Protocol Title Feasibility of PET/CT to detect the oral/pulmonary distribution of nicotine following e-cigarette use

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OBJECTIVES

Aim 1: To establish the methodology required for the evaluation of the biodistribution (*in vivo* deposition, absorption and transport) of carbon 11 (^{11}C) labeled nicotine following e-cigarette use

Aim 2: To determine the cranio-pharyngeal/pulmonary distribution of nicotine following e-cigarette use

Aim 3: To determine the potentially lowest required dose using the next generation digital PET/CT technology to provide detailed or accurate oral/pulmonary distribution data following e-cigarette use

BACKGROUND AND RATIONALE

Electronic cigarettes (e-cigarettes) are marketed as non-combustible nicotine delivery devices. Typically, the device works by the user placing a refill cartridge/solution (containing carrier solvents and nicotine) into the device. An airflow sensor or power button is then used to activate a battery that turns on an atomizer. The atomizer then produces an aerosol from the liquid containing the nicotine. This aerosol is typically, but inaccurately, referred to as a vapor, so the term vaporizer is commonly used to describe the device. It must be noted; however, that since fine particulates (solid, liquid, and gas) are formed, the atomization of the solvent results in an aerosol and not a vapor. This aerosol simulates cigarette smoke in that the aerosol is delivered into the mouth and respiratory tree by inhalation. The remaining (non-absorbed) aerosol is then exhaled.

E-cigarette manufacturers claim that e-cigarettes are safer than conventional cigarettes and that they may aid in smoking cessation. As a result, e-cigarettes are gaining popularity and use in the general public as a replacement for traditional cigarettes. However, little is known about the benefits and health risks of e-cigarette use. Although e-cigarettes have been shown to have negligible effect on carbon monoxide exposure and heart rate, they contain a variety of glycerols mixed with variable concentrations of nicotine. In addition, other substances are present in the refill solutions and/or resulting vapor, such as tobacco specific nitrosoamines, cotinine, aldehydes, metals, volatile organic compounds, tobacco alkaloids, and other pharmacologically active ingredients (although in concentrations much lower than in combustible traditional cigarettes). In addition, studies have shown that during the atomization process, there is a large range in the size of the particulate matter formed that is dependent upon both the device and refill solution used. It is well know that inhaled particulate matter may induce inflammation and may eventually lead to an increase in a variety of health risks including cardiovascular disease and emphysema.

Although e-cigarettes are not currently regulated, studies have indicated that the e-liquid products should be regulated to ensure consistent nicotine delivery and to prohibit the use of ethylene glycol and other excipients that currently may be present at potentially harmful amounts (high risk category). Additionally, only minimal valid data are available related to the biodistribution (deposition of nicotine and/or aerosol excipients) following inhalation. The evaluation of nicotine and aerosol deposition in the body will help to determine if the particulates formed during the atomization process may induce any serious health risks. Additionally, these data may be compared to traditional cigarette smoking to determine if e-cigarettes are truly a safer alternative to conventional cigarettes. In order to provide these data, quantitative methods need to be developed that allow for accurate *in vivo* biodistribution determination following inhalation. Radioactive carbon (^{11}C) biodistribution studies have been reported in the literature for traditional cigarettes. Here, cigarettes are laced with ^{11}C-nicotine, and the biodistribution following inhalation

is evaluated using positron emission tomography (PET). The aim of the current study is to establish the methodology required to evaluate the biodistribution of ^{11}C-nicotine labeled refill solution following e-cigarette inhalation using digital PET/CT and/or conventional PET/CT. Once established, these methods will be applied to determine the biodistribution of e-cigarette aerosol.

RESEARCH DESIGN AND METHODS

Methods Overview

Potential participants will be identified using advertisements including brochures and online postings. After participants are identified, their eligibility will be determined using survey tools.

All eligible participants will first have a screening visit at the WCIBMI for study participation. During this initial visit, subjects will be informed about the study in detail, and the relevant consent form will be reviewed and signed. If they agree to participate, they will go through a full dress rehearsal. Up to 10 volunteers will only participate in the dress rehearsal. All other volunteers will have a dress rehearsal without radiation exposure on day 1, and then on a second day, they will participate in the full imaging study using ^{11}C-nicotine.

During the imaging study, S-nicotine will be labeled with ^{11}C and placed in the cartridge of an e-cigarette. At the time of initial inhalation, the S-nicotine will be formulated using of ^{11}C purified by HPLC.

There will be two different dosage groups:

A, 3mCi dosage of ^{11}C-nicotine placed into the e-cigarette

or

B, 9 mCi dosage of ^{11}C-nicotine placed into the e-cigarette

The target dose will be the 3mCi dosage. Current investigational research by other groups uses dose levels of 10-20mCi. Based on our simulations, we predict that the 3mCi dose level will be sufficient but we will not know. Therefore we are including a second dose level group as an alternate which will still be below dose levels reported by other groups.

Subjects will take a maximum of 10 puffs (1 puff per 30 seconds) from the e-cigarette while positioned in the PET/CT system.

Dynamic PET/CT imaging will be performed for a maximum of 60 minutes following inhalation.

The subject will be placed in the PET camera in order to generate axial images of the following regions: head/neck (e.g., brain, oral cavity, and throat) and thorax (e.g., trachea, lungs).

From the PET/CT images, quantitative radioactivity deposition will be determined, and the biodistribution and uptake/clearance will be evaluated.

PET data will be acquired in listmode and subsequently used for simulation to determine the potentially lowest dose feasible using the next generation digital PET/CT technology.

Recruitment

Potential participants will be identified through brochures, contact with vape shops, and social media postings. Brochures will be distributed at vape shops, and social media postings will be made in interest groups. A link to an informational video will also be provided in the brochure. To determine eligibility, participants will sequentially complete two eligibility surveys.

Survey 1

The brochures and social media postings will include a link to Eligibility Survey 1. Survey 1 will collect sensitive, medical information, but will be completely anonymous. Medical information will be collected solely for the purposes of determining participant eligibility and will be unlinked from any identifying participant information as well as future collected study data.

Survey 2

Once participants are determined eligible by Survey 1, they will be directed to complete Survey 2 to confirm their eligibility. Survey 2 will ask participants questions about their vaping habits. If they are confirmed eligible from the survey, they will also be asked to provide their name and contact information (e-mail). Study personnel will then contact eligible participants to schedule a time for them to come to the WCIBMI for their initial visit. Responses from Survey 1 and Survey 2 will be completely unlinked to maintain anonymity about participants' medical information.

Selection Criteria

Inclusion Criteria

- Males and females of 18 years of age or older
- Current regular user of e-cigarettes (use at least once daily for the past 30 days) with nicotine strength > 6 mg/ml.
- Healthy medical history
- Abstinent from any tobacco/nicotine use for 4 hours prior to imaging

Exclusion Criteria

- Not a regular user of e-cigarettes
- Pregnant or lactating (only excluded from imaging study)
- Prisoner
- Incapable of giving informed consent
- Being claustrophobic
- Unable to lie flat on the scanner for extended periods of time
- Unstable medical condition such as heart disease, uncontrolled hypertension, thyroid disease, diabetes, renal or liver impairment, or glaucoma
- Prostatic hypertrophy, stroke, ulcer in the past year
- Psychiatric conditions such as schizophrenia, adult ADHD, or bipolar disorder
- Current or regular use of psychiatric medications such as tranquilizers, antipsychotics and anti-depressants
- Use of medications that are inducers of CYP2A6 (a nicotine metabolizing enzyme) such as rifampicin, dexamethasone, phenobarbital, and other anti-convulsion drugs
- Unable to communicate in English
- Current use of smokeless tobacco, tobacco cigarettes (5 or more per day). Occasional use of pipes or cigars is permitted if subject abstains for the week prior to the study
- Older than 80 years

Participant Procedures

Cold – Volunteers – No imaging - full dress rehearsal without radiation exposure (Single day participation)

In order to optimize the procedural setup of our imaging methodologies, we will recruit up to 10 cold volunteers to only complete a dry run / dress rehearsal of the study. These volunteers will be taken through the study process without the use of the ^{11}C–nicotine e-cigarette and without performing any imaging. The volunteers will eligible to join the full imaging study.

Imaging Volunteers – full study with imaging session (Two-day participation)

A maximum of 30 subjects will be included in the imaging portion of this study. Similar to the cold / dress rehearsal volunteers, these participants will complete an initial dress rehearsal visit at the WCIBMI to walk through the study procedures. However, the full study participants will also return to the WCIBMI on a second date to complete the imaging study with the ^{11}C-nicotine e-cigarette. Bergstrom et al. (Clinical Pharmacology and Therapeutics, 1995; 57(3):309-17) has reported a relative percent deviation of 34% in the total dose released following 5 minutes of aerosol inhalation (15.4 +/- 5.3%). Assuming an alpha value of 0.05 and a power of 0.8, a sample size of at least 8 subjects is required to observe significant differences in the dose released. One of the aims of this study is to evaluate the next generation PET/CT technology that may exhibit different variance. As a result, larger populations are likely required in order to evaluate a significant difference between the groups. If the power is set to 0.95 to account for this, then the number of subjects required per group is 14. In order to ensure significance and to account for any subjects that do not comply, a total of 30 subjects is requested. Subjects may enroll up to two times (i.e., repeat the imaging study with ^{11}C-nicotine one additional time), but the second enrollment must be at least one week after the first visit.

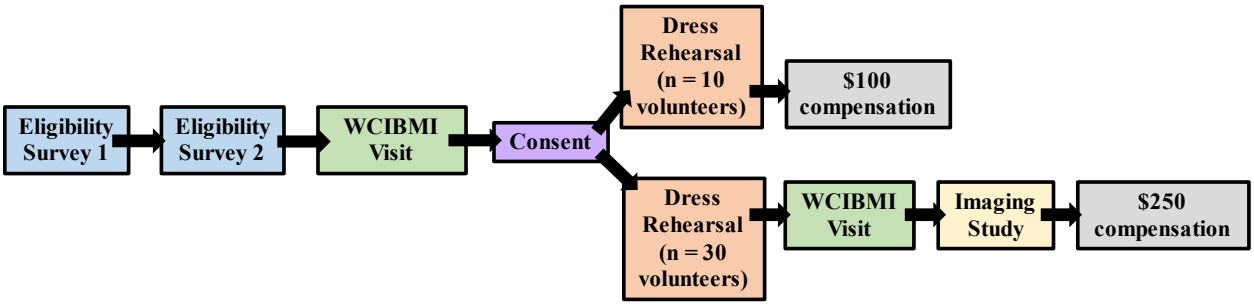

Figure: Schematic of participant procedures. After determined eligible via eligibility surveys, participants will be invited to the WCIBMI to complete the relevant consent forms. If they are participating in the full study, they will first participate in the dress rehearsal, and then study personnel will schedule a time for the participant to return to the WCIBMI and complete the full, imaging portion of the study. If they are participating in only the dress rehearsal, they will not receive any radiation or imaging, and they will receive compensation at the end of the dress rehearsal.

Measurement / Instrumentation

PET/CT imaging will be predominately performed on the next generation digital photon counting PET/CT (Vereos); however, the mobile conventional PET/CT (Gemini TF 64) that is parked on the Martha Morehouse facility will serve as a backup PET/CT imaging system in case of any technical reasons. Our main focus is to utilize the digital PET system, but we need and want the

option to perform imaging on the conventional PET/CT system in case there is a technical issue causing unavailability of the digital PET system.

Both systems are able to acquire using listmode with time-of-flight encoding.

Both systems have an internal closed circuit video system that will record every aspect during the subjects' presence in the room. Access to these video recordings will be restricted to authorized study personnel and used only for the purpose of reviewing the procedure and correlating placement and timing of the e-cigarette and ^{11}C-nicotine inhalation with the acquired PET images.

The PET acquisition will be initiated prior to the placement of the prepared e-cigarette in the field of view.

Prior to the imaging examination using the ^{11}C-nicotine prepared e-cigarette, the subject will be trained using a full dress rehearsal with no radioactivity.

For the imaging examination, the subjects will be supine on the imaging bed proximal to the PET camera while they are instructed when and how to vape. Subjects will vape normally and exhale freely into a directional airflow, ensured by a fan and a plastic bubble placed on the backside of the PET system combined with a suction vacuum pulling the air through a HEPA filter. Once vaping is completed, the e-cigarette will be taken from the subject, and the remaining residual ^{11}C-nicotine activity within the e-cigarette will be determined using a well counter. The delivered dose will then be determined as the total activity in the e-cigarette liquid prior to vaping minus the decay corrected activity in the e-cigarette liquid after vaping.

Immediately and during the vaping, the dynamic and continuous PET imaging will be performed using multiple bed positions to image from the vertex of the skull to the abdomen.

A very low dose CT attenuation scan (0.8 mSv) will be acquired for attenuation correction and anatomic correlation prior to and after the emission scanning.

Schematic of the PET/CT for e-cigarette vape/aerosol trapping

The diagrams below show the experimental setup. As has been reviewed with the OSU Radiation Safety Officer (RSO), we will trap the exhaled ^{11}C-nicotine e-cigarette vape only via positive airflow through the PET gantry and subsequent suction through a HEPA filter vacuum system.

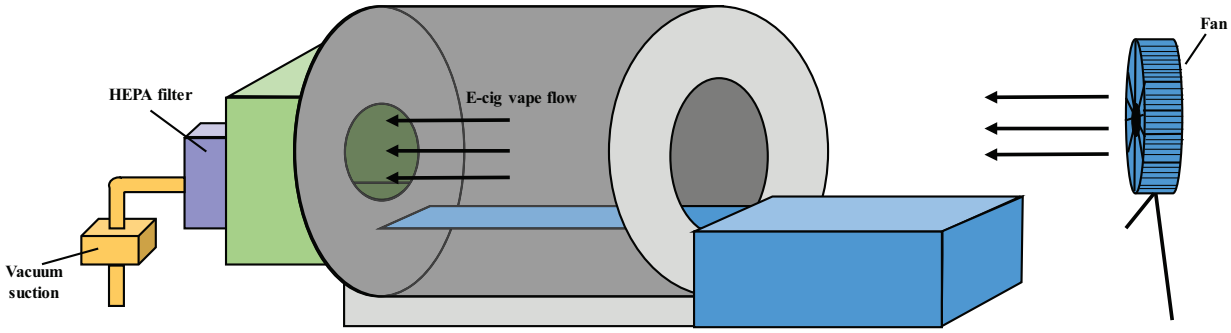

Figure: Schematic of experimental setup. The green compartment behind the PET/CT device will be composed of a transparent, PVC, bag-like enclosure that is taped to the device and the collector system with the HEPA filter. A fan that is placed on one side of the PET/CT patient table will initiate the directional air flow.

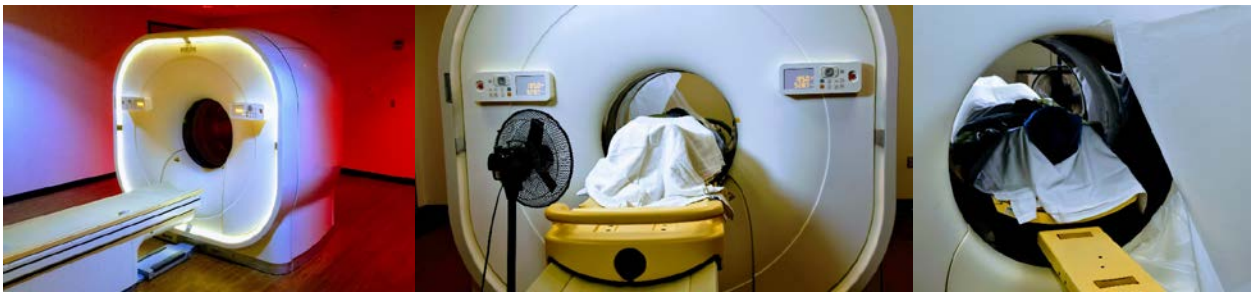

Figure: Subject positioned head-first on PET/CT system. A test subject will be positioned on the system with the fan for directional air flow on one side (center). The image on the far right depicts the plastic enclosure open to show the rear view of the subject position.

^{11}C-nicotine Dosage

As has been reviewed in a broad collaboration with Medical Physics and Radiation Safety and agreed in consensus with the RSO, the maximum dose that we may mix into the vape fluid is 9 mCi of ^{11}C-nicotine. Initially, we will use a dosage of 3 mCi (Dose level A); however, if this dosage does not yield adequate image quality, the dosage will be raised to 9 mCi (Dose level B) for subsequent participants. Due to this low dose approach and the short half-life of ^{11}C (i.e., 20.3 min), the directional airflow with a HEPA filter is sufficient for removing and absorbing the residual vape/aerosol. After a subject completes an ^{11}C-nicotine vaping session, the PET/CT imaging area will be considered radioactive until 20 half-lives (i.e., 406 min) have passed. The imaging area will be marked as "*Do not access*" until the next morning.

Schematic of an e-cigarette

The following figure details the components of the e-cigarette. During the dress rehearsal, a user manual will be provided to the subject and will be reviewed for the subjects participating in imaging.

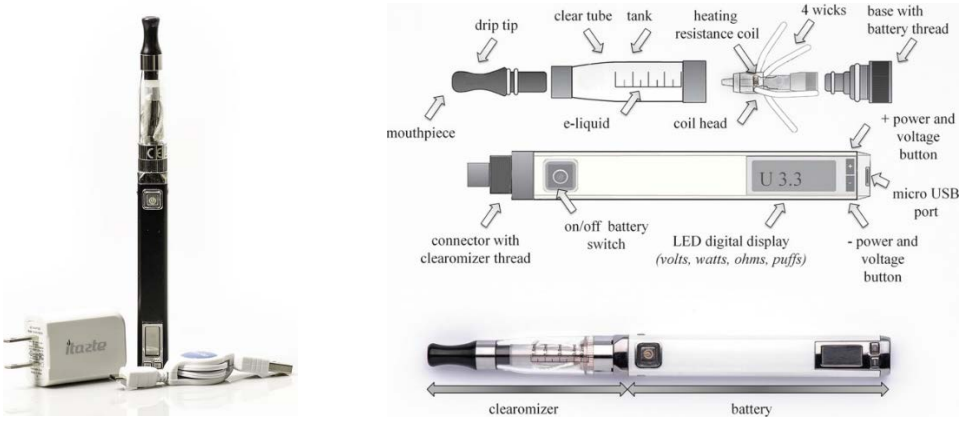

Figure: E-cigarette schematic. The e-cigarette system that will be used is Itazte VV (left). The e-cigarette components are detailed in the schematic of a generic e-cigarette (right; source: Wikimedia.org).

DETAILED STUDY PROCEDURES

Screening Visit – Dress Rehearsal

Once the participant has been identified and indicated interest in participation, they will be invited to the WCIBMI to see the facilities, experience being in the PET/CT device (i.e., to determine if the subject is claustrophobic), and sign the informed consent and HIPAA if they remain interested in participating.

For those subjects that have indicated their interest to participate in the imaging study, the following additional steps will occur during the dress rehearsal visit:

- Participants will be informed about the further study details, the consent form will be reviewed and signed, and an imaging date will be scheduled.
- Participants will be given the e-cigarette that will be used in the study so that they can get accommodated to the device and practice vaping in the supine position.
- For female subjects participating in the imaging procedure, urine will collected during the screening visit to confirm that they are not pregnant.

All subjects participating in the imaging study will be required to have a full dress rehearsal at the WCIBMI prior to their imaging procedures in order to practice and be familiar with the study facilities/procedure: the gowning into hospital scrubs and protective gear, the proper posture for PET scanning, and the e- cigarette vaping and exhaling procedure. There will be no PET/CT imaging or ^{11}C-nicotine radiation exposure during the dress rehearsal.

Subjects who initially agree only to the dress rehearsal are eligible to add the subsequent imaging study without an additional dress rehearsal unless requested by the PI. The subject will complete appropriate consent documentation of their expanded participation. The dress rehearsal and subsequent ^{11}C-nicotine PET/CT imaging examination will follow the same steps.

Subject Gowning

The subject will wear hospital scrubs and personal protective gear in order to minimize any ^{11}C radioactive deposits from the exhaled vape. The subject will also wear two sets of gloves. The gowning will be guided and monitored by research project personnel.

Figure: Subject gowning procedure. Still pictures from a training video demonstrate the subject gowning procedure with personal protective gear.

Subject Positioning on the PET/CT System

After gowning, the subject will be placed on the PET/CT system. A knee support pad and a head-holder will be available and used to improve subject comfort and minimize subject motion.

Once the subject is comfortably positioned, the surview scan will be performed using CT only, and the PET/CT imaging field will be planned. Once the imaging field is determined, an attenuation CT scan of the imaging field will be acquired.

The e-cigarette will be brought to the PET/CT suite in a transport box. Once all preparations are completed for the PET image acquisition, the subject will be given the e-cigarette.

Figure: Subject positioning procedure. These still pictures from a training video demonstrate the subject positioning procedure on the PET/CT system, including the process of handing over the e-cigarette (lower center), and the process for the subject to hold the e-cig during vaping (lower right).

E-cigarette Vaping

The following figure presents still pictures from the training video to demonstrate the handover of the e-cig to the subject, how the subject should handle the e-cig, and the process of vaping as instructed with up to 10 puffs.

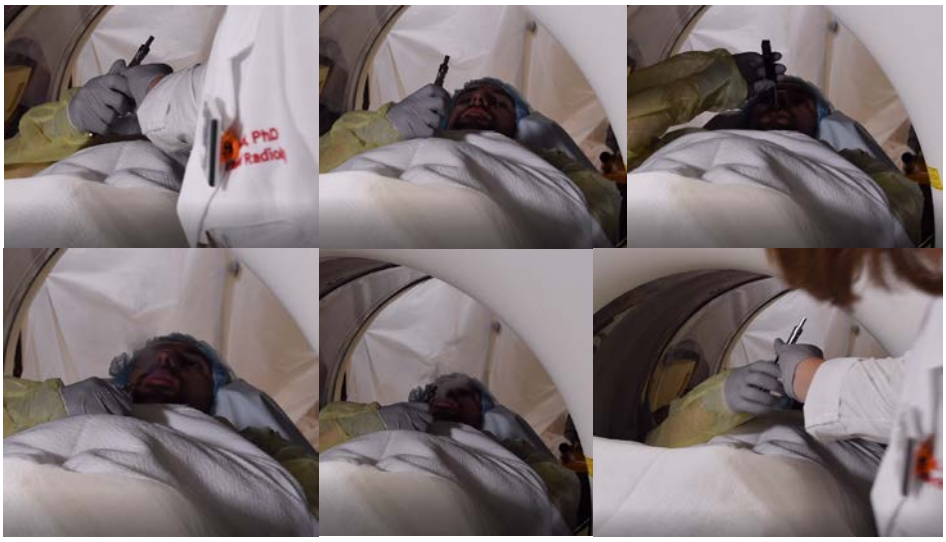

Figure: ^{11}C-nicotine e-cigarette puffing procedure. Still pictures from a training video demonstrate the handling of the c-cig in the PET/CT system, the handover to the subject, handling of the e-cigarette during vaping, and return of the device to staff.

Subject De-gowning

The following figure presents still pictures from the training video to demonstrate the de-gowning procedure of the participant.

Figure: Subject de-gowning procedure. Still pictures from a training video demonstrate the participant de-gowning procedure. The participant will remove and dispose of all personal protective gear, and study personnel will subsequently evaluate the radiation levels of the participant.

Imaging Visit

All subjects will be contacted 24-48 hours prior to the imaging study to confirm their availability and continued participation. The procedures detailed for the dress rehearsal will also be followed for the imaging session. The subject will again disrobe and change into hospital scrubs. The subject

will wear full-body disposable personal protective equipment (PPE) including a gown, hairnet, double latex gloves, and foot coverings.

The subject will be positioned on the PET/CT during the transport of the ^{11}C- e-cig material from Kinnear to the WCIBMI (15 minutes prior to study of vaping).

Once the ^{11}C-nicotine e-cigarette enters the PET/CT facility, it will be placed into the facility's dose calibrator for a secondary dose confirmation and then transported directly to the PET/CT imaging room. The subject will then be instructed to start inhalation (maximum 10 puffs at 30 second intervals) while positioned in the PET/CT system. Afterwards, the used e-cigarette will be removed from the imaging system by a staff member, and its remaining residual ^{11}C-nicotine activity determined using a dose calibrator.

PET imaging will be performed for a maximum of 60 minutes following onset of ^{11}C-nicotine inhalation/vaping. After PET imaging is completed, the subject will be de-gowned, and the radiation levels will be determined. The subject will be asked to stay in the patient waiting/recovery area for up to 120 minutes (2 hours). Prior to release, the subject will be surveyed again to ensure that any exhaled breath/air is at background radioactivity levels. Additionally, all subjects will be contacted within 24 – 48 hours after release from the study to ensure that they are feeling well and have not experienced any adverse reaction to the study. Any adverse reactions will be recorded and reported immediately to the PI.

The medical records for the subjects will not be obtained for this study. Participants will be asked to provide medical information in an eligibility survey only to confirm their eligibility. Responses to this survey will be entirely anonymous and confidential. In addition, there will be no personal data recorded other than the subject's gender, age, ethnicity, weight, height, and length of time that they have been smoking and/or using e-cigarettes. All subject data will use coded identifiers in all imaging files with subject numbers replacing the typically patient name fields.

Imaging

All PET/CT imaging will be performed using the Vereos digital PET/CT and/or the mobile conventional Gemini PET/CT systems located at the WCIBMI. The subject will lay supine and therefore must be able to inhale/vape in this position. The subject will be placed in the PET camera as to generate axial slices in the following regions: head/neck (e.g., brain, oral cavity, and throat) and thorax (e.g., trachea, lungs). CT image acquisition will be at the lowest dosages possible for accurate anatomic localization of ^{11}C radioactivity on the PET images (80KV, 30mAs, 3D dose modulated). Dynamic PET images will be acquired over a time period of up to 60 minutes following the start of ^{11}C-nicotine inhalation/vaping. The total PET imaging scan time will not exceed 60 minutes per subject. The total time in the imaging suite will be a maximum of 85 minutes for each subject (15 minutes prior to scanning, <5 minutes for inhalation, <2 minutes to remove e-cigarette, and up to 60 minutes scanning). Once the subject leaves the imaging suite, the imaging suite will be surveyed. If the radioactivity is above background levels, the suite will be locked and the PET/CT imaging area will be considered radioactive until 20 half-lives (i.e., 406 min) have passed. The imaging area will be marked as "*Do not access*" until the next morning and subsequently surveyed no sooner than every 30 minutes until background levels of radioactivity are achieved.

Optimal PET imaging parameters will be utilized for each PET/CT system and, as a result, may differ between the conventional Gemini and next generation digital Vereos systems. However,

time of flight acquisition with lost mode recording will be used on both systems. For example, the digital Vereos system is able to acquire PET images with faster timing resolution and with lower voxel sizes compared to the conventional Gemini system. The PET image acquisition parameters, however, will be held constant on each individual system.

^{11}C Labeling

^{11}C-labeled S-nicotine will be produced at the Kinnear Road facility following a gas-phase method developed by the WCIBMI team (Gosh A. et al, Applied Radiation & Isotopes, 2017, submitted) according to applicable production guidelines for inhalants. In short, purified ^{11}C-labeled S-nicotine will be diluted with the e liquid. The ^{11}C-labeled S-nicotine concentration, radiochemical purity, and specific activity will be determined. Appropriate amount of the e liquid containing ^{11}C-labeled nicotine will be loaded in the e-cigarette cartridge. The radioactivity in the cartridge will be determined, and the e-cigarette will be placed in a lead syringe holder, which will be delivered to the WCIBMI imaging facility at Kenny Road.

^{11}C Dose

The total radioactivity in the cartridge of the e-cigarette will not exceed 9 mCi delivered to the patient. Initially, we will use a dosage of 3 mCi (Dose level **A**); however, if this dosage does not produce adequate image quality, the dosage will be raised to 9 mCi (Dose level **B**) for subsequent participants. The release of radioactivity is expected to be 15 +/- 5% during the inhalation phase (Bergstrom et al, Clinical Pharmacology and Therapeutics, 1995; 57(3):309-17). The total delivered dose to the subject will be determined and recorded.

Data Analysis

All PET images will be reconstructed to generate quantitative images for absolute determination of radioactivity distribution. Corrections for the decay of ^{11}C will be applied. The deposition will be shown in 3D and co-localized relative to the anatomy (as provided by the CT images). The total radioactivity will be determined in the head/neck and thorax (e.g., brain, oral cavity, trachea, esophagus, bronchi, lungs, and other relevant soft tissues like muscle). Analysis of other tissues within the imaging field of view may be performed if ^{11}C activity is observed.

The biodistribution, uptake, and clearance will be evaluated in the specified tissues. The results obtained using conventional PET/CT will be compared to the results obtained using the new digital technology. The percent variance in the total radioactivity will be determined at each time point for each tissue.

We are not performing intra-individual comparator examinations. We are focusing on assessing the new generation digital technology and have comparable historical benchmark data available for comparison.

Compensation

Each subject who participates will be eligible for compensation.

The volunteers who participate in the dress rehearsal only (i.e., no ^{11}C-nicotine exposure and no PET/CT imaging) will receive a $100 Visa gift card.

Subjects who participate in the complete study (i.e., dress rehearsal and ^{11}C-nicotine PET/CT imaging) will receive a $250 Visa gift card, which will be provided to them after completion of all study requirements.

By law, payments to subjects are considered taxable income.

Incidental Findings

Imaging incidental findings will not be observable in this study as the PET and CT imaging will not be of diagnostic quality.

RISKS, BENEFITS, SAFETY, AND CONFIDENTIALITY

Risks

Overall, the risk/benefit ratio of this study is minimal since the probability and magnitude of harm or discomfort anticipated in the study are not greater than what is ordinarily encountered during the performance of a routine PET/CT.

Minimal risk exists during any PET imaging. Risks are mainly due to potential impingement by the moving table and falling off the imaging table.

Radiation Risk

Dose Level A (3 mCi ^{11}C-nicotine Dosage)

Participants will receive a e-cigarette with an total dosage of 3 mCi from the ^{11}C-nicotine, of which only a fraction will be inhaled by the participant. For the 3 mCi dosage, the PET radiotracer will yield minimal radiation exposure of 0.64 mSv.

The attenuation CT will add 0.8 mSv EDE to the overall dose exposure. For the 3 mCi ^{11}C-nicotine dosage, the combined and total EDE is 1.44 mSv. On average, a U.S. resident receives an annual radiation exposure from natural sources of about 3.6 mSv. The 1.44 mSv EDE is equivalent to 4.8 months or 146 days of natural radiation exposure. The calculated corresponding BEIR VII risk estimate may range from about one in 48,906 (0.002%) to about one in 20,469 (0.005%).This is considered minimal risk.

9 mCi ^{11}C-nicotine Dosage

If necessary, the ^{11}C-nicotine dosage may be raised to 9 mCi. Only a fraction of this dosage will be inhaled by the participant. From this dosage, participants will receive a minimal radiation exposure of 1.90 mSv.

The attenuation CT will add 0.8 mSv EDE to the overall dose exposure, so the combined and total EDE is 2.70 mSv for the 9 mCi ^{11}C-nicotine dosage. The 2.70 mSv EDE is equivalent to 9 months or 274 days of natural radiation exposure. The calculated corresponding BEIR VII risk estimate may range from about one in 11,575 (0.0086%) to about one in 4,845 (0.021%).

For both ^{11}C-nicotine dosage levels, we can compare this possible extra cancer risk to other risks (over a lifetime) that everyone is subject to in everyday life. For example, the chances of a person dying of cancer with no extra radiation exposure are about one in 4. The chances of dying in a car crash are about one in 82, and the chances of being killed by a car while crossing the street are about one in 730.

Participants who only complete the dry run portion of the study will have no radiation exposure or risk.

Adverse Reactions

Overall, there is minimal risk for serious adverse reactions as a consequence of enrolling in this study. There will be no IV injections. The ^{11}C-nicotine will be inhaled as part of the e-cig vaping process. Adverse reactions will be assessed and reported as required.

Inconvenience

The subjects may experience inconvenience due to the study procedures. Inconvenience will be addressed through monetary compensation of participants.

Benefits

There will be no direct benefits to the subjects for participating in the study; however, this study will further scientific knowledge about the health risks of e-cigarettes and the sites of nicotine deposition following e-cigarette use.

INTERNAL VALIDITY

Image data will be evaluated at a minimum following standard published procedures. This includes but is not limited to visual evaluation of uptake, normal tissue, and imaging artifacts, semi-quantitative analysis, blinded reader analysis, and using various methods for image post-processing. This should ensure internal and external validity of the data and avoid study bias. Functional and molecular imaging read outs will be developed according to established methodologies.

Managing and verifying the internal validity is an important task in this exploratory research program in order to develop the appropriate methodology to be validated in prospective clinical trials.

As there is an opportunity that the staff member administering the facility experience can potentially bias the subject, we will perform training sessions with the staff prior to them administering the facility experience. We will also perform from time to time an observational assessment by a study member participating in the subject facility experience with the task to observe the team member administering the facility experience in order to ensure that no bias or systematic errors occur.

In this exploratory study, we will have to constantly assess the potential sources of systematic errors or bias in order to ensure that we may derive conclusions that warrant generalization to other contexts.

We will assess the different factors impacting internal validity of the data sets we generate. For the specific factors we will address the following considerations. The following text uses material presented at https://en.wikipedia.org/wiki/Internal_validity and is hereby specifically acknowledged.

Temporal Precedence

Potential lack of clarity how one experience may influence a subsequent experience as that might be a cause and effect relationship.

Confounding

A major threat to the validity of causal inferences is confounding. Observations in one variable may relate to another manipulated variable. Where spurious relationships cannot be ruled out, hypothesis would have to be appropriately developed.

Selection Bias

Selection bias refers to the problem that, at pre-test, differences between groups exist that may interact with the independent variable and thus be 'responsible' for the observed outcome. Researchers and participants bring to the experiment a myriad of characteristics, some learned and others inherent. For example, sex, weight, hair, eye, and skin color, personality, mental capabilities, and physical abilities, but also attitudes like motivation or willingness to participate.

During the selection step of the research study, if an unequal number of test subjects have similar subject-related variables there is a threat to the internal validity. If subjects in two groups to be compared are not alike with regard to the independent variable, but similar in one or more of the subject-related variables, it may jeopardize the internal validity.

Self-selection to participate in this research can have a negative effect on the interpretive power of the dependent variable, this is especially known for online surveys where individuals of specific demographics opt into the test at higher rates than other demographics.

History

Events outside of the study/experiment or between repeated measures of the dependent variable may affect participants' responses to experimental experiences. Often, these are large scale events (natural disaster, political change, etc.) that affect participants' attitudes and behaviors such that it becomes impossible to determine whether any change on the dependent measures is due to the independent variable, or the historical event.

Maturation

Subjects may change during the course of the experiment or even between measurements. Both permanent changes, such as physical growth and temporary ones like fatigue, provide "natural" alternative explanations; thus, they may change the way a subject would react to the independent variable. So upon completion of the study, the researcher may not be able to determine if the cause of the discrepancy is due to time or the independent variable.

Repeated Testing

Repeatedly measuring the participants may lead to bias. Participants may remember the answers or may be conditioned to know that they are being tested. Repeatedly taking (the same or similar) tests usually leads to score gains.

Instrument Change

The instrument used during the testing process can change the experiment, an aspect that we will managed via device quality control to the largest extent possible. This also refers to observers being more concentrated or primed, or having unconsciously changed the criteria they use to make judgments. This can also be an issue with self-report measures such as facility perceptions given at different times. In this case the impact may be mitigated through the use of retrospective pretesting. If any instrumentation changes occur, the internal validity of the main conclusion is affected.

Differential Attrition

This error occurs if inferences are made on the basis of only those participants that have participated from the start to the end. However, participants may have dropped out of the study before completion, and maybe even due to the study or experiment itself. If this attrition is

systematically related to any feature of the study, the administration of the independent variable, the instrumentation, or if dropping out leads to relevant bias between groups, a whole class of alternative explanations may be possible that account for the observed differences.

Selection-maturation Interaction

This occurs when the subject-related variables, color of hair, skin color, etc., and the time-related variables, age, physical size, etc., interact. If a discrepancy between the two groups occurs between the testing, the discrepancy may be due to the age differences in the age categories.

Experimenter Bias

Experimenter bias occurs when the individuals who are conducting an experiment inadvertently affect the outcome by non-consciously behaving in different ways to members of control and experimental groups. It is possible to eliminate the possibility of experimenter bias through the use of double blind study designs, in which the experimenter is not aware of the condition to which a participant belongs.