure (44). Levels of 1-hydroxypyrene (***1-HOP***) is a urinary metabolite of pyrene and an established biomarker of PAH uptake. To quantify urinary 1-HOP levels, the glucuronide will be hydrolyzed and the total 1-HOP level will be measured as previously described (45). All measured biomarkers of exposure will be adjusted for ***creatinine*** levels due to variations in urine excretion rates between study visits. Creatinine will be measured according to the Jaffe rate method using an automated urine analyzer. Urine samples from female subjects will be tested for ***pregnancy*** and women testing positive will be excluded.

Saliva Analysis:

We will measure the concentration of secreted inflammatory mediators in saliva to assess differential cytokine/chemokine production induced after exposure to different flavored tobacco

products. We will use the magnetic bead-based multiplex assay platform using Luminex technology. This multiplexing approach will enable us to measure up to 500 different analytes in relatively low sample volumes. For human saliva samples, Milliplex® Map Human Cytokine/ Chemokine Magnetic Bead Panel - Premixed 41 Plex - Immunology Multiplex Assay kit (Millipore Cat. #: HCYTMAG60PM X41BK) will be used. This will allow us to determine 41 different cytokines/chemokines in each sample. Additionally, samples will be analyzed for selected inflammatory biomarkers using commercially available ELISA kits for PGE2, prostanoids, leukotriene in saliva samples.

Oral Rinse Analysis:

We will characterize the microbiota by analyzing 16S rRNA (bacteria) and ITS1 gene (fungi) and the virome (including human papillomaviruses) using NGS. Additionally, we will expand traditional microbiome cross-sectional community profiling into mechanistic association by leveraging the study's prospective design and complementing it with functional information obtained through Bioinformatic algorithms (PICRUSt) and shotgun metagenomics. This approach will quantify differences in secreted and other microbial gene products; connect them to annotated pathways and make them available for downstream analyses. Thus we will analyze inferred biochemical functions that are related to inflammation and subsequent oral and respiratory related outcomes. Samples collected will be stored in -80°C freezer and kept at Roswell for analysis.

Upon completion of the study, biospecimen samples will be shipped to:

University of Rochester Medical Center
School of Medicine and Dentistry
Department of Environmental Medicine (SMD)
601 Elmwood Ave, Box 850
Rochester, NY 14642
Phone: (585) 276-3000

All EBC, plasma, nasal epithelium, urine, and saliva samples will be shipped to Biomarkers, Genomics & Epigenomics (BGE) Core, Rochester, New York, for analysis.

- The Biomarkers, Genomics & Epigenomics (BGE) Core will only analyze the samples collected by Roswell Park Comprehensive Cancer Center study staff.
- All samples will be de-identified before shipment and analysis results will only be sent to appropriate staff including the Investigator, Statistician, and Clinical Research Coordinator.

Note: All investigator or analyzing research laboratories housing research samples need to maintain current Temperature Logs and study-specific Sample Tracking and Shipping Logs. The Principal Investigator/Laboratory Manager must ensure that the stated lab(s) have a process in place to document the receipt/processing/storage/shipping of study-related samples/specimens. This is required for all studies collecting specimens.

If there are remaining samples after all analysis has been done and the participant checks yes on the consent document to having any extra samples stored for future studies, then the samples will be kept in a -70°C or below freezer in Dr. Maciej Goniewicz's Laboratory listed above. If there are remaining samples and the participant checks no on the consent document to having any extra

samples stored, then the extra samples of that participant will be immediately destroyed once they are taken off study or completed.

16 MEASUREMENT OF EFFECT

N/A

16.1 Solid Tumors

N/A

16.2 Hematologic Tumors

N/A

16.3 Other Response Parameters

N/A

17 SAFETY EVALUATION

17.1 Safety Monitoring

Subjects will be monitored for safety during study sessions by trained laboratory staff. Participants are able to use the study products at home outside of the laboratory and can stop product use at any time without being withdrawn from the study completely. Subjects will be given phone numbers for research associates, the study nurse and supervising physicians to call if there are adverse effects or other study-related problems. Subjects will be advised that if they have any adverse effects and/ or wish to stop participating in the study for any reason, they are free to do so. Vital signs will also be monitored continuously throughout the study sessions.

17.2 Unanticipated Problems

An Unanticipated Problem (UP) is any incident, experience, or outcome that meets all of the following criteria:

- Unexpected (in terms of nature, severity, or frequency) given:
  - The research procedures that are described in the study-related documents, including study deviations, as well as issues related to compromise of participant privacy or confidentiality of data.
  - The characteristics of the participant population being studied.
- Related or possibly related to participation in the research (possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research).
- Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized and if in relation to an adverse event is also deemed Serious as defined below:

17.3 Serious Adverse Events

A serious adverse event (SAE) is any adverse event (experience) that in the opinion of either the investigator or sponsor results in **ANY** of the following:

- Death
- A life-threatening adverse event (experience). Any AE that places a participant or participants, in the view of the Investigator or sponsor, at immediate risk of death from the reaction as it occurred. It does NOT include an AE that, had it occurred in a more severe form, might have caused death.
- Inpatient hospitalization or prolongation of existing hospitalization (for > 24 hours).
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
- A congenital anomaly or birth defect.
- Important Medical Event (IME) that, based upon medical judgment, may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed above.

17.4 Reporting Unanticipated Problems

Unanticipated problem reporting will begin at the time of participant consent. The Reportable New Information (RNI) Form will be submitted to the CRS Quality Assurance (QA) Office within 1 business day of becoming aware of the Unanticipated Problem. After review, the CRS QA Office will submit the RNI to the IRB.

When becoming aware of new information about an Unanticipated Problem, submit the updated information to the CRS QA Office with an updated Reportable New Information Form. The site Investigator or designated research personnel will report all unanticipated problems to the IRB in accordance with their local institutional guidelines.

18 DATA MANAGEMENT AND CONFIDENTIALITY

To ensure data security and confidentiality, all assessments will be administered by trained research study staff, and blood draws will be performed by the study nurse or a certified phlebotomist.

All questionnaires will be administered using iPad with REDCap software at each visit.

Data management activities will be performed using REDCap (Research Electronic Data Capture). REDCap is a secure web-based application that supports data capture and management for research studies. In the event of REDCap being inaccessible for a study visit, paper copies of the questionnaires will be administered to the participant at that point in time and data entry will be performed by the study staff after the study session is completed. Data can be entered and changed only by study staff members with the rights to do so.

The data collected from this study will be organized by a unique identification number assigned to the participant so that their identity will be available only to project staff and will remain completely confidential. The clinical research coordinator, staff and PI of the study will have access to the data records. All study records will be kept electronically in Roswell Park Comprehensive Cancer Center maintained and secured equipment. Contact information will be stored in a separate limited-access Excel file on the Roswell Prevention server (\\CancerPrev\CancerPrev$\HealthBehavior\TobaccoEpidemiology). Data collected and housed at UR is specific to their own subjects.

- Access to the data by all necessary research personnel will be through a password-protected network computer. At no time will data be downloaded on a thumb drive or other portable media or laptop.
- Participant identity will not be disclosed in the event of publication or sharing of data.
- Any paper files containing identifiers (if applicable to the research) will not be taken off Roswell Park premises; they will be kept in a locked filing cabinet in a secure building on the Roswell Park campus.
- Identifiable Private Information will not be re-used or disclosed for purposes other than research use.
- There are no outside entities to which Identifiable Private Information will be disclosed.

Any data, specimens, forms, reports, and other records that leave the site will be identified only by a participant identification number (Participant ID, PID) to maintain confidentiality. All records will be kept in a limited access environment. All computer entry and networking programs will be done using PIDs only.

18.1 Data Collection

Data management activities are performed using REDCap.

18.2 Maintenance of Study Documents

Essential documents will be retained per Roswell Park's policy for 6 years from the study termination date. These documents could be retained for a longer period, however, if required by the applicable local regulatory requirements or by an agreement with Roswell Park.

18.3 Revisions to the Protocol

Roswell Park may make such changes to the protocol as it deems necessary for safety reasons or as may be required by the U.S. FDA or other regulatory agencies. Revisions will be submitted to the IRB/ERC for written approval before implementation.

18.4 Termination of the Study

It is agreed that, for reasonable cause, either the Roswell Park Investigators or the Sponsor, may terminate this study, provided a written notice is submitted within the time period provided for in the Clinical Trial Agreement. In addition, Roswell Park may terminate the study at any time upon immediate notice if it believes termination is necessary for the safety of participants enrolled in the study.

18.5 HIPAA Authorization

The minimum personally identifiable data that are needed for the research to be conducted are participant name and phone number / email to contact the participant and date of birth to verify age for eligibility. Participants are not assigned a MRN through their participation in this study, therefore HIPPA authorization is not necessary.

19 STATISTICAL PLAN

- **Analysis of Exhaled Breath (EBC):**

  Biomarkers, Genomics & Epigenomics (BGE) Core. A commercial enzyme immunoassay kit (Cayman) along with analysis on selected samples by GC-MS will be used to measure the levels of F_2-Isoprostanes, lipid peroxidation (MDA & 4-HNE), 8-hydroxyl-2-deoxyguanosine (8-OHdG), TBARS, GSH/GSSG, and myeloperoxidase (MPO) activity.

- **Nasal Epithelium Brushing Biopsies:** Nasal epithelial cells will be sent to ***Biomarkers, Genomics & Epigenomics (BGE) Core*** for transcriptomic analysis using the nCounter Inflammation v2 expression panel from NanoString (NanoString). We will assess the expression of 255 genes inflammation-related targets. Additionally, we will also use Human v3 miRNA Assays for profiling miRNAs. NanoString data will be normalized and processed using NanoString data analysis software (nSolver).

- **Plasma Analysis:** Samples will be transferred to ***Biomarkers, Genomics & Epigenomics (BGE) Core*** for analysis of: 1) oxidative/carbonyl stress; 2) inflammatory mediators (IL-1, IL-6, PGE2, C-reactive protein-CRP, fibrinogen); 3) anti-inflammatory lipid mediators [e.g., resolvin D1], and 4) microparticles/exosomes: ***Oxidative/carbonyl stress:*** We have established methodologies for determination of oxidative/carbonyl stress and inflammation (13,25-30) in plasma using ELISA (R&D Systems). Plasma samples will be used for determination of oxidative stress including lipid peroxidation products (malondialdehyde, MDA and 4-hydroxy-2-nonenal, 4-HNE), protein carbonylation by the OxyBlot™ kit, advanced glycation end-products (AGEs) by ELISA/ELISPOT kits (Cell Biolabs), 8-hydroxy-2-deoxyguanosine [8-OHdG] assays (Cell Biolabs/Enzo), and levels of reduced and oxidized GSH by enzymatic recycling method (29). The levels of pro-resolving lipid mediators by lipidomics facility and anti-inflammatory mediators (resolvin D1, resolvin E1, and IL-10) will be measured using high performance liquid chromatography-tandem electrospray ionization mass spectrometry (HPLC-ESI-MS-MS) (31,32). ***Inflammatory responses*** include cyclooxygenase 2 (COX-2), PGE2 (EIA/ELISA kits, Cayman, R&D/Enzo Life), NF-κB-dependent pro-inflammatory cytokines by the Luminex™ 100 (13,25)] as well as IgG and IgE measured by EIA. ***Anti-inflammatory lipid mediators:*** Lipid mediator concentrations will be determined by mass spectrometry as described previously (31,32) or by EIA (PGE2, PGD2, and TxB2) according to the manufacturer's protocol. ***Microparticles and exosome assays:*** Currently, no information is available on the biological effects of ENDS on functional characteristics of exosomes in humans. We will determine the functional characteristics of exosomes and their cargos in the above recruited subjects. We will isolate and characterize MPs and exosomes from human plasma from users of flavored ENDS and correlate them with other toxicity biomarkers and lung function. The following methods will be used to isolate MPs and exosomes from human plasma samples from ENDS users: 1) Ultracentrifugation and 2) ExoQuick plasma precipitation kit (33-35). We will use nanoparticle tracking analysis for size and concentration analysis of the isolated exosomes using NanoSight NS300. Additionally, we will also perform electron microscopy analysis to confirm their size, morphology, and other minute structures on their surface. Furthermore, we will also perform phenotypic

characterization of exosomes using specific protein markers by FACS analysis (35-38). Plasma will be also analyzed for ***nicotine and cotinine*** using UPLC-MS/MS (39).

- **Urine Analysis:** Samples will be transported to ***Biomarkers, Genomics & Epigenomics (BGE) Core*** and stored in the cryovials at -80°C until analyzed. **Cotinine and Total Nicotine Equivalents (TNE)** are markers of either smoking or vaping and of environmental tobacco smoke exposure (PhenX). Cotinine in urine samples will be measured by gas chromatography mass spectrometry (GC-MS)(40). ***NNAL*** (PhenX) is tobacco-specific biomarker and will be used to verify tobacco use self-reported status reported by study subjects. NNAL will be measured by using liquid chromatography linked to tandem mass spectrometry (UPLC-MS/MS)(41). A commercial enzyme immunoassay kit (Cayman) along with analysis on selected samples by GC-MS will be used to measure the levels of ***F2α-isoprostanes*** (42). Urinary 3-hydroxyproply mercapturic acid, ***3-HPMA*** will be assayed to determine total acrolein exposure (43). Levels of 1-hydroxypyrene (***1-HOP***) is a urinary metabolite of pyrene and an established biomarker of PAH uptake. To quantify urinary 1-HOP levels, the glucuronide will be hydrolyzed and the total 1-HOP level will be measured as previously described (44). All measured biomarkers of exposure will be adjusted for ***creatinine*** levels due to variations in urine excretion rates between study visits. Creatinine will be measured according to the Jaffe rate method using an automated urine analyzer. Urine samples from female subjects will be tested for ***pregnancy*** and women testing positive will be excluded.

- Respiratory symptoms changes in ENDS users in each arm will be first examined using summary statistics and frequency distributions. Univariate general linear models and Chi-square tests (or Fisher's exact tests for small cell counts) will be used to evaluate the unadjusted differences from baseline to 90 days after across arms. Linear mixed effects model for normally distributed variables and/or generalized estimating equation (GEE) model for non-normally distributed variables will be used to examine the adjusted differences across groups. Both linear mixed effects model or GEE model will include the effect of time, arm, and interaction between time and arm with potential confounding variables such as gender, age, BMI, marijuana and tobacco use, and daily average consumption of flavors**.** The variance-covariance matrix in the linear mixed effects model and GEE model will be used to account for the within-subject correlations. The type of variance-covariance matrix will be selected through likelihood ratio tests. AIC values will be used to evaluate the goodness of fit of the models. Linear contrasts in both models will be used to evaluate the longitudinal changes in respiratory symptoms across arms. The estimated coefficients and their corresponding 95% CIs will be used to measure the magnitude of changes across arms. Residual diagnostic plots will be used to examine the satisfaction of normality assumption for linear mixed effects models and potential outliers and influential data values for both models. In case of over 5% of missing data, missing patterns will be evaluated and multiple imputations will be conducted to impute the missing data. Sensitivity analysis will also be conducted to examine the effect of imputation. All analysis will be conducted using either proc mixed or proc genmod procedure in SAS v9.4 with familywise error rate strongly controlled by the Bonferroni method (52-54).

19.1 Sample Size Determination

Power analysis was conducted to calculate the sample size needed to evaluate respiratory symptom changes in ENDS users switching from flavors of potentially high-toxicity to flavors of potentially low-toxicity with at least 80% power and 5% significance level. Based on a recent randomized, controlled, open-label multicenter study with three similar arms (51), we estimated LS mean ratio (%) (95% CI) in oxidative stress (8-epi-PGF2α, pg/mg Cr) between Arm 1 vs. Arm 2 of 87.3% (78.2; 97.5, $p<.02$). The estimated pooled standard deviation was 49.5 pg/mg Cr. The sample size ratio we plan to recruit is 2:2:1 for Arms 1-3, respectively. According to the repeated measurement model with the assumption of within-subject correlation of 0.8, we need 135 subjects in total for 80% power at 5% significance level using the proc power procedure in SAS v9.4 (SAS Institute Inc.). Based on conservative attrition rates of 25.0% in Arms 1-2, and 62.5% in Arm 3, we will need 72 subjects in each arm. Therefore, we will need 216 subjects in total to maintain at least 80% power at 5% level of significance.

19.2 Randomization

During the first visit, subjects will be randomized to the one of the three study arms using a randomization function in statistical analysis software R.

19.3 Demographics and Baseline Characteristics

We do not expect clinically important gender or race/ethnicity differences for this study, and we will not be powered to detect effects by demographic subgroups. Still, descriptive statistics (as appropriate: n, percent, mean, median, min, max) will be used to summarize demographic and baseline characteristics.

19.4 Primary Analysis

To evaluate respiratory symptoms in ENDS users switching from banned flavors of potentially high-toxicity profile to non-banned flavors of potentially low-toxicity profile, respiratory symptom changes in ENDS users in each arm will be first examined using summary statistics and frequency distributions.

Participants that drop out of the study prior to completing the four study sessions will be treated as non-evaluable.

19.5 Secondary Analysis

We will assess biological responses (e.g. stress response, cytokines concentrations, microbiota) in response to the different study products.

20 PROVISIONS TO MONITOR THE DATA TO ENSURE THE SAFETY OF SUBJECTS

Subjects will be monitored for safety during product use sessions by trained laboratory staff. Participants are able to use the study products ad lib and can stop product use at any time without being withdrawn from the study completely. Subjects will be given phone numbers for research associates and supervising physicians to call if there are adverse effects or other study-related problems. Subjects will be advised that if they have any adverse effects and/ or wish to stop

participating in the study for any reason, they are free to do so. Vital signs will also be monitored continuously throughout the session.

The data collected from this study will be organized by a unique identification number assigned to the participant so that their identity will be available only to project staff and will remain completely confidential. The clinical study coordinator and staff and PI of the study will have access to the data records. All study records will be kept electronically in Roswell Park Comprehensive Cancer Center maintained and secured equipment. Any data, specimens, forms, reports, and other records that leave the site will be identified only by a participant identification number (Participant ID, PID) to maintain confidentiality. All records will be kept in a limited access environment. All computer entry and networking programs will be done using PIDs only. Information will not be released without written authorization of the participant.

21 VULNERABLE POPULATIONS

N/A

22 COMMUNITY-BASED PARTICIPATORY RESEARCH

N/A

23 SHARING OF RESULTS WITH SUBJECTS

No results will be shared with study participants or with parties outside of the research team.

24 SETTING

All study sessions will take place in The Health Behavior Human Exposure Lab located at Roswell Park Comprehensive Cancer Center. The research team will identify and recruit potential participants from the Western New York region. The research team running study subjects will include a certified registered nurse and/or a certified phlebotomist.

25 PROVISIONS TO PROTECT THE PRIVACY INTERESTS OF SUBJECTS

Participants will be given phone numbers for research associates, the study nurse, and supervising physicians to call if there are adverse effects or other study-related problems. Participants will be advised that if they have any adverse effects and/ or wish to stop participating in the study for any reason, they are free to do so.

The data collected from this study will be organized by a unique identification number assigned to the participant so that their identity will be available only to project staff and will remain completely confidential. The clinical study coordinator and staff and PI of the study will have access to the data records. All study records will be kept electronically in Roswell Park Comprehensive Cancer Center maintained and secured equipment.

Any data, specimens, forms, reports, and other records that leave the site will be identified only by a participant identification number (Participant ID, PID) to maintain confidentiality. All records will be kept in a limited access environment. All computer entry and networking programs will be done using PIDs only. Information will not be released without written authorization of the participant.

26 RESOURCES AVAILABLE

Investigators on this protocol have the education and research training and experience to conduct this study safely and appropriately. All data and specimen collection will be done by trained clinical research coordinators, research associates, registered nurses or certified phlebotomists working on this study.

All study procedures will be performed in the:

Health Behavior Human Exposure Lab
Gratwick Basic Science Building (GBSB)
Laboratory location: 3rd Floor, Room 3920
Laboratory phone: 716-845-3312

The laboratory is 10' x 10.5" x 11'8" space equipped with a sink, multiple infusion chairs, and an Axis Communications video camera. In addition, the laboratory also has specialized mechanical exhausts and ducts designed to keep the room under negative air pressure at all times.

- **Sample Preparation Equipment:** Sample preparation equipment includes clinical centrifuges, vortex mixers, freezers, UV-visible spectrometer, microbalances, Labconco RapidVap Vertex evaporator and Labconco CentriVap evaporator.
- **Puffing Topography Monitors**: Dr. Goniewicz Lab is equipped with 15 CRESS Pocket puffing topography monitors. The CReSS Pocket is a portable and autonomous version of the widely used CReSS Lab (Clinical Research Support System for Laboratories) reference system. This battery-operated device automatically measures smoking behavior parameters or characteristics including date, time, start and end of smoking, puffs per cigarette, puff volume and puff duration. The monitors allow for acquiring behavioral information in the smoker's natural setting over several weeks. Measurements are collected and stored in on-board memory for up to four weeks at a time. The stored data can easily be downloaded onto a Windows computer. A built-in verification process ensures privacy and reliability of the data and restricts unauthorized access.
- **Expired Carbon Monoxide (expCO) Monitors:** The Goniewicz Lab is equipped with four monitors that measure expired CO (PhenX). Expired CO will be measured to confirm current cigarette smoking status using Micro+ Smokerlyzer® (Bedfont, UK). Smokerlyzer® uses electrochemical sensor to detect CO in a concentration range 0-500ppm and with a sensor sensitivity of 1ppm. The repeatability of tests is <±5% with an accuracy of 2ppm/±5%. Response time is less 30 seconds. The sensor drift is <5% per annum.
- **Fractional Exhaled Nitric Oxide (FeNO) Monitor:** FeNO is an established biomarker for airway inflammation and it will be measured in participants' breath using a FDA-cleared monitor NIOX VERO® (Aerocrine) according to ATS guidelines. FeNO has been used to monitor airway inflammation to account for persistent and/or high allergen exposure as a factor associated with higher levels of FeNO. NIOX VERO® is a portable monitor with measurement range of FeNO from 5 to 300 ppb. The patient exhalation time is 10 seconds and measurement time is 1 min. Accuracy of measurements is ± 5 ppb or max 10% and precision <3 ppb of measured value <30 ppb or <10% of measured value ≥30 ppb. The instrument has memory capacity of 15,000 measurements.

- **Spirometer**. Goniewicz Lab is equipped with a MicroLoop system (CareFusion), a hand-held spirometer that completely complies with ATS/ERS 2005 standards. The primary measures will be forced vital capacity (FVC) and the forced expiratory volume in 1 second (FEV1). Tests includes: Forced (FVC): FEV1, FVC, PEF, FEV1/FVC, FEV6, VC, FEV.75, FEV3, FEV.75/VC, FEV.75/FVC, FEV1/VC, (FER), FEV3/VC, FEV3/FVC, FEV.75/FEV6, FEV1/FEV6, FEF25 (MEF75), FEF50 (MEF50), FEF75 (MEF25), FEF25-75 (MMEF), FEF50/VC, FEF50/FVC, MMEF/FVC (FEF25-75/FVC), FIV1, FIVC, PIF, FIV1/FIVC (FIR), FIF25 (MIF75), FIF50 (MIF50), FIF75 (MIF25), R50 (FEF50/FIF50), MET25-75, FET, MVV (ind); Relaxed (SVC): VT (TV), Ti, Te, Ti/Ttot, EVC, IVC, IC, VT/Ti (TV/Ti), IRV, ERV, FR. Volume range is 0.1–8 L and the flow range of 0.2–15 L/s. Accuracy is ±3% according to ATS recommendations. The MicroLoop system is compatible with PC software (Windows® 2000, XP, 7).
- **Strength of Respiratory Muscles Monitore**d. Goniewicz Lab is equipped with a Micro RPM (Respiratory Pressure Meter; CareFusion), a handheld diagnostic instrument designed for rapid assessment of inspiratory and expiratory muscle strength. The unit can measure the maximum inspiratory and expiratory mouth pressures, MIP and MEP, and the Sniff Nasal inspiratory Pressure, SNIP. The system is intended for use with pediatric and adult patients over the age of 3 years in hospitals, physician offices, laboratories and occupational health testing environments. The unit is easy to operate and battery powered. The unit is connected to a PC running PUMA software that provides a real time display of pressure/time curves, overlay of successive curves, predicted values, patient database, incentive display, maneuver quality check, and maneuver variability measurement.
- **Computer Systems:** The laboratory contains seven computers that control analytical systems. Software that meets the needs of the laboratories for word processing, data analysis and analytical chemistry are available. All instrument data systems are networked to a server and PCs for data analysis and preparation of reports.

27 PRIOR APPROVALS

This study is funded by NIH grant 1U54CA228110-01 (O'Connor and Goniewicz; MPI)

28 COMPENSATION FOR RESEARCH-RELATED INJURY

If the subject believes they have been injured as a direct result of their participation in this research study, they will be advised to notify the Roswell Park Patient Advocate at (716) 845-1365 or the Study Doctor at (716) 845-8541.

Medical diagnosis and treatment for the injury will be offered, and a determination will be made regarding appropriate billing for the diagnosis and treatment of the injury. A financial counselor (716-845-3161) will be able to provide an explanation of coverage and to answer questions the subject may have regarding study related billing.

The subject is not prevented from seeking to collect compensation for injury related to malpractice, fault, or blame on the part of those involved in the research.

29 ECONOMIC BURDEN TO SUBJECTS

Participants will be responsible for transportation to and from the research site and any costs that might entail. The remainder of the study will be conducted at no financial cost or obligation to the participant.

30 CONSENT PROCESS

The Investigator (or IRB approved designee) is responsible for obtaining written consent from each participant in accordance with ICH-GCP guidelines using the approved informed consent form, before any study specific procedures (including screening procedures) are performed. The informed consent form acknowledges all information that must be given to the participant according to ICH-GCP, including the purpose and nature of the study, the expected efficacy and possible side effects of the treatment(s), and specifying that refusal to participate will not influence further options for therapy. Any additional information that is applicable to the study must also be included. Additional national or institutionally mandated requirements for informed consent must also be adhered to. The participant should also be made aware that by signing the consent form, processing of sensitive clinical trial data and transfer to other countries for further processing is allowed.

The Investigator shall provide a copy of the signed consent form to the participant and the signed original shall be given to the CRS Regulatory Research Associate for the Regulatory Binder. A copy of the signed consent form must be filed in the participant file. At any stage, the participant may withdraw from the study and such a decision will not affect any further treatment options. The consenting process will take place at the first visit for every participant. The consent MUST be signed by every participant before any study procedure is performed.

The Roswell Park SOP: Informed Consent Process for Research (HRP-090) will be followed.

31 PROCESS TO DOCUMENT CONSENT IN WRITING

The Roswell Park SOP: Written Documentation of Consent (HRP-091) will be followed.

32 DRUGS OR DEVICES

Subjects randomized to **Arm 2** will be asked to use new assigned tobacco flavor regularly for 90 days and not use any alternative or additional flavors during this time. Study subjects will be provided with tobacco flavored products similar to what they normally use. Participants using MOD devices will be provided free-based tobacco refill solution 4mg nicotine. Study subjects using POD devices will be receiving Juul device including Virginia Tobacco refill pods 5% nicotine. Study subjects using disposable devices will be provided Hyde device Gold flavor 5% nicotine. Subjects in **Arm 3** will be asked to refrain from using any ENDS for 90 days and instead only use the provided 'tobacco free' nicotine pouches. The oral nicotine pouch, ROGUE, will contain 3mg of nicotine. We will implement strict product dispensation: weekly supply of solutions between visits 1 and 2, monthly supply between visits 2, 3 and 4 will be provided to subjects during study visits or by mail (with delivery confirmation). Subjects will be asked to return all used and unused refill bottles, pods, devices, pouches and will be asked to document any deviations.

The primary psychoactive ingredient in the study products is nicotine. All products are currently available for commercial purchase and will be purchased by the research team either online or at

local stores (e.g., tobacco/vape shops, convenience store). These nicotine-containing products will be stored in a cool, dark location in laboratories or offices that are locked and only accessible to those with a key. When participants are randomized to an arm at Visit#1, the laboratory staff will provide the participant with the appropriate amount of product that will last until Visit#2, which is 7 days after Visit#1. At Visit#2, participants will receive enough product to last until scheduled Visit#3 and at this session participants will receive enough product to last until Visit#4 (if at any time in between visits participants are running low in supply, they will be instructed to notify study staff so that they may provide them with more product either by pick up or by mail).

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34 APPENDICES/ SUPPLEMENTS

Appendix A INVESTIGATOR STUDY ELIGIBILITY VERIFICATION FORM INCLUSION CRITERIA

Participant Name: _____

Participant ID: _____

Title: A Randomized, Parallel-group Open-label Trial of ENDS Users Switching from Flavors of Potentially High-toxicity Profile to Flavors of Potentially Low-toxicity Profile (CRoFT_3.2)

INCLUSION CRITERIA				
Yes	**No**	**N/A**	**All answers must be "Yes" or "N/A" for participant enrollment.**	**Date**
☐	☐	☐	1. Age ≥ 21 years and ≤55 years.	
☐	☐	☐	2. ENDS users as determined by: (a) using banned flavored ENDS with nicotine such as fruit, candy, dessert flavors, and/or any product that indicates such flavors (b) using ENDS daily, regularly for the past 6 months (self-reported).	
☐	☐	☐	3. No smoking tobacco or using smokeless tobacco for the past 6 months.	
☐	☐	☐	4. Subjects should be free of acute respiratory illness within the proceeding 30 days prior to recruitment (self-reported).	
☐	☐	☐	5. Participant must understand the investigational nature of this study and sign an Independent Ethics Committee/Institutional Review Board approved written informed consent form prior to receiving any study related procedure.	
☐	☐	☐	6. After receiving information about the NYS flavor ban, participants must still not want to continue vaping or stop using their flavor for the next 90 days.	

Investigator Signature: _____ **Date:** _____

Printed Name of Investigator: _____

Appendix B INVESTIGATOR STUDY ELIGIBILITY VERIFICATION FORM EXCLUSION CRITERIA

Participant Name: _____

Participant ID: _____

Title: A Randomized, Parallel-group Open-label Trial of ENDS Users Switching from Flavors of Potentially High-toxicity Profile to Flavors of Potentially Low-toxicity Profile (CRoFT_3.2)

EXCLUSION CRITERIA				
Yes	**No**	**N/A**	**All answers must be "No" or "N/A" for participant enrollment.**	**Date**
☐	☐	☐	1. Individuals with health conditions and therapies that may affect immune responses and levels of inflammatory markers, including allergic rhinitis, aspirin/NSAID therapy, asthma, any use of inhaled corticosteroid or injectable steroid, immunodeficiency (HIV or other), Guillain-Barre Syndrome, COPD, or fever/respiratory illness within 30 days prior to entry into study (self-reported).	
☐	☐	☐	2. Pregnant or nursing female participants (self-reported on telephone screener, pregnancy test on Visit#1)	
☐	☐	☐	3. Unable to communicate in English.	
☐	☐	☐	4. Unable or unwilling to follow protocol requirements.	
☐	☐	☐	5. Self-report having active, untreated medical/psychiatric conditions.	
☐	☐	☐	6. History of serious side effects from nicotine or from any nicotine replacement therapies.	
☐	☐	☐	7. Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.	
☐	☐	☐	8. Vulnerable populations, such as cognitively impaired adults, individuals who are not yet adults, pregnant women, and prisoners.	
☐	☐	☐	9. Any condition which in the Investigator's opinion deems the participant an unsuitable candidate to receive study drug.	
☐	☐	☐	10. After receiving information about the NYS flavor ban, participants who report that they are thinking about quitting or stop using their flavor within the next 90 days.	

Participant meets all entry criteria: ☐ **Yes** ☐ **No**

If "NO", do not enroll participant in study.

Investigator Signature: _____ **Date:** _____

Printed Name of Investigator: _____

Appendix C Recruitment Materials (Print Ad Copy)

ROSWELL PARK®
COMPREHENSIVE CANCER CENTER

RESEARCH PARTICIPANTS NEEDED

Researchers at Roswell Park are searching for **e-cigarettes users who are willing to participate in a research study.**

Participation involves 4 sessions that last 45 to 60 minutes each.

Must be **21-55 years old daily e-cigarette user to participate**.

Participants will be compensated for their time.

If interested, please contact **[716-845-3456]** and ask for the **FLAVOR** study

Appendix D Telephone Screening Questionnaire

Date	☐☐/☐☐/2022	
Participant number	☐☐☐☐☐☐☐☐☐☐	
Can you read and write in English? *(must be yes)*	Yes ☐	No ☐
What is your date of birth? *(age ≥ 21 years ≤ 55 years)*	_____/_____/_____	
Do you use re-fillable e-cigarettes every day or occasionally? *(must be every day)*	Every day ☐	Occasionally ☐
Have you used flavoured e-cigarettes regularly for the past 6 months *(must be yes)*	Yes ☐	No ☐
Do you use flavoured e-liquid containing nicotine to re-fill your device? *(must be yes)*	Yes ☐	No ☐
What flavors do you currently use? *(must be fruit, candy, dessert, or any combination)*		
Are you currently aware that your flavored product falls within the NYS flavor ban on vaping products? The ban became effective in May 2020 and prohibits the sale of flavored vaping products. Do you think you will quit vaping or stop using your flavored product in the next 90 days?	Yes ☐	No ☐
Check inclusion criteria. "Are you currently…."	**YES**	**NO**
In the past 6 months, have you smoked tobacco or used smokeless tobacco.	☐	☐
Pregnant/breastfeeding *(females only)*	☐	☐
In the past 30 days, have you had any acute respiratory illness?	☐	☐
History of respiratory allergy (house, dust, pollen), health conditions and therapies that may affect immune responses and levels of inflammatory markers, including: allergic rhinitis, aspirin/NSAID therapy, asthma, immunodeficiency (HIV or other), Guillain-Barre Syndrome, COPD, or fever/respiratory illness within past 30 days.	☐	☐
Are your currently being treated for any medical conditions (such as unstable heart disease, uncontrolled hypertension, thyroid disease, diabetes, renal/liver impairment, or glaucoma)	☐	☐
Are you currently being treated for any psychiatric conditions (such as schizophrenia or bipolar disorder) or do your currently use psychiatric medications (such as major tranquilizers and antidepressants)?	☐	☐
Are you currently being treated for any illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements?	☐	☐
Have you ever had any history of serious side effects from nicotine or from any nicotine replacement therapies?	☐	☐

NEED TO ANSWER 'NO' TO ALL CRITERIA. IF ANSWERED 'YES' TO ANY OF ABOVE: INELIGIBLE

How did you hear about our study? _____

If Ineligible:

Unfortunately we won't be able to conduct the study with you. However, we have studies running all the time so would you mind leaving us your name and number to contact you about other studies?

Name	Phone Number

We thank you for your interest and if you have any friends or family that may be interested in the study, feel free to pass along our number. Again we are sorry that it is not going to work. Goodbye.

If Eligible:

Great— it looks like you're eligible for the study. Can I have your first and last name and phone number in case we get disconnected?

Participant Name	Phone Number

Thank you. Now I'd like to give you some more information before asking whether you'd like to make an appointment for study Visit#1. The purpose of this study is to determine whether or not e-cigarette flavors may have adverse effects on the respiratory system when inhaled. In order to accomplish this, we ask that you come to 4 visits, over a 90 day period. We will randomize you into a specific group where you may be asked to switch to using a different e-liquid flavor over the 90 days, continue using your regular flavor over the 90 days on study or you might be in the group where you will be asked to stop vaping for 90 days and use an oral nicotine product provided. At Visit#1 you will be randomized into one of the three groups. The group selection is random based on a computer program so you cannot pick which group you wish to be in. At each visit you will fill out questionnaires about your e-cigarettes use and exposure, provide breath, urine and blood samples as well as provide us a small sample of your e-liquid flavor. After completion of each visit, please be sure to vape as usual over the next month before your next scheduled visit. In addition, we ask that you refrain from smoking tobacco and marijuana, use of smokeless tobacco and drugs of abuse during whole study and refrain from eating or drinking 1hr before each visit.

Is this something you think you are interested in doing?

If Answer No:

Thank you for your time. If you have any friends or family that may be interested in this study, feel free to pass along our number. Again thank you for your time. Goodbye.

If Answer Yes:

Great! I would also like to mention that this research has received ethics clearance from the Roswell Park Cancer Comprehensive Cancer Center Review Board and all the information you provide will be kept strictly confidential. Only the investigators directly associated with the study will have access to this information and it

will be destroyed once the study is completed. This study is funded by the National Institute of Health (NIH) and the investigators have no affiliations with any tobacco company or maker of nicotine products.

I realize I've given you a lot of information- do you have any questions about the study?

Would you like to make an appointment for Visit#1?

If no:

Thank you and goodbye.

If yes:

Great.

We will be conducting the sessions at Roswell. These are scheduled for [*time to be determined*] on [*day of week to be determined*]

DATE: _____ Time: _____

We anticipate that each study visit will take approximately 30-45 minutes. Remember to continue to vape as you normally do, but refrain from smoking tobacco, marijuana, use of smokeless tobacco and drugs. Also, please refrain from eating or drinking 1hr before each visit.

We will make a reminder call closer to your visit date, but feel free to call us if you have any questions or concerns before your visit.

Thank you!

Appendix E Demographics, Medical History and Drug Use

1. What is your birthdate?
   MM/DD/YYYY_____;
   [ ] Don't Know [ask follow-up question];
   [ ] Refused

2. [Follow-up question if "don't know":]
   About how old are you?
   AGE _____;
   [ ] Don't Know
   [ ] Refused

3. What is your biological sex assigned at birth?
   [ ] Female
   [ ] Male
   [ ] Intersex
   [ ] None of these describe me (optional free text)
   [ ] Prefer not to answer

4. What terms best express how you describe your gender identity? (Check all that apply)
   [ ] Man
   [ ] Woman
   [ ] Non-binary
   [ ] Transgender
   [ ] None of these describe me, and I'd like to consider additional options (if selected see below (4b) for branching question)
   [ ] Prefer not to answer

4b. Are any of these a closer description to your gender identity?
   [ ] Trans man/Transgender Man/FTM
   [ ] Trans woman/Transgender Woman/MTF
   [ ] Genderqueer
   [ ] Genderfluid
   [ ] Gender variant
   [ ] Questioning or unsure of your gender identity
   [ ] None of these describe me, and I want to specify_____

5. Are you…
   [ ] Married
   [ ] Divorced
   [ ] Widowed
   [ ] Separated
   [ ] Never married
   Or
   [ ] A member of an unmarried couple
   [ ] Refused

6. What race or races do you consider yourself to be? Please select one or more.

CHECK ALL THAT APPLY.

[ ] 1 AMERICAN INDIAN OR ALASKA NATIVE
[ ] 2 ASIAN [GO TO Q2]
[ ] 3 BLACK OR AFRICAN AMERICAN
[ ] 4 NATIVE HAWAIIAN OR PACIFIC ISLANDER [GO TO Q3]
[ ] 5 WHITE
[ ] 6 OTHER
[ ] 99 DON'T KNOW
[ ] 77 REFUSED

7. Do you consider yourself to be Hispanic, Latino, or of Spanish origin?

[ ] 1 YES [ask follow-up question]
[ ] 2 NO
[ ] 7 REFUSED
[ ] 9 DON'T KNOW

8. READ IF NECESSARY: Where do your ancestors come from?
[ ] Puerto Rican
[ ] Cuban/Cuban American
[ ] Dominican Republic
[ ] Mexican/Mexican American
[ ] Central/South American
[ ] Other Latin American
[ ] Other Hispanic or Latino

9. What is your best estimate of the total income of all family members from all sources, before taxes, in [last calendar year?
[ ]Less than $10,000
[ ]$10,000 to $14,999
[ ]$15,000 to $19,999
[ ] $20,000 to $24,999
[ ]$25,000 to $29,999
[ ] $30,000 to $34,999
[ ]$35,000 to $39,999
[ ]$40,000 to $49,999
[ ] $50,000 to $74,999
[ ]$75,000 to $99,999
[ ]$100,000 to $149,999
[ ]$150,000 to $199,999
[ ]$200,000 or more
[ ]Prefer not to say

10. What is the highest grade or level of school you have completed or the highest degree you have received?
    [ ] 8TH GRADE;
    [ ] 12TH GRADE, NO DIPLOMA;
    [ ] HIGH SCHOOL GRADUATE;
    [ ] GED OR EQUIVALENT;
    [ ] SOME COLLEGE, NO DEGREE;
    [ ] ASSOCIATE DEGREE
    [ ] BACHELOR'S DEGREE (EXAMPLE: BA, AB, BS, BBA);
    [ ] MASTER'S DEGREE or Higher
    [ ] REFUSED;
    [ ] DON'T KNOW

11. We would like to know about what you do --are you working now, looking for work, retired, keeping house, a student, or what?
    [ ] 1 WORKING NOW
    [ ] 2 ONLY TEMPORARILY LAID OFF, SICK LEAVE OR MATERNITY LEAVE
    [ ] 3 LOOKING FOR WORK, UNEMPLOYED
    [ ] 4 RETIRED
    [ ] 5 DISABLED, PERMANENTLY OR TEMPORARILY
    [ ] 6 KEEPING HOUSE
    [ ] 7 STUDENT
    [ ] 8 OTHER (SPECIFY):_____

12. Are you pregnant now?

    [ ] YES
    [ ] NO

13. Do you currently take any of the following pain-relieving medications **regularly** (at least once a week)?	No	If yes, then please indicate total tablets per week									
	0 or <1 per week	1-2	3-4	5-6	7-8	9-10	11-12	13-14	15-21	22-28	29+
'Baby' or low-dose aspirin											
Aspirin or aspirin-containing product (Bayer®, Bufferin®, Excedrin®)											
Ibuprofen (Advil®, Motrin®)											
Naproxen, ketoprofen or other non-steroidal (Aleve®, Feldene®, Indocin®, Naprosyn®, Orudis®, Relafen®)											
Cox-2 inhibitor (Celebrex®, Vioxx®)											
Acetaminophen (Aspirin-free Excedrin®, Tylenol®, Tempra®)											

Never took regularly or did not stop use	**Yes, I stopped regular use**	**Condition improved**	**Don't work**	**I had side effects**	**I heard about side effects**	**Drug no longer available**	**Other**

14. In the past 3 years, please indicate if you have taken either of the following types of medications.	**No**	**Yes, regularly (daily for at least 2 months)**	**Yes, but not regularly**
Statin medications such as lovastatin (Mevacor®), atorvastatin (Lipitor®), rosuvastain (Crestor®), pravastatin (Pravachol®), simvastatin (Zocor®), fluvastatin (Lescol®)			
Steroid medication in pill form such as prednisone, dexamethasone (Decadron®), solumedrol (Medrol dose-pack®)			

15. Have you EVER used any of these medicines or drugs? *Read list. (If "YES" to any drug category, ask:* **Which ones?**) *Record specific drug(s) used.*

1 [ ] Sedatives or tranquilizers, for example…barbs, downers, Am'-bee-en, Lunesta, phenobarbital, pentobarbital, Hal'-see-on, Tuinal, Nembutal, Seconal, Librium, Valium, Xanax, benzodiayz'-a-peens, tranks, Ativan.
2 [ ] Painkillers, for example…methadone, codeine, Demerol, Vy'-ko-din, Oxi-kon'-tin, opium, oxy, Per'-ko-set, Dill-odd'-id, Per'-ko-dan, morphine.
3 [ ] Mariwa'-na, including THC, for example…weed, pot, dope, hashish, Mary Jane, joint, blunt.
4 [ ] Cocaine or crack, for example…blow, rock, snow.
5 [ ] Stimulants, for example…Add'-erall, Concerta, Sy'-lert, Pro-vig'-il, Ritalin or Dexedrine, speed, amphetamine, methamphetamine, uppers, bennies, pep pills, crystal, crank.
6 [ ] Club drugs, for example…MDMA, ecstasy, GHB, Ro-hip'-nol, kett'-amine, Special K, XTC, roofies.
7 [ ] Hallucinogens, for example…LSD, acid, PCP, mescaline, pay-o'-tee, sillosy'-bin, mushrooms, angel dust, cactus.
8 [ ] Inhalants or solvents, for example…nitrous oxide, lighter fluid, gasoline, cleaning fluid, glue, poppers, whippets.
9 [ ] Heroin, for example…smack, black tar, poppy.
10 [ ] Any OTHER medicines or drugs, for example…steroids, Elavil, Thorazine, or Haldol.

Is at least one category marked in 13?

1 [ ] Yes - *Classify as ever (drug) use*
2 [ ] No - *Classify as non (drug) user*

NOW we are going to ask about FRUIT-FLAVORED drinks WITH ADDED SUGAR.

16. How often did you drink FRUIT-FLAVORED DRINKS with sugar (such as Kool-Aid &r e.g., Hi-C & reg;, lemonade, or cranberry cocktail)? Do NOT include diet drinks.

*Read if necessary: INCLUDE Gatorade &r e.g., and other sports drinks with added sugar. INCLUDE Tampico & reg;, Sunny Delight ® and Twister & reg;. Do NOT include 100% fruit juices or soda. Do NOT include yogurt drinks or carbonated water.

[ ] Never
[ ] 1-3 times last month
[ ] 1-2 times per week
[ ] 3-4 times per week
[ ] 5-6 times per week
[ ] 1 time per day
[ ] 2 times per day
[ ] 3 times per day
[ ] 4 times per day
[ ] 5 or more times per day
[ ] Refused
[ ] Don't know

17. During the past month, how often did you drink 100% FRUIT JUICE, such as orange, mango, apple, and grape juices? Do NOT count fruit drinks.

*Read if necessary: INCLUDE only 100% pure juices. Do NOT include fruit drinks with added sugar, like Kool-Aid & reg;, Hi-C & reg;, lemonade, cranberry cocktail, Gatorade & reg;, Tampico & reg, and Sunny Delight & reg;.

[ ] Never
[ ] 1-3 times last month
[ ] 1-2 times per week
[ ] 3-4 times per week
[ ] 5-6 times per week
[ ] 1 time per day
[ ] 2 times per day
[ ] 3 times per day
[ ] 4 times per day
[ ] 5 or more times per day
[ ] Refused
[ ] Don't know

18. During the past month . . . How often did you eat FRUIT? COUNT fresh, frozen, or canned fruit. Do NOT count juices.

*Read if necessary: Include fruits such as apples, bananas, applesauce, melon, berries, fruit salad, mangos, papayas, oranges, and grapes.

[ ] Never
[ ] 1-3 times last month
[ ] 1-2 times per week
[ ] 3-4 times per week
[ ] 5-6 times per week
[ ] 1 time per day
[ ] 2 times per day
[ ] 3 times per day
[ ] 4 times per day
[ ] 5 or more times per day
[ ] Refused
[ ] Don't know

Appendix F History of Tobacco Use

1. Which of the following **electronic nicotine products** have you used? Choose all that apply.

1 [ ] E-cigarette including vape pens, hookah pens, personal vaporizers, and mods
2 [ ] E-cigar
3 [ ] E-pipe
4 [ ] E-hookah
5 [ ] Something else (SPECIFY)
-8 [ ] DON'T KNOW
-7 [ ] REFUSED

2. Please think about the *E-cigarette* you use most of the time.

Is your ***E-cigarette*** rechargeable?

1 [ ] Yes
2 [ ] No
-8 [ ] DON'T KNOW
-7 [ ] REFUSED

ASK: Past 30 day "not light" e-product users.

3. Does your ***E-cigarette*** use a tank system?

1 [ ] Yes
2 [ ] No
3 [ ] I don't know
-8 [ ] DON'T KNOW
-7 [ ] REFUSED

ASK: Past 30 day "not light" e-product users.

4. How many milliliters of e-liquid does your tank system hold?

1 [ ] |__||__|
2 [ ] I don't know
-8 [ ] DON'T KNOW
-7 [ ] REFUSED

ASK: Past 30 day "not light" e-product users that use refillable e-products (Q6 = 1).

5. Does your ***E-cigarette*** use cartridges?

1 [ ] Yes
2 [ ] No
-8 [ ] DON'T KNOW

-7 [ ] REFUSED

ASK: Past 30 day "not light" e-product users.

6. Can you refill your **"*E-cigarette*"** cartridges with "e-liquid"?

1 [ ] Yes
2 [ ] No
-8 [ ] DON'T KNOW
-7 [ ] REFUSED

ASK: Past 30 day "not light" e-product users.

DISPLAY: If Q5 = 1, display" *E-cigarette* cartridges", else display "*E-cigarette* ".

7. Can you change the voltage on your ***E-cigarette***?

1 [ ] Yes
2 [ ] No
3 [ ] I don't know
-8 [ ] DON'T KNOW
-7 [ ] REFUSED

ASK: Past 30 day "not light" e-product users that use a rechargeable e-product (Q2 = 1, -8 or -7).

8. Do you change the voltage on your ***E-cigarette****?*

1 [ ] Yes
2 [ ] No
3 [ ] I don't know
-8 [ ] DON'T KNOW
-7 [ ] REFUSED

ASK: Past 30 day "not light" e-product users that can change their voltage (Q7 = 1).

9. How long have you used E-cigarettes?

   _____ Years

   _____Months
10. When you first started using e-cigarettes, were they flavored to taste like menthol, mint, clove, spice, fruit, chocolate, alcoholic drinks, candy or other sweets?
    [] Yes
    [] No
    [] Don't know
    [] Refused

11. Which flavor did you first start using? Choose all that apply.

[] Menthol or Mint
*Name:*_____if checked participant prompted to write in name

[] Clove or Spice
*Name:*_____if checked participant prompted to write in name

[] Fruit
*Name:*_____if checked participant prompted to write in name

[] Chocolate
*Name:*_____if checked participant prompted to write in name

[] An alcoholic drink (wine, cognac, margarita, or other cocktail)
*Name:*_____if checked participant prompted to write in name

[] Candy or other sweets
*Name:*_____if checked participant prompted to write in name

[] Tobacco Flavor

[] Unflavored

[] Some other flavor
*Name:*_____if checked participant prompted to write in name

[] Don't know
[] Refused

12. Check all the flavors you most commonly use? (check all that apply)
    [] Menthol or Mint
    [] Clove or Spice
    [] Fruit
    [] Chocolate
    [] An alcoholic drink (wine, cognac, margarita, or other cocktail)
    [] Candy or other sweets
    [] Tobacco Flavor
    [] Unflavored
    [] Some other flavor
    [] Don't know
    [] Refused

13. Out of all the flavors you most commonly use, which would you say is the one you use most often? (only check one answer)
    [] Menthol or Mint
    [] Clove or Spice
    [] Fruit
    [] Chocolate
    [] An alcoholic drink (wine, cognac, margarita, or other cocktail)

[] Candy or other sweets
[] Tobacco Flavor
[] Unflavored
[] Some other flavor
[] Don't know
[] Refused

14. Out of all the flavors you most commonly use, which one flavor is your absolute favorite? (only check one answer)
[] Menthol or Mint
[] Clove or Spice
[] Fruit
[] Chocolate
[] An alcoholic drink (wine, cognac, margarita, or other cocktail)
[] Candy or other sweets
[] Tobacco Flavor
[] Unflavored
[] Some other flavor
[] Don't know
[] Refused

15. What level of nicotine do you most commonly use?
[] 0-2 mg
[] 3-5 mg
[] 6-11 mg
[] 12-17mg
[] 18-23 mg
[] 24 or more mg
[] Other
*Please Specify:*_____if checked participant prompted to write in details

16. If your most commonly used nicotine level was not available in the flavor you use most often, would you…
[] choose a different flavor in your regular nicotine level
[] choose your most commonly used flavor in a lower nicotine level
[] choose your most commonly used flavor in a higher nicotine level
[] go somewhere else to try and find your most commonly used flavor and nicotine level

17. If you typically use NON tobacco and NON menthol flavor, and tobacco and menthol flavors were the only flavors commercially available for e-cigarettes would you..
[] use tobacco flavor
[] use menthol flavor
[] make my own flavors
[] buy flavors that are not tobacco or menthol illegally, which are unregulated
[] I already use tobacco and/ or menthol flavors
18. How would you say you feel about vaping tobacco flavored e-liquid?
[] like it a lot
[] like it a little

[] could tolerate it
[] don't like it at all
[] would rather smoke cigarettes

19. How would you say you feel about vaping menthol flavored e-liquid?
    [] like it a lot
    [] like it a little
    [] could tolerate it
    [] don't like it at all
    [] would rather smoke cigarettes

20. Have you ever tried a flavor(s) that made you feel sick?
    [] Yes
    [] No

21. If yes, please list all the flavors that ever made you feel sick.
    1_____
    2_____
    3_____
    4_____
    5_____

For each above flavor listed, the participant will be promoted to check all that apply to the below symptoms they may have felt when using the specific flavor.

□ Cough
□ Coughing stuff up
□ Coughing interfering with sleep
□ Sore throat
□ Scratchy throat
□ Hoarseness
□ Runny Nose
□ Plugged nose
□ Sneezing
□ Headache
□ Body aches
□ Feeling "run-down"
□ Sweats
□ Chills
□ Feeling feverish
□ Feeling dizzy
□ Feeling tired
□ Irritability
□ Sinus pain
□ Sinus pressure
□ Sinus drainage
□ Swollen glands
□ Plugged ears
□ Ear discomfort

□ Watery eyes
□ Eye discomfort
□ Head congestion
□ Chest congestion
□ Chest tightness
□ Heaviness in chest

22. In your ENTIRE LIFE, have you ever:
    a. Smoked at least 50 cigars?
        1 [ ] Yes
        2 [ ] No
        3 [ ] Don't Know / Refused
    b. Smoked a pipe at least 50 times?
        1 [ ] Yes
        2 [ ] No
        3 [ ] Don't Know / Refused
    c. Used snuff, such as Skoal®, Skoal Bandit® or Copenhagen® at least 20 times?
        1 [ ] Yes
        2 [ ] No
        3 [ ] Don't Know / Refused
    d. Used chewing tobacco, such as Redman®, Levi Garrett® or Beechnut® at least 20 times?

        1. [ ] Yes

        2. [ ] No

        3. [ ] Don't Know / Refused

23. Have you ever smoked a cigarette, even one or two puffs?

        1 [ ] Yes
        2 [ ] No [GO TO Q6]
        -8 [ ] DON'T KNOW [GO TO Q6]
        -7 [ ] REFUSED [GO TO Q6]

    ASK: All respondents
24. Do you now smoke cigarettes…
        1 [ ] Every day
        2 [ ] Some days
        3 [ ] Not at all [GO TO Q3]
        -8 [ ] DON'T KNOW [GO TO Q3]
        -7 [ ] REFUSED [GO TO Q3]

    ASK: Respondents who have ever smoked a cigarette (Q1 = 1)

25. In the past 30 days, have you smoked a cigarette, even one or two puffs?

1 [ ] Yes
2 [ ] No
-8 [ ] REFUSED
-7 [ ] DON'T KNOW

ASK: Respondents who do not currently smoke every day or some days (Q2 = 3)

26. How many cigarettes have you smoked in your entire life? A pack usually has 20 cigarettes in it.

1 [ ] 1 or more puffs but never a whole cigarette
2 [ ] 1 to 10 cigarettes (about ½ pack total)
3 [ ] 11 to 20 cigarettes (about ½ pack to 1 pack)
4 [ ] 21 to 50 cigarettes (more than 1 pack but less than 3 packs)
5 [ ] 51 to 99 (more than 2 ½ packs but less than 5 packs)
6 [ ] 100 or more cigarettes (5 packs or more)
-8 [ ] DON'T KNOW
-7 [ ] REFUSED

ASK: Respondents who have ever smoked a cigarette (Q1 =1)

27. About how long has it been since you completely quit smoking cigarettes?

1 [ ] I___I___I DAYS
2 [ ] I___I___I MONTHS
3 [ ] I___I___I YEARS
-8 [ ] DON'T KNOW
-7 [ ] REFUSED

ASK: Former users (IF Q1 = 1 AND Q4 = 5 AND Q2 = 3) and Experimental Former users (IF Q1 = 1 AND Q4 = 1, 2, 3, 4 AND Q2 = 3). Else go to next section (Q6).

Appendix G History of Switching from Tobacco to ENDS

1. Did you **ever use** or **switch** from tobacco cigarettes to E-cigarettes to reduce your health risk?
   [ ] Yes
   [ ] No
   [ ] Refused
   [ ] Don't know

2. Have you **ever** stopped smoking for one day or longer **because you were trying to quit smoking?**

   [ ] Yes
   [ ] No
   [ ] Refused
   [ ] Don't know

3. In your **whole life**, how many times have you stopped smoking for one day or longer **because you were trying to quit smoking**?

   [ ] 1-994 times
   [ ] 995+ times
   [ ] Refused
   [ ] Don't know

Appendix H Use of Flavored Product

To help us evaluate your product use while on study, we need to get an idea of what your use pattern was like in the past __30__ days. To do this, we would like you to fill out the attached calendar.

✓ Filling out the calendar is not hard!

✓ Try to be as accurate as possible.

✓ We recognize you won't have perfect recall. That's OKAY.

✓ WHAT TO FILL IN

•The idea is to record exactly what you used for **each day** on the calendar.

•On days when you **did not use any product**, not even one, you should write a "0."

It's important that something is written for every day, even if it is a 0".

✓ YOUR BEST ESTIMATE

•We realize it isn't easy to recall things with 100% accuracy.

•If you are not sure whether you used 1 or 2 or more products or whether you used them on a Thursday or a Friday, give it your best guess! What is important is that 2 or more products is very different from 1 product. The goal is to get a sense of how well you are able or not able to adhere to the study product provided throughout the time on study.

✓ HELPFUL HINTS

•If you have an appointment book you can use it to help you recall your use.

•Holidays such as Thanksgiving and Christmas are marked on the calendar to help you recall your smoking. Also, think about how much you use products personal holidays & events such as birthdays, vacations, or parties.

•If you have **regular patterns to your product use**, you can use these to help you recall your use..

✓ COMPLETING THE CALENDAR

•A blank calendar is attached. Write in the product(s) you used on **each day and what flavors they were**.

• The time period we are talking about on the calendar is

from _____ to _____.

•In estimating the number of flavors, be as accurate as possible.

•DOUBLE CHECK THAT <u>ALL</u> DAYS ARE FILLED IN BEFORE RETURNING THE CALENDAR.

• Before you start look at the **SAMPLE CALENDAR**.

✓ SAMPLE CALENDAR

2000	SUN	MON	TUES	WED	THURS	FRI	SAT
S E P T						1 20	2 0
	3 20	4 Labor Day 20	5 23	6 28	7 21	8 20	9 23
	10 20	11 20	12 20	13 28	14 25	15 0	16 24
	17 20	18 20	19 20	20 20	21 22	22 22	23 24
	24 21	25 22	26 26	27 24	28 23	29 0	30 22

1. Based on the calendar you just filled out, what was the most common flavor you used in the past 30 days? (Please only type in one flavor)
   Subject prompted to fill in answer _____

2. In the past 30 days, what was your **favorite** flavor that you used? (Please only type in one flavor)
   Subject prompted to fill in answer _____

3. In the past 30 days, what level of nicotine did you most commonly use?
   [] 0-2 mg
   [] 3-5 mg
   [] 6-11 mg
   [] 12-17mg
   [] 18-23 mg
   [] 24 or more mg
   [] Other
   *Please Specify:*_____if checked participant prompted to write in details

4. If your most commonly used nicotine level was not available in the flavor you use most often in the last 30 days, would you…
   [] choose a different flavor in your regular nicotine level
   [] choose your most commonly used flavor in a lower nicotine level

[] choose your most commonly used flavor in a higher nicotine level
[] go somewhere else to try and find your most commonly used flavor and nicotine level

Appendix I:Flavor Preference

1. When you first started using e-cigarettes, were they flavored to taste like menthol, mint, clove, spice, fruit, chocolate, alcoholic drinks, candy or other sweets?
   [] 1 Yes
   [] 2 No
   [] -8 Don't Know
   [] -7 Refused

2. Which flavor did you first start using? If multiple flavors were mixed together, choose all that apply.

   [] Menthol or mint
   *Name:*_____
   [] Clove or spice
   *Name:*_____
   [] Fruit
   *Name:*_____
   [] Chocolate
   *Name:*_____
   [] An alcoholic drink (such as wine, cognac, margarita or other cocktails)
   *Name:*_____
   [] Candy or other sweets
   *Name:*_____
   [] Tobacco flavor
   [] Unflavored
   [] Some other flavor
   *Name:*_____

   [] -8 Don't Know
   [] -7 Refused

3. Which flavors have you used in the past 30 days (since your last visit)? Choose all that apply.

   [] Menthol or mint
   *Name:*_____
   [] Clove or spice
   *Name:*_____
   [] Fruit
   *Name:*_____
   [] Chocolate
   *Name:*_____
   [] An alcoholic drink (such as wine, cognac, margarita or other cocktails)
   *Name:*_____
   [] Candy or other sweets
   *Name:*_____

[] Tobacco flavor
[] Unflavored
[] Some other flavor
*Name:*_____

[] -8 Don't Know
[] -7 Refused

4. Which flavor [is/was] it? If multiple flavors were mixed together, choose all that apply.

[] Menthol or mint
*Name:*_____
[] Clove or spice
*Name:*_____
[] Fruit
*Name:*_____
[] Chocolate
*Name:*_____
[] An alcoholic drink (such as wine, cognac, margarita or other cocktails)
*Name:*_____
[] Candy or other sweets
*Name:*_____
[] Tobacco flavor
[] Unflavored
[] Some other flavor
*Name:*_____

[] -8 Don't Know
[] -7 Refused

5. In the past 30 days, have you tried a new flavor that you have never had before?

[] Menthol or mint
*Name:*_____
[] Clove or spice
*Name:*_____
[] Fruit
*Name:*_____
[] Chocolate
*Name:*_____
[] An alcoholic drink (such as wine, cognac, margarita or other cocktails)
*Name:*_____
[] Candy or other sweets
*Name:*_____
[] Tobacco flavor
[] Unflavored

[] Some other flavor
*Name:*_____

[] -8 Don't Know
[] -7 Refused

6. Do you still use the new flavor you tried in the past 30 days?
   [] Yes
   [] No

7. If no, why did you stop using it?
   [] Did not like taste
   [] I felt sick (if this box is checked-participant will be prompted to check how they felt-(see below for the symptoms)
   [] other _____
   - □ Cough
   - □ Coughing stuff up
   - □ Coughing interfering with sleep
   - □ Sore throat
   - □ Scratchy throat
   - □ Hoarseness
   - □ Runny Nose
   - □ Plugged nose
   - □ Sneezing
   - □ Headache
   - □ Body aches
   - □ Feeling "rundown"
   - □ Sweats
   - □ Chills
   - □ Feeling feverish
   - □ Feeling dizzy
   - □ Feeling tired
   - □ Irritability
   - □ Sinus pain
   - □ Sinus pressure
   - □ Sinus drainage
   - □ Swollen glands
   - □ Plugged ears
   - □ Ear discomfort
   - □ Watery eyes
   - □ Eye discomfort
   - □ Head congestion
   - □ Chest congestion
   - □ Chest tightness
   - □ Heaviness in chest
   - □ Lack of energy
   - □ Loss of appetite

8. Have you ever tried a flavor(s) that made you feel sick? ***(only asked on first visit)***
   [] Yes
   [] No

9. If yes, please list all the flavors that ever made you feel sick. ***(only asked on first visit)***
   1_____
   2_____
   3_____
   4_____
   5_____

For each above flavor listed, the participant will be promoted to check all that apply to the below symptoms they may have felt when using the specific flavor.

□ Cough
□ Coughing stuff up
□ Coughing interfering with sleep
□ Sore throat
□ Scratchy throat
□ Hoarseness
□ Runny Nose
□ Plugged nose
□ Sneezing
□ Headache
□ Body aches
□ Feeling "run-down"
□ Sweats
□ Chills
□ Feeling feverish
□ Feeling dizzy
□ Feeling tired
□ Irritability
□ Sinus pain
□ Sinus pressure
□ Sinus drainage
□ Swollen glands
□ Plugged ears
□ Ear discomfort
□ Watery eyes
□ Eye discomfort
□ Head congestion
□ Chest congestion
□ Chest tightness
□ Heaviness in chest
□ Lack of energy
□ Loss of appetite

Appendix J Use of Regular Brand

1. About how long has it been since you last took a puff from an e-cigarette? (If it was earlier today, enter 1 day).

   [ ] I___I___I MINUTES
   [ ] DON'T KNOW
   [ ] REFUSED

2. In the last 30 days (since your last visit) where did you purchase your e-liquid from? Check all that apply.

   [] Online *Name:*_____if checked participant prompted to write in name
   [] I make my own
   [] Local vapor shop *Name:*_____if checked participant prompted to write in name
   [] Local Native Territory *Location:*_____if checked participant prompted to write in name
   [] Other *Please Specify:*_____if checked participant prompted to write in details

3. In the last 30 days (since your las visit) about how much e-liquid did you purchase?
   (For example if you bought two 15mL bottles then you purchased 30mL total, if you purchased a 30mL bottle and a 15mL bottle then you bought 45mL total)

   I bought approximately _____mL

4. Did you use up all the e-liquid you purchased in the last 30 days?

   [] Yes
   [] No

5. In the last 30 days (since your last visit), what level (s) of nicotine were in your e-liquid that you purchased? Check all that apply.

   [] 0-2 mg
   [] 3-5 mg
   [] 6-11 mg
   [] 12-17mg
   [] 18-23 mg
   [] 24 or more mg
   [] Other *Please Specify:*_____if checked participant prompted to write in details

Appendix K Personal History of Respiratory Symptoms/Diseases (past 24 hours and past 30 days) *issued 2 times to the participant

Content of the Wisconsin Upper Respiratory Symptom Survey (WURSS-44)

Symptoms	Symptoms	Symptoms	Functional impairments
1. How sick do you feel? [Gt]	12. Body aches **[A]**	23. Swollen glands **[A]**	***34. Think clearly*** **[F]**
2. Cough **[C]**	13. Feeling "run down" **[Ti]**	24. Plugged ears **[E]**	35. Speak clearly **[F]**
3. Coughing stuff up **[C]**	14. Sweats **[Sw]**	25. Ear discomfort **[E]**	***36. Sleep well*** **[F]**
4. Cough interfering with sleep **[C]**	15. Chills **[Sw]**	26. Watery eyes **[O]**	***37. Breathe easily*** **[F]**
5. Sore throat **[Th]**	16. Feeling feverish **[Sw]**	27. Eye discomfort **[O]**	***38. Walk, climb stairs, exercise*** **[F]**
6. Scratchy throat **[Th]**	17. Feeling dizzy **[O]**	***28. Head congestion*** **[O]**	***39. Accomplish daily activities*** **[F]**
7. Hoarseness **[Th]**	***18. Feeling tired*** **[Ti]**	***29. Chest congestion*** **[Ch]**	***40. Work outside the home*** **[F]**
8. Runny nose **[N]**	19. Irritability **[O]**	30. Chest tightness **[Ch]**	***41. Work inside the home*** **[F]**
9. Plugged nose **[N]**	20. Sinus pain **[Si]**	31. Heaviness in chest **[Ch]**	***42. Interact with others*** **[F]**
10. Sneezing **[N]**	21. Sinus pressure **[Si]**	32. Lack of energy **[Ti]**	***43. Live your personal life*** **[F]**
11. Headache **[Si]**	22. Sinus drainage **[Si]**	33. Loss of appetite **[O]**	***44. Compared to yesterday [Gy]***

Items selected for WURSS-21 are in bold italics

Directions for items (2 – 33): "Please rate the average severity of your cold symptoms over the last 24 hours or 30 days by marking the appropriate circle for each of the following symptoms."

Response options range 0 to 7, with 0 = Do not have, 1 = Very mild, 3 = Mild, 5 = Moderate, 7 = Severe

Directions for items (34 – 43): "Over the last 24 hours or 30 days, how much has your cold interfered with your ability to..."

Response options are 0 to 7, with 0 = Not at all, 1 = Very mildly, 3 = Mildly, 5 = Moderately, 7 = Severely

Factor analysis for original validation study identified 10 domains: C = Cough; Th = Throat; N = Nasal; A = Aches; Ti = Tired; Si = Sinus/headache; Sw = Sweats/chills/fever; E = Ears; Ch = Chest; F = Functional/activity

Gt = Global severity today; Gy = Global change since yesterday; O = Did not fit within any domain

Appendix L Quality of Life as Affected by Respiratory Condition (past 30 days)

St. George's Respiratory Questionnaire (SGRQ)

Please tick in one box to show how you describe your current health:

□ Very Good □ Good □ Fair □ Poor □ Very Poor

Part 1

Questions about how much chest trouble you have had over the past 3 months.

Please tick (x) one box for each question:

	Most days a week	Several days a week	A few days a month	Only with chest infections	Not at all
1. Over the past 3 months, I have coughed:	☐	☐	☐	☐	☐
2. Over the past 3 months, I have brought up phlegm(sputum):	☐	☐	☐	☐	☐
3. Over the past 3 months, I have had shortness of breath:	☐	☐	☐	☐	☐
4. Over the past 3 months, I have had attacks of wheezing:	☐	☐	☐	☐	☐

5. During the past 3 months how many severe or very unpleasant attacks of chest trouble have you had? Please tick (x) one:

More than 3 attacks ☐
3 attacks ☐
2 attacks ☐
1 attack ☐
No attacks ☐

6. How long did the worst attack of chest trouble last? (Go to question 7 if you had no severe attacks) Please tick (x) one:

A week or more ☐
3 or more days ☐
1 or 2 days ☐
Less than a day ☐

7. Over the past 3 months, in an average week, how many good days (with little chest trouble) have you had? Please tick (x) one:

No good days ☐
1 or 2 good days ☐
3 or 4 good days ☐
Nearly every day is good ☐
Every day is good ☐

8. If you have a wheeze, is it worse in the morning? Please tick (x) one:

No ☐
Yes ☐

Part 2

Section 1	
How would you describe your chest condition?	Please tick (x) one:
	The most important problem I have ☐
	Causes me quite a lot of problems ☐
	Causes me a few problems ☐
	Causes no problem ☐
If you have ever had paid employment.	Please tick (x) one:
	My chest trouble made me stop work altogether ☐
	My chest trouble interferes with my work or made me change my work ☐
	My chest trouble does not affect my work ☐

Section 2		
Questions about what activities usually make you feel breathless these days.	Please tick (x) in each box that applies to you **these days**:	
	True	False
Sitting or lying still	☐	☐
Getting washed or dressed	☐	☐
Walking around the home	☐	☐
Walking outside on the level	☐	☐
Walking up a flight of stairs	☐	☐
Walking up hills	☐	☐
Playing sports or games	☐	☐

Section 3		
Some more questions about your cough and breathlessness these days.	Please tick (x) in each box that applies to you **these days**:	
	True	False
My cough hurts	☐	☐
My cough makes me tired	☐	☐
I am breathless when I walk	☐	☐
I am breathless when I bend over	☐	☐
My cough or breathing disturbs my sleep	☐	☐
I get exhausted easily	☐	☐

Section 4		
Questions about other effects that your chest trouble may have on you these days.	Please tick (x) in each box that applies to you **these days**:	
	True	False
My cough or breathing is embarrassing in public	☐	☐
My chest trouble is a nuisance to my family, friends or neighbors	☐	☐
I get afraid or panic when I cannot get my breath	☐	☐
I feel that I am not in control of my chest problem	☐	☐

	True	False
I do not expect my chest to get any better	☐	☐
I have become frail or an invalid because of my chest	☐	☐
Exercise is not safe for me	☐	☐
Everything seems too much of an effort	☐	☐

Section 5

Questions about your medication, if you are receiving no medication go straight to section 6.

Please tick (x) in each box that applies to you **these days**:

	True	False
My medication does not help me very much	☐	☐
I get embarrassed using my medication in public	☐	☐
I have unpleasant side effects from my medication	☐	☐
My medication interferes with my life a lot	☐	☐

Section 6

These are questions about how your activities might be affected by your breathing.

Please tick (x) in each box that applies to you **because of your breathing**:

	True	False
I take a long time to get washed or dressed	☐	☐
I cannot take a bath or shower, or I take a long time	☐	☐
I walk slower than other people, or I stop for rests	☐	☐
Jobs such as housework take a long time, or I have to stop for rests	☐	☐
If I walk up a flight of stairs, I have to go slowly or stop	☐	☐
If I hurry or walk fast, I have to stop or slow down	☐	☐
My breathing makes it difficult to do things such as walk up hills, carrying things upstairs, light gardening such as weeding, dance, play bowls or play golf	☐	☐
My breathing makes it difficult to do things such as carry heavy loads, dig the garden or shovel snow, jog or walk at 5 miles per hour, play tennis or swim	☐	☐
My breathing makes it difficult to do things such as very heavy manual work, run, cycle, swim fast or play competitive sports	☐	☐

Section 7

We would like to know how your chest <u>usually</u> affects your daily life.

Please tick (x) in each box that applies to you **because of your chest trouble**:

	True	False
I cannot play sports or games	☐	☐
I cannot go out for entertainment or recreation	☐	☐
I cannot go out of the house to do the shopping	☐	☐
I cannot do housework	☐	☐
I cannot move far from my bed or chair	☐	☐

Here is a list of other activities that your chest trouble may prevent you doing. (You do not have to tick these, they are just to remind you of ways in which your breathlessness may affect you):
Going for walks or walking the dog
Doing things at home or in the garden
Sexual intercourse
Going out to church, pub, club or place of entertainment
Going out in bad weather or into smoky rooms
Visiting family or friends or playing with children
Please write in any other important activities that your chest trouble may stop you doing:

. . . . . .
.. . .
. . . . . .
.. . .
.

Now would you tick in the box (one only) which you think best describes how your chest affects you:

It does not stop me doing anything I would like to do ☐
It stops me doing one or two things I would like to do ☐
It stops me doing most of the things I would like to do ☐
It stops me doing everything I would like to do ☐

Thank you for filling in this questionnaire. Before you finish would you please check to see that you have answered all the questions.

Appendix M Secondhand Exposure

1. Not counting decks, porches, or garages, during the past 30 days, that is, on how many days did **someone other than you** smoke tobacco inside your home while you were at home?

   [ ] NUMBER OF DAYS _____
   [ ] NONE
   [ ] DON'T KNOW/NOT SURE
   [ ] REFUSED

2. Not counting decks, porches, or garages, to your knowledge, during the past 30 days, has anyone, including yourself, smoked tobacco inside your home when he or she was not supposed to?

   [ ] YES
   [ ] NO
   [ ] DON'T KNOW/NOT SURE
   [ ] REFUSED

3. To your knowledge, during the past 30 days, that is, has anyone, including yourself, used tobacco products of any kind, including electronic cigarettes, at your work when he or she was not supposed to?

   [ ] YES
   [ ] NO
   [ ] DON'T KNOW/NOT SURE
   [ ] REFUSED

4. During the past 30 days, that is, on how many days did you ride in a vehicle where **someone other than you** was smoking tobacco?

   [ ] NUMBER OF DAYS_____
   [ ] NONE
   [ ] DON'T KNOW/NOT SURE
   [ ] REFUSED

5. Not counting times while you were at work, during the past 30 days, that is, on how many days did you breathe the smoke from **someone else** who was smoking in an indoor or outdoor public place?

   [ ] NUMBER OF DAYS_____
   [ ] NONE
   [ ] DON'T KNOW/NOT SURE
   [ ] REFUSED

6. Not counting times while you were at work, to your knowledge, during the past 30 days, has anyone, including yourself, used tobacco of any kind in an indoor or outdoor public place when he or she was not supposed to?

   [ ] YES
   [ ] NO
   [ ] DON'T KNOW/NOT SURE
   [ ] REFUSED

Appendix N House Rules about Vaping and Smoking

1. For tobacco products that are burned, such as cigarettes, cigars, pipes, or hookah, which statement best describes the rules about smoking a tobacco product inside your home?

   [ ] It is not allowed anywhere or at any time inside my home
   [ ] It is allowed in some places or at sometimes inside my home
   [ ] It is allowed anywhere and at any time inside my home
   [ ] DON'T KNOW
   [ ] REFUSED

   ASK: All respondents

2. Now think about other tobacco products that are not burned, like smokeless tobacco, dissolvable tobacco, and electronic cigarettes. Which statement best describes the rules about using these products inside your home?

   [ ] It is not allowed anywhere or at any time inside my home
   [ ] It is allowed in some places or at some times inside my home
   [ ] It is allowed anywhere and at any time inside my home
   [ ] DON'T KNOW
   [ ] REFUSED

3. At your workplace, is smoking in **indoor** areas…?

   [ ] Always allowed
   [ ] Allowed only at some times or in some places
   [ ] Never allowed
   [ ] DON'T KNOW/NOT SURE
   [ ] REFUSED

4. Not counting motorcycles, in the vehicles that you or family members who live with you own or lease, is smoking…

   [ ] Always allowed in all vehicles
   [ ] Sometimes allowed in at least one vehicle
   [ ] Never allowed in any vehicle
   [ ] RESPONDENT'S FAMILY DOES NOT OWN OR LEASE A VEHICLE
   [ ] DON'T KNOW/NOT SURE
   [ ] REFUSED

Appendix O EBC – RT (Respiratory Research)

Note: Participants should not eat or drink anything for 1 hour before EBC collection.

Step 1: Condense Exhaled Breath Using the RTube

1-1 Prepare the cooling sleeve before taking samples:

1. Place the aluminum sleeve into a plastic bag to prevent moisture from freezing to the inside of the sleeve and contaminating the sample.
2. Place the sleeve into a freezer of the appropriate temperature, depending on the exhaled substance of interest. Home freezers work well for stable compounds.
3. Allow the sleeve to cool to the appropriate temperature. While transporting the sleeve over long distances, store in an ice chest filled with ice or frozen packets.

1-2 Prepare the RTube: The RTubes are sent ready to use for most applications; however, when studying compounds in EBC that are found diffusely in the laboratory setting as contaminants, extra precautions should be taken. These include, but are not limited to, nitrate and nitrite. We recommend that you wash the condensing chamber with ultrapure deionized water which meets or exceeds specification ASTM D 1193 for Type I and type II Reagent Grade Water and allow them to dry fully before use to eliminate any possible contamination.

1-3 Fill out label located on the side of the collection Tube and note the location of the red arrow located on the label as this should be pointing up during the collection.

1-4 Place the blue insulating cover over the aluminum cooling sleeve to protect your hand and keep the aluminum cold.

1-5 Place the aluminum sleeve over the outside of the collection chamber.

1-6 The patient should immediately begin breathing in and out through the mouthpiece with the red arrow pointing upward. In some cases participants will have a very shallow breathing pattern and should be encouraged to exhale hard enough to "hear their breath flowing through the top of the RTube." The one way valve will direct the exhaled air through the cooling sleeve where the sample will be collected. After extended periods of storage, the RTube may present some resistance upon the initial exhalation attempt. This is normal and easily corrected using the pre-use verification procedure here.

1-7 Collection times should be standardized for each application. We recommend a five to seven minute collection time for most applications.

1-8 After sample collection has been completed and the sample stored properly, the patient should dispose of the mouthpiece and place the cooling sleeve back into the protective bag and refreeze.

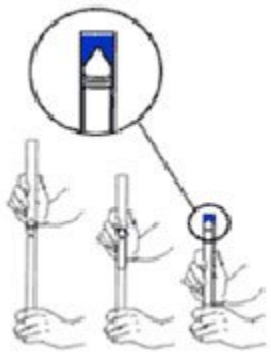
Step 2: Store/Transport Condensate

2-1 Detach mouthpiece and discard. Place the cap on the end of the RTube opposite the blue duckbill valve and near the red arrow. Optionally, the opposite end can be also capped in addition to the end with the red arrow. Additional caps are available by request, please call for more information.

Freeze the sample at the appropriate temperature or leave at room temperature if appropriate for the compounds of interest. When studying stable compounds a sample can be stored at room temperature or in a home/laboratory freezer until needed. If packaged properly and kept out of high heat, the sample can be shipped

by mail with no contamination or loss of sample fluid. In fact, the RTube is so simple to use that the unsupervised subject should be able to perform all sampling from their hospital bed, at their workplace, or from the comfort of their own home. When studying common laboratory contaminates the sample should be removed from the collector and stored in the freezer as soon as possible.
Be sure to remove the cap on the lower end (opposite the red arrow) if so equipped PRIOR to thawing. The RTube incorporates a pressure relief to ensure sample protection during the thawing process which will only function if the lower cap is removed. Keep the upper cap in place until sample is fully thawed and ready for analysis.

Step 3: Use the Plunger to Collect EBC into a Pool at the Top of the RTube



3-1 In the laboratory, remove the cap and pool the sample by plunging the one-way duckbill valve toward the top of the Tube for easy collection. Simply place the RTube over the top of Standard Plunger and push down until the RTube touches the base of the Standard Plunger. Ensure the red arrow marked “UP” is properly oriented during this process. As the RTube is pushed down over the Plunger the nose on the end of the Plunger engages the duckbill valve and strokes it the entire length of the condensation surface much like a syringe. The result is a neatly presented .5mL – 1.5mL pool of pure EBC ready for analysis.

Syringe-style plunger pools condensate near the top of the RTube for easy sample collection.

Appendix P Spirometry

1. Setup the device according to CareFusion Manual instructions and enter the participants specifications.

2. When the device is ready, you will say to the participant:

   "*You are going to take a deep breath, as deep as you can, and then you will place the white mouthpiece into your mouth making sure your lips are entirely sealed around the mouthpiece.* You *will blow out all your breath as quick as you can completely emptying your lungs. I will instruct you through the whole process as you are exhaling."*

Note: Use the provided nose strips to seal the nostrils so that no air leaks out during the process. Air leakage will hinder final results.

3. Once the nose clips are on the participant, click the start button on the computer and instruct the participant to take a very deep breath and have them place the mouth piece into their mouth and tell them to blast the air out of their lungs. Make sure to coach them to keep going until the graph reads well on the screen.

4. Repeat step three 2 more times with the participant.

5. If the 3 breath sample values are consistent with each other, the test is complete. If the samples are inconsistent, then they need to be repeated again.

Appendix Q Nasal Epithelium Sampling

1. Label 1.5mL Eppendorf tube with subject code.
2. Aliquot 200µL buffer Qiagen RNAlater into the Eppendorf.
3. Welcome the subject, ask him or her to sit on a chair, and comfort him/her by explaining what the purpose is and what you are going to do and showing them the curette.
4. Explain the four possible health effects which he/she can expect
    a. Your eyes may tear up.
    b. You may sneeze.
    c. There may be some mild pain, similar to having blood drawn.
    d. Your nose may bleed a little bit, but normally any bleeding ends in a minute or two.
5. Ask the subject to blow their nose thoroughly, both sides.
6. With the curette still inside the plastic, bend it forward ~ 12 degrees and then open it.
7. Unpack the nasal speculum and explain for what purpose you use it.
8. Ask the subject to tilt his or her head back so you can see up the nose and breath through the mouth.
9. Gently insert the speculum into the nostril and open it. Make sure that the point follows the line of the nostril and doesn't poke into the side of the nostril.
10. Use the curette with your dominant hand.
11. Scrape the outer side of the of the nose three times.
12. Withdraw the curette and speculum and put the nasal speculum on the table like in the indicated figure, to prevent contamination.
13. Look to see if you have collected any material (little white flecks).
14. Immerse the tip of the curette in the buffer and collect the sample, by tapping on the scraper with your middle or ring finger, make sure all the tissue comes off the curette.
15. The cells will dissolve quickly in the buffer, so it has to be checked in the scoop if there are cells present. When you doubt the amount of cells, it is worthwhile to take a new sample.
16. Each curette is scraped across the inner surface of the nose 3X and then tapped into the RNA-later
17. Each sample consists of 3 curettes, each scraped 3 times.
18. Since the cells are dissolving right away when you add them to the buffer, no homogenization has to be done and the cell samples can be stored in the -80ºC freezer.

Step 9: Asking the subject to tilt his head (arrow) and breath through the mouth (red circle)

St
wi
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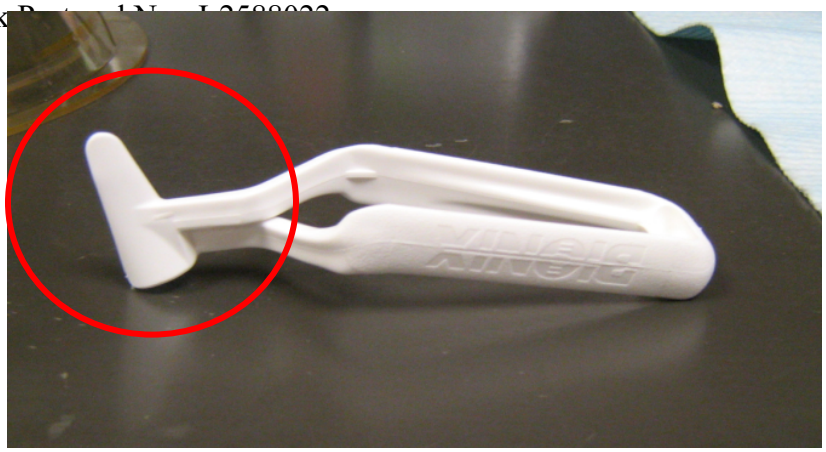


Step 11: Put the nasal speculum on the table, with its functional side upwards (red circle)

Appendix R Exhaled FeNO

Preparation for measurement

1. Lift the breathing handle from the holder and remove the handle cap.
2. Obtain a new patient filter. Attach the patient filter to the breathing handle. Make sure to twist the patient filter in place until it clicks.

Note: Store the patient filters in the original box prior to use.
Note: Do NOT use sharp objects to open the packaging for the patient filter. Do not touch the filter membrane.
Note: Patient filters should be used immediately after opening.
Note: There is a risk of leakage if the filter is not correctly attached to the breathing handle and this may result in incorrect measurement values.
Note: Do not switch OFF the instrument during measurement procedure.

3. Give the breathing handle to the patient and guide the patient to provide a breath sample as described in the next section.

Measurement

1. Empty the lungs by breathing out thoroughly.
2. Close the lips around the mouthpiece on the patient filter so that no air leakage occurs.
3. Inhale deeply through the patient filter to total lung capacity. During inhalation, the cloud on the display moves upwards.

Note: The procedure is activated by inhaling air from the handle or by pressing the start measurement button.

4. Exhale slowly through the filter while keeping the cloud within the limits as indicated on the display (the white lines).
5. The instrument display and audio signals guide the user to the correct exhalation pressure. A continuous sound indicates correct pressure with a frequency proportional to the pressure. An intermittent high frequency sound - too strong pressure
   An intermittent low frequency sound - too weak pressure
   Exhalation with: Pressure correct Pressure too strong Pressure too weak
6. Exhale until the cloud has passed the flag.
7. The instrument will analyze the sample and generate a result in approximately one minute.

Note: Do not exhale or inhale through the patient filter during the analysis process

Results
The result is then displayed:

(A) Patient ID - if applicable
(B) FeNO value in ppb (parts per billion)
(C) Measurement mode 10s/6s
(D) Measurement sequence number
(E) OK - returns to main view

Appendix S MIP/MEP

Operation – Mouth Pressures (PImax/MIP + PEmax/MEP)

- Insert the battery into the compartment at the rear of the MicroRPM.
- Fit the required Pressure Valve Assembly ('Inspiratory' for PImax (MIP), 'Expiratory' for PEmax (MEP)) into the MicroRPM; insert a new Bacterial Filter into the Pressure Valve Assembly and then the Rubber Flanged Mouthpiece onto the Bacterial Filter

PImax (MIP) Test

1. Slide the MicroRPM switch from 'Off' to 'MIP/MEP', whilst applying no pressure to the mouthpiece. Rotating segments will be displayed whilst the unit performs an auto-zero function.
2. When the MicroRPM is ready a 'beep' will be heard and '0' displayed.
3. To perform the test instruct the subject to insert the mouthpiece into the mouth, ensuring the flange is positioned over the gums and inside the lips, whilst the 'bite blocks' are between the teeth.
4. The subject should then exhale to RV (Residual Volume), lungs empty, then perform a 'Mueller' maneuver, a forced inhalation against the MicroRPM with as much effort as possible for as long as possible (minimum 2 seconds).
5. The display will report the result, the maximum average inspiratory pressure sustained over a 1 second period of the test, in centimeters of water (cmH2O). Ideally, the subject should repeat this test 3 times to ascertain a best value.

PEmax (MEP) Test

1. Slide the MicroRPM switch from 'Off' to 'MIP/MEP', whilst applying no pressure to the mouthpiece. Rotating segments will be displayed whilst the unit performs an auto-zero function.
2. When the MicroRPM is ready a 'beep' will be heard and '0' displayed.
3. To perform the test instruct the subject to insert the mouthpiece into the mouth, ensuring the flange is positioned over the gums and inside the lips, whilst the 'bite blocks' are between the teeth.
4. The subject should then inhale to TLC (Total Lung Capacity), lungs full, then perform a 'Valsalva' maneuver, a forced exhalation against the MicroRPM with as much effort as possible for as long as possible (minimum 2 seconds).
5. The display will report the result, the maximum average expiratory pressure sustained over a 1 second period of the test, in cmH2O. Ideally, the subject should repeat this test 3 times to ascertain a best value. To repeat either the PImax or PEmax tests the MicroRPM must firstly be returned to the 'Off' position.

Operation – SNIP (Sniff Nasal Inspiratory Pressure)

1. Insert the battery into the rear of the MicroRPM.
2. Fit the Nasal Probe Adapter into the MicroRPM and then attach the correct size Nasal Probe. The correct size (1-4) can be ascertained by fitting a Nasal Probe to the unit, then firmly inserting the Nasal Probe into a nostril.
3. Instruct the subject to block the open nostril with a finger and then to attempt a sniff. The correct Nasal Probe size has been selected once there is no leakage around the Nasal Probe.

SNIP Test

1. Slide the MicroRPM switch from 'Off' to 'SNIP', whilst applying no pressure to the Nasal Probe. Rotating segments will be displayed whilst the unit performs an auto-zero function.
2. When the MicroRPM is ready a 'beep' will be heard and '0' displayed.

3. To perform the test, instruct the subject to insert the chosen Nasal Probe firmly into a nostril, whilst ensuring the other nostril remains open throughout the test.
4. The subject should then breathe normally and at the end of a normal tidal expiration, FRC (Functional Residual Capacity), be instructed to perform a short, sharp voluntary sniffing maneuver with as much effort as possible.
5. The display will report the result, the peak inspiratory nasal pressure, in cmH2O. On subsequent tests the MicroRPM will continue to display the highest SNIP value, overwriting previous values. Ideally, the subject should repeat this test 10-15 times to ascertain the highest value.

Appendix T Saliva Sampling

1. The patient removes the swab from the Salivette® (see Figs. 1 & 2)
2. Instruct the participant to place the swab in the mouth and chew it for 60 seconds to stimulate salivation (see Fig. 3).
3. Next have the participant return he swab with the absorbed saliva to the Salivette® (see Fig. 4)
4. Replace the stopper (Fig. 5)
5. Centrifuge for 2 minutes at 1,000 x g yields a clear saliva sample in the conical tube (Fig. 6)
6. Particles and mucous strands are collected in the specially designed tip of the Salivette® tube (Fig. 7)
7. The closed insert containing the swab is then hygienically disposed. The saliva recovered can now be used for analysis (Fig. 8)

Appendix U Oral Health

1. How often do you brush your teeth?
   [ ] Never [ ] 2 times a day
   [ ] < once a week [ ] 3 times a day
   [ ] 1-2 times a week [ ] > 3 times a day
   [ ] Every other day [ ] Once a day
   [ ] Don't know [ ] Refused

2.. What material do you use with the tooth brush?
   [ ] Nothing
   [ ] Toothpaste
   [ ] Other _____ (Specify) let participant write in answer if selected
   [ ] Don't know
   [ ] Refused

3. Do your gums bleed when you wash your teeth?
   [ ] No
   [ ] Sometimes
   [ ] Always or almost always
   [ ] Don't know
   [ ] Refused

4. How often do you use mouthwashes?
   [ ] Never [ ] 2 times a day
   [ ] < once a week [ ] 3 times a day
   [ ] 1-2 times a week [ ]> 3 times a day
   [ ] Every other day [ ] Once a day
   [ ] Don't know [ ] Refused

5. How many natural teeth do you have? (The adult mouth normally has **32 teeth, 16 on top and 16 on bottom).**
   [ ] _____ **(subtract from 32 the number of missing or artificial teeth)**
   [ ] Don't know
   [ ] Refused

6. Do you have any removable dentures? Choose all that apply (can choose more than one)
   [ ] a partial denture
   [ ] a full upper denture
   [ ] a full lower denture
   [ ] Don't know
   [ ] Refused

7. About how long has it been since you last visited a dentist? Include all types of dentists, such as, orthodontists, oral surgeons, and all other dental specialists, as well as dental hygienists.
   [ ] Between 1 – 6 months
   [ ] Between 6 – 12 months
   [ ] More than 1 year ago
   [ ] Never seen a dentist
   [ ] Don't know
   [ ] Refused

8. Do you have any fillings?
   [ ] yes If yes, how many fillings? _____ let participant write in answer if selected
   [ ] no
   [ ] Don't know
   [ ] Refused

9. Have you taken any antibiotic medications in the last month?
   [ ] yes, but not currently
   [ ] yes, currently
   [ ] no
   [ ] Don't know
   [ ] Refused

10. Do you currently take an inhaled corticosteroid (e.g. Beclomethasone dipropionate (Qvar), Budesonide (Pulmicort), Budesonide/Formoterol (Symbicort), Fluticasone (Flovent), Fluticasone INH powder (Arnuity Ellipta)?
    [ ] yes
    [ ] no
    [ ] Don't know
    [ ] Refused

Appendix V Oral Rinse

Supplies

SCOPE® mouthwash: Travel size Scope mouthwash bottle is used to rinse mouth.

Saline solution: An alternative to Scope mouthwash, it will be used for participants with open oral ulcers or in recovering alcoholics concerned about the alcohol content.

Medicine cup: A 4.5 oz. sterile medicine cup used for pouring in the mouthwash.

Specimen container: A 5 oz. sterile specimen container used for spitting in the mouthwash and for storing.

Gloves: The dental examiner will use a new pair of gloves before the exam begins.

The procedures to collect the Oral Rinse sample are as follows:

1. Put on gloves and ask the participant:

   "Do you have any sores in your mouth that might hurt when you use a mouthwash with some alcohol in it, such as Scope?"

2. Pour 10mL of SCOPE® mouthwash, or the alternative saline solution, into a medicine cup, making sure not to touch the rim of the cup.
3. Hand the medicine cup with the mouthwash to the participant and explain to the participant:

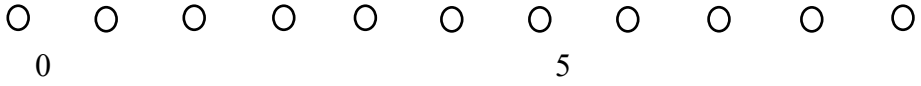
   "We are going to ask you to swish some Scope mouthwash around your mouth, gargle, and then spit the mouthwash into a cup. First, you will rinse your mouth with the mouthwash for 5 seconds and then gargle for 5 seconds. You will do this three times and then spit the mouthwash into a cup. Do you have any questions about this test?"

4. Have the participant put the mouthwash, or saline, into their mouth when ready and then instruct them through the swish gargle cycles using a timer.
5. At the end of the 30 seconds, open the specimen container and hand it to the participant. Hold the top lid down to avoid contaminating it.
6. Have the participant spit the mouthwash into the specimen container when done gargling. Participants are allowed to touch the rim of the container when they spit the mouthwash.
7. Take the specimen collection cup from the participant being careful not to touch the rim.
8. Seal the specimen container properly to prevent leakage.
9. Place the participant label on the specimen collection container until processing.

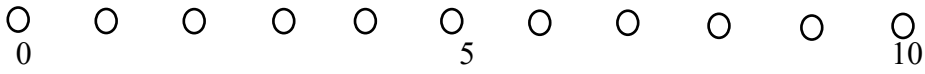
Appendix W Subjective Feelings About COVID-19

The coronavirus to me feels ...

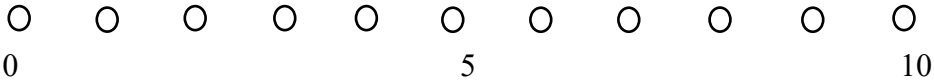
1. **Far away from me** **Close to me**



2. **Old** **New**

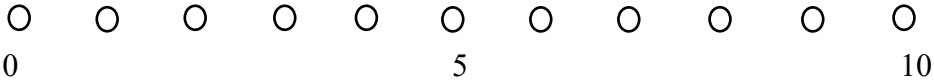


3. **Spreading slowly** **Spreading fast**

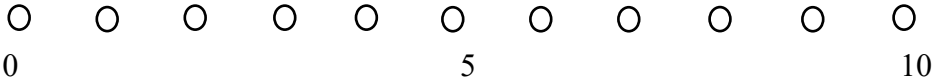


4. **Something I almost never think about** **Something I think all the time**

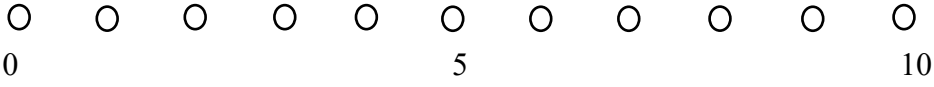
5. **Not fear-inducing** **Fear-inducing**



6. **Not media hyped** **Media hyped**



7. **Not worrying** **Worrying**



8. **Something I am able to combat with my own action** **Something that makes me feel helpless**

0 5 10

9. Not stressful **Stressful**

O O O O O O O O O O O

0 5 10

Appendix X COVID-19 Screening and Vaccine Questionnaire

1.) Have you had a Temp over 100.4 in the past 10 days?
YES NO

2.) Have you had any Respiratory Issues in the past 10 days?
YES NO

3.) Have you had any Chills in the past 10 days?
YES NO

4.) Have you had any Loss of Taste in the past 10 days?
YES NO

5.) Have you had any Loss of Smell in the past 10 days?
YES NO

6.) Have you had any Muscle Aches in the past 10 days?
YES NO

7.) Have you had a Positive COVID-19 Test in the past 10 days?
YES NO

8.) Have you had "Close Contact" with Confirmed or Suspected COVID-19 Case in the past 10 days?
YES NO

9.) Have you ever tested positive for COVID-19?
YES NO
When? _____

10.) Have you received the COVID-19 vaccine?
YES NO
When? _____
What brand?
- Moderna
  - (both doses?)
    YES NO
- Pfizer
  - (both doses?)
    YES NO
- Johnson & Johnson

11.) If NO to question 10, Are you scheduled to receive the vaccine?

YES NO
When? _____

12.) Have you received a booster shot?

YES NO
When? _____
What brand?

- Moderna
- Pfizer
- Johnson & Johnson

Appendix Y Expired CO

Expired CO Instructions

Pre-Test Instructions
Explain the following before starting the test:

- **Step 1 – Define the Sample Needed**
  Say: *"The goal of the test is for you to empty your lungs, by slowly pushing out as much air as possible."*

- **Step 2 – Explain Breath Hold and Countdown**
  Say: *"When the test begins, you are going to take a deep breath in and I'm going to hand you the tester to hold while the tester counts down for a few seconds. Once started, please don't talk until the test is finished."*

- **Step 3 – Describe the Exhalation Process**
  Say: *"When the countdown reaches level 2, you are going to place your mouth over the mouthpiece and exhale slowly, pushing all of the air out of your lungs."*

During the Test

- **Step 1 – Breath Hold**
  Say: *"Take a deep breath in and hold it.*"
  Start the tester's countdown as they breathe in, then hand them the tester to hold.

- **Step 2 – Countdown**
  As they hold their breath, say:
  Don't put your mouth on the mouthpiece until you're ready to exhale and remember not to talk until the test is finished."
  Watch the countdown to know when the subject should be blowing into the tester.

- **Step 3 – Exhalation**
  When the countdown reaches level 2, say:
  "Put your mouth over the mouthpiece and begin exhaling slowly. Push all of the air out nice and slowly; try and empty your lungs."
  When the subject is finished exhaling deeply and completely, they can hand the tester back to you.

Appendix Z Marijuana/Cannabis Use

For the next few questions, we would like to ask you about marijuana/cannabis, which includes a joint, pot, weed, hash, or oil.

1. When was the last time you used marijuana/cannabis?
    - I have never used marijuana/cannabis
    - Earlier today
    - Not today but sometime in the past 7 days
    - Not in the past 7 days but in the past 30 days
    - Not in the past 30 days but sometime in the past 6 months
    - Not in the past 6 months but sometime in the past 12 months
    - 1 to 4 years ago
    - 5 or more years ago
    - Don't know
    - Refused

Respondents who used Marijuana in the past 30 days move to question #2 & #3

2. In the last 30 days, how often did you use marijuana/cannabis?
    - Once or twice
    - Once or twice a week
    - 3 or 4 times a week
    - 5 to 6 times a week
    - Everyday
    - Don't Know
    - Refused
3. In the last 30 days did you…

    (Yes/No Checklist)
    - Smoke marijuana/cannabis WITHOUT tobacco
    - Smoke marijuana/cannabis WITH tobacco in a joint or blunt
    - Use a waterpipe/bong to smoke marijuana/cannabis
    - Use a vaporizer to heat dried marijuana/cannabis leaves or herb
    - Use an e-cigarette to vape marijuana/cannabis oil or liquid
    - Eat or drink marijuana/cannabis in a food or a beverage
    - Use marijuana/cannabis extracts, including oil, wax or shatter
    - Us another form of marijuana/cannabis (please specify):_____
    - Don't know
    - Refused

Appendix AA NYS Flavor Ban Educational Document

We would like to inform you that the current flavor(s) you are vaping fall within the NYS policy described below. In short, the sale of such flavor(s) are prohibited within the state.

SECTION 1399-MM-1

Sale of flavored products prohibited

Public Health (PBH) CHAPTER 45, ARTICLE 13-F

§ 1399-mm-1. Sale of flavored products prohibited. 1. For the purposes of this section "flavored" shall mean any vapor product intended or reasonably expected to be used with or for the consumption of nicotine, with a distinguishable taste or aroma, other than the taste or aroma of tobacco, imparted either prior to or during consumption of such product or a component part thereof, including but not limited to tastes or aromas relating to any fruit, chocolate, vanilla, honey, candy, cocoa, dessert, alcoholic beverage, mint, wintergreen, menthol, herb or spice, or any concept flavor that imparts a taste or aroma that is distinguishable from tobacco flavor but may not relate to any particular known flavor. A vapor product intended or reasonably expected to be used with or for the consumption of nicotine, shall be presumed to be flavored if a product's retailer, manufacturer, or a manufacturer's agent or employee has made a statement or claim directed to consumers or the public, whether expressed or implied, that such product or device has a distinguishable taste or aroma other than the taste or aroma of tobacco.

2. No vapor products dealer, or any agent or employee of a vapor products dealer, shall sell or offer for sale at retail in the state any flavored vapor product intended or reasonably expected to be used with or for the consumption of nicotine.

3. Any vapor products dealer, or any agent or employee of a vapor products dealer, who violates the provisions of this section shall be subject to a civil penalty of not more than one hundred dollars for each individual package of flavored vapor product intended or reasonably expected to be used with or for the consumption of nicotine sold or offered for sale, provided, however, that with respect to a manufacturer, it shall be an affirmative defense to a finding of violation pursuant to this section that such sale or offer of sale, as applicable, occurred without the knowledge, consent, authorization, or involvement, direct or indirect, of such manufacturer. Violations of this section shall be enforced pursuant to section thirteen hundred

ninety-nine-ff of this article, except that any person may submit a complaint to an enforcement officer that a violation of this section has occurred.

4. The provisions of this section shall not apply to any vapor products dealer, or any agent or employee of a vapor products dealer, who sells or offers for sale, or who possess with intent to sell or offer for sale, any flavored vapor product intended or reasonably expected to be used with or for the consumption of nicotine that the U.S. Food and Drug Administration has authorized to legally market as defined under 21 U.S.C. § 387j and that has received a premarket review approval order under 21 U.S.C. § 387j(c) et seq.

Appendix BB GERD-HRQL Questionnaire

GERD Health-Related Quality of Life (GERD-HRQL) Questionnaire

Scale: 0=No Symptoms 1=Noticeable, but not bothersome 2=Noticeable, bothersome, but not everyday
3=Bothersome daily 4=Bothersome and affects daily activities 5=Incapacitating to do daily activities

Questions (Circle One):

1. How bad was the heartburn?	0	1	2	3	4	5
2. Heartburn when lying down?	0	1	2	3	4	5
3. Heartburn when standing up?	0	1	2	3	4	5
4. Heartburn after meals?	0	1	2	3	4	5
5. Does heartburn change your diet?	0	1	2	3	4	5
6. Does heartburn wake you from sleep?	0	1	2	3	4	5
7. Do you have difficulty swallowing?	0	1	2	3	4	5
8. Do you have pain while swallowing?	0	1	2	3	4	5
9. Do you have gassy or bloating feeling?	0	1	2	3	4	5
10. If you take reflux medication, does this affect your daily life?	0	1	2	3	4	5