Synthetic Cooling Agents in Combustible Cigarettes: A Pilot Study

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I. Objectives

Menthol cigarettes contribute largely to the huge burden that smoking put on public health in the US.[1-3] Nationally representative data show that ~43% of adults who smoke use menthol cigarettes with disproportionate trends among racial and gender minorities worsening persistent tobacco-related health disparities (e.g., 80% and 50% among Black and Hispanic adult smokers, respectively).[4-6] Moreover, menthol cigarettes were shown to be "starter products" among youth,[7,8] inducing more nicotine dependence,[8,9] prolonging cigarette smoking,[10,11] and making smoking cessation harder, especially at a younger age.[12,13] The US Food and Drug Administration, lagging behind regulatory agencies in Canada and the European Union, recently proposed a rule to specifically ban menthol in cigarettes to reduce smoking prevalence and related health inequities.[14] However, local US jurisdictions (states like California and Massachusetts and cities like San Francisco, CA and *Columbus, OH – effective Jan 2024*) have already enacted their own menthol ban. Yet the tobacco industry responded with lobbying, legal action (e.g., suing California State), and the introduction of synthetic cooling agents to cigarettes that could impart the same cooling effects as menthol. **Hence, assessing the impact of synthetic cooling agents on cigarette <u>abuse liability</u> is timely and critical.**

Synthetic cooling agents like Wilkinson Sword compounds (e.g., WS-3) may serve as an effective replacement to menthol. Like menthol, WS-3 elicits a cooling sensation by chemically activating the transient receptor potential melastatin-8 (TRPM8) in trigeminal nerves in the buccal cavity when inhaled and gives a deceptive cooling sensation.[15-17] WS-3 could elicit the same impact as menthol in terms of smoothness-enhancing and bitterness-reducing of cigarette smoke leading to increased uptake among groups that are more sensitive to sensory impacts, like adolescents and young adults (AYAs).[18,19] Indeed, synthetic cooling agents are currently widely used in electronic cigarettes (ECs), and these ECs are commonly used by AYAs.[20,21] In addition, chemical analyses examining synthetic cooling additives in EC e-liquid have found that these cooling agents can be added at much higher concentrations than menthol without inducing harsh or aversive sensory effects.[22] In response to the California menthol restriction, R.J. Reynolds introduced cigarettes with synthetic cooling agents that produce a cooling sensation without violating the menthol flavor restriction.[23] These cooling agent non-menthol cigarettes are almost indistinguishable from their corresponding menthol cigarettes in terms of marketing and packaging. **There is a need to systematically assess if these new cooling agent cigarettes impart the same cooling perception among menthol smokers which will void any menthol restriction.**

Using a cross-over double-blinded study design, we will study the abuse liability (including sensory characteristics and appeal) of newly introduced cooling agent non-menthol cigarettes. Established young adult smokers will complete 3 clinic visits in which they smoke *ad-lib* one of three randomly assigned cigarettes that differ either by menthol or synthetic cooling agent content. The study cigarettes will include RJR brands Newport non-menthol box (control), Newport box (menthol), and Newport non-menthol mix box (cooling agent). Subjective effects and objective measures related to abuse liability and acute pulmonary effects (i.e., spirometry and nasal epithelial lining fluid [NELF]) will be evaluated. At each clinic visit, participants will also complete a forced choice task for a second ad-lib smoking session choosing between their usual brand cigarette or the study cigarette.

Aim 1. Assess the content of synthetic cooling agents in newly introduced Newport cooling agent non-menthol, menthol, and traditional non-menthol cigarettes. <u>H1a</u>: synthetic cooling agents will be present at high levels in cooling agent non-menthol cigarettes, <u>H1b</u>: synthetic cooling agents are present at low levels in other RJR cigarettes, and <u>H1c</u>: synthetic cooling agents are transferred intact to smoke, increasing the abuse liability of these new cigarettes, especially among young users.

Aim 2. Assess the subjective effects and abuse liability of Newport cooling agent non-menthol cigarettes. The cooling power of WS-3 is 150% that of menthol, hence, <u>H1a</u>: participants who smoke cigarettes with synthetic cooling agents will report greater cooling effects, more satisfaction, and greater demand in the

forced choice task, and H1b: puffing topography will be more intense (greater total puff volume) than the control and menthol conditions. H1c: Cigarettes with cooling agents (i.e., menthol or WS-3) will be more appealing than the control.

Exploratory Aim 3. Examine acute changes to pulmonary health associated with Newport cooling agent cigarette use. H3: Compared to menthol cigarettes, participants who smoke cigarettes with synthetic cooling agents will have decreased pulmonary functioning, increased markers of respiratory inflammatory and oxidative stress (e.g., IL-6, IL-8, 8-isoprostane, malondialdehyde), and decreased antiviral host-defense mediators (IFNγ, IL6, and IL12p40), as measured via NELF samples.

II. Background and Rationale

Menthol, a cooling agent that increases nicotine dependence among smokers. Menthol is a natural chemical compound extracted from peppermint and other plants in the *Mentha* genus.[24] Due to its cooling effect, menthol is ubiquitously used in food, pharmaceuticals, cosmetics, toothpaste, and other consumer products.[25] Menthol was first added to tobacco cigarettes in the 1920s and, currently, menthol cigarettes make up 37% of the US cigarette market.[26,27] The cooling sensation that menthol exerts balances the aversive sensation of nicotine and tobacco smoke,[28] leading to increased palatability which explains the widespread use of menthol in cigarettes by the tobacco industry.[29-32] It is estimated that menthol cigarettes are responsible for 10.1 million new smokers, 3 million life years lost, and 378,000 premature deaths in the US from 1980 to 2018.[33] The tobacco industry understood and capitalized on the impact of menthol on cigarette use, initiation among youth, and prolonged nicotine dependence.[26,28,31,32,34] The industry manipulated menthol content in "starter products" to hook lifelong users at a younger age.[7,34,35] The industry understood that menthol could act through different biological mechanisms to reinforce nicotine addiction, yet menthol's chemosensory properties are the most important.[36,37] Menthol elicits a cooling sensation as it chemically activates the transient receptor potential melastatin-8 (TRPM8).[15,16] Menthol stimulates the TRPM8 in trigeminal nerves in the buccal cavity when inhaled and gives a deceptive cooling sensation.[38] These sensory properties reinforce smoking and nicotine dependence among smokers,[10,11,13,39,40] and are associated with higher nicotine intake from cigarettes.[41,42] Puff volume, puff duration, and puffing frequency were reported to be significantly higher when smoking menthol cigarettes.[43-45] This may explain the higher retention of toxicants when users smoked menthol cigarettes compared to matching non-menthol cigarettes.[41,45] The impact of menthol on nicotine addiction is more pronounced among adolescents and young adults (AYAs),[4,46] increases nicotine dependence,[8,9] and makes it harder for them to quit smoking compared to non-menthol smokers, especially at a younger age.[12,47,48] *The detrimental effect of menthol cigarettes on public health could be replicated in any tobacco product with cooling additives that evades tobacco regulation. In a recent example, following the California menthol restriction, R.J. Reynolds introduced cigarettes with synthetic cooling agents that produce a cooling sensation without violating the menthol flavor restriction.*[23]

The positive impact of menthol cigarette restrictions. Longitudinal data from Canada showed that a nationwide menthol ban helped some menthol smokers quit smoking, yet the majority of pre-ban menthol smokers either switched to non-menthol cigarettes (59%) or managed to continue smoking menthol cigarettes (20%).[49] Moreover, the ban did not significantly reduce smoking among adults or initiation among youth.[50] These observations were also reported among EU menthol smokers after a similar ban.[51,52] In the US, simulation and empirical studies have suggested that a federal menthol cigarette ban would benefit public health,[53-56] but showed that a big proportion of menthol smokers may switch to other tobacco products, similar to observed trends in other nations.[57-61] Also, data from the US and Canada showed that local menthol restrictions could help smokers quit and benefit public health.[62,63] Hence, *local and federal menthol restrictions are beneficial to individual and public health. However, recently new synthetic cooling agents which have been added to cigarettes to impart the same cooling effects as menthol. These synthetic cooling agents do not impart a characterizing flavor of menthol, hence circumventing the menthol restriction. This study will provide much needed data to understand how these products may changes user behavior, the toxicant profile of these products, and data from this study will provide much needed scientific data to regulatory agencies regarding the*

regulation of these new tobacco products.

Industry tactics to undermine menthol restrictions. Historically, the tobacco industry responded to tobacco control measures by legal actions. These maneuvers were intended either to halt regulations that could help reduce tobacco use in the population (hence the industry profit) or at least delay the implementation of these regulations.[64] Another tactic used by the industry was to modify tobacco products. For example, after the publishing of the Surgeon General report in 1964 that linked tobacco smoking to lung cancer and other diseases, the tobacco industry introduced light cigarettes and filtered cigarettes as "safer" alternatives to unfiltered regular cigarettes.[65] In response to the menthol restriction in Canada and the EU, the tobacco industry introduced flavor accessories that could be used by customers to add menthol and other flavors to their cigarettes.[66] The industry also introduced cigarettes with menthol-filled capsules in the filters to be crushed by the consumer during smoking. Another innovation, unique to the US to the best of our knowledge, was the introduction of synthetic cooling agents (i.e., WS-3) to cigarettes.[23] These cigarettes are known as "cool non-menthol cigarettes." *There is a need to assess the abuse liability and toxicity of synthetic cooling agents added to products labeled as non-menthol cigarettes and evaluate their potential to circumvent local and federal menthol restrictions.*

Innovation. This is the first study to systematically examine cool non-menthol cigarettes with synthetic cooling agents. As such, using novel methodologies developed by our study team, we will first determine the type and level of synthetic cooling agents added to these cigarettes. We will also examine how these additives differ from their menthol and non-menthol counterparts in terms of chemical profile and nicotine content. It is well established that positive sensory experiences are linked to continued and more frequent use of tobacco products.[67-69] Because these new cigarette products recently emerged in the US marketplace, little is known about how these products may change appeal, use patterns, and the sensory experience. Thus, the findings which will support an R01 submission (Fall 2023), will provide the first data on how the addition of synthetic cooling agents changes the appeal and abuse liability potential of combustible cigarettes. Further, data on the inhalation safety of synthetic cooling agents are lacking. As such, we will be collecting the first assessment of how synthetic cooling agents added to cigarettes may alter the respiratory toxicity profile of combustible cigarettes. While this study incorporates the expertise of our research team, it also provides a foundational training opportunity for Dr. Tackett (PI) to learn new techniques (i.e., forced-choice task, additive profile of tobacco products) while expanding her training in human laboratory research and the respiratory assessment of tobacco products.

Preliminary Studies.
Given that these cool non-menthol cigarettes arrived on the US marketplace in January 2023, little is known about these products. However, data from a recently completed project provide some insight into how synthetic cooling agents alter e-cigarette (EC) use among young people. Drs. Tackett, El Hellani, and Wagener, and Prof. Brinkman recently completed an observational clinical trial (2021 – 2022) among 85 young adult (aged 18 – 25) current EC users (daily or weekly use for at least 3 months). This study examined naturalistic differences in nicotine dependence, vaping preferences (flavor, device type), and puffing topography parameters [using participants' own device; total puff count, puff volume (mL), puff duration (sec), inter-puff interval (sec), puff velocity (mL/s), and total puff volume (mL)]. Most participants began vaping regularly at age 18 (71.8%; Mage = 18.5 years; SD = 2.2 years), had used ECs for about 3.6 years (61%; SD = 1.8 years), and reported moderate nicotine dependence (Hooked on Nicotine Checklist (HONC) total score,[70] range 0 – 10, M = 5.3, SD = 3.4)). Most of the participants were using ECs preferred vaping ice variant products (60%, n = 51) which are flavors with synthetic cooling agents added (e.g., Blue Razz Ice, Lush Ice). Young adults who used ice flavors had greater puff duration (3.42 vs. 2.84 seconds for non-ice flavors). *These data highlight emerging evidence that the use of synthetic cooling agents is common in ECs, use is prevalent among young adults, and vaping synthetic cooling agent e-liquid is associated with increased puff duration.*

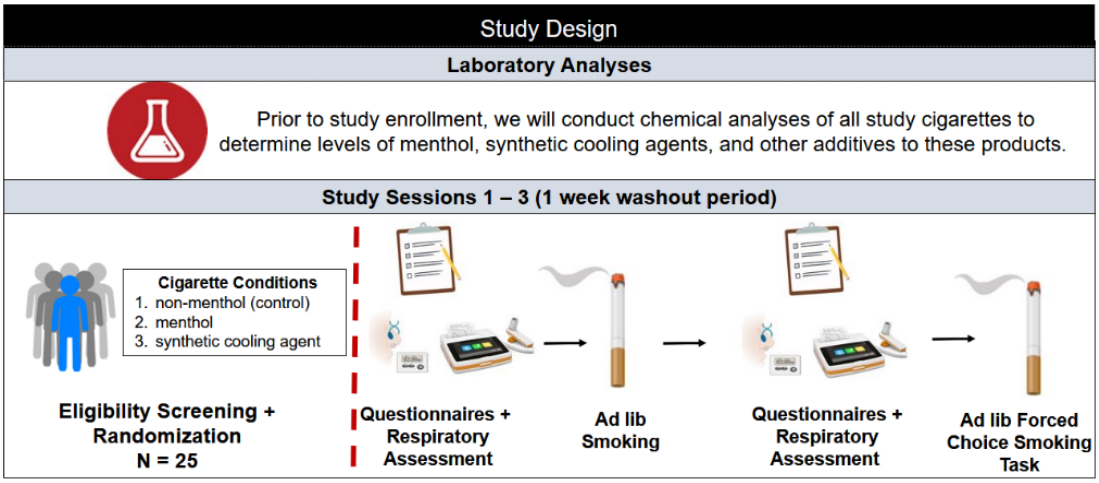
III. Procedures

A. Research Design.

Overview: These pilot data are essential to supporting the R01 application as this is the first-time new cigarette products have emerged on the marketplace with synthetic cooling agents added. Evidence is needed to provide the FDA with guidance on how to regulate cigarette products with synthetic cooling agents and how this changes the abuse liability and appeal of these products as well as their potential to serve as a menthol replacement in the event of a menthol flavor restriction in the United States (and Ohio). Our team is uniquely suited to conduct both the behavioral and chemical analyses of these products.

Proposed Study Design (Figure 1). Prior to study enrollment, we will conduct chemical analyses for each study cigarette to determine the levels of additives including menthol and synthetic cooling agent levels (e.g., WS-3, WS-23). Using a cross-over double-blinded study design, we will study the abuse liability (including sensory characteristics and appeal) of newly introduced cool non-menthol cigarettes. Established young adult smokers (N=25; Ages 21 – 29) will complete 3 clinic visits in which they smoke *ad-lib* three matched cigarettes from the same brand that differ by menthol or synthetic cooling agent content. Participants will arrive at the session at least 12 hours nicotine abstinent. The study cigarettes will include Newport non-menthol box (control), Newport box (menthol), and Newport non-menthol mix box (WS-3). Subjective effects (e.g., cooling perceptions, appeal, satisfaction) and objective measures related to abuse liability (i.e., cigarette puffing topography,) and pulmonary effects (i.e., spirometry and nasal epithelial lining fluid [NELF]) will be evaluated. Finally, participants will be presented with a second ad-lib smoking task (i.e., forced-choice task) where the participant will have the opportunity to choose between their usual brand cigarette and the study cigarette which will determine if the study product is preferred over their usual brand cigarette.

Figure 1. Study Design.



B. Sample

Study Procedures. Chemical Analyses. Tobacco samples will be extracted with isopropanol containing carvacrol as an internal standard. Nicotine, menthol, WS-3, WS-5, WS-12, and WS-23 will be quantified by GC-MS. The four synthetic cooling agents are selected based on information from the RJR website,[71] or recently published data on other types of tobacco products.[72] Briefly, ~1 g of tobacco will be extracted with 50 mL of the extraction solution. After shaking for 2 hours, a 1-µL aliquot will be analyzed by GC-MS using selected ion monitoring (SIM) and measurements will be conducted in triplicate (n = 3).

Participant Screening and Enrollment:
Phone and online screens will determine preliminary eligibility. Participants will complete an in-person screening prior to completing the informed consent. Following the informed consent, participants will complete a urine pregnancy test and twelve-hour nicotine abstinence (assessed via self-report, and eCO monitor) to confirm eligibility. If eligible, participants will then complete informed consent. Ineligible participants will be informed that they cannot participate and paid $10 for their time. We will recruit 25 young adults (aged 21 – 29 years) established cigarette users (see inclusion criteria below, i.e., restricting our age range to young adults who can legally purchase these products). In our most recent studies recruiting young adult EC users (e.g., 20YVNR35490079; K01HL148907), we averaged 12 participant sessions per week and 80% retention (20% potential dropout rate). We will conduct analyses to examine attrition bias for those who complete the study and their non-complete counterparts. When able, we will collect data from participants who leave the study prior to completing (e.g., ask about time constraints, reasons for not returning, etc.).If we assume a similar recruitment rate, the proposed study can be completed in 3 months of data collection plus 9 months for clinical data analysis (3 months), NELF analysis (1 month), R01 submission (2 months), and data sharing/dissemination (3 months). Women, Children, Race/Ethnicity. We will recruit a sex-balanced sample. According to U.S. Census data, the racial composition of individuals living in Columbus is 53.9% Non-Hispanic White, 29% Non-Hispanic Black or African American, 5.9% Non-Hispanic Asian, 3.9% Hispanic White, and 3.7% Non-Hispanic multiracial. We expect that our distributions will be similar.

Eligibility Requirements. Inclusion criteria: (1) aged 21 – 29 years; (2) established cigarette user (at least 100 lifetime cigarettes and currently use ≥5 cigarettes/day or weekly use over the past 3 months); (3) willing to provide informed consent and abstain from using tobacco products 12 hours prior to the three lab sessions; and (4) read and speak English. Exclusion criteria: 1) recently COVID-19+ (defined as a positive test in the past 30 days) or a recent COVID-19 hospitalization (within the past 6 months); 2) self-reported unstable or significant psychiatric conditions (past and stable conditions will be allowed; this will be confirmed via self-report); 3) history of cardiac event or distress within the past 3 months; 4) are currently pregnant, planning to become pregnant, or breastfeeding (will be verified with urine pregnancy test); 5) currently attempting to quit using combustible tobacco products; and (6) have suffered from any serious lung disease or infection (e.g. , tuberculosis, cystic fibrosis, or lung cancer) in the past 30 days.

Recruitment Feasibility and Retention. We will recruit our sample from a combination of resources, including the CTR databases of past participants (N = 1952; n = 1377 within our age range). The only demographic variables collected on the screener are age, gender, and sex assigned at birth. For gender 351 (26%) identify as male, 985 (72%) identify as female, and 40 (3%) are another non-binary gender.). Participants will primarily be recruited for this study through flyers/social media. Our recruitment language will contain a brief overview of our study, a link to an online screener, as well as contact information (email, phone) for our lab. Young adults who are interested in participating can fill out the screener online through the link or over the phone. In the screener, we will ask individuals to provide us with their preferred form of contact (home phone, cell phone, email). We will make it

clear in the screener that providing this information gives us permission to contact the individual about further participation in the study if eligible.

Retention: Participants who drive will be provided with free parking, $80 for each visit that they attend ($240 total), and an additional bonus of $50 for protocol compliance (attending all 3 visits) for a possible total of $290. To be more inclusive and equitable to participations who do not have access to reliable transportation, we are also budgeting up to $25/visit to use Lyft/Uber rideshare services. We will also facilitate retention by: 1) obtaining multiple sources of contact; 2) offering weekend appointments, and 3) reminder calls/texts/emails.

C. Measurement / Instrumentation

To facilitate adherence to the 12-hour nicotine abstinence (verified via exhaled carbon monoxide [abstinence range = 3–4 ppm])[73], efforts will be made to complete all study visits in the morning hours (~7:00 AM–11:00 AM), near the participant's typical waking time. Randomization to condition order will be completed prior to study Visit 1. To ensure eligibility, pregnancy exclusion will be confirmed with a urine test (pregnancy tests will be completed at each visit throughout the study). Questionnaires will provide basic demographic and tobacco use history information. Participants will complete the pre-smoking respiratory assessments (spirometry and NELF collection). Spirometry will be completed in the standing position.[74] *Nasal Epithelial Lining Fluid (NELF)*[75] samples will be collected as described before [75-77]. Briefly, the absorbent matrix cut to fit within the nasal passages will be inserted into the inferior nasal turbinate, and the matrix will be clamped in place with a padded nose clamp for 2 min after which the strips will be removed and placed into microfuge tubes and frozen.[77] Samples will be extracted for assessment of inflammatory mediators (cytokines and chemokines; e.g., IL-6, IL-8), mediators of host defense status (IP-10, IL-1β, IFNγ)[76] via multi-plex (Mesoscale Diagnostics). Then, participants will move to our negative pressure room to complete the first smoking session. *Puffing Topography* will be measured with a validated cigarette puff topography device (eTOP®) that records puff count, puff duration, inter-puff interval, puff flow rate, puff volume, and total puff volume. Participants will be instructed to smoke the study cigarette. Then, questionnaires (i.e., pre/post smoking) will capture specific behavioral and subjective effects associated with abuse liability, specifically as it relates to cooling perception (Table 1). *Appeal and Sensory Quality*[18,78,79] will require participants to rate each product on visual analog scales (range, 0-100; liking and willingness to use again) and sensory characteristics (e.g., olfactory response, somatosensory responses, flavor intensity (very intense – no intensity), sensation intensity (very intense – no intensity), pleasantness of taste + sensation (very pleasant – very unpleasant, cooling power/intensity). The *Drug Effects Questionnaire (DEQ)*[80] will be used to rate acute responses to the cigarette on visual analog scales (range, 0 "not at all" – 100 "extremely"). Items assess liking/wanting (the average of "I feel good cigarette effects," "I want more of that cigarette I received," "I feel the cigarette strength," and "I like the cigarette effect"). *Modified Cigarette Evaluation Questionnaire (mCEQ)*[81] will be used to measure domains of reinforcement (vaping satisfaction, psychological rewards, and aversion; e.g., "Was the product satisfying?" on a 7-point scale (e.g., 1 "not at all", 4 "moderately", 7 "extremely"). Nicotine withdrawal will be assessed using the empirically validated 15-item version of the *Minnesota Nicotine Withdrawal Scale (MNWS).*[82] The *Cigarette Purchase Task*[83] will assess economic demand (puffs) via questionnaire. During this questionnaire, participants will be asked to indicate the quantity of puffs they would hypothetically purchase and use if it was available at incrementally increasing prices ranging from $0 (free) to $100. Participants will be instructed to assume they were purchasing cigarette puffs for their consumption in 24 hours. Finally, participants will be presented with a second ad-lib smoking task (i.e., forced-choice task) where the participant will have the opportunity to choose between their usual brand cigarette and the study cigarette which will determine if the study product

is preferred over their usual brand cigarette.

Table 1. Study Measures and Outcome Variables			
	Study Session		
Measures	**1**	**2**	**3**
Sociodemographic measures	X		
Tobacco use history/nicotine dependence	X	X	X
Exhaled carbon monoxide	X	X	X
Respiratory Assessment (pre/post smoking)			
Spirometry	X	X	X
Nasal epithelial lining fluid (NELF)	X	X	X
Abuse Liability			
Smoking Topography	X	X	X
Appeal and Sensory Characteristics	X	X	X
Drug effects/liking	X	X	X
Modified Cigarette Evaluation Questionnaire (mCEQ)	X	X	X
Cigarette Purchase Task	X	X	X
Minnesota Nicotine Withdrawal Scale	X	X	X
Product Comparison			
Forced Choice Task	X	X	X

D. Detailed Study Procedures

Synthetic cooling agents (WS-3 and WS-23) added to cigarettes have the potential to increase the appeal and toxicity of combustible cigarettes as they are poised to serve as a flavorless menthol replacement. Importantly, these products benefit from an existing data gap, because evidence that may be needed to inform regulatory actions or product standards for the protection of public health is sorely lacking. This timely and innovative pilot study will address two key unknowns about synthetic cooling agents added to cigarettes. First, we will determine the amount and type of additives in these new cigarette products, while also examining non-menthol and menthol cigarettes. Next, using a double-blind randomized crossover trial, we will examine the impact of synthetic cooling agents on the appeal and abuse liability among young adult smokers (aged 21 – 29; N = 25). Participants will complete three clinic visits in which they will be randomized to smoke (first session) a same-brand cigarette in one of three variations: 1) non-menthol cigarette (control condition), 2) menthol cigarette, or 3) synthetic cooling agent cigarette. Questionnaires will assess appeal and sensory characteristics, and puffing topography will be collected during each session to examine differences in puffing behavior by cigarette type. To assess economic demand for the study product, participants will also complete a forced choice task for a secondary ad-lib smoking session where they can choose between the participant's usual brand cigarette and the study cigarette. Finally, as an exploratory aim, we will examine potential acute differences in pulmonary function (i.e., spirometry) and inflammation (i.e., nasal epithelial lining fluid [NELF]) before and after the first smoking session. Findings will constitute preliminary data to support a larger R01 application which will be aimed at informing FDA rulemaking surrounding the use of synthetic cooling agent additives in combustible cigarettes.

Phone Screening: All advertisements (see “Recruitment and Informed Consent” procedures below)

will have a phone number for RAs, QR code, and a direct link to the study screener. Trained RAs will provide a brief description of the study, screen the for eligibility while coding the answers into REDCap, and for eligible participants, schedule the first visit and provide directions to the study site. To provide some control over daily fluctuations in nicotine craving and withdrawal and to facilitate adherence to the 12-hour nicotine abstinence, efforts will be made to complete all study visits in the morning hours (~8:00–11:00 AM), near the participant's typical waking time.

Study Randomization and Blinding Procedures: Each participant will receive a randomized ordering developed using a random sequence generator. The Random sequence generator will be conducted by the study PI. Staff who will **not** interact with participants will prepare the study products in the randomized order sequence prior to each visit. Participants and data collection staff will be blinded to the order/conditions administered at each trial. The sequence list and subsequent participant ID numbers will be kept separate from data collection staff and will only be accessible in REDCap by staff who will not be interacting with participants.

NELF Collection and Training: Data collection staff will be trained by Dr. Tackett to conduct the NELF collection procedures. A training video will also be provided as an ad-hoc reference for study staff. Pre-made NELF collection kits will be provided by The University of North Carolina at Chapel Hill from Dr. Meghan Rebuli (collaborator and colleague of Dr. Tackett).[76,77,84] To collect the sample, the nares are briefly moistened with 0.1 mL of 0.9% sterile, normal saline solution. Pre-cut Leukosorb strips are inserted into the nare at the inferior nasal turbinate along the anterior side until the indicator mark reaches the base of the nare. A padded nose clip will be applied to maintain the strip in the nose and to ensure maximum surface area of the strip with the nasal mucosa. The strip remaines in the nare for 2 minutes. Strips containing NELF are then removed from the nostril, placed in microcentrifuge tubes, and frozen at −80 °C until analysis. These procedures have been utilized in Dr. Tackett's (PI) previous remote data collection studies by participants themselves via video conferencing software (e.g., zoom). Eight-five percent of participants in these past studies reported self-collection of this sampling procedures as "easy."[84]

Study Visit 1: To ensure eligibility pregnancy exclusion will be confirmed with a urine test (pregnancy tests will be completed at each visit throughout the study). We will also ask participants to provide an exhaled breath sample to determine adherence to 12-hour nicotine abstinence (exhaled carbon monoxide [eCO]).

Informed consent will be obtained, and participants will provide basic demographic and product use history information. At the start of each visit, we will assess participants' height, weight, and blood pressure. Next, participants will complete computer-based spirometry testing and airwave oscillometry, which will measure central obstruction, peripheral obstruction, and dynamic collapse. Participants will also provide the first of two NELF samples. Then, participants will move to our negative pressure vaping room to complete the first smoking session and will be randomized to use the first product of the three conditions (i.e., non-menthol control; menthol; synthetic cooling agent). The study RA will connect the study to the topography device. ***Puff Topography*** will be measured with a validated puff topography device.[85] The device uses a pressure transducer integrated into a plastic holder to produce measures of puff count, puff duration, inter-puff-interval, puff flow rate, average puff volume, and total puff volume. Participants will be instructed to smoke ad libitum. Next, participants will repeat the respiratory assessment, provide the final NELF sample, and complete ***questionnaires*** *(see table 1)* to capture specific domains (i.e., perceived effects, behavioral) associated with abuse liability. ***Lastly***, participants will return to the negative pressure rooms to participant in the final smoking session. Participants will complete a ***forced choice task***, in which they will choose if they would prefer to smoke their usual brand cigarette, or the study cigarette provided to them during this

session.

Study Visits 2 - 3: All procedures will be followed as outlined above in study session 1.

Protection Against Risk:
The research protocol calls for current tobacco users. Those who participate in this study will be asked questions about nicotine and tobacco product use. Questionnaires, respiratory assessments, and user topography procedures are all non-invasive and involve minimal risk to study participants. Potential risks are as follows: a) loss of confidentiality or privacy, and b) potential for undermining smoking cessation.

Recruitment and Informed Consent: At first contact, all participants will be screened according to the studies inclusion/exclusion criteria. Those who are eligible will be given a brief verbal overview of the study and invited to participate. Informed consent (including a description of the nature, purpose, risks, and benefits of the study) will take place through oral explanation of the study. The voluntary nature of the study and the participant's right to withdraw at any time will be stressed during the consent process; a copy of the informed consent will be provided to the participant in written form at the time of consent for them to keep. Informed consent will be collected by IRB-approved study personnel. Recruitment script and materials (e.g., advertisements), consent forms and all study procedures will be approved by the Institutional Review Board. All participants will provide consent before any study data is collected.

There are minimal risks associated with this protocol. The protocol requires young adult (aged 21 – 29 years) current tobacco users to undergo 12 hours of nicotine abstinence on three occasions and to smoke three different types of commercially available cigarettes. The risk of side effects and adverse events are very low. These products are available at a variety of stores nationwide, without a prescription. Nevertheless, all participants will be screened for general medical precautions (e.g., pregnancy, cardiovascular disease) and monitored for adverse events during the study period. Study personnel will assess for adverse events via self-report at all visits. Any serious adverse events will be reported following guidelines outlined in the Data Safety and Monitoring Plan. We will withdraw participants who have a serious adverse event or become pregnant or begin to breastfeed. Identifying information will never be reported in any publication. Nicotine abstinence can lead to withdrawal symptoms that include irritability, anxiety, restlessness, hunger, and difficulty sleeping. The effects can be uncomfortable but are not dangerous. The risk of undermining cessation is also potential risk; however, we will only recruit users not currently engaged in a cessation attempt, and we will provide all participants at the end of the study with a referral for cessation services (e.g., Quit Line).

Protection against loss of confidentiality and privacy will be maintained by numerically coding all data, disguising identifying information, and keeping data locked in file drawers or in a secure, password-protected database. Only study research assistants and the PI will have the information that connects participant's name and ID number. All electronic data will be numerically coded and stored in a password protected database, on a password protected computer in a secure research space. Participant information will be accessible only to research staff, who are pledged to confidentiality and complete training in the ethical conduct of research (i.e., both HIPAA and CITI trainings). Identifying information will not be reported in any publication.

Whereas no assurance can be made to an individual participant that s/he will personally benefit from this research, the experience should be beneficial. The immediate benefits of this research are

scientific in nature, which in the long-term should benefit society as a whole. The study will also benefit young adult tobacco users as a group by providing information as to the abuse liability of cigarette products using synthetic cooling agents; and serve as evidence to inform regulatory action that improves public health. Overall, it is expected that the potential benefits to participants in the proposed study outweigh the potential risks.

This study is an innovative investigation that will have important public health implications. Currently, no human studies have objectively examined the behavioral (abuse liability, appeal, and user topography) implications of synthetic cooling agents added to cigarettes. Understanding this impact, will help immediately inform the science base needed for the FDA to implement appropriate product-specific regulations.

Adverse Events:

In an effort to meet the NIH policy for Data and Safety Monitoring, we have created a system for oversight of the project. Oversight of internal monitoring of the participants' safety will be conducted by the PI, Dr. Alayna Tackett. Drs. Wagener, El Hellani, and Brinkman (Co-I's) also will participate in the development and administration of the plan. All of the investigators of the application have extensive experience with behavioral research studies with human subjects. Investigators will meet regularly about the project, at which time they will evaluate the progress of the study, review data quality, recruitment, and study retention, and examine other factors that may affect the ability of the project to be completed successfully. The team will review the rates of adverse events to determine any changes in participant risk. They will also conduct a more in-depth review of the study once per year. A brief report will be generated once a year for the study record and forwarded to The Ohio State University's Institutional Review Board. The investigators will be available to meet outside of the regular meetings, if necessary, to discuss concerns regarding a particular participant or any problems that may arise for participants. If necessary, they will make appropriate recommendations for changes in the protocol. Dr. Tackett will conduct daily oversight of participant safety. She will meet weekly with staff to review participants' experiences with the study, including adverse events (we expect that the risk of adverse events is low given the study's protocol and population). Any adverse events that are observed will be immediately reported to Dr. Tackett. All serious adverse events will be reported to The Ohio State University's IRB immediately by telephone and by written report within 24 hours of our receipt of information regarding the event. The investigative team will review all serious or unexpected adverse events and provide recommendations. We will inform NIH of any significant action taken as a result of these reviews.

Adverse Event Monitors:

- Study Team
  - Alayna Tackett, PhD (expertise: clinical psychology, tobacco regulatory science, youth/young adult tobacco use)
  - Theodore Wagener, PhD (expertise: clinical psychology, tobacco regulatory science, tobacco product constituents, toxicant exposure)
  - Marielle Brinkman, BS (expertise: tobacco regulatory science, chemistry, tobacco product constituents, toxicant exposure)
  - Ahmad El Hellani, PhD (expertise: organic chemistry, tobacco regulatory science, toxicant exposure)
- Biostatistician
  - Alice Hinton, PhD (expertise: biostatistics, longitudinal data analyses, clinical research)
- External Monitor:

- Dharini Bhammar, MBBS, PhD (expertise: obesity, respiratory function and exercise physiology, physiological assessments of pulmonary and metabolic data)

Adverse Event Reporting Timelines:

- All staff are required to notify Dr. Tackett of any adverse events immediately.
- In accordance with current standard procedure, Dr. Tackett will notify the IRB of all Serious Adverse Events within 24 hours.
- In accordance with new policy and procedure, Dr. Tackett will notify the NIH of all Serious Adverse Events within 2 business days.

All observed or volunteered adverse events will be recorded on the adverse event page(s) of the Adverse Event Form. Exacerbation of pre-existing illness is defined as a manifestation (sign or symptom) of the illness that indicates a significant increase in the severity of the illness as compared to the severity noted at the start of the study. It may include worsening or increase in severity of signs or symptoms of the illness, increase in frequency of signs and symptoms of an intermittent illness, or the appearance of a new manifestation/complication.

For all adverse events, Dr. Tackett will pursue and obtain information adequate both to determine the outcome of the adverse event and to assess whether it meets the criteria for classification as a serious adverse event. For all adverse events, sufficient information should be obtained by Dr. Tackett to determine the causality of the adverse event (i.e., other illness). Dr. Tackett is required to assess causality and indicate that assessment on the Case Report Form. Follow-up of the adverse event is required if the adverse event or its sequelae persist. Follow-up is required until the event or its sequelae resolve or stabilize at a level acceptable to the investigator or his/her designated representative.

All serious adverse events will be reported immediately to Dr. Tackett then to IRB and the NIH. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious adverse events when, based upon appropriate medical judgment, they may jeopardize the patient/subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Any serious adverse event or death must be reported immediately independent of the circumstances or suspected cause if it occurs or comes to the attention of the principal investigator at any time during the study through the last follow-up visit required by the protocol. Any serious adverse event occurring at any other time after completion of the study must be promptly reported. For all serious adverse events, the investigator is obligated to pursue and provide information as requested in addition to that on the Adverse Event Form. In general, this will include a description of the adverse event in sufficient detail to allow for a complete medical assessment of the case and independent determination of possible causality. Information on other possible causes of the event, including concomitant medications and illnesses, must be provided. The investigator's assessment of causality must also be provided. Dr. Tackett will ensure that information reported immediately by telephone or other means and information entered in the Adverse Event Form are accurate and consistent.

We will monitor for risk of using cigarettes by screening participants for general medical precautions. The most likely adverse event (potential for nicotine overdose) is anticipated to be rare and mild based on Dr. Tackett, Wagener, and Brinkman previous studies, and will be handled quickly (i.e., advice to participant to reduce or eliminate nicotine use). Dr. Tackett and study personnel will be available for any questions that participants may have about ECs. Any adverse events, breaks of

confidentiality, or any other data or safety issues that arise will be discussed immediately between study personnel, CO-I's and Dr. Tackett.

E. Internal Validity

Quality assurance: Procedures in place to ensure the validity and integrity of the data. 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. All investigators and staff associated with this project have been trained, and new hires will be trained on human research ethics in accordance with the requirements of the local institutions during initial project approval.

Drs. Tackett will train and closely monitor the Research Assistants on the procedures to be used in this study. Such monitoring will consist of frequent in-person discussion of study visits at the beginning of the study and less frequent monitoring as the study progresses.

Data will be collected in a consistent manner across all years of the study. Standard operating procedures will be developed. We will conduct analyses to examine attrition bias for those who complete the study and their non-complete counterparts. When able, we will collect data from participants who leave the study prior to completing (e.g., ask about time constraints, reasons for not returning, etc.).

Data collection/entry/transmission/analysis: Many subjective measures will be administered using RedCap, and thus, all data are entered directly into a RedCap database. These will be identified by Subject Identifier. Forms will include programming features to ensure valid data (i.e., input masks, validation criteria, skip logic) and will be stored at the Ohio State University.

F. Data Analyses

The goal of this pilot is to determine the role of synthetic cooling agents added to cigarettes and how this may circumvent the potential removal of menthol in tobacco products. Given this is a pilot study to generate data to support an R01 application, we are underpowered. However, we do expect to observe significant mean differences for ratings of sensory and appeal ratings. As such, a sample size of 25 subjects has 60-40% power to detect large differences. Statistical analyses will be performed using SAS 9.4 and an alpha of .05. Additionally, between-subjects and within-subjects effects will be considered using mixed effects and multilevel modeling techniques in SAS 9.4 which allow for proper treatment of the interdependence of repeated observations within individuals.

Aim 1. Ingredients will be quantified using the analytical method described above. Head-to-head comparison will be performed using multivariable regression analysis between the different cigarette types tested. Tobacco samples will be extracted with isopropanol containing carvacrol as an internal standard. Nicotine, menthol, WS-3, WS-5, WS-12, and WS-23 will be quantified by GC-MS. The four synthetic cooling agents are selected based on information from the RJR website,[71] or recently published data on other types of tobacco products.[72] Briefly, ~1 g of tobacco will be extracted with 50 mL of the extraction solution. After shaking for 2 hours, a 1-µL aliquot will be analyzed by GC-MS using selected ion monitoring (SIM) and measurements will be conducted in triplicate (n = 3).

Aim 2. The impact of cooling effects, satisfaction, demand via the forced choice task (H2a), puffing topography (H2b), and appeal (H2c) will be examined using mixed-effect regression modeling. For

count outcomes (e.g., frequency/quantity of puffs), negative binomial regression models will be fit to estimate rate ratios (RRs) with 95% confidence intervals (CI). For hypotheses, the synthetic cooling agent cigarette will be compared to the menthol and control non-menthol condition. For all statistical tests performed, we will construct 95% CIs for mean differences and pairwise comparisons will be adjusted for multiple testing procedures. All statistical tests will be two-tailed with a significance level of $p<0.05$.

Exploratory Aim 3. The pulmonary effects will be analyzed with linear mixed effect regression models for each of the outcomes (e.g., spirometry: FEV_1, FVC, FEV1/FVC; NELF: IL-6, IL-8, IFNv, IL12p40) where cool non-menthol and menthol cigarettes will be compared to the control non-menthol condition. We will also examine differences by topography outcomes (e.g., puff volume, duration, frequency, inter-puff intervals) and subsequent changes in pulmonary health.

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Appendix A. Nasal epithelial lining fluid (NELF) Sample Procedure Schematic