Tobacco
Research Center

UCSF

Short-Term Cardiovascular Effects of E-Cigarettes: Influence of Device Power

(TCORS-Study 1)

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BACKGROUND

Cardiovascular Effects and Biomarkers

The CV system is sensitive to toxicants in tobacco smoke—toxicants that may also be present in e-cigarette and HNB aerosols. From 1965-2009, smoking caused 7.0 million deaths from CVD compared to 5.8 million from cancer and 3.2 million from respiratory diseases in the U.S. The relationship between tobacco smoke exposure and risk of CVD increases sharply for people who smoke only a few cigarettes per day.

An understanding of how cigarette smoking causes CVD informs our approach to assessing the potential for e-cigarette and HNB products to cause CVD. Cigarette smoking produces both chronic (accelerated atherosclerosis) and acute (acute myocardial infarction, stroke, sudden death) CVD, with overlapping mechanisms. Mechanisms are important to understand as this guides selection of biomarkers. Mechanisms of smoking-induced CVD include oxidative stress, inflammation, endothelial damage and dysfunction, thrombogenesis, hemodynamic stress, and arrhythmogenesis (the latter two thought to be mediated by sympathetic nervous system (SNS) stimulation) (**Figure 1**). Studies suggest that e-cigarette use may cause vascular injury, oxidative stress, decrease in flow-mediated dilation, and heart rate and blood pressure have been shown to increase immediately after e-cigarette use.

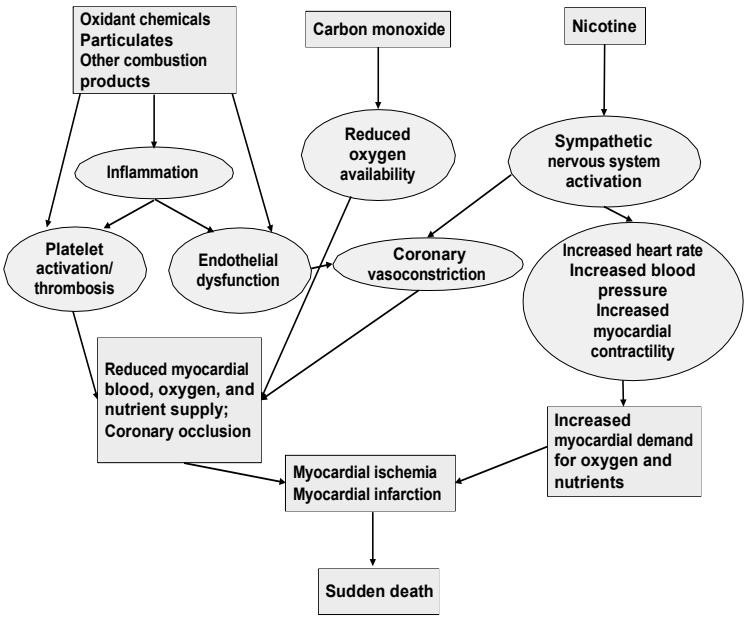


Figure 1 Summary of mechanisms by which tobacco smoke constituents cause an acute cardiovascular event.

Constituents of smoke of most concern for CVD include reactive chemicals (carbonyls such as acrolein), nicotine, particles, and carbon monoxide. Acrolein is of concern because risk estimates suggest that it accounts for a majority of the total non-cancer risk of smoking, as much as 88.5%. Exposure to acrolein is associated with increased CVD risk, even in nonsmokers. Nicotine causes SNS stimulation exhibited as increased heart rate and catecholamine release (particularly epinephrine), and has effects on vascular function. SNS stimulation may contribute to CV events in people with pre-existing CVD, including sudden death due to arrhythmia. SNS stimulation also contributes to a more atherogenic lipid profile and insulin resistance and diabetes, which are important CVD risk factors. Depending on device and use conditions, e-cigarettes produce nanoparticles consisting of metals, metal oxides, and other

compounds. Epidemiologic studies have long linked exposure to particles in ambient air to increased risk of CVD, cancer, and respiratory diseases.

We propose to measure biomarkers of exposure to potential CV toxicants (acrolein, nicotine, and CO). We will also measure biomarkers of relevant pathogenesis mechanisms related to acute CVD (oxidant injury, inflammation, platelet activation, endothelial dysfunction and SNS stimulation; SNS stimulation is measured as increased heart rate and blood pressure and urine catecholamines). Specific biomarkers with citations are summarized in **Table 3**. These biomarkers were selected since they have been shown to change rapidly after smoking, so we can assess short-term effects, and are known to be correlated with CVD risk. Our studies of different e-cigarette power and HNB products will include all of the biomarkers presented in **Table 3**. Since e-liquid pH is expected to influence primarily nicotine exposure and pharmacokinetics, the e-liquid pH study will focus on the effects of nicotine exposure and pharmacokinetics on hemodynamic parameters and not on the other CVD biomarkers.

Table 3: Cardiovascular Risk Biomarkers

Function	Biomarkers
Sympathetic nervous system stimulation	Heart Rate
	Urinary catecholamines
Oxidative stress	Urinary F2-isoprostane
Platelet activation / Thrombosis	Urinary 11-dehydro-thromboxane B2 (11-dTXB2)
Endothelial dysfunction	Plasma Vascular Endothelial Growth Factor (VEGF)
	Plasma Intracellular Adhesion Molecule-1 (ICAM-1)
Inflammation	Plasma interleukin 6 (IL-6)

E-cigarette Power is related to Nicotine Delivery and Toxicant Generation

E-cigarette electrical power (P), which is a function of battery voltage (V) and atomizer (coil) resistance (R) ($P = V^2/R$), may be one of the most important determinants of e-cigarette health effects. The heat supplied by the atomizer to raise the temperature of the e-liquid is a product of electrical power and length of time electricity is applied to the atomizer. The temperature of the heating e-liquid influences nicotine delivery, the type and amount of chemicals generated in the aerosol, and possibly aerosol particle size. In one study, the average power used by users of 2nd generation (pen-shaped) e-cigarettes and 3rd generation (variable voltage or wattage) e-cigarettes ranged from 9 to 76 W, with some individuals using as much as 160 W. Users of high-powered e-cigarettes typically use low nicotine content e-liquids but have similar or higher plasma nicotine levels than users of less powerful devices who use high nicotine content e-liquids. This is indicative of the large amounts of aerosol delivered to users of high-powered e-cigarettes and raises concerns about exposure to toxicants and contaminants in e-liquids. Further, propylene glycol (PG), vegetable glycerin (VG), and flavorants thermally decompose to chemicals such as formaldehyde and acrolein and reactive oxygen species in a temperature-dependent manner. Elevated biomarkers of acrolein exposure have not been reported in e-cigarette users. This is most likely because studies have not characterized acrolein exposure in users of high-powered e-cigarettes.

1 INTRODUCTION AND STUDY DESIGN

1.1 RESEARCH QUESTION

What are the effects of electronic cigarette device power?

1.2 SPECIFIC AIMS

1.2.1 Specific Aim #1

Determine the impact of e-cigarette device power on nicotine pharmacology, systemic exposure to toxic volatile organic compounds (VOCs), and short-term cardiovascular effects.

Hypothesis 1a: Systemic nicotine exposure and subjective measures of sensation in the throat, reward, and satisfaction will increase with increasing power.

Hypothesis 1b: Mercapturic acid metabolites of VOCs, particularly acrolein, will increase with e-cigarette power.

Hypothesis 1c: CV effects increase with higher power, and are manifested as changes in hemodynamic parameters, hormonal release, and biomarkers of endothelial function, platelet activation, inflammation, and oxidative stress.

2 STUDY DESIGN

2.1 DESIGN SUMMARY

This is a single-site, randomized, crossover study of experienced adult e-cigarette users to assess nicotine exposure, toxicant exposure, and the short-term CV effects of e-cigarette power. Three power levels will be assessed on all participants: 10, 35, and 70 watts.

2.2 STUDY TIMELINE

- Preparation for this study began 06/23/2017
- Application submitted to the IRB: 11/27/2017
- Approval date: 01/31/2018
- Expiration date: 01/30/2019
- Estimated start date:
- Estimated end date:

2.3 ELIGIBILITY

Potential participants will express interest in participation by filling out the secure REDCap survey hyperlink from advertisements, the UCSF Tobacco Research websites (e.g., Facebook), or by emailing the UCSF Tobacco Research Center email address. Participants will be contacted to take part in a confidential email screening to determine if they are eligible to come in for an in-person screening visit. At the in-person screening visit, participants will fill out case report forms (TC & EC use, medical history, etc.), the Clinical Research Coordinator (CRC) will take physiological measurements (blood pressure, height, weight, expired CO, etc.), and the participants will provide sample collections (urine and saliva). The participant's study chart will be reviewed by the Study Physician in order to determine study eligibility.

All individuals interested in participating and who meet the inclusion/exclusion criteria will be invited to be part of the study. Inclusion criteria are described below.

2.3.1 Inclusion Criteria

☐ Healthy on the basis of medical history and limited physical examination.
- Heart rate < 105 BPM*
- Systolic Blood Pressure < 160 and > 90*
- Diastolic Blood Pressure < 100 and > 50*
- Body Mass Index ≤ 38.0 (at investigator's discretion for higher BMI if no other concurrent health issues)

**considered out of range if both machine and manual readings are above/below these thresholds*

- ☐ Use e-cigarettes on at least 25 days in the past 30 for at least 3 months and have not used another tobacco product in the past 30 days (or no use per month)
- Age: ≥ 21 years old
- Age: ≤ 70 years old
- ☐ Any race/ethnicity
- ☐ Any sex

2.3.2 Exclusion Criteria

- ☐ Used tobacco products other than e-cigarettes in past 30 days
- ☐ Expired carbon monoxide of over 5 ppm at screening
- ☐ Medical
    - Heart disease
    - Seizures
    - Cancer
    - Thyroid disease (~~not hypo or hyper,~~ okay if controlled with medication)
    - Diabetes
    - Hepatitis B or C or Liver disease
    - Glaucoma
    - Kidney disease or urinary retention
    - History of stroke
    - An ulcer in the past year
    - Active use of an inhaler for asthma or COPD
- ☐ Psychiatric conditions
    - Current or past schizophrenia, and/or current or past bipolar disorder
    - Major depression, current or within the past year
    - Major personality disorder
    - Participants with current or past minor or moderate depression and/or anxiety disorders will be reviewed by the study physician and considered for inclusion
    - History of psychiatric hospitalizations are not exclusionary, but study participation will be determined as per study physician's approval
- ☐ Drug/Alcohol Dependence
    - Alcohol or illicit drug dependence within the past 12 months with the exception of those who have recently completed an alcohol/drug treatment program
    - Positive toxicology test at the screening visit (THC & prescribed medications okay)
    - Opioid replacement therapy (including methadone, buprenorphine, or other)
- ☐ Positive urine cannabis is not exclusionary but participant must report use of cannabis in any form on not more than 2 times per week to be eligible
- ☐ Psychiatric medications

- Current regular use of any psychiatric medications with the exception of SSRIs and SNRIs and current evaluation by the study physician that the participant is otherwise healthy, stable, and able to participate.

☐ Medications
- Use of medications that are inducers of nicotine metabolizing enzyme CYP2A6 (Example: rifampicin, carbamazepine, phenobarbital, and other anticonvulsant drugs).
- Concurrent use of nicotine-containing medications
- Any stimulant medications (example: Adderall) generally given for ADHD treatment

☐ Other/Misc. Chronic Health Conditions
- Oral thrush
- Fainting (within the last 30 days)
- Other "life threatening illnesses" as per study physician's discretion

☐ Pregnancy
- Pregnancy (self-reported and urine pregnancy test)
- Breastfeeding (determined by self-report)

☐ Concurrent participation in another clinical trial
☐ Inability to communicate in English
☐ Planning to quit smoking or vaping within the next 60 days

2.3.3 Eligibility Determination

After completion of in-person screening procedures, the CRC will complete an Eligibility Checklist which lists all inclusion/exclusion criteria and the participant's status based on responses in the case report forms. The Project Manager and CRC will review the Eligibility Checklist after the saliva cotinine results are available.

The Project Manager and CRC will refer to the Study Physician or Principle Investigator if the participant:

☐ Currently takes any medications on schedule or as needed
☐ Has current or past depression and/or anxiety disorders
☐ Has any psychiatric hospitalizations in medical history

The Study Physician will review the information and sign off on the Eligibility Checklist or indicate that the participant is ineligible. If none of the above conditions are present, the CRC and Project Manager will sign off on Eligibility Checklist without Study Physician review.

The participant will be notified of eligibility via phone or email after this review.

2.3.4 Reassessment of Eligibility

Eligibility may need to be reassessed if the following conditions occur: the orientation visit is out of window (e.g. 90 days past from screening visit), and/or 30 days have passed since the time of the screening visit.

2.3.4.a Outside Orientation Visit Window

If the participant is outside of their orientation visit window of 90 days, the screening visit will be repeated. The original screening visit data will remain in the study chart, however it will not be used for analysis. The participant will repeat all visit procedures, forms, and provide a saliva sample to be re-sent for analysis.

2.3.4.b 30 Days Past Screening Visit

If 30 days have passed since the screening visit, in order to ensure that no significant changes have occurred in a participant's current medical status and smoking/vaping habits, the CRC will review some eligibility items. This will occur if the participant is more than 30 days, but less than 90 days outside of their screening visit window. The CRC will ask the following over the phone:

- if there are any new medications
- if they have seen a physician for any medical/psychological reason
- if they are still using their e-cigarettes as indicated at the in-person screening visit

If any significant changes have occurred, the CRC and Project Manager will meet to discuss and complete another Eligibility Checklist based on current participant responses.

2.4 IDs & Assignment

2.4.1 Study IDs

Participants who are eligible for an in-person screening visit will be assigned a unique Study ID upon consent to participate in the study. This Study ID will begin with the "####" to refer to the CTSI-5B study number, which is how the study is referred to at the research ward. These numbers (to be determined after in-service meeting with CTSI-5B) will be followed sequentially starting from 001. For example, the first participant enrolled will have a Study ID of ####001.

The Screening Visit Log will keep track the REDCap IDs, and Study IDs (if consented).

2.5 PARTICIPANT WITHDRAWAL

2.5.1 Dropout Definition

If a participant declares he/she is no longer interested in completing the study, he/she will be considered a "dropout." Once given "dropout" status, the participant can no longer contribute to the study.

2.5.2 Dropout Compensation

If participant decides to stop participating in the study prior to starting the inpatient portion of the study, he/she will only receive compensation for the Screening Visit ($30) and no compensation will be given for the inpatient portion.

If a participant decides to drop out during the inpatient study, compensation will be pro-rated for completed visits.

2.5.3 Lost to Follow-Up

If a participant is unable to be contacted and does not respond to calls from the Clinical Research Coordinator, he/she will be considered "lost to follow-up" (LTFU). If a participant is considered LTFU, every effort will be made to contact him/her.

2.5.3.a Contact Attempts

After three unsuccessful phone and email contact attempts, a letter of intent to contact will be mailed to the participant's home address. This letter will inform the participant that they will be formally withdrawn from the study by the date that is 90 days past the last point of contact. It will also include details on the remaining study compensation available if procedures were continued. If the participant fails to contact the CRC after this outreach, and 90 days have passed since last communication, he/she will be considered "lost to follow-up". No further outreach will be conducted. Once given "LTFU" status, the participant can no longer contribute to the study.

2.5.3.b Returning to Visit Procedures

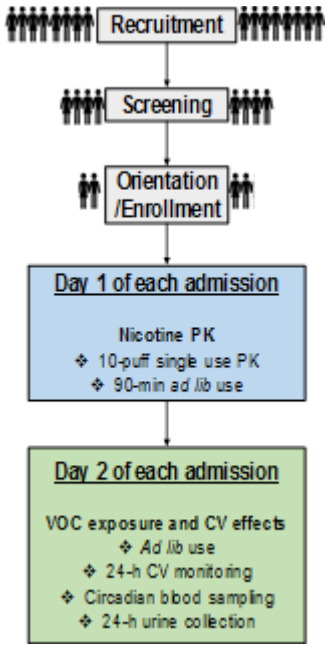
If a formerly lost participant returns, before starting the inpatient portion of the study, the reassessment of eligibility steps outlined in Section 2.3.4 of this manual will be followed. If the participant is LTFU during some point in his/her inpatient portion, there will not be the option to repeat this.

3 STUDY DESIGN

3.1 VISIT SCHEDULE

The study will consist of 3 Study Arms, for 2 in-patient nights each.

The Study Visit chart below describes the schedule of visits and procedures.



3.2 SCHEDULE OF ASSESSMENTS

3.2.1 Visit Windows

Table 1 describes the schedule of visits in relation to the start point of the Phone/Email Screen. Ideally, participant assessments will fall within the designated windows.

Table 1

Assessment Point	Maximum Time allowed from Phone/Email Screen	Maximum Time allowed from Screening Visit
Phone/Email Screen		
Screening Visit	30 days	
Orientation	120 days	90 days

Every effort should be made to enroll and run eligible participants. Thus, if participants are outside of their screening visit or orientation visit windows, reassessment of eligibility will occur as described in Section 2.3.4.

4 RECRUITMENT METHODS

Our methods of recruitment will be as follows:

- Postings to craigslist (paid and free sections)
- Flyers posted in mostly institutional settings (colleges, career centers, churches) and left at vape and smoke shops
- Newspaper ads (typically Bay Guardian, SF Weekly, East Bay Express)
- Blogs, google+ and social networking sites (e.g., Facebook, Reddit, etc)
- Contact of prior callers or study participants who have given permission for re-contact as documented either in the consent form for the prior study or in our phone screening database.

Individuals who respond to recruitment postings/flyers will be screened initially by REDCap survey to determine potential eligibility. Based on this initial assessment they may be asked to come to the UCSF Tobacco Research Center for an in-person screening visit. Additionally, participants from previous studies at the UCSF Tobacco Research Center, who appear to be eligible for the study will be re-contacted and asked if they are interested in participating.

4.1 PRELIMINARY REDCAP SCREENING

Individuals who see the ad will be prompted to click on a hyperlink which will lead them to a preliminary screen on REDCap, or email the UCSF Tobacco Coordinator email address for more information.

The preliminary REDCap screen is a short survey in which the participants provide the following information:

- Contact information
- E-Cigarette/Tobacco Cigarette product use
- Prescription Medication Use
- Exclusionary medical conditions

This REDCap survey is programmed to generate a report of eligible participants. The information for eligible participants will be exported weekly from REDCap into an Excel sheet. The CRC will use this information to call only valid prospects to complete a comprehensive confidential phone screen.

Eligible participants may be contacted by phone or email if any follow-up clarification questions are needed.

Eligible participants will be given 3 available appointment options from which they may choose to come in for an in-person screening visit. Participants will then respond with their desired appointment option, and will be scheduled for an in-person screening visit.

5 STUDY VISITS AND PROCEDURES

5.1 SCREENING VISIT

Participants will undergo a 1-2 hour in-person screening visit to determine study eligibility. During this visit, participants will complete a basic physical assessment: height, weight, heart rate, blood pressure, expired carbon monoxide, urine toxicology test, and pregnancy test (if applicable), saliva collection, and a Screening Packet containing questions regarding medical history and product use. Photographs of the participants' e-cigarette and e-liquid will be taken.

The schedule of procedures for the screening visit will occur in the order as follows:

5.1.1 Consent

Upon initiation of the screening visit, the CRC will greet the participant and ask him/her to read the first line of the consent form aloud to confirm literacy. The CRC will instruct the participant to read each page of the consent document and initial at the bottom of each page indicating content understanding. The participant will be asked to refrain from signing the consent form until the CRC returns to discuss the consent form and answer any questions the participant may have regarding the study. The CRC will leave the room for approximately 10 minutes to allow the participant an adequate amount of time to read the consent form. The CRC will return, answer any questions, and present a brief PowerPoint presentation highlighting important aspects of the consent document. Consent will be obtained via participant and CRC signatures at the conclusion of the presentation. Three copies of the consent document will be signed and dated by both the participant and the CRC. The CRC should retain the initialed copy and the second copy, and the third copy will be given to the participant for their records. The participant will also be asked to read and sign a copy of the Bill of Rights to ensure that the participant is aware of his/her rights in participating in a research study.

5.1.2 Urine Collection

After consent, the participant will be asked to provide a urine collection to test for drug use and pregnancy testing (if female).

If the participant has a positive toxicology screen (marijuana is okay), he/she will be dismissed and given the option to rescreen within 30 days.

5.1.3 Vital Assessment

The following physiological measures will be assessed:

- Height and Weight
- Blood pressure and heart rate
- Expired carbon monoxide (CO)

If the participant's blood pressure is out of range, it will be re-tested at the end of the visit manually, following the saliva collection. If it is still out of range, they will

be considered ineligible. If the participant's expired CO levels are not ≥ 5 ppm, he/she will be dismissed from the visit and not paid.

5.1.4 Screening Packet

The screening packet will then be administered. It is broken into four main parts:

- **Section 1: Personal Data Form (Page 1)**
  - This section contains the participant's contact information as well as an Emergency Contact.
- **Section 2: Demographics (Pages 2-3)**
  - This section contains information regarding the participant's gender, age, ethnicity, race, and education.
- **Section 3: Nicotine Product Use (Pages 4-22)**
  - This section contains information regarding participant's use of nicotine replacement therapy, tobacco and e-cigarette product use, and drug/alcohol use.
- **Section 4: Medical History (Pages 23-28)**
  - This section contains information regarding the participant's medical history, including exclusionary medical and psychiatric conditions.
  - If any symptoms are endorsed on the Medical History in Section 8, the CRC will follow up with the participant asking:
    - How often and when was the last time you experienced these symptoms?
    - Do you ever take medication for this? If so, how often?

Notes on the Medical History follow-up will be recorded at the bottom of the last page of the form (page 16).

5.1.5 Saliva Collection

After the participant completes the Screening Packet, he/she will be asked to provide a saliva collection.

5.1.6 End of Screening Visit

At the end of the screening visit, the participant will be asked to fill out a "Certificate of Participation" in order to receive compensation for the screening visit. The participant will be dismissed and the CRC will inform him/her that he/she will be contacted in 1-2 weeks with eligibility results from the screening visit.

5.1.7 Post-Processing

The participant's urine will be discarded and the CRC will store the participant's saliva in the freezer at the UCSF Tobacco Research Center lab. The saliva samples will be labeled with the Study ID and transferred to the Benowitz Lab at the end of the day on Friday afternoons. The CRC will complete a Screening Log on the Shared Lab Drive filling in information on the samples dropped off. Cotinine results will be expected the following Tuesday.

The CRC will complete the Screening Visit Log and enter data from the Screening Packet into the "TCORS-Study1 Screening Packet" REDCap database. Data entry should occur within 48 hours of the screening visit.

The CRC will submit the participant's check request after confirming the participant's vaping status (COT ≥ 30 ng/ml). If the participant's cotinine is < 30 ng/ml, they will not be compensated for their screening visit.

5.2 PREPARATION FOR INPATIENT PORTION

5.2.1 5B SETUP PAPERWORK

After study dates have been confirmed with the participant and the research ward, an admission request will be submitted via secure email to the research ward. The CRC will then prepare CTSI-5B Set-up paperwork for the admissions.

The set-up paperwork consists of the following:

5.2.1.a Coversheet

- The coversheet contains general information regarding the study and supplies needed.
- CRC will place a label with the participant's name and study ID, and edit the admission and set-up drop-off dates.

5.2.1.b Consent Form

- An original signed copy of the consent form from the Screening Visit will be included in the set-up.

5.2.1.c Nurses Flow Sheet

- The Nurses Flow Sheet contains study procedures and time points for each day of the inpatient admission.
- CRC will update this form with participant's TC product brands or SREC assignment.

5.2.1.d Outpatient Form

- The outpatient form has PHI filled out by the participant at the screening visit with name, SSN, date of birth, and contact information.

5.2.1.e MD Orders

- The MD Orders are pre-signed by the Study Physician that will conduct the physical during the admissions.
- CRC will write in the admission date and pencil in the participant's name on the top right corner.

5.2.1.f Admit PE

- The Admit PE will be filled out by the Study Physician as they perform the history and physical.
- CRC will pencil in participant's name in the top right corner.

5.2.1.g Adverse Event Form

- The Adverse Event form is included in the set-up should any adverse events occur during the course of the study.
- CRC will change the study ID.

5.3 ORIENTATION

Participants will attend an Orientation Visit during which the e-cigarette device power level order will be determined and study procedures will be described in detail again. The following will occur:

5.3.1 Product Assignment

Three e-cigarette device power levels will be assigned to all participants: 10, 35, and 70 watts. The order of the assignment will be randomized, single-blinded.

E-liquid Selection: We will obtain the e-liquid from AVAIL, a leading premium e-liquid manufacturer and retail business which works with academic centers to provide research grade products that come with certificates of analysis confirming nicotine content, lack of diacetyl (<38 ppm) and acetyl propionyl (<100 ppm). We will purchase the most popular e-liquid from AVAIL at the time of the study. Currently, it is Mardi Gras, a mixed berry medley (personal communication, AVAIL), and will contain 3 mg/mL (0.3%) nicotine and 20/80 PG/VG. We chose 0.3% nicotine because this is the typical nicotine strength of e-liquids used in high-powered e-cigarettes. While researchers often request 70/30 PG/VG e-liquids for studies, the most popular retail e-liquids are 20/80 PG/VG (personal communication, AVAIL).

E-cigarette Device: The delivery device will be CUPTI™ made by KangerTech. It is a variable wattage all-in-one device with operating wattage of 7.0 – 75.0 W, which is inclusive of the three power levels we intend to study. We chose this device because KangerTech is a popular brand and their devices function reproducibly within models. It is important to note that e-cigarettes continue to evolve and therefore, the final choice of e-cigarette used in the study will depend on what is popular at the time we begin data collection.

5.3.2 Overnight Abstinence

Participants will need to abstain from using any e-cigarette device the night before each hospital admission, starting at 10 PM.

5.4 STUDY VISITS

5.4.1 STUDY ARM #1

5.4.1.a In-Patent Day #1

- Participants will be admitted to the hospital research ward at about 7 A.M. on the first morning of the 2-day inpatient visit after overnight abstinence from e-cigarettes starting at 10 PM.

- At the time of admission to the hospital ward, expired CO will be measured. Those with expired CO of 5 ppm or over will be sent home.
- Participants will be in a hospital-approved smoking room with negative pressure and a fan ventilating to the outside.
- At about 8 AM, an intravenous catheter will be placed in the forearm for blood sampling and light breakfast will be served.

Standardized E-Cigarette Vaping Session

- At 9 AM, the participant will vape the study e-cigarette at the assigned power in a standardized protocol: one 4-second puff every 30 seconds for a total of 10 puffs.
- We are standardizing puff duration to minimize within- and between-subject variations in vaping topography.
- After each puff, participants will exhale into a gas trap to determine amount of nicotine exhaled.
- E-cigarettes will be weighed before and after use to determine the amount of nicotine inhaled.
- After this standardized session, the participant will abstain from using e-cigarettes for a 4-hour period.

4-hr Abstinence

- Heart rate will be measured before and at 5, 10, 15, 20, 25, and 30 minutes after product use.
- Withdrawal, craving and reward will be assessed before and immediately after the standardized session and at 2 and 4 hours after.
- Blood nicotine levels will be measured before and then at 2, 5, 15, 30, 45, 60, 90, 120, 180 and 240 minutes after completing product use.
- At the end of the 4-hour abstinence period, participants will be allowed 90 minutes of ad libitum access to the e-cigarette at the same assigned power level as during the standardized session.

90- minute *ad libitum* session

- Blood samples will be collected before and every 15 minutes from the beginning of the session.
- Withdrawal, craving, and reward will be assessed before and immediately after the session ends.
- Vaping topography (puff number, puff duration, and inter-puff interval) will be measured via digital video recordings that allow frame-by-frame analysis

5.4.1.b In-Patient Day #2

- On the second inpatient day (after overnight abstinence starting at 10 PM), participants will wear a 24-hour ambulatory blood pressure and heart rate recorder to assess circadian blood pressure, heart rate pattern, and heart rate variability (8 AM-8 AM).
- Participants will be free to vape the assigned product as they would like from 8 AM to 12 midnight (hospital policy does not allow vaping after midnight).
    - The time of each puff will be recorded by the participant using a mobile app (Nomie) or paper log if app is unavailable.
- The e-cigarette will be weighed before and at the end of the day to determine the amount of e-liquid consumed, including if re-filled.
- Questionnaires to assess reward and craving and other nicotine withdrawal symptoms will be completed 4 times (8 AM, 12 noon, 4 PM and 8 PM).
- 24-Hour urine will be collected for assessment of various toxicant biomarkers and effects biomarkers.
- Blood will be sampled every four hours from 8 AM to midnight and at 8 AM the next day before discharge.
    - Nicotine will be measured in all blood samples and cardiovascular effects biomarkers will be measured in the 8 AM (pre-vaping) and 12 PM blood samples.
- Participants will be discharged in the morning after.

5.4.2 STUDY ARM #2

5.4.2.a In-Patent Day #1

- Participants will be admitted to the hospital research ward at about 7 A.M. on the first morning of the 2-day inpatient visit after overnight abstinence from e-cigarettes starting at 10 PM.
- At the time of admission to the hospital ward, expired CO will be measured. Those with expired CO of 5 ppm or over will be sent home.
- Participants will be in a hospital-approved smoking room with negative pressure and a fan ventilating to the outside.
- At about 8 AM, an intravenous catheter will be placed in the forearm for blood sampling and light breakfast will be served.

<u>Standardized E-Cigarette Vaping Session</u>

- At 9 AM, the participant will vape the study e-cigarette at the assigned power in a standardized protocol: one 4-second puff every 30 seconds for a total of 10 puffs.
- We are standardizing puff duration to minimize within- and between-subject variations in vaping topography.

- After each puff, participants will exhale into a gas trap to determine amount of nicotine exhaled.
- E-cigarettes will be weighed before and after use to determine the amount of nicotine inhaled.
- After this standardized session, the participant will abstain from using e-cigarettes for a 4-hour period.

4-hr Abstinence

- Heart rate will be measured before and at 5, 10, 15, 20, 25, and 30 minutes after product use.
- Withdrawal, craving and reward will be assessed before and immediately after the standardized session and at 2 and 4 hours after.
- Blood nicotine levels will be measured before and then at 2, 5, 15, 30, 45, 60, 90, 120, 180 and 240 minutes after completing product use.
- At the end of the 4-hour abstinence period, participants will be allowed 90 minutes of ad libitum access to the e-cigarette at the same assigned power level as during the standardized session.

90- minute *ad libitum* session

- Blood samples will be collected before and every 15 minutes from the beginning of the session.
- Withdrawal, craving, and reward will be assessed before and immediately after the session ends.
- Vaping topography (puff number, puff duration, and inter-puff interval) will be measured via digital video recordings that allow frame-by-frame analysis

5.4.2.b In-Patient Day #2

- On the second inpatient day (after overnight abstinence starting at 10 PM), participants will wear a 24-hour ambulatory blood pressure and heart rate recorder to assess circadian blood pressure, heart rate pattern, and heart rate variability (8 AM-8 AM).
- Participants will be free to vape the assigned product as they would like from 8 AM to 12 midnight (hospital policy does not allow vaping after midnight).
  - The time of each puff will be recorded by the participant using a mobile app (~~TimeJot~~ Nomie) or paper log if app is unavailable.
- The e-cigarette will be weighed before and at the end of the day to determine the amount of e-liquid consumed, including if re-filled.
- Questionnaires to assess reward and craving and other nicotine withdrawal symptoms will be completed 4 times (8 AM, 12 noon, 4 PM and 8 PM).
- 24-Hour urine will be collected for assessment of various toxicant biomarkers and effects biomarkers.

Blood will be sampled every four hours from 8 AM to midnight and at 8 AM the next day before discharge.

5.4.3 STUDY ARM #3

5.4.3.a In-Patent Day #1

- Participants will be admitted to the hospital research ward at about 7 A.M. on the first morning of the 2-day inpatient visit after overnight abstinence from e-cigarettes starting at 10 PM.
- At the time of admission to the hospital ward, expired CO will be measured. Those with expired CO of 5 ppm or over will be sent home.
- Participants will be in a hospital-approved smoking room with negative pressure and a fan ventilating to the outside.
- At about 8 AM, an intravenous catheter will be placed in the forearm for blood sampling and light breakfast will be served.

<u>Standardized E-Cigarette Vaping Session</u>

- At 9 AM, the participant will vape the study e-cigarette at the assigned power in a standardized protocol: one 4-second puff every 30 seconds for a total of 10 puffs.
- We are standardizing puff duration to minimize within- and between-subject variations in vaping topography.
- After each puff, participants will exhale into a gas trap to determine amount of nicotine exhaled.
- E-cigarettes will be weighed before and after use to determine the amount of nicotine inhaled.
- After this standardized session, the participant will abstain from using e-cigarettes for a 4-hour period.

<u>4-hr Abstinence</u>

- Heart rate will be measured before and at 5, 10, 15, 20, 25, and 30 minutes after product use.
- Withdrawal, craving and reward will be assessed before and immediately after the standardized session and at 2 and 4 hours after.
- Blood nicotine levels will be measured before and then at 2, 5, 15, 30, 45, 60, 90, 120, 180 and 240 minutes after completing product use.

- At the end of the 4-hour abstinence period, participants will be allowed 90 minutes of ad libitum access to the e-cigarette at the same assigned power level as during the standardized session.

90- minute *ad libitum* session

- Blood samples will be collected before and every 15 minutes from the beginning of the session.
- Withdrawal, craving, and reward will be assessed before and immediately after the session ends.
- Vaping topography (puff number, puff duration, and inter-puff interval) will be measured via digital video recordings that allow frame-by-frame analysis

5.4.3.b In-Patient Day #2

- On the second inpatient day (after overnight abstinence starting at 10 PM), participants will wear a 24-hour ambulatory blood pressure and heart rate recorder to assess circadian blood pressure, heart rate pattern, and heart rate variability (8 AM-8 AM).
- Participants will be free to vape the assigned product as they would like from 8 AM to 12 midnight (hospital policy does not allow vaping after midnight).
  - The time of each puff will be recorded by the participant using a mobile app (~~TimeJot~~ Nomie) or paper log if app is unavailable.
- The e-cigarette will be weighed before and at the end of the day to determine the amount of e-liquid consumed, including if re-filled.
- Questionnaires to assess reward and craving and other nicotine withdrawal symptoms will be completed 4 times (8 AM, 12 noon, 4 PM and 8 PM).
- 24-Hour urine will be collected for assessment of various toxicant biomarkers and effects biomarkers.

Blood will be sampled every four hours from 8 AM to midnight and at 8 AM the next day before discharge.

5.4.4 PARTICIPANT SCHEDULE

Study Arm #1: Power level 10, 35, or 70 watts (computer randomized, single-blinded)	
Day 1	**Day 2**
HOSPITAL	
• **Standardized Session** • **4-hr abstinence and blood draws** • **Followed by 90 min Free use session w/ video monitoring**	• **Free use** • **24-hr CV monitoring** • **Circadian blood draws** • **24-hr urine collection**

Study Arm #2: 1 of the other 2 remaining power levels (computer randomized, single-blinded)	
Day 1	**Day 2**
HOSPITAL	

• Standardized Session • 4-hr abstinence and blood draws • Followed by 90 min Free use session w/ video monitoring	• Free use • 24-hr CV monitoring • Circadian blood draws • 24-hr urine collection

Study Arm #3: Remaining power level (computer randomized, single-blinded)	
Day 1	Day 2
☐---------- HOSPITAL ----------☐	
• Standardized Session • 4-hr abstinence and blood draws • Followed by 90 min Free use session w/ video monitoring	• Free use • 24-hr CV monitoring • Circadian blood draws • 24-hr urine collection

5.5 PARTICIPANT COMPENSATION

Participants will be compensated according to Table 2:

Table 2

Screening Visit	In-Patient Day #1	In-Patient Day #2	In-Patient Day #3	In-Patient Day #4	In-Patient Day #5	In-Patient Day #6	Completion Bonus
$30*	$240	$240	$240	$240	$240	$240	$360
Total Compensation: $1830							

*Compensation for the screening visit will be contingent upon a negative toxicology drug screen (THC is okay) and a cotinine of ≥ 30 ng/ml.

Compensation for participants who drop-out and do not make it through all portions of the study is described in Section 2.5.2.

6 ADVERSE EVENTS AND PROTOCOL DEVIATIONS

6.1 SAFETY MONITORING

During the inpatient stay, participants will be monitored by the CRC and the nursing staff who will directly contact the Study Physician in case of any subsequent adverse events. If the participants experience adverse effects, the Study Physician will evaluate this with them and decide if they should be withdrawn from this study.

6.2 REPORTING ADVERSE EVENTS

Reporting of serious adverse events will follow the current requirements of the UCSF Human Research Protection Program's Institutional Review Board (IRB), with the concurrent reporting to the Nurse Manager at the CRC. Specifically, the following will be reported within five (5) working days of the Principal Investigator's (PI) awareness, in writing:

- All serious adverse events associated with the study procedures and/or
- Any incidents or problems involving the conduct of the study or patient participation, including problems with the recruitment and/or consent process. The PI will provide a discussion of such events to the IRB on an annual basis during study renewal.
- Any incidents or questionable adverse events are discussed at the weekly staff meetings with the PI.

The standard adverse event grading scale will be used to report any potential adverse advents from phlebotomy, study drug, or other study procedures:

- **Grade 1 - Mild AE:** did not require treatment
- **Grade 2 - Moderate AE:** resolved with treatment
- **Grade 3 - Severe AE:** resulted in inability to carry on normal activities and required profession medical attention
- **Grade 4 - Life-threatening or disabling AE**
- **Grade 5 - Fatal AE**

The supervising Study Physician will be notified of all Grades 2 through 5 AEs. Additionally, the PI will be notified of all grades 4 and 5 AEs. Grades 3 through 5 AEs

will be reported to the CCRC and the CHR. Grades 4 and 5 will be reported within 24 hours. Grade 3 will be reported within 20 days. Grade 2 will be included in the yearly progress report. Grade 1 will not be reported.

6.3 PROTOCOL DEVIATIONS & VIOLATIONS

Protocol deviations are defined as an event that deviates from the defined study protocol, but does not pose any risk to the participant or harm to the quality of the study data. For example, a follow-up call may be conducted outside of the stipulated window, or a section of questions on a questionnaire may be missed due to a mistake in branching. Deviations will be recorded on a protocol deviation sheet and reviewed and signed by the PI.

Protocol violations are events that deviate from the defined study protocol, but put the participant at risk and/or cause harm to the study data. Protocol violations will be recorded on a protocol violation sheet, reviewed and signed by the PI and reported to the IRB.

7 DATA COLLECTION AND MANAGEMENT

7.1 DATA COLLECTION FORMS

All of the following measures will be collected.

Screening Process:

- UCSF Tobacco Research Center Online Screen

In-Person Screening Visit:

- TCORS-Study 1 Screening Packet

Inpatient Study Visits:

- Minnesota Nicotine Withdrawal Scale (MNWS)
- Questionnaire on Smoking Urges-Brief (QSU-brief)
- Modified Cigarette Evaluation Questionnaire (mCES), further modified for e-cigarettes
- Appeal and Sensory Effects Questionnaire
- Perceptions of Risk Questionnaire

8 DUTIES AND RESPONSIBILITIES OF STAFF

8.1 PRINCIPAL INVESTIGATOR

The Principal Investigator is responsible for study design and oversight of implementation, data analysis, and manuscript preparation.

8.2 CO-PRINCIPAL INVESTIGATORS

The Co-Principal Investigators are responsible for study design and oversight of implementation, data analysis, and manuscript preparation, with the assistance of the Principal Investigator.

8.3 STUDY PHYSICIAN

The Study Physician is responsible for medical study chart review and eligibility determination, medical history and physical examination at SFGH admissions, and other medically related expertise.

8.4 PROJECT MANAGER

The Project Manager is responsible for overall functioning of study coordination with CHR protocols; monitoring of study budget and coordination with departmental administrative personnel; supervision of research associates; management of clinic facilities.

8.5 CLINICAL RESEARCH COORDINATOR

The CRC will oversee study logistics including consenting and screening participants, conducting study visit procedures, coordinating inpatient admissions and procedures with nursing staff at the research ward, coordinating specimen testing with laboratory, overseeing participant reimbursement, maintaining study charts and data entry.