PROTOCOL TITLE:

Biomarkers of Exposure and Effect in SREC Users

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REVISION HISTORY

Revision #	Version Date	Summary of Changes	Consent Change?
1	12/20/17	Minor changes to schedule of measures and administrative corrections.	Yes
2	05/30/18	Clarified inclusion/exclusion criteria and made editorial updates. Minor changes to schedule of measures.	Yes
3	07/30/18	Changes to schedule of measures and made editorial updates	Yes
4	09/14/18	Research related injury language was updated per IRB request, data sharing language was updated per IRB request, minor change to schedule of measures table.	Yes
5	10/11/18	Changed bonus payment structure for usual brand group, aligned primary/secondary aims with SAP & DSMP	Yes
6	05/14/19	Minor changes to study measures table	No
7	09/13/21	Reduced sample size, reduced study duration, changed study product, updated COVID procedures,	Yes
8	03/29/22	Revised study visit and procedure processes, noted that SREC. flavors will be used, subject payment section revised	Yes
9	04/25/22	Revised protocol from three arms to one arm (removed NRT and control group). Removed several subjective measures. Updated pod dispensing calculation.	Yes
10	06/13/22	Revised inclusion criteria	No
11	10/05/22	Updated flavor dispensing process, revised eligibility criteria, adjusted quit-date timeframe and added ability to withdraw participants who have not completely switched by week 2	Yes
12	2/20/2023	Clarified SAE reporting requirements. Updated flavor dispensing process, buccal cell collection process, and compliance session dates. Removed outdated	No

		references to surveys that were not being collected and added exclusion criteria for marijuana use.	
13	4-25-2023	Per IRB request, updated recruitment numbers to reflect enrollment versus completer goals for the study. Per IRB request, language in the consent form was updated.	Yes
14	07/28/2023	Per IRB request, updated monitoring responsibility text. Removed Hanna Vanderloo from study information.	No
15	10/30/2023	Per IRB request, updated exclusion criteria to exclude persons with medical history of thromboembolic events and/or cerebrovascular events, or medical diagnosis that LMP believes is high risk for thromboembolic events and/or cerebrovascular events.	No
16	03/13/2024	Following HRPP audit, the protocol and consent form are being revised to address findings. Clarified biosample tracking language and procedures in the protocol. Revised explanation of circumstances in which clinical health data like BP would be shared with clinicians in the protocol and consent. Updated the consent to include new language on the use of deidentified data and samples of participants in future research. Added section 4.6 detailing product destruction procedures.	Yes

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ABBREVIATIONS/DEFINITIONS

- AE: Adverse effect
- BP: Blood pressure
- BPM: Beats per minute
- CES: Cigarette Evaluation Scale
- CESD: Centers for Epidemiological Studies-Depression
- CO: Carbon monoxide
- CPD: Cigarettes per day
- FDA: Food and Drug Administration
- FTND: Fagerström Test for Nicotine Dependence
- HMPMA: 4-hydroxybut-2-ylmercapturic acid
- 2-HPMA: 2-Hyrdoxypropylmercapturic acid
- 3-HPMA: 3-Hydroxypropylmercapturic acid
- HR: Heart rate
- ITP: Investigational Tobacco Product
- ITR: Interactive Text Response
- ITRS: Interactive Text Response System
- NIDA: National Institute for Drug Abuse
- NMR: Nicotine metabolite ratio
- NNAL: 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanol
- NNN: *N*′-Nitrosonornicotine
- PAH: Polycyclic aromatic hydrocarbons
- PheT: Phenanthrene tetraol
- PS-ECDI: Penn State Electronic Cigarette Dependence Index
- SAE: Serious adverse event
- SREC: Standardized Research E-cigarette
- TNE: Total Nicotine Equivalents
- VOC: Volatile organic compounds

STUDY SUMMARY

Study Title	**Biomarkers of Exposure and Effect in SREC Users**
Study Design	Within-subjects, repeated measures study
Primary Objective	Measure biomarkers of tobacco constituents and inflammation in smokers who will switch for six weeks to the Standardized Research E-cigarette (SREC) developed by the National Institute on Drug Abuse.
Secondary Objective(s)	Collect and analyze information on behavioral aspects and a comprehensive panel of subjective measures, including patterns and topography of SREC use.
Research Intervention(s)/Investigational Agents	Standard Research E-cigarette (SREC) developed by the National Institute on Drug Abuse
IND/IDE # (if applicable)	IU0000806, IU0000807, IU0000935, IU0000937, IU0000939/P00128
Investigational Drug Services # (if applicable)	NA
Study Population	Cigarette smokers
Sample Size (number of abstinent completers)	30
Study Duration for Individual Participants	A total 11 weeks: 1 week baseline period, 6 weeks of assigned product use and a follow-up at week 10

1.0 Objectives

Given the exponential growth of the prevalence of e-cigarette use in the United States, particularly among the youth and cigarette smokers,[1-3] understanding the potential toxicity associated with these devices is paramount. For instance, some e-cigarettes may be exposing users to harmful constituents, and the number of such constituents identified in various e-cigarettes continues to grow, including toxic chemicals formed from the e-liquid base components propylene glycol and glycerol.[4-7] Therefore, better assessment of harmful exposures and resulting macromolecular alterations in e-cigarette users are critical for characterizing electronic cigarettes.

This study is focused on characterizing the toxic and carcinogenic potential of the Standardized Research E-cigarette (SREC) developed by the National Institute on Drug Abuse. In the environment of continuously changing e-cigarette market, SREC was developed as a model e-cigarette that will remain available for an extended period of time and can be used as a bridging element in various studies aimed at evaluating the value and limitations of e-cigarettes as tobacco risk reduction tools.

Our overall goal is to generate initial reference data on chemical exposures and associated effects in smokers switching to SREC. Biomarkers of exposure and effect are important tools in assessing harmful effects. In smokers, biomarkers of certain tobacco toxicants and carcinogens, such as tobacco-specific *N*-nitrosamines (TSNA), polycyclic aromatic hydrocarbons (PAH), and volatile organic compounds (VOC) have been related to both the levels of exposure and the risk of cancer. In addition, certain chemicals identified in some e-cigarettes can potentially cause oxidative stress and inflammation, and result in DNA damage – effects that are strongly implicated in the etiology of chronic diseases, including cancer and cardiovascular disease.[8-11] Therefore, it is also important to assess relevant biomarkers of effect, such as DNA adducts and inflammatory markers. Lastly, our recent data indicate that the potent oral and esophageal carcinogen *N*′-nitrosonornicotine (NNN), which is present only in trace levels in e-cigarettes, can be formed in saliva of e-cigarette users,[12] and this process needs to be carefully examined.

In the proposed study, over the 6-week experimental phase, subjects will be asked to completely switch from smoking to SREC. The specific aims and hypothesis are as follows:

Primary Aim 1: To analyze a panel of exposure biomarkers in the urine of smokers switched to SREC and compare the exposure profiles against baseline measures (while smoking) and the available data on the levels of these biomarkers in non-users of tobacco/nicotine products. The biomarkers will include total nicotine equivalents (TNE, biomarker of nicotine), total NNAL and total NNN (TSNA biomarkers), PheT (PAH biomarker), and mercapturic acids HMPMA, 2-HPMA, and 3-HPMA (biomarkers of VOC).

Hypothesis: There will be significant reductions in exposure in switchers to SREC, to the levels typically found in non-users of nicotine/tobacco products.

Secondary Aim 1: To compare formaldehyde-DNA adducts and oxidative DNA adduct 8-oxo-dG in DNA from oral cells and leukocytes, a comprehensive panel of circulating cytokines and chemokines, and the urinary biomarkers of oxidative damage and

inflammation 8-iso-PGF-2α and PGEM in smokers switched to SREC against the baseline levels and the available data on non-users of nicotine/tobacco products. In a subset of study participants, a comprehensive panel of circulating cytokines and chemokines will be also analyzed in plasma.

Hypothesis: The levels of biomarkers of effect in SREC switchers will be lower than at baseline (while smoking cigarettes), but higher than those found in non-users of nicotine/tobacco.

Secondary Aim 2: To analyze salivary NNN and the NNN-derived HPB-releasing DNA adducts in oral cell in smokers who completely switch to SREC, and compare to baseline measures (while smoking) and the available data on the levels of these biomarkers in non-users of tobacco/nicotine products. Only verified switchers to SREC (as determined by analyzing urinary biomarkers in Aim 1) will be included in these analyses.

Hypothesis: Levels of salivary NNN and HPB-releasing DNA adducts will be lower after switching to SREC than at baseline, and higher than in non-users of nicotine/tobacco.

For all Aims, data on the levels of biomarkers in non-users of tobacco/nicotine products will be retrieved from our recently completed study. In addition, data on smokers from the same study will be used as a reference, to verify that baseline levels of biomarkers assessed in our participants in Aims 1-3 are representative of the typical levels found in smokers.

Collectively, this study will contribute to better understanding of the long-term health effects that may be associated with e-cigarette use.

2.0 Background

Electronic cigarettes (e-cigarettes) offer a promise of a less harmful alternative to smoking tobacco cigarettes. Compared to more than 7,000 constituents that have been identified in tobacco and cigarette smoke, the number of constituents that may be present in e-liquid and e-cigarette aerosol is relatively small. Furthermore, the levels of harmful constituents that have been measured in some e-cigarette aerosols are generally much lower when compared to cigarette smoke.[13] However, emerging data including our preliminary studies suggest that e-cigarette use may pose certain health risks. For instance, aerosol of e-cigarettes operated at high voltage may contain substantial amounts of formaldehyde, a genotoxic agent that has been classified by the International Agency for Research on Cancer (IARC) as carcinogenic to humans.[14] In addition, our preliminary data indicate that e-cigarette users may experience elevated oxidative damage and inflammation. Lastly, while the reported levels of the tobacco-specific oral and esophageal carcinogen *N*′-nitrosonornicotine (NNN) are extremely low in e-cigarettes, endogenous formation of this carcinogen has been demonstrated in laboratory animals,[15] is possible in humans,[16;17] and our preliminary data show that it can be found in saliva of e-cigarette users.[12] Better assessment of such exposures and resulting macromolecular alterations are critically important in characterizing an electronic cigarette device and can provide critical insights into the potential long-term health effects of e-cigarette use. Given that popularity of e-cigarettes has grown exponentially since they were first introduced in the U.S., particularly among the youth and cigarette smokers,[1-3] such research is highly significant.

In this proposal we will use a panel of relevant and novel biomarkers in smokers who will switch for six weeks to the Standard Research E-cigarette (SREC) developed by the National Institute on Drug Abuse. We will monitor our study subjects for the levels of tobacco constituent metabolites in urine (biomarkers of exposure, *Aim 1*), DNA adducts and inflammatory markers (biomarkers of effect, *Aim 2*), and will investigate NNN formation and the resulting DNA damage in the oral cavity (*Aim 3*). This panel of biochemical measures will be accompanied by a comprehensive panel of subjective measures, including patterns and topography of SREC use, altogether generating valuable reference data for SREC.

Tobacco constituent biomarkers

Nicotine is the major known addictive agent of tobacco and cigarette smoke,[22] and a key component of most e-cigarette liquids. Nicotine exposure is measured by analyzing total nicotine equivalents (TNE), the sum of urinary total nicotine, nicotine-*N*-oxide, total cotinine, and total 3'-hydroxycotinine. TNE account for 73 – 96% of the nicotine dose[22] and are an outstanding monitor of nicotine uptake.[22] Tobacco-specific *N*-nitrosamines 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and NNN are likely causative agents for cancers of the lung, pancreas, oral cavity, and esophagus in tobacco users,[23-25] and are classified as human carcinogens by the International Agency for Research on Cancer (IARC).[24;25] NNN and NNK are present at relatively high levels in cigarette smoke, but only trace levels have been measured in e-cigarettes.[13;26] Human exposure to NNK is measured by analyzing the sum of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) and its glucuronides in urine; this validated and widely applied biomarker is referred to as total NNAL.[27-29] Total NNAL correlates with cigarettes per day, nicotine equivalents in urine, and cotinine,[30;31] and is an effective uptake marker for NNK.[32] Human exposure to NNN is measured by analyzing the sum of unchanged NNN and its *N*-glucuronide excreted in urine (total NNN).[33;34] Consistent with the known carcinogenicity of NNK and NNN, several nested case-control studies in smokers have demonstrated that urinary total NNAL is related to lung cancer and urinary total NNN is related to esophageal cancer risk.[35-39] Thus, urinary NNAL and NNN are biomarkers of both exposure and cancer risk. Polycyclic aromatic hydrocarbons (PAH) are formed during the incomplete combustion of organic matter, always occur as mixtures, and many are potent carcinogens or toxicants in laboratory animals.[40;41] PAH are widely accepted as major contributors to lung cancer in smokers,[41-44] and only trace levels of a few PAH have been reported in e-cigarettes.[45] 1-Hydroxypyrene (1-HOP) is an established biomarker of PAH exposure.[34] Certain volatile compounds such as acrolein, crotonaldehyde and propylene oxide are important toxicants and carcinogens in cigarette smoke,[29] and can also be also formed upon heating and vaporization of propylene glycol and glycerin in e-cigarette aerosol. Acrolein is an intense irritant, causes cilia-toxicity in the lung, and is proposed to be a lung carcinogen,[46;47] crotonaldehyde is a potent irritant and forms DNA adducts in the human lung,[48] and propylene oxide is an IARC Group 2B carcinogen. Acrolein, crotonaldehyde, and propyene oxide are excreted in the urine as 3-hydroxypropylmercapturic acid (3-HPMA), 3-hydroxy-1-methylpropylmercapturic acid (HMPMA), and 2-hyrdoxypropylmercapturic acid (2-HPMA), respectively. These biomarkers can be readily measured by established validated methods.[49-52] The structures of these urinary tobacco constituent biomarkers are illustrated in Figure 1.

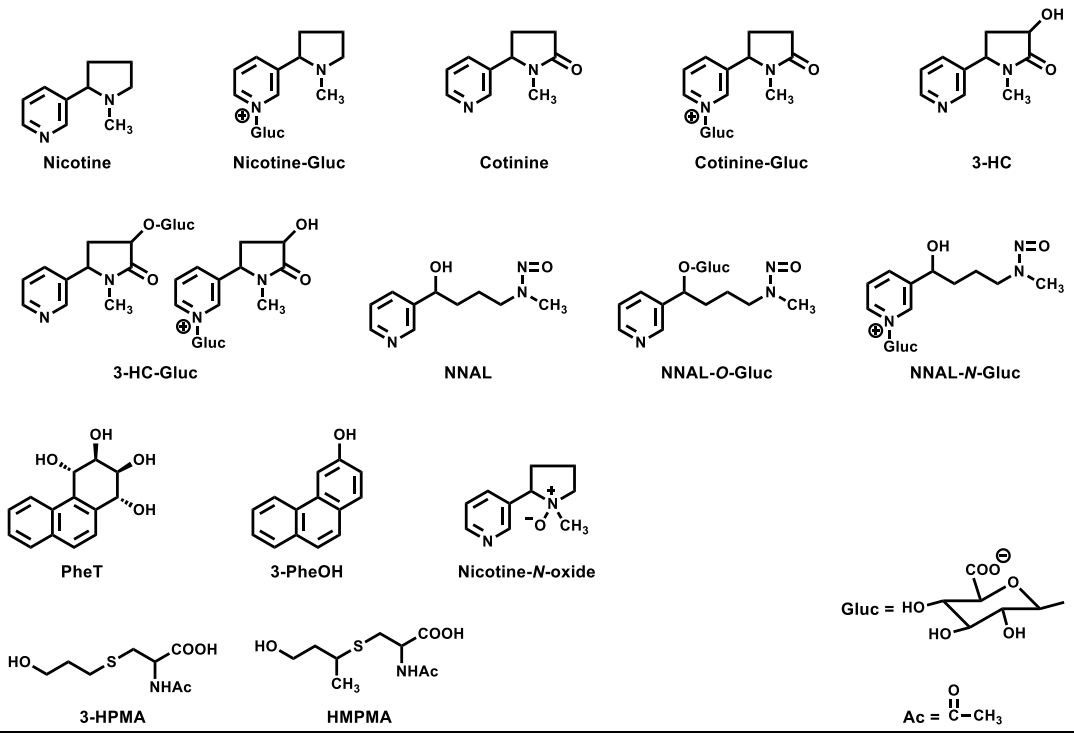

Figure 1. Urinary biomarkers to be analyzed in this study

In our preliminary published study on carcinogen and toxicant biomarkers in urine of 28 e-cigarette users who quit smoking 2-36 months before study entry (median 9 months), levels of NNAL, 1-HOP, 2-HPMA, 3-HPMA, and HMPMA were significantly lower in the urine of e-cigarettes users than in smokers.[53] In studies by Ruiz et al.[54] and Goniewicz et al.[55], in which cigarette smokers switched to e-cigarette use for 5 days and 2 weeks, respectively, significant reductions in the levels of these biomarkers were also observed. The findings of these three studies are summarized in Table 1. While all three studies show general decrease in toxicant and carcinogen exposures in e-cigarette users as compared to smokers, the inconsistencies in study designs (e.g., recruitment of current e-cigarette users vs. switching from smoking to e-cigarettes for various periods of time), the differences in e-cigarette brands and duration of use, and reported biomarker units make it difficult to clearly define these exposures in e-cigarette users and generalize the results to other e-cigarette products.

Formaldehyde-derived DNA adducts

As with most chemical carcinogens, DNA damage is critical in formaldehyde carcinogenesis because DNA adducts, if unrepaired, can cause mutations in critical genes such as *K-RAS* and *TP53*, leading to loss of normal cellular growth control mechanisms and cancer. Our group was the first to demonstrate the presence of a specific formaldehyde-DNA adduct, *N6*-hydroxymethyldeoxyadenosine (N^6-$HOCH_2$-dAdo), in DNA from smokers' leukocytes. Measurement of this adduct in humans showed that its levels are related to smoking, with remarkable differences in N^6-$HOCH_2$-dAdo being observed between smokers and nonsmokers.[56;57] These adducts can be measured in DNA extracted from either blood or oral cells.[58]

Table 1. Reported reductions in tobacco-related urinary biomarkers in e-cigarette users.

Study	Our preliminary study[53] 28 e-cigarette users who quit smoking 2-36 months before study entry; a variety of e-cigarette brands used.		Ruiz et al.[54] 15 smokers who completely switched to researchable Blu e-cigarettes with tobacco-flavored e-liquid containing 24 mg/mL nicotine.		Goniewicz et al.[55] 20 smokers who switched to M201 Mild e-cigarettes with tobacco-flavored cartridges containing 11 mg nicotine (only 9 complete switchers).	
Biomarker	Level in e-cigarette users	Estimated % of smokers' levels	Level after 5 days of e-cigarette use	% of baseline smoking level	Level after 2 weeks of e-cigarette use	% of baseline smoking level
NNAL	0.02 pmol/mL	1.3	174 ng/24h	40	80 ng/g	35
1-HOP	0.38 pmol/mL	43	94 ng/24 h	30	746 ng/g	96
2-HPMA	141 pmol/mL	35	Not analyzed	Not analyzed	21 µg/g	47
3-HPMA	1100 pmol/mL	19	214 µg/24 h	14	410 µg/g	44
HMPMA	705 pmol/mL	14	71 µg/24 h	13	616 µg/g	33

Inflammation and oxidative damage

Inflammation involves infiltration of lymphocytes, macrophages, and neutrophils into tissues under stress, and induces lipid peroxidation, resulting in the generation of a spectrum of reactive oxygen and nitrogen species capable of causing extensive damage to DNA and proteins, and leading to toxic and mutagenic events.[59;60] Chronic inflammation and oxidative stress are important interlinked contributing factors in the pathogenesis of cigarette smoke-associated diseases, including lung cancer.[61-66] Inflammation leads to the induction of the immediate-early response gene prostaglandin G/H synthase-2 (COX-2).[67] The product of COX-2, prostaglandin E_2, is metabolized to PGEM, a urinary metabolite which has been associated with cancer development in a variety of studies.[68] Levels of PGEM are reported to be significantly higher in current smokers than in former smokers than in never-smokers, which is thought to reflect inflammation in the lung due to induction of COX-2.[69;70] Oxidative stress-induced peroxidation of arachidonic acid results in the production of F_2-iosprostanes, among which urinary 8-*iso*-$PGF_{2\alpha}$ is an accepted biomarker of oxidative damage.[71;72] Many studies consistently report that levels of 8-*iso*-$PGF_{2\alpha}$ are elevated in smokers.[34;73-75]

In our preliminary study, we have found that levels of 8-*iso*-$PGF_{2\alpha}$ and PGEM are similar in the urine of e-cigarette users and cigarette smokers (same subjects as in Table 1). The median levels of 8-iso-PGF2α were 0.80 pmol/mL urine (range 0.13-2.17, N=28) in e-cigarette users, the same as 0.80 pmol/mL (range 0.18-2.74, N=83) in cigarette smokers from a recently completed study. For PGEM, the median values (in pmol/mL) were 32.1 (5.0-184, N=28) in e-cigarette users vs. 31.4 (range 3.0-149, N=86) in cigarette smokers. This is in contrast with the expected decrease to normal levels after 2 weeks of smoking cessation.[71;76-78] Our subjects used various e-cigarette brands and e-liquid types, and it is not clear whether the elevated levels of inflammatory and oxidative markers were due to exposures to high levels of formaldehyde from their e-cigarettes. Measurement of 8-*iso*-$PGF_{2\alpha}$ and PGE-M in the proposed study, in which subjects will use SREC e-cigarettes containing low levels of formaldehyde (0.1 µg per puff) and operating at a relatively low voltage (3.30 V), will eliminate this potential contributing factor and allow to examine

the potential capacity of the low-formaldehyde e-cigarette aerosol to sustain the high levels of systemic inflammatory state in former smokers.

Oxidative DNA damage is likely involved in tumor promotion and other harmful effects of smoking[79;80]. Measurement of DNA adducts can offer a direct assessment of such damage. Direct oxidation of guanine produces 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxo-dG), which is widely used as a biomarker of oxidative stress.[81] We recently developed a highly sensitive LC-MS/MS method for the analysis of 8-oxo-dG in very small amounts of DNA,[82] and will use this method to assess oxidative DNA damage in our study subjects. Circulating cytokines and chemokines, such as CRP, TGFα, IL6, IL8, and others, are well-established biomarkers associated with lung cancer and cardiovascular disease risk.[83] These systemic inflammation markers will be also assessed to provide a comprehensive oxidative and inflammatory profile in our study subjects.

Endogenous formation of NNN

Human exposure to NNN can result from two sources: in addition to the direct intake from tobacco-containing products, this carcinogen can also be formed endogenously from nornicotine, which is a tobacco constituent and also a nicotine metabolite. For instance, co-administration of nornicotine and nitrite led to the formation of NNN in laboratory animals,[15] and we previously reported evidence of endogenous NNN formation in some users of oral nicotine replacement therapy products such as nicotine gum or lozenge.[16;17] This process is likely to occur in the oral cavity where metabolically-formed nornicotine can be excreted by salivary glands and react with nitrite, which is formed in oral cavity via the bacterial reduction of dietary nitrate.[84-87] In agreement with this hypothesis, we recently reported that NNN can be formed from nornicotine in human saliva without deliberate addition of any other substance.[88] We also conducted preliminary investigation of NNN formation of NNN in saliva of e-cigarette users. Salivary NNN and urinary tobacco biomarkers, including total NNN, were analyzed in 20 e-cigarette users (various e-cigarette brands, 3 to 36 months reported duration of exclusive e-cigarette use) and compared to smokers and nonsmokers. The geometric mean of NNN in saliva of e-cigarette users was 2.60 pg/mL, ranging from non-quantifiable (LOQ) to 76.0 pg/mL, while in smokers, salivary NNN ranged from LOQ to 739.0 pg/mL. Approximately 80% of smokers had salivary NNN in the range of levels found in e-cigarette users (Figure 2). Consistent with a previous report,[53] very low levels of urinary total NNN were present in only 5 out of 20 e-cigarette users (ranging from 0.001 to 0.01 pmol/mL urine), and only trace levels of NNN were found in e-cigarette liquids. Together, our findings demonstrate that NNN is formed in the oral cavity of e- cigarette users. However, there were important limitations in this preliminary study. We did not analyze NNN content in specific e-cigarettes/e-liquids used by the study subjects. Therefore, even though the results of our and other studies consistently indicate that levels of NNN are minimal in e-cigarette liquids and aerosols, we could accurately quantify the potential contribution of e-cigarette-derived NNN to the amount of this carcinogen measured in saliva of e-cigarette users. In addition, we did not collect information on secondhand smoke exposure in nonsmokers or e-cigarette users, and such exposure could have been

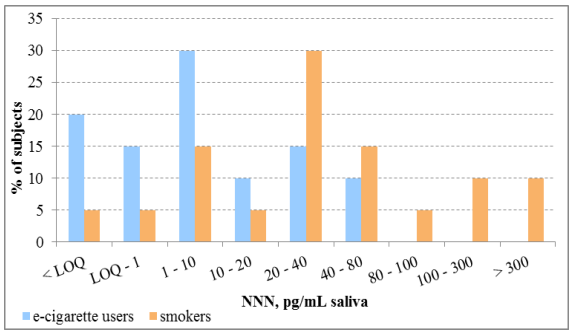

Figure 2. Distribution of salivary NNN in e-cigarette users and smokers.[12]

responsible for the presence of trace levels of tobacco biomarkers in some e-cigarette users in our study. The proposed study will address these limitations and will help to better understand the factors affecting the extent of NNN formation in the oral cavity of e-cigarette users.

HPB-releasing DNA adducts in oral cells

Metabolic activation of NNN and NNK leads to the formation of pyridyloxobutyl (POB)−DNA adducts.[23;89] Under strong acid hydrolysis conditions, the majority of POB−DNA adducts decompose to release 4-hydroxy-1-(3-pyridyl)-1-butanone (HPB).[90;91] HPB-releasing adducts may cause mutations in critical genes associated with cancer, and the importance of these adducts in NNN and NNK carcinogenesis has been demonstrated.[23;42] We recently developed a highly sensitive high resolution LC-MS/MS assay for the measurement of HPB-releasing DNA adducts in oral cells – a non-invasive source of DNA and a promising surrogate tissue for assessing NNN- and NNK-induced molecular alterations in the aerodigestive tract.[92;93] The method was tested by analyzing oral samples from 65 smokers, including 30 head and neck squamous cell carcinoma (HNSCC) patients and 35 cancer-free controls. These analyses revealed a remarkable difference between the two groups: the median HPB-releasing DNA adduct level was 6.6 times greater for those with HNSCC than for smokers without HNSCC ($p = 0.002$).[93] Additional multivariate regression adjusting for gender, age and alcoholic frequency showed persistent significant difference in HPB-releasing adduct levels between cases and controls with a ratio of geometric means equal to 20.0 (95% CI=2.7-148.6) (*unpublished data*). Together, these findings suggest that levels of HPB-releasing DNA adducts in oral cells may be an independent predictor of HNSCC in smokers. Therefore, in Aim 3, we will also investigate whether HPB-releasing DNA adducts will be present in oral cells after complete switching to SREC (due to NNN formation in saliva). The lifespan of buccal cells is approximately 7-11 days (from formation to shedding), and we expect that only new cells, not previously exposed to cigarette smoke, will be collected in complete switchers at weeks 4 and 6.

3.0 Study Endpoints/Events/Outcomes

3.1 Primary Endpoints

- Changes in biomarkers of various toxicant and carcinogen exposures at 4 and 6 weeks after participant switched to the SREC device (Aim 1).

3.2 Secondary Endpoints

- Changes in inflammatory biomarkers and DNA adducts at 4 and 6 weeks after switching to the SREC device (Aim 2).
- Levels of NNN and nornicotine in saliva and HPB-releasing DNA adducts in oral cells of complete switchers to SREC.

Biomarkers that will serve as primary endpoints are summarized in Table 2. For all biomarkers, the potential changes relative to baseline (% change) will be assessed.

Table 2. Carcinogen, toxicant, and inflammatory biomarkers to be analyzed in this study

Biomarkers*	Source	Matrix
Aim 1 – Biomarkers of exposure		
TNE	Nicotine	Urine
Total NNAL	NNK	Urine
Total NNN	NNN	Urine
PheT	PAH	Urine
2-HPMA	Propylene oxide	Urine
3-HPMA	Acrolein	Urine
HMPMA	Crotonaldehyde	Urine
CEMA	Acrylonitrile	Urine
Aim 2 – Biomarkers of effect		
N^6-$HOCH_2$-dAdo	Formaldehyde	Oral cells, leukocytes
8-oxo-dG	Oxidative stress, inflammation	Oral cells, leukocytes
Cytokines and chemokines	Oxidative stress, inflammation	Plasma
8-*iso*-$PGF_{2\alpha}$	Oxidative stress	Urine
PGE-M	Inflammation	Urine
Aim 3 – NNN formation in oral cavity		
NNN	Nornicotine nitrosation	Saliva
Nornicotine	Nornicotine or nicotine metabolism	Saliva
HPB-releasing DNA adducts	NNN and NNK metabolic activation	Oral cells

* Abbreviations: TNE, total nicotine equivalents; NNAL, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; NNN, *N*′-nitrosonornicotine; PheT, phenanthrene tetraol; 2-HPMA, 2-hyrdoxypropylmercapturic acid; 3-HPMA, 3-hydroxypropylmercapturic acid; HMPMA, 4-hydroxybut-2-ylmercapturic acid; N^6-$HOCH_2$-dAdo, N^6-hydroxymethyl-deoxyadenosine; 8-oxo-dG, 8-oxo-7,8-dihydro-2′-deoxyguanosine; 8-*iso*-$PGF_{2\alpha}$, 9,11,15-trihydroxyprosta-5,13-dien-1-oic acid; PGE-M, 11α-hydroxy-9,15-dioxo-2,3,4,5-tetranorprostane-1,20-dioic acid; HPB, 4‑hydroxy-1-(3-pyridyl)-1-butanone.

- Product use: For cigarettes, cigarettes per day will be assessed based on a daily entries into an Interactive Text Response System (ITRS). For SREC, number of bouts and estimated number of puffs will be assessed based on self-reported daily

ITR entries, and amount of e-liquid consumed will be assessed by difference in pod weights before and after use.

- Cigarette avoidance: the number of cigarette avoidance days, defined as no tobacco cigarettes smoked in the past 24 hours based on daily ITR entries, and the rate of cigarette avoidance, calculated as the proportion of cigarette avoidance days out of the total number of days in the product use period (approximately 42 days for those who complete the study).

3.3 Exploratory Endpoints

- Scores on subjective measures:
  - *Fagerstrom Test for Nicotine Dependence* (FTND);[94]
  - *Wisconsin Index of Smoking Dependence Motives* is a multidimensional measure of dependence based on theoretically grounded motives for drug use and intended to reflect mechanisms underlying dependence; subscales have been found to be related to smoking heaviness and relapse;[95]
  - *Product Evaluation Scale* is a 7-point Likert-type scale that includes items from the Cigarette Evaluation Scale[98] and from a scale that was used to evaluate various medicinal nicotine products[97] to assess psychological reward, satisfaction and aversiveness (Cigarette Evaluation Scale will be used at baseline);
  - *Product Expectancies Scale;*
  - *Drug Effects/Liking Questionnaire*;[99]
  - *E-Cigarette Purchase Task*

3.4 Safety Endpoints

- *Adverse Events Scale* assesses the nature, severity, duration, action taken, and outcome of adverse events related to tobacco product use;
- Potential adverse consequences: Change in mental (CESD) or physical health (heart rate, blood pressure, exhaled CO level, weight, Respiratory and Global Health Questionnaire, Health Changes Questionnaire, Adverse Events)
- Change in alcohol, substance use (Alcohol Use Questionnaire, Drug Use Questionnaire)
- Other tobacco product use

4.0 Study Intervention/Investigational Agent

4.1 Description of Product

Standardized research e-cigarette (SREC). The device and the disposable pods with liquids are manufactured by the NJOY company. The use of SREC is subject to the FDA Investigational Tobacco Product (ITP) review and will be used only after such review is completed. The device operates using a rechargeable 400 mAh lithium-ion battery and uses sealed disposable 1.9-mL pods with e-liquid containing 5% w/w nicotine. Flavors that will be used include tobacco, menthol, watermelon and blueberry. The device uses a battery that can be recharged via a micro USB port. The e-liquid and the aerosol are well-characterized in terms of chemical impurities and by-products (such as aldehydes), which are minimal.

4.2 Product Handling

Participants will receive the SREC products free of charge. The product will be used *ad libitum*. SREC will be purchased directly from NJOY, and all packaging will be labeled "for investigational use only." The products will be stored at Tobacco Research Programs at 717 Delaware SE in locked cabinets. Only authorized study coordinators will have access to the products and will dispense them to study participants.

4.3 ITP for SREC

An Investigational Tobacco Product application has been submitted for the use of SREC in this study. The communication from the FDA Center for Tobacco Products stated that there are no concerns with the proposed investigational use of SREC as described in this protocol (FDA STN # IU0000806, IU0000807, IU0000935, IU0000937, IU0000939/P00128).

4.4 Biosafety

Not applicable

4.5 Stem cell research

Not applicable

4.6 Product Disposal

Any unused, expired or returned products will be disposed of in collaboration with the U of M Department of Environmental Health and Safety – Regulated Waste. All disposed product will be counted, logged and weighed prior to disposal. A disposal professional from Environmental Health and Safety will collect the products slated for disposal after the last participant completes the study. The disposal log and destruction trail will be stored on Box. See SREC.Product.Disposal.SOP.V1 for additional details.

5.0 Procedures Involved

5.1 Study Design

Smokers will be asked to completely switch to SREC for 6 weeks. Study participants will be assessed at baseline and weeks 1, 2, 3, 4, and 6 for complete switching from cigarettes to SREC, as well as SREC use patterns and subjective measures. The potential changes in the levels of various toxicant and carcinogen exposures, inflammatory biomarkers, DNA adducts, and oral NNN formation will be assessed at baseline and at 4 and 6 weeks. The proposed clinical trial procedures and measures have been used in a number of studies we have conducted.[101-103]

5.2 Study Procedures

Smokers will undergo a screening visit, a baseline period to collect data and biological samples for the assessment during usual brand cigarette smoking, five clinic visits over the 6-week assessment period after product assignment, and a follow-up visit at 4 weeks after the end of the study. Study visits and measures are summarized in Table 3.

***Screening**:* The purpose of this visit is to inform the subjects about the study protocol, obtain informed consent, and further screen for eligibility (after being pre-screened over the telephone or online). In-person procedures will be completed curbside at the research clinic or socially distanced in the clinic to minimize contact between the study subject and coordinator. After obtaining informed consent, which will be done remotely using a secure video-conferencing link, screening questionnaires will be completed to collect demographic data and smoking history. Additionally, we will administer medical, and medication history questionnaires. Those who meet our preliminary eligibility will have a Baseline 1 visit scheduled. Our Licensed Medical Professional will review these health questionnaires for consistency and eligibility for the study. A reminder call or text will be sent to the participant prior to this visit.

***Baseline period**:* (Baseline 1) Once the participant arrives to the clinic visit (University of Minnesota – Tobacco Research Programs, 717 Delaware St. SE), their vitals will be collected. Next, participants will be handed a carbon monoxide (CO) device and asked to blow into the machine. Participants of childbearing potential will be asked to provide a urine sample to test for pregnancy. Vitals collection, CO measurement and confirmation of pregnancy will be the final criteria to determine the subject's eligibility. If eligible, the participants will be shown how to complete the daily ITR and given a urine cup and instructions on how to collect their first morning void urine the morning of their next visit. They will be asked to return to the clinic one week later for Baseline 2. Upon arriving to Baseline 2, the first morning void urine will be collected and the daily ITR reviewed. Vitals and CO will be collected. Participants will be given a plastic cup and asked to rinse their mouth with tap water. This is done in preparation for the oral sample collection (saliva and buccal cells), which will occur at the end of visit, at least 20 minutes after the rinse. Participants will also be asked to complete several online surveys.

Participants will be provided counseling throughout the study to help them quit the use of cigarettes. Once assigned to SREC, the study coordinator will work with the participant to set a quit date approximately 3-5 days after assignment. In the period leading up to the quit date, participants will be encouraged to try the product and use it regularly to ensure the product and the study are a good fit for them. This approach is incorporated to maximize the likelihood that participants who proceed with the study will comply with the requirements. One day before the set quit date, the coordinator will schedule a zoom meeting with the participant to go over ways to quit and discuss the incentive program we are using to help them quit. Approximately one day after the set quit date, the coordinator will contact the participant to see how they are managing and discuss ways to address any barriers to quitting they are experiencing. Additional counseling will be available throughout the study as needed for each individual participant.

Product dispensing and use instructions: Participants will be instructed to stop smoking and completely switch to SREC, using it *ad libitum*. At the baseline 2 visit, they will receive two devices with the USB cable, and sufficient number of refill pods to last until the next visit. Participants who smoke <10 cigarettes per day (CPD) will receive 4 pods, 10-20 CPD will receive 6 pods and 20+ CPD will receive 8 pods. Participants will be asked to contact the study staff if (s)he uses more than one pod on two days during the week, to supply them with additional pods. Participants will be allowed to choose a primary flavor they want to use and will be given a sample of one alternative flavor to try

if they choose. They will be allowed to request a different flavor if they decide they no longer like the current flavor. Prior to dispensing to participants, each pod box will be labeled with a unique ID, weight of on pod per batch will be recorded, and the pods will be placed in an envelope. The envelope will be marked with the dates of the week during which they will be used (e.g., *"Start: January 22, 2021. End: January 29, 2021"*). Subjects will be instructed on how to use the SREC device and told to completely empty a pod before opening a new one. They will be asked to save all the used pods and put them back in the envelope. At the next clinic visit, which will occur one week later, they will be asked to bring back the envelope with used and unused pods. The returned pods will be counted and weighed to record the consumption. The unused pods will be re-dispensed to the same subject to use in the following week. Participants will be asked to return devices and all remaining pods at the end of the study.

Blood collection. Participants may opt in to have their blood drawn during this study. To attenuate the influence of diurnal variations and the influence of feeding status on the levels of inflammatory cytokines, the baseline visit and visits at weeks 4 and 6 will be held in the mornings. *If a subject cannot come in the morning for the clinic visit at baseline, weeks 4 or 6, the visit will be scheduled for a later time of the same day, and the subject will be offered to come for a blood draw in the morning of the day prior, or up to two days after, that visit*. Participants will be asked to fast (10 hours) prior to the scheduled visit. By opting in, participants are agreeing to have their blood drawn in the morning (before 11:00 am) under fasting conditions. Two 10 mL EDTA tubes will be drawn.

Intervention period: The study calendar is based off of the quit date and subjects will come to the clinic weekly for 4 weeks and at week 6. Biological samples will be collected at weeks 4 and 6 after the start of the assigned product use. Prior to the biological sample clinic visit, the participant will collect their first morning void urine. Once the participant arrives to the clinic, their first morning void urine will be collected. Next, they will be asked to rinse their mouth with tap water in preparation for the buccal cell collection. At each of the clinic visits, participants will provide a breath CO. If participant reports smoking or the CO is high (> 6 ppm), a brief compliance counseling session will be conducted to discuss any challenges the participant is facing. Tools to deal with these challenges will be provided. Their vitals will also be collected. Participants will be asked to complete the relevant online surveys (see **Table 3**). The ITR data will also be reviewed for completeness. Used and unused SREC pods will be collected, recorded, and new envelopes with the products will be dispensed to last until the next visit, as outlined below.

At week 1, subjects will receive one envelope with enough product to use until their next scheduled visit at week 2. The envelope will be marked with the start and end date. The envelope will be brought back to the clinic when they come to the next scheduled visit so that weekly use of the product can be assessed. Similarly, at the weeks 2 and 3 visits, subjects will receive one envelope with products for the use until their next scheduled visit. At week 4 they will receive two envelopes with enough product to last until their week 6 visit. Subjects will be instructed to use the products from the corresponding envelopes during each week and place all used and unused products back in the same

envelopes at the end of each week. Again, subjects will be told to use the entire pod before starting a new one. The number of dispensed SREC pods will be adjusted based on the participant's daily use. Participants will continue to record their product use and if applicable, cigarettes smoked. Daily SREC use will be reported using number of bouts of e-cigarette use, estimated number of puffs per bout reported in the ITRS, and the total weight of consumed e-liquid (based on the difference of pod weights before and after use). Daily use regular cigarettes will be also recorded using the ITRS.

If a participant is unable to achieve cessation (confirmed biologically either via CO or urine) within the first two weeks of the study, the participant will be withdrawn. This allows us to maximize the number of complete switchers in the study.

***Follow up assessment*:** The purpose of the follow-up visit is to assess the potential changes in smoking status or patterns in study participants. Week 10 will be done remotely using a secure video-conferencing link. Online surveys will be completed. Those continuing to smoke will be strongly advised to quit and treatment resources will be provided if requested (Clearing the Air manual).

All in person visits may be switched to curbside or fully virtual if COVID restrictions require. The visit procedures will remain the same.

Table 3. Schedule of visits and measures

Study period	**Screen**	**Baseline 1**	**Baseline 2**	**Product use**					**F/U**
WEEK	**92**	**91**	**0**	**1**	**2**	**3**	**4**	**6**	**10**
Visit window		1 week		Can be done anytime during the week			-1 day, + 3 days	-1 day, + 3 days	-1 day, + 3 days
Biological samples and biomarker measures									
Alveolar carbon monoxide (CO)		x	x	x	X	x	x	x	
Vital signs		x	x	x	X	x	x	x	
Urine Pregnancy tests*		x		x			x	x	
NicCheck Test (if applicable)		x	x						
First morning void urine (for urinary biomarkers according to Aims 1-3)			x				x	x	
Blood collection (morning, fasting)*******			x				x	x	
Oral cells			x				x	x	
Saliva			x				x	x	
Subjective measures									

Demographics Questionnaire	x								
Tobacco Use History and Exposure	x								
Nicotine Dependence (FTND)**			x						
E-Cigarette Dependence (PS-ECDI)								x	
Environmental Tobacco Smoke Exposure			x				x		
Occupational & Environmental Toxicants			x				x		
Brief Medical History	x								
Concomitant Medications	x		x	x	x	x	x	x	
Prime MD	x								
CESD	x						x	x	
Alcohol Use Questionnaire***	x				x			x	
Recreational Drug Use Questionnaire***	x				x			x	
Respiratory and Global Health			x	x	x	x	x	x	
Health Changes Questionnaire			x	x	x	x	x	x	X
Adverse Events Scale			x	x	x	x	x	x	X
ITR product use recording		x	x	x	x	x	x	x	
Timeline Follow Back (TLFB)*****			x	x	x	x	x	x	
Product Evaluation Scale			x	x	x		x	x	
Purchasing Task******			x					x	
Drug Effects/Liking****			x	x	x	x	x	x	
Tobacco Use Status									X

* Pregnancy tests may also be administered at clinic visits if last menstruation reported being more than 35 days ago.
** All subjects will be evaluated at baseline.
*** Use over the past 30 days at weeks 2 and 6.
**** Regular cigarettes will be evaluated at baseline for all subjects; at the following visits only SREC will be evaluated.
*****To be done if participant reports using other tobacco products on the daily ITR or if there is missing data in the ITRS
****** At baseline, the cigarette purchase task will be done. At week 6, a product specific purchasing task will be performed.
******* Participants may opt-in to have their blood drawn

5.3 Study Duration

There will be approximately 11 weeks of participation for each subject (1-2 weeks between screening and the baseline period, 6 weeks of treatment, and 4 weeks follow-up). Over approximately 18 months, male and female smokers (N= up to 120) will be recruited for eligibility screening with the goal of 30 compliant completers (total abstinence for 6 weeks).

5.4 Individually Identifiable Health Information

Information about each subject will be entered into a database by the Study Coordinator. Each subject will be coded with a unique number, and only these coded ID's will be entered into the database. All raw data will be kept in locked file cabinets.

Only the Study Coordinator and Principal Investigators will have access to individually identifiable private information about subjects. Coded ID's will be used throughout the study by all the researchers involved. Because this study uses a research e-cigarette and we are required to submit an Investigational Tobacco Product application through the Food and Drug Administration, the records may potentially be monitored by this governmental agency. This information will be provided to the IRB and will be included in the human consent form. While all the samples and information will be collected specifically to achieve the goals of this proposal, de-identified individual subject data and back-up samples may be available to other researchers for research purposes after our study is complete. Consent will be obtained from subjects to allow de-identified biosamples to be stored in a biorepository for future analyses of biomarkers or genotyping.

5.5 Use of radiation

Not applicable

5.6 Use of Center for Magnetic Resonance Research

Not applicable

6.0 Data and Specimen Banking

6.1 Storage and Access

Biomarker specimens will be collected and stored at the study site *(University of Minnesota - Tobacco Research Programs, 717 Delaware St. SE)* until delivery to the Masonic Cancer Center's Dr. Irina Stepanov laboratory for storage and analysis. Sample location will be logged in REDCap during initial collection and processing and following the transfer from 717 Delaware to Dr. Stepanov's lab. All biosamples transferred from 717 Delaware St. SE to Dr. Stepanov's lab will be logged on the day of their transfer both outgoing from 717 and received by the lab. Samples that are not used for the primary analysis of study biomarkers will be banked for future use. The banked samples will be stored until analyses and destroyed if it is determined they are no longer needed. The samples, which may also include DNA or RNA, may be stored up to a maximum of 10

years from the study's end. A subject has the right to withdraw consent at any time by informing the Principal Investigator by following the instructions provided in the consent and HIPAA documents. If this occurs, any remaining identifiable research sample(s) will be destroyed.

6.2 Data

Data will be stored in a secure database (RedCap) at the study site at the University of Minnesota, Masonic Cancer Center. Only study personnel directly involved with the study will have access to the linked records. The samples that will be transferred to the laboratory or stored for future analyses will be de-identified. We will not be transferring data that has any identifying information over the internet.

Biomarker samples (blood, urine, oral and buccal cells) that are banked after the completion of the primary analyses will be stored at the Masonic Cancer Center Stepanov laboratory located at the Cancer and Cardiovascular Research Building for future use.

6.3 Release/Sharing

No identifying information will be shared with outside investigators. If used in any collaborative efforts beyond the scope of this study, any shared data will be de-identified. However, records for the study may be reviewed by other investigators in the Tobacco Research Programs, for instance with the goal of ensuring that participants are enrolled in only one study at a time or that they meet the eligibility criteria to participate and to maintain their safety. The records may also be reviewed by departments at the University with appropriate regulatory oversight or a representative of the funding agency, National Institutes of Health, and the Food and Drug Administration.

7.0 Sharing of Results with Participants

Information on the biomarker measurements will not be shared with participants. However, results of clinical measures, such as blood pressure or heart rate, may be shared with participants if they ask about it or if such measures are out of normal range. If a participant will request to share these clinical measures with their clinician, they can provide a written request that this clinical information be shared. No biomarker measurements will be shared with clinicians.

8.0 Study Population

8.1 Inclusion Criteria

a) Male or female smokers who are 21-65 years of age and are willing to stop smoking and completely switch to e-cigarettes;
b) Report smoking ≥ 5 cigarettes daily;
c) Biochemically confirmed regular smoking status by a CO level ≥ 6 ppm, (if < 6, then NicCheck test of 6 or greater);
d) Smoking daily for at least 1 year;

e) No unstable and significant medical or physical conditions as determined by the licensed medical professional (to ensure safety of the subject, to minimize the effects of poor health on biomarker measures and to maximize compliance to study procedures);
f) No unstable pulmonary illness (COPD, asthma, COVID, current bronchitis) or exacerbation of symptoms in the past 3 months as determined by the licensed medical professional;
g) Subjects are in stable, good mental health (i.e. not currently, within the past 3 months, experiencing unstable or untreated psychiatric diagnosis, including substance abuse) as determined by the licensed medical professional;
h) Subjects who are not taking medications that affect relevant metabolic enzymes (as noted on the excluded medication list);
i) Women who are not pregnant or nursing or planning to become pregnant;
j) Subject has provided written informed consent to participate in the study.

8.2 Exclusion Criteria

a) Regular tobacco or nicotine product use (including nicotine replacement) (> 9 days in last 30 days) other than cigarettes;
b) Currently (within the last week) using nicotine replacement or other products for cessation purposes;
c) Significant immune system disorders, respiratory diseases, kidney or liver diseases or any other medical disorders that may affect biomarker data;
d) Excessive drinking (5 or more drinks daily) or problems with drinking or drugs (self-report of binge drinking alcohol or treatment for drug or alcohol abuse within last 3 months); to be assessed by PI or licensed medical professional;
e) Failure to agree to take adequate protection to avoid becoming pregnant during the study;
f) Vital signs outside of the following range (participants failing for vital signs will be allowed to re-screen once):
    - Systolic BP greater than or equal to 160 mm/hg
    - Diastolic BP greater than or equal to 100 mm/hg
    - Systolic BP below 90 mm/hg and symptomatic (dizziness, extreme fatigue, difficulty thinking, inability to stand or walk, feeling faint)
    - Diastolic BP below 50 mm/hg and symptomatic (dizziness, extreme fatigue, difficulty thinking, inability to stand or walk, feeling faint)
    - Heart rate greater than or equal to 105 bpm
    - Heart rate lower than 45 bpm and symptomatic (dizziness, extreme fatigue, difficulty thinking, inability to stand or walk, feeling faint)
g) Expired air carbon monoxide (CO) level greater than 80 ppm;
h) Self-reported allergies to propylene glycol, lactic acid or vegetable glycerin;
i) Adverse reactions when previously using electronic cigarettes;
j) Unable to read for comprehension or completion of study documents (determined during the consenting/screening process);
k) Unstable living environment that would compromise the ability to attend visits, sequester study products or complete study procedures outside of visits.

l) Smoked marijuana use >9 times per month, and/or unwillingness to abstain from smoked marijuana during switch to study product (non-combusted forms of marijuana are acceptable)
m) Medical history of thromboembolic events and/or cerebrovascular events, or medical diagnosis that the medical professional believes indicates a high risk of thromboembolic or cerebrovascular events.

8.3 Screening

Cigarette smokers will be first pre-screened for eligibility over the telephone or online. If subjects meet the initial eligibility criteria for the study, they will be asked to complete a remote screening visit (completed via secured video-conferencing meeting link) where the entire study will be explained in detail, informed consent will be obtained and the screening questionnaires will be completed.

9.0 Vulnerable Populations

No vulnerable populations will be recruited.

10.0 Local Number of Participants

We will recruit up to 120 smokers in order to complete at least 30 participants 20 achieving complete abstinence.

11.0 Local Recruitment Methods

11.1 Recruitment Process

Cigarette smokers will be recruited through the University of Minnesota Tobacco Research Programs. A variety of media will be used that will foster the recruitment across a spectrum of age, education and socioeconomic status, and race/ethnicity.

Cigarette smokers will contact the Tobacco Research Programs and will be pre-screened for eligibility over the telephone or in REDCap. During this pre-screening, information will be obtained on where the subject heard about the study, their geographic location, and basic demographics. This data will provide information on the primary media avenue, radio or television station, advertisement placements, internet location or domain responsible for recruiting subjects. We will use this information in our recruitment efforts.

11.2 Source of Participants

Primary recruitment via advertisements from Minneapolis-St. Paul metro area.

11.3 Identification of Potential Participants

Participants will self-identify first in response to advertisements and contact the study coordinator. Then, they will be screened over the phone or online for eligibility.

11.4 Recruitment Materials

Subjects will be recruited through printed flyers and advertisements through a variety of media outlets and the internet.

11.5 Payment

Subjects will receive $25 for screening, $30 for the Baseline and Intervention visits ($20 for the visit and $10 for transportation expenses). Those who consent to the blood draw will receive an additional $20 for each blood draw. In order to maximize overall compliance, participants will be paid a $600 bonus,). Subjects will be told that the bonus is contingent on compliance and this will be monitored via their daily ITR responses, product accountability, completion of forms, and submission of biological samples, and CO $\leq$ 6 ppm. The maximum amount they can earn is $925.

12.0 Withdrawal of Participants

12.1 Withdrawal Circumstances

If a participant is unable to achieve cessation (confirmed biologically either via CO or urine) within the first two weeks of the study, the participant will be withdrawn. Additionally, a study participant may be discontinued from the study if investigators determine that this is the best decision in order to protect his/her safety. If a participant either withdraws from the study or the investigators decide to discontinue a participant due to an adverse event (AE) or serious adverse event (SAE), the participant will have appropriate follow-up assessments and if necessary, referrals will be made for medical care. The participant experiencing an AE/SAE will be followed until the problem resolves, stabilizes, or is clearly unrelated to the study product. Any AE that remains open will be reviewed and closed at the Week 10 follow-up interview.

For the participant's protection, they will be withdrawn immediately from the study if any of the following occur:

1) Cardiovascular disease (CVD) event: Typically include*s* MI (heart attack), PTCA (angioplasty/stenting), bypass surgery, stroke, or peripheral vascular disease. Less common CVD problems would be new cardiac arrhythmias (e.g., new atrial fibrillation) or new valvular disease (e.g., mitral or aortic regurgitation).
2) DVT/PE (deep vein thrombosis/pulmonary embolism, i.e., blood clots in the venous system).
3) Suicide Attempt: A participant will be withdrawn if he/she attempts suicide at any time during participation in the study.
4) Psychiatric Hospitalization: A participant will be withdrawn if he/she is hospitalized for psychiatric reasons at any time during participation in the study.
5) Pregnancy: If a participant becomes pregnant, she be immediately withdrawn from the study. In addition, the licensed medical professional will follow-up after delivery to ask questions about the health of the baby.
6) Expired breath carbon monoxide increase: A participant will be withdrawn from the study if the average of the 2 (or 3) CO readings is 100 ppm or greater.

7) Respiratory Illness Event: A participant will be withdrawn if they develop a severe (hospitalized or the symptom had a significant impact on his/her activities) respiratory illness that is related or possibly related to the SREC product as determined by the licensed medical professional.

The following will be monitored and can lead to the participant being withdrawn by the Principal Investigators or Medical Monitor:

1) Blood pressure (BP) or heart rate (HR) changes: If any of the following occur post-enrollment:
   1) BP is at or above 160 systolic/100 diastolic; 2) BP is below 90 systolic/50 diastolic and the participant is experiencing symptoms listed on the 'Blood Pressure and Heart Rate Symptom Checklist; 3) HR is at or above 105 bpm; 4) or below 45 bpm and the participant is experiencing symptoms listed on the 'Blood Pressure and Heart Rate Symptom Checklist.
2) Medication changes: If a participant begins taking any of the exclusionary medications or other medications that could potentially have a smoking-drug interaction post- enrollment, the licensed medical professional will determine how best to monitor and minimize potential risks (including withdrawal if warranted). We will also recommend that a letter be provided to the participant to give to their physician (with their consent) making them aware of the potential changes in smoking that could occur because of participation in the study.
3) Any hospitalization or debilitation in which participation in the study could be detrimental to the recovery process. This will be self-reported by the participant and reviewed by the investigators and medical professional to determine if continued participation in the study is appropriate.
4) If a participant is behaving in an inappropriate or threatening manner, admits to lying about eligibility criteria, including omitting previous medical diagnoses and medications, is participating in other smoking research studies that could affect the primary outcome measures, does not follow study instructions, etc., then he/she can be withdrawn at the investigators' discretion.
5) If there is reason to believe the participant is sharing large quantities of the study product with other people.

If a participant chooses to withdraw from the study at any time, we will ask them to return to the clinic to return study product and to receive their final study payment.

12.2 Withdrawal Procedures

Subject will be informed about the withdrawal at the visit and data collection will stop.

12.3 Termination Procedures

No additional procedures will be conducted if subjects are withdrawn.

13.0 Risks to Participants

13.1 Foreseeable Risks

The foreseeable risks for this study are minimal. Potential risks for all participants include: emotional discomfort, breach of confidentiality, nicotine withdrawal symptoms, effects of returning to regular smoking, effects of smoking the SREC e-cigarettes, risks associated with smoking, risks to fetuses, and changes in blood pressure/heart rate and pain/bruising at the site of the phlebotomy.

1. Survey Questionnaires: The interviews will include questions about medical history, drug and alcohol use, and questionnaires about mood. Answering these personal questions could make the subject feel uncomfortable.
2. Breach of Confidentiality: The risk of the interview is loss of privacy if other people find out the results.
3. Obtaining Blood Pressure: The blood pressure cuff may cause minimal discomfort. In obtaining blood pressure, researchers may find out the subject has abnormal blood pressure and must refer them to their primary care physician.
4. Smoking Cigarettes: All cigarettes are detrimental to a person's health and can lead to severe or fatal medical problems including:
    a. Cardiovascular Diseases: Coronary heart disease, heart attack, stroke, peripheral vascular disease, reduced blood circulation, abdominal aortic aneurysm
    b. Respiratory Diseases: Emphysema, bronchitis, tuberculosis and chronic airway obstruction
    c. Cancers: Lung, bladder, liver, colon, cervical, esophageal, kidney, larynx, mouth, pancreatic, throat, stomach cancers and acute myeloid leukemia
    d. Diabetes
    e. Immune function, rheumatoid arthritis
    f. Other Health Risks Associated with Smoking: Including but not limited to infertility, ectopic pregnancy, lower bone density in postmenopausal women, hip fracture in women, male sexual dysfunction; age-related macular degeneration, blindness and cataracts
5. Use of SREC e-cigarette: The side effects of SREC may be similar to other commercially available vaping devices. Such devices can expose users to several chemicals, including nicotine, carbonyl compounds, and volatile organic compounds, known to have adverse health effects. The health effects and potentially harmful doses of heated and aerosolized constituents of e-liquids, including solvents, flavorants, and toxicants, are not completely understood. E-cigarette aerosol is not harmless "water vapor" although it generally contains fewer toxicants than combustible tobacco products. Specific potential known risks include:
    a. Refill pods that will be used in this study contain nicotine. Nicotine is an addictive chemical. It is possible that this experience could lead to long-term use of e-cigarettes after the trial is over.
    b. Nicotine may contribute to some of the disease associated with smoking
    c. The most common side effects related to e-cigarette use are changes in taste, mucus in throat/sinus, dry mouth, dry cough, throat irritation, sore

throat, mouth ulcers, dizziness, headache, and nausea, difficulty breathing, heart palpitations, vomiting, abnormal heart rate, stomach discomfort, rash, chest pain.

d. Although uncommon, batteries from e-cigarettes have exploded/ignited and injured users.
e. E-cigarettes can overheat and present a minor burn risk if the button is turned on repeatedly.
f. Ingestion of e-liquids containing nicotine can cause acute toxicity and possible death if e-liquids containing nicotine are consumed. Participants will be directed to keep device and all e-liquid refill cartridges away from pets and children.
g. On rare occasions, allergic reactions have occurred after using e-cigarettes.
h. Severe lung disease has been associated with using e-cigarette/vaping products. The CDC and FDA continue to investigate, but the majority of cases have involved the use of cannabis oil, such as THC, obtained on the black market. In addition, one of the chemicals of concern is the addition of Vitamin E acetate to e-liquid cartridges and pods.

6. Dual Use of Tobacco or Nicotine Products: There is also the chance of use of more than one product (e.g., continued smoking among participants) and/or continued use of the products. Cessation of all tobacco products will be strongly recommended to the subjects both at the end of the experimental phase and at follow-up.

7. Smoking Withdrawal: Participants may experience smoking withdrawal symptoms during this study. The symptoms can be uncomfortable but are typically of minimal risk. Smoking withdrawal symptoms include:

   a. Anger, irritability, frustration
   b. Anxiousness, nervousness
   c. Depressed mood or sadness
   d. Desire or craving to smoke
   e. Difficulty concentrating
   f. Increased appetite, hunger or weight gain
   g. Insomnia, problems sleeping or awakening at night
   h. Restlessness
   i. Impatience
   j. Constipation
   k. Dizziness
   l. Coughing
   m. Dreaming or nightmares
   n. Nausea
   o. Sore Throat

8. Returning to Regular Smoking: It is possible that if participants return to smoking their usual brand of cigarette at the end of the study they may experience mild and transient nausea, dizziness, and lightheadedness.

9. Changes in Blood Pressure and/or Heart Rate: Smoking and nicotine can affect the cardiovascular system which may result in changes in blood pressure and/or heart rate.
10. Changes in Mood, Emotions and Psychiatric Symptoms: Smoking and nicotine can affect a person's mood and emotions and are associated with psychiatric disorders including major depressive disorder, general anxiety disorder, bipolar disorder and eating disorders. Any changes in nicotine use or cigarette consumption could adversely affect mood, emotions and the symptoms related to psychiatric conditions in some individuals.
11. Risk to Fetus: Smoking during pregnancy can lead to miscarriage, preterm delivery, stillbirth, low birth weight, problems with the placenta, birth defects such as cleft palate, sudden infant death syndrome (SIDS), early childhood behavioral problems, altered lung development and possible propensity for addiction. Although unknown, similar effects could occur with the use of vaping devices.
12. Smoking and Oral Contraceptives in Women: Women who smoke and are over the age of 35 should not take oral contraceptives that contain estrogen without consulting their physician. Smoking while using oral contraceptives can increase the risk of having a cardiovascular event such as a heart attack or stroke. Additionally, there is a potential risk of thrombosis associated with hormonal therapy (including contraceptives) and smoking.
13. Smoking and Medications: Quitting smoking can greatly benefit participants' health. However, changes in smoking can lead to changes in how well some medications work. Participants should disclose all medications they are taking. We also recommend that participants discuss any planned or actual changes in how much they smoke with their doctor, especially if they are taking any medications for psychiatric, cardiovascular, or other serious diseases.
14. Unforeseen risks: There may be additional unforeseen short and long-term risks of participation.

Because smoking causes a multitude of diseases, if at any time the smoker wants to quit smoking, this decision will be encouraged and supported. At the end of study participation, subjects will be strongly encouraged to stop use of all tobacco products and to set a quit date and provided with a treatment resources and referral to different treatments.

13.2 Reproduction Risks:

Pregnant women will not be recruited. If participants choose to be sexually active, they should use an appropriate "double barrier" method of birth control (such as female use of a diaphragm, intrauterine device (IUD), or contraceptive sponge, in addition to male use of a condom) or the female should be using prescribed "birth control" pills, injections, or implants. Female participants with child-bearing potential will be tested for pregnancy at the baseline visit and monthly thereafter, if applicable. If a participant becomes pregnant during the study, she will be withdrawn from the study. Approximately 30 days after being withdrawn or having a positive pregnancy test, the research staff will call the

participant to confirm her due date. The licensed medical professional will follow-up with the participant after delivery to ask questions about the baby's health.

13.3 Risks to Others:

Second-hand exposure from SREC or usual brand cigarettes may be a risk to others.

14.0 Potential Benefits to Participants

Whereas no assurance can be made to an individual subject that he/she will personally benefit from such research, the experience should not impose any significant risk. Subjects will have the opportunity to learn about factors that may be associated with cigarette smoking. Quitting tobacco will be strongly recommended to our subjects and cessation materials will be provided. Referrals to community resources will also be made.

15.0 Statistical Considerations

15.1 Data Analysis Plan

The goals of this study are: to determine the effect of switching to SREC on biomarkers of exposure and effect (assessed at 4 and 6 weeks); to determine levels of salivary NNN and HPB-releasing DNA adducts in SREC users (assessed in the verified complete switchers at weeks 4 and 6); and to determine important moderators for SREC use and exposure/effect measures.

Demographic and smoking history variables will be summarized and compared to national survey data for the general population of smokers. Baseline covariates will be summarized to inform the comparison data retrieval on non-users of nicotine/tobacco and smokers from recently completed studies. Continuous measures will be summarized by mean (SD), median (range) or other summary measures. The distribution of biomarker and cytokine levels is usually highly right-skewed and will be analyzed in the log scale and reported as geometric means and 95% confidence intervals. Categorical covariates will be summarized by contingency tables.

The primary endpoints are the weeks 4 and 6 for (1) toxicant and carcinogen exposure biomarkers; (2) inflammatory biomarkers; and (3) DNA adducts adjusted for the baseline value. The analysis of our primary endpoints will use linear mixed models to account for potential intra-subject correlation due to repeated measurements at 4 and 6 weeks from a single individual. With two repeat observations, the within subject correlation structure will be compound symmetry. Residual plots and other diagnostic methods will be used to check model assumptions. The mean model for each hypothesis will include the overall mean and an effect for time (4 or 6 weeks) Therefore, our primary analysis for all endpoints will only adjust for the baseline biomarker level (for precision). Due to the multiple biomarkers being tested in this exploratory trial, a reduced alpha level of 0.001 will be used to determine statistical significance for the time effect. An additional analysis will be completed adjusting for important factors such as environmental toxicant exposures, stress and health to determine the effect their inclusion has on the association between group and biomarkers. The secondary endpoints include the amount of product

used (cigarettes per day, number of e-cigarette episodes and puffs per day and the number of smoke-free days (cigarette avoidance). These outcomes are considered counts and will be analyzed as Poisson or negative binomial variables. Product use by visit will be analyzed by generalized estimating equation (GEE) models, which account for the multiple visits for each individual. Smoke-free days across the entire study period (approximately 42 days) will be analyzed by a Poisson or negative binomial regression. These analyses will include an offset term to adjust for the number of days reported by visit and in total. Covariates will include baseline patient characteristics that may affect patterns of use. Exploratory endpoints include the subjective measures which will be collected up to 6 times during the study period. Measures providing a score will be analyzed using a linear mixed model. The fixed effect is visits. AEs and SAEs (safety endpoints) will be tabulated. Compliance to the assigned treatment is a concern and we are considering the possibility that the non-compliance rate could be as high as 50%, despite our efforts to keep it at low as possible. Complete switching will be biochemically verified; therefore the analysis will be repeated with only those identified as completely switching to SREC. Estimates and confidence intervals of the degree of reduction in outcome variables can be determined for those that successfully switched to the study product. In addition, we will summarize compliance and evaluate the correlation between compliance and baseline covariates to identify potential predictors of treatment compliance. Finally, we will complete a sensitivity analysis that accounts for missing information using multiple imputation with the Markov Chain Monte Carlo (MCMC) method carried out in PROC MI in SAS. Missing values are imputed using regression models developed from baseline covariates. Values can be imputed for both continuous and categorical variables.[104] Following imputation, standard statistical methods can be applied. A final single assessment of time effect will be obtained from combining the results across the imputed datasets using PROC MIANALYZE in SAS.

15.2 Power Analysis

Based on our previous experience, we estimate the inter-subject coefficient of variation (CV) for the biomarkers listed in Table 1 to be between 30% and 40%. Biomarkers will be analyzed in the logarithmic scale; as a result, the standard deviation (SD) of the log of the level of these biomarkers is approximately equal to the CV in their original scale. For purposes of our power calculations, we will set the SD for each biomarker at 0.4 and assume a correlation of 0.5 between baseline and 6-week biomarker levels. The major hypotheses will be tested including only smokers who completely switch from smoking to SREC use. If a conservative estimate of only 50% of the 30 enrolled subjects achieving complete switching to SREC is applied, then 15 subjects would have greater than 90% power to detect a 45% decrease in the biomarker level in 6 weeks with a paired t-test. However, we incorporated measures to maximize the complete switching (as described in the Study procedures section) and will aim to have at least 20 subjects achieving this goal. The significance level was set at 0.001 due to multiple biomarkers being tested.

15.3 Data Integrity

A variety of measures will be taken to ensure data accuracy and completeness. The regular research team meetings will include discussions of proper methods for data

collection, transmission, and storage, limiting data collection to those in protocol required to answer a research question, de-identifying data, and encryption methods.

A comprehensive data dictionary will be created to specify definitions and value codes for all variables that will be entered into REDCap and other study databases. Electronic forms for the collection of subjective measures via REDCap will include programming features to ensure valid data (i.e., input masks, validation criteria, skipout logic) and will be stored on the University of Minnesota, HIPAA compliant, computing system. Double entry will be used for all other de-identified data entered into REDCap. Biological specimens will be labeled with barcode labels that incorporate the participant ID. The secured biospecimen website will identify the location of each sample at the clinic (prior to submission to the laboratory) and in the Stepanov laboratory.

Oversight of the product distribution will be conducted by the Project Manager in collaboration with the co-Principal Investigators. A Product Tracking database will be created. This database will be used to track product purchases, product inventory, and assignment of study product to participants.

Quality control procedures will be conducted for all data collected, including analysis of missing data, and logic checks for out of range and other anomalous values. Queries will be made regarding such data issues, with documentation of any changes made in the data.

16.0 Confidentiality

All investigators and staff associated with this project have been trained, and new hires will be trained, on human research ethics and Good Clinical Practice in accordance with the requirements of the University of Minnesota.

Only the Study Coordinator and Principal Investigators will have access to individually identifiable private information about subjects. Coded ID's will be used throughout the study by all the researchers involved. Because this study uses a research e-cigarette and we are required to submit an Investigational Tobacco Product application through the Food and Drug Administration, the records may potentially be monitored by this governmental agency. This information will be provided to the IRB and will be included in the human consent form.

17.0 Provisions to Monitor the Data to Ensure the Safety of Participants

17.1 Data Integrity Monitoring

Oversight for quality control and adherence to protocol procedures will be conducted by the Project Manager in collaboration with the co-Principal Investigators. A start-up meeting with whole research team will take place prior to participant enrollment. During this meeting, there will be training on the study protocol, standard operating procedures, equipment and data collection platforms. The co-Principal Investigators will closely monitor the research staff on the procedures to be used in this study. Such monitoring will consist of frequent in-person discussion of study visits and other procedures to make sure that all protocol procedures are followed, and regular research team meetings to

provide updates on study progress and review the data collection process, the results from data monitoring and other issues of concern.

Standard operating procedures will be developed for consistent implementation of the protocol. Study Coordinator will be administering all measures during clinic visits and entering the information about each subject into a database. Each visit will have a checklist of all measures that need to be obtained and the order by which they will be administered. Project Manager will be directly supervising the Study Coordinator and periodically review protocol compliance and implementation, and adherence to good clinical practice procedures.

The Study Coordinator will go over the questionnaire instructions and will be available to the participant to answer any questions he/she may have. Questionnaires will be reviewed for completeness while the participant is present. Other questionnaires are completed on the computer, so that participants cannot accidentally skip a question. However, they may choose not to answer questions. Several biochemical measures (expired breath CO and urine pregnancy) will be analyzed immediately, while the participant is present. If necessary (e.g., if the sample volume is insufficient for analysis), the Study Coordinator can gather another sample immediately and re-analyze.

Once a participant has completed all study procedures and all open events have been closed, the PI will review the participant's binder and sign a form indicating study completion for that participant.

17.2 Data Safety Monitoring

CTSI is responsible for periodically monitoring this study. While participating in the trial, AEs and concomitant medications will be assessed at every study visit and vital signs will be obtained periodically. AEs will typically be identified during the administration of the Health Changes Questionnaire and Respiratory and Global Health Questionnaire, and in some cases during the administration of the CES-D. Other events may be identified from physiological study measures or by spontaneous reports during assessments.

Assessment of Questionnaire Items

- Health Changes Questionnaire: If the participant answers 'YES' to Questions 1, 2, or 3, the interviewer will assess for an 'Adverse Event.'
    1) *Have you had any negative changes in your health since your last visit?*
    2) *Have you had any changes in medication since your last visit?*
    3) *Since your last visit, have you received any form of medical care?*
- Respiratory Health Questionnaire: If the participant indicates 'YES' to Question 6 regarding having a cold or flu the interviewer will administer the 'Adverse Event Log.'
    6) *In the past week, have you experienced any health problems, such as cold, flu, or other respiratory illness that would affect these respiratory symptoms?*
- CES-D: If the participant scores 16 or higher and are not already being monitored for depression, an 'Adverse Event' will be recorded and the licensed medical

professional will provide information regarding follow-up. If there is already an open event, information will be added to the existing 'Adverse Event.'

Assessment of Physiological Data

- CO level: An 'Adverse Event' will be recorded if the average of two consecutive measurements in the same visit is:
  - CO is greater than 50 ppm if CO at Baseline is < 20 ppm
  - CO is greater than 60 ppm if CO at Baseline is 20 – 34 ppm.
  - CO is greater than 70 ppm if CO at Baseline is 35 – 49 ppm.
  - CO is greater than 80 ppm if CO at Baseline is 50 – 64 ppm.
  - CO is greater than 90 ppm if CO at Baseline is 65 – 80 ppm.
- Blood Pressure:
  - The 'Adverse Event Log' and 'Blood Pressure and Heart Rate Symptom Checklist' will be completed if an automatic and subsequent manual blood pressure measurement during the same visit is at or above 160 systolic or 100 diastolic.
  - The 'Adverse Event Log' and 'Blood Pressure and Heart Rate Symptom Checklist' will be completed if an automatic and subsequent manual blood pressure measurement during the same visit is below 90 systolic or 50 diastolic and the participant is experiencing symptoms listed on the 'Blood Pressure and Heart Rate Symptom Checklist.'
- Heart Rate:
  - The 'Adverse Event Log' and 'Blood Pressure and Heart Rate Symptom Checklist' will be completed if an automatic and subsequent manual heart rate measurement during the same visit is at or above 105 bpm.
  - The 'Adverse Event Log' and 'Blood Pressure and Heart Rate Symptom Checklist' will be completed if an automatic and subsequent manual heart rate measurement during the same visit is below 45 bpm and the participant is experiencing symptoms listed on the 'Blood Pressure and Heart Rate Symptom Checklist.'

Adverse Events Communicated by Participants

The occurrence of AEs will be sought by non-directive questioning of the participant at each visit during the study. AEs also may be detected when the participant volunteers them during or between visits or through physical examination, laboratory test, or other assessments.

Review and Reporting of Adverse Events and Serious Adverse Events

Co-Principal Investigators with oversight from Sharon Allen, M.D. (Medical Monitor) and Jane Schulz (Nurse Practitioner) will review all AEs and assess whether they are related to the study product.

An AE is defined as the appearance or worsening of any undesirable sign, symptom, or medical condition occurring after starting the study procedures even if the event is not considered to be related to the study product. Medical conditions/diseases present before starting the study are only considered adverse events if they worsen after starting the study product. Abnormal laboratory values or test results constitute adverse events only if they induce clinical signs or symptoms, are considered clinically significant, or require therapy; there are no plans for active monitoring of laboratory tests as part of this project. Withdrawal symptoms are considered adverse event if the symptom had a significant impact on the participant's daily life, caused a major disruption of functioning, or took any medication for it.

To the extent possible, each adverse event will be evaluated to determine:

1. the severity grade (mild, moderate, severe)
2. its relationship to the study product used (suspected/not suspected)
3. its duration (start and end dates or if continuing at final exam)
4. outcome (resolved/improved/unchanged/worsened; study product temporarily interrupted or permanently discontinued due to this adverse event; concomitant medication taken; non-drug therapy administered)
5. whether it constitutes a SAE

Information about all SAEs will be collected and recorded on the project's Serious Adverse Event Report Form. A SAE is defined an undesirable sign, symptom or medical condition which:

1. is fatal or life-threatening;
2. requires or prolongs hospitalization;
3. results in persistent or significant disability/incapacity;
4. constitutes a congenital anomaly or a birth defect;
5. is medically significant, in that it may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above.

Events not considered to be SAEs are hospitalizations that are:

1. elective or pre-planned, for a pre-existing condition that is unrelated to the products under study and did not worsen;
2. for general care, and/or overnight observation;
3. treatment on an emergency, outpatient basis for an event not fulfilling any of the definitions of serious given above and not resulting in hospital admission.

Safety evaluation will be performed on all accrued participants for whom SREC was dispensed. The assessment of safety will be based on the frequency of AEs and severity grade of AEs. Other safety data (e.g. CO, vital signs) will be considered as appropriate.

SAEs occurring after the participant has signed the consent form and until 4 weeks after the participant has stopped use of study product that are unexpected and at least possibly related will be reported. SAEs occurring more than 4 weeks after study discontinuation need only be reported if a relationship to study product is suspected. All AEs will be summarized by presenting, for each treatment group, the number and percentage of participants who experienced any AE, the number reporting AEs in each body system and

the number of AEs by type. Any other information collected (e.g., severity or relatedness to study medication) will be listed as appropriate. A summary of clinically relevant toxic events, such as AEs leading to death or rated as SAEs, those with a suspected relationship to study product, or AEs requiring further medication or non-drug therapies will be provided. Reports will be reviewed regularly by the study investigators and the NIDA Scientific Officer.

Data Safety Monitoring Plan administration

The Co-Principle Investigators will be responsible for monitoring the safety of this trial, executing the DSM plan for this project and complying with the reporting requirements.

Investigators will meet at least monthly throughout this project to review any recruitment, data quality, and safety issues. Specifically, they will assess enrollment information, demographics and characteristics of the participants, the expected versus actual recruitment rates, quality assurance or regulatory issues that may have occurred during the past month, protocol violations, and review AEs to determine if there are any changes in participant risk. Investigators are available to meet outside of the scheduled meetings, if concerns regarding a particular participant or another problem should arise.

The DSM report will include treatment group information. This includes enrollment information, demographics and characteristics of the participants, the expected versus actual recruitment rates, quality assurance or regulatory issues that may have occurred during the year, a summary of AEs and SAEs, protocol violations, and any actions or changes to the protocol. Also included will be any and all actions by the IRB.

18.0 Provisions to Protect the Privacy Interests of Participants

18.1 Protecting Privacy

It will be made clear to participants that all information obtained during assessments is confidential and that no information will be shared with the participants' clinicians unless the participant requests this in writing. The only information that will be shared with clinicians upon such request by a participant would be clinical measures such as blood pressure or heart rate. Biomarkers analyzed for the study will not be shared.

While all the samples and information will be collected specifically to achieve the goals of this proposal, de-identified individual subject data and back-up samples may be made available to other researchers for research purposes after our study is complete. We will obtain permission from subjects to allow de-identified biosamples to be stored in a biorepository for future analyses of biomarkers or genotyping.

18.2 Access to Participants

There will be no access to medical records or any other sources of private information about the participating subjects.

19.0 Compensation for Research-Related Injury

19.1 Compensation for Research-Related Injury

The study poses minimal risk to participants. In the event that this research activity results in an injury, treatment will be available, including first aid, emergency treatment and follow-up care as needed. Care for such injuries will be billed in the ordinary manner, to the participant or the participant's insurance company. The participants will be asked to let the study physicians know right away if they think that they have suffered a research related injury.

19.2 Contract Language

Not applicable

20.0 Consent Process

20.1 Consent Process (when consent will be obtained)

The consenting process will take place remotely via a secured video-conferencing meeting invitation. Potential participants will be told the nature of the research during pre-screening and then at the screening visit. They will be told they may discontinue participation at any time and will not be discriminated against if they choose to do so. Interested subjects will be provided considerable time to review the consent form, consider whether or not to participate, and have any questions answered by the coordinator. Participants will be required to demonstrate an understanding of the study purpose and procedures prior to signing the consent form. Assessment of the subject's understanding will be completed via questions by a slideshow presentation. The consent form must be signed before the research is started. Immediately after signing, the participant will receive an email with a signed copy of the consent form.

The electronic consent forms will be stored in a REDCap database with restricted access for essential study personnel only. The electronic informed consent (eIC) will be built us the 'UMN e-Consent HRP-592-TEMPLATE-Biomedical'. The template will be customized to match the written informed consent form exactly. The electronic signatures obtain in the outline above are intended to be the equivalent of handwritten signatures. Therefore, the electronic signatures will occur in accordance with the predicated rule (e.g. approved, reviewed and verified) as outlined in the Food and Drug Administration's CFR part 11.

20.2 Waiver or Alteration of Consent Process

Not applicable

20.3 Non-English Speaking Participants

Not applicable

20.4 Participants Who Are Not Yet Adults

Not applicable

20.5 Cognitively Impaired Adults, or adults with fluctuating or diminished capacity to consent

Not applicable

20.6 Adults Unable to Consent:

Not applicable

21.0 Setting

21.1 Research Sites

The study will be conducted at the University of Minnesota, Twin Cities.

- Subject recruitment and sample collection will take place at Tobacco Research Programs (717 Delaware St. SE, Minneapolis, MN 55414).
- Biochemical analyses will be carried out in the Masonic Cancer Center (2231 Sixth Street SE, Minneapolis, MN 55455).

22.0 Multi-Site Research

Not applicable

23.0 Resources Available

The study will be conducted at the University of Minnesota's Tobacco Research Programs housed at the Delaware Clinical Research Unit at 717 Delaware St. SE Minneapolis, MN. Dr. Dorothy Hatsukami serves as the Director for this Program. We have a Research Project Manager (Andrew Egbert) who oversees all research and is responsible for logistics of implementing the protocols and standard operating procedures. We also have an Administrator who ensures the smooth operation of the daily activities of the Program. In addition, the Program has a registered nurse practitioner and many research project coordinators. The shared space at the Delaware Clinical Research Units includes a shared waiting room with a receptionist, 7 physical exam rooms (two dedicated to the Tobacco Research Programs), 1 phlebotomy room, 5 interview rooms, 2 day hospital rooms, an infusion room, 1 smoking laboratory with one way observation room, laboratory space for processing blood, urine processing laboratory, a locked medication supply room, locked protocol room for subject files, cubicles for data entry, management and analyses, locked supply storage and access to three conference rooms. Two restrooms are in the clinical space for urine collections. We have dedicated space for our biorepository with key card access containing ten -20 freezers. We also have access to all of the resources of the University of Minnesota for our use, as needed.

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