VERSION NUMBER	VERSION DATE	REASON FOR CHANGE (high-level)
V1	*October 3, 2022*	*Initial Submission*
V2	*November 16, 2022*	*Requested revisions; updated to template v12*
V3	*December 22, 2022*	*Requested revisions per DCP concurrence review 12/5/22*
V4	*February 5, 2023*	*Request revisions per DCP concurrence review 1/9/23*
V5	*March 21, 2023*	*Requested revisions per DCP concurrence review 2/21/23; added option of remote consent*
V6	*April 28, 2023*	*Requested revisions per DCP concurrence review 4/14/23 incl. removing Appendix E/F to be stand alone documents.*
V7	*May 31, 2023*	*Requested revisions per DCP concurrence review 5/15/23 incl revision of table of contents, clarification of 24-hr urine collection on the day of flashover training, and addition 24-hr urine questionnaire to Appendix E.*
V8	*August 14, 2023*	*Updates per CIRB review 7/27/23. General cleanup/formatting improvements.*
V8.1	*January 11, 2024*	*The entry and end-of-study brief physical exams have been removed. Firefighters undergo an extensive annual physical exam as part of their occupational duties, which includes fire training. Consequently, the removal of the brief physical exams does not present any issues regarding eligibility evaluation or participant; safety for an intervention lasting 7-10 days with*

		broccoli seed and sprout extract. Moreover, eliminating the physical exam will ease the scheduling challenges encountered during the enrollment of the initial cohort of participants; minor updates as requested per DCP consensus review dated 9/5/23; general cleanup;Added information re: new gear rollout at TFD; update
V8.2	*October 7, 2024*	*The first cohort of six participants completed the study under protocol versions 8.0 and 8.1. The urine samples were analyzed for levels of the benzene metabolite, SPMA (the primary study endpoint), at baseline and after flashover training. Under a different grant, we assessed SPMA levels after a different fire training setting (burn room training). The collective information suggested that measuring the benzene metabolite after either training fire is not a viable endpoint to evaluate the effects of BSSE on the detoxification of carcinogens due to the low and variable benzene exposure during the training fires. Additionally, we unblinded treatment assignment and found that the intervention received prior to the flashover training could not explain the low and*

		variable benzene exposure after the flashover training. *Based on the available information, we propose an alternative design to assess changes in urinary acetaminophen mercapturate after acetaminophen administration as a surrogate biomarker for the upregulation of glutathione conjugation, the primary pathway responsible for the formation of SPMA from benzene. Using acetaminophen metabolism as a surrogate for assessing upregulation of Phase II detoxification pathways has been studied previously.* *We also propose a longer, 12-week intervention period to allow for the evaluation of the effects of BSSE on epigenetic modifications, such as miRNA and DNA methylation, observed in incumbent firefighters. In addition, extending the intervention period provides the unique opportunity to explore the effects of BSSE on the detoxification of carcinogens, including benzene and polycyclic aromatic hydrocarbons (PAHs), to which firefighters are exposed during real world firefighting activities, such as actual fire and/or fuel leaks.*

V8.3	*January 22, 2025*	*Requested revisions per DCP concurrence review 11/5/24 for greater clarity for urine collection and questionnaires, change in LAO PI, and addition of Appendix F; updated template from v12 to v17.*
V8.4	*February 28, 2025*	*Updates per DCP consensus review.*
V8.4	*May 6, 2025*	*Updates per CIRB review; updated version date per CIRB review.*
V8.5	*November 12, 2025*	• *The protocol was updated to DCP Version 18 (April 10, 2025)* • *Due to the stringency of fire fighter schedule we needed to adjust visit schedules. This includes expanding allowable visit windows and timing of urine collections to be considered evaluable for the study endpoints.* • *Clarified need to obtain end of study measures while participant on study agent.* • *Clarified medical history to require month and year and allow use of 15th of the month for data collection if participant cannot recall the exact day of their annual physical.* • *Replaced post fire/fuel questionnaire with new abbreviated participant facing questionnaire (appendix F)* • *Added non-participant facing incident report for data collection (appendix H)* • *General protocol cleanup*

COVER PAGE

DCP Protocol #: *UAZ22-11-01*
Local Protocol #: *STUDY00003544*

Phase II Randomized, Double-Blind, Placebo-Controlled Trial of Broccoli Seed and Sprout Extract (BSSE) to Evaluate Detoxification of Carcinogens in Firefighters

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SCHEMA

Phase II Randomized, Double-Blind, Placebo-Controlled Trial of Broccoli Seed and Sprout Extract (BSSE) to Evaluate Detoxification of Carcinogens in Firefighters

Study Population: Healthy, non-smoking incumbent firefighters

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Visit 1/Screening: Informed consent, clinical evaluation (medical history including date (month and year required, if date cannot be recalled 15th of month will be used) of last physical exam, height/weight, vital signs), baseline symptoms, blood draw for clinical labs and for research tests, concomitant medications (conmeds) and supplement use, Karnofsky performance status, baseline tobacco/alcohol and COVID assessment, pregnancy test (if applicable). Participants using any supplements containing active compounds in cruciferous vegetables must cease taking these supplements at least 14 days prior to Baseline Visit. Participants must refrain from using non-study acetaminophen (or acetaminophen containing products) for 72 hours before the Baseline Visit.

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Randomization: 66 participants will be randomized 1:1 to the following intervention:
BSSE OR matched placebo

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Visit 2/Baseline (can occur up to 12 weeks from screening visit**):** Vital signs, conmeds, pregnancy test (if applicable), any change in health status since visit 1 screen (if change reported, participant will be re-evaluated for eligibility as clinically indicated), a single dose of acetaminophen administration (2 x 500 mg), urine collection before acetaminophen dosing and for 8 (-1 and + 2) hours post acetaminophen dosing. Pre-Acetaminophen Spot Urine Questionnaire will be completed. Staff will provide urine collection supplies for the 8-hr urine collection, an Adverse Event (Symptom)/New Medicaton diary and Intake Calendar including instructions for use of study agent (BSSE). The 8-hr urine will be collected by the Tucson Fire Department (TFD) research liaisons or our study staff within 24 hours of completion. Staff will dispense study agent, and urine collection supplies for Post-Fire Spot Urine Questionnaire if fire/fuel leak activity occurs during the intervention period.

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Study Agent Intervention Period: Participants take BSSE or matched placebo 4 tablets once daily for 12 weeks based on randomization assignment. The intervention period allows a window of −14 days to +7 days. Participants to collect a spot urine within 8 ± 2 hours post each fire/fuel leak activity and complete Post-Fire/Fuel Leak Spot Urine Questionnaire. The Spot Urine and Post-Fire/Fuel Leak Spot Urine Questionnaires will be collected by the TFD research liaisons or study staff within 24 hours of completion. Interim phone/email contact at week 1 (+7 days), 4±7 days, 8±7 days to assess adverse events (AEs), study agent compliance, and adequate completion of Adverse Event (Symptom)/New Medication Diary and Intake Calendar

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Visit 3/End-of-Intervention: Occurs at 12 weeks with an allowable window of −14 to +7 days while the participant remains active on intervention.**):** Weight, vital signs, assess conmeds, collect study agent, review adverse events (AEs), collect Intake Calendar and Adverse Event (Symptom)/New Medication Diary, assess agent compliance, blood draw for clinical labs and research tests, Karnofsky Performance Status, follow-up tobacco/alcohol and COVID assessments, a single dose of acetaminophen administration (2 x 500 mg), spot urine collection before acetaminophen dosing. Pre-acetaminophen Spot Urine Questionnaire will be completed. Staff will provide urine collection supplies for the 8 (-1 and +2) hours post-acetaminophen urine collection. The 8-hr cumulative urine will be collected by the TFD research liaisons or our study staff within 24 hours of completion.

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Follow-up: Telephone or email assessment of AEs 2 weeks ±7 days post intervention

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Endpoints:

Primary Endpoint: Change in urinary excretion of acetaminophen mercapturate after 12 weeks of study agent intervention, assessed within the allowable window of 14 days before to 7 days after the 12-week time point.

Secondary Endpoints: Change in urinary excretion of acetaminophen glucuronide after 12 weeks of study agent intervention within the allowable window of 14 days before to 7 days after the 12-week time point, and assessment of the safety and tolerability of BSSE.

Exploratory Endpoints: Glutathione S-Transferase Mu 1 (GSTM1) and Glutathione S-Transferase Theta 1 (GSTT1) genotypes and interaction with detoxification; urinary metabolomics; urinary excretion of mercapturic acid of benzene after fire/fuel leak activities; urinary excretion of metabolites of polycyclic aromatic hydrocarbons (PAHs) after fire/fuel leak activities; epigenetic modifications (microRNA and DNA methylation)

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1. OBJECTIVES

This is a phase II, randomized, double-blind, placebo-controlled trial to determine if BSSE intervention can upregulate Phase II detoxification pathways in firefighters.

1.1 Primary Objective

- To determine whether BSSE increases the urinary excretion of mercapturic acid of acetaminophen, a surrogate for detoxification of the carcinogen benzene in healthy, incumbent firefighters.

1.2 Secondary Objectives

- To evaluate the effects of BSSE on urinary excretion of acetaminophen glucuronide, a surrogate for detoxification of polycyclic aromatic hydrocarbons (PAHs).
- To evaluate the safety and tolerability of BSSE.

1.3 Exploratory Objectives

- To determine whether the *GSTM1* and *GSTT1* genotypes are important genetic modulators of BSSE-induced detoxification of carcinogens.
- To evaluate the effects of BSSE on urinary metabolomics.
- To determine the effects of BSSE on the urinary excretion of the mercapturic acid of benzene after fire/fuel leak activities.
- To determine the effects of BSSE on the urinary excretion of metabolites of PAHs after fire/fuel leak activities.
- To determine the effects of BSSE on epigenetic modifications (blood microRNA and DNA methylation).

2. BACKGROUND

2.1 Cancer Risk Among Firefighters

There are approximately 1.06 million active firefighters in the United States[1]. Firefighters are routinely exposed to carcinogens during the course of their daily duties particularly from smoke exposure arising from active fire rescue, structural or incidental firefighting or combustion[2]. There is an expansive number of toxic components prevalent in smoke. Various elements including soot, polycyclic aromatic hydrocarbons (PAH), gases, and volatile organic compounds (VOCs) among others are exposures that firefighters encounter after close proximity to flames and after changing their protective equipment[3]. All fires release toxic and carcinogenic substances including benzene, 1,3-butadiene, and formaldehyde[4]. Additionally, newer building materials and furnishings that combust and pyrolyze result in significantly more toxicants from smoke than wildland fires or fires from buildings predating newer construction materials. Burgess et al. estimated the time firefighters spent in structural fires in two cities in Arizona including Tucson and Phoenix; total firefighter activity was 47.6 hours/year and 50 hours/year in Phoenix and Tucson, respectively, displaying significant exposure to potential carcinogens[5]. Wood-fueled fires remains a leading hazard that firefighters encounter globally. Wood smoke emits numerous compounds including PAHs and methylphenols (MP)[6]. These compounds, many of which are known carcinogens, increase the risk of cancer in firefighters.

Several studies to date have demonstrated that firefighters are at an increased risk of developing various malignancies including melanoma, multiple myeloma, acute myeloid leukemia, prostate, kidney, brain, and respiratory tract cancers among others[2, 7, 8]. An extensive pooled cohort study of approximately 30,000 United

States firefighters from San Francisco, Chicago, and Philadelphia from 1950-2009 showed excess cancer mortality and incidence predominantly in the digestive (including colon) and respiratory tracts as well as the kidneys in male firefighters, and increased bladder cancer among women firefighters. This was the first study to also report an elevated mortality and incidence of malignant mesothelioma among US firefighters[9]. A study of 34,796 male and 2,017 female career firefighters in Florida from 1981 to 1999 demonstrated an increased risk of bladder, testicular, and thyroid cancers in male firefighters and cervical and thyroid cancers in female firefighters, as well as Hodgkin disease[10]. The Florida Firefighter Cancer Registry evaluated 109,009 career firefighters from 1981-2014 and reported an increased risk of thyroid, prostate, testicular, and melanoma cancers in male firefighters younger than age 50 while female firefighters were noted to have an increased risk of thyroid and brain cancer[11].

While several studies have established carcinogen exposure with an increased risk of malignancy among firefighters, there remains limited data pertaining to the cellular mechanistic drive. Epigenetic modifications including DNA methylation (DNAm), histone modification, and dysregulated microRNA (miRNA) expression are established in carcinogenesis with regulation of oncogenes and tumor suppressor genes. Studies of Tucson firefighters have demonstrated epigenetic modification associated with occupational exposures[12]. Jeong et al. examined blood from 52 incumbent and 45 new recruit nonsmoking firefighters for miRNA expression and found at least a 1.5-fold significant difference between groups with 9 microRNA identified[13]. Among incumbent firefighters, two of the three microRNAs with increased expression had activities in line with cancer promotion, and six of the miRNA with decreased expression had activities in line with tumor suppression. Similarly, Jung et al performed a longitudinal evaluation of whole blood miRNA expression in 52 new recruits prior to live-fire training and 20-37 months later taking into account cumulative fire-hours, fire-runs and time since most recent fire[14]. The study identified an increase in oncogenic miRNA and decrease in tumor suppressive miRNA with exposure. DNAm has also been shown as a cellular mechanism by which cancer risk is increased among firefighters. Zhou, et al. obtained peripheral blood samples from 45 incumbent and 41 new recruits non-smoking male firefighters which were subsequently analyzed for DNAm[15]. Genome-wide methylation was able to identify accurately the incumbent from new recruits as well as by the years of service among incumbents. The Sirtuin signaling pathway, p53 signaling, and 5' AMP-activated protein kinase (AMPK) signaling were identified as potential mechanisms associated with increased cancer risk among firefighters. Beyond epigenetic changes, genotoxicity has been reported in Danish firefighters[16].

Several studies have utilized the training fire setting to evaluate the potential health hazard from smoke exposure in firefighters. An Australian training facility evaluated human resources records and self-reported data in a retrospective cohort and showed that individuals in the high probability of exposure group had an increased risk of overall cancers, testicular cancer, and melanoma relative to the general population[17]. Fire departments in Ontario where firefighter training exercises took place in burn houses examined air samples and skin and urine specimens of 28 firefighters prior to and after training fire exposure[6]. With wood as the primary fuel source employed in the training exercise, MP and PAH metabolites were measured. The study noted that air samples demonstrated 5 times greater concentration of MP than PAH whereas skin wipe samples from multiple body sites showed evidence of whole-body smoke exposure. Urine samples displayed several MPs and deconjugated PAH metabolites including hydroxynaphthalene, hydroxyfluorene, and hydroxyphenanathrene, among others. Despite large between-subject variations in operational roles, the creatinine-normalized levels of these markers remained significantly elevated. Recently, Rosting et al measured urine and air benzene and toluene concentrations pre- and post- fire drill in 9 firefighters. The study detected the benzene metabolite, s-phenylmercapturic acid (SPMA) and toluene metabolite s-benzylmercapturic acid (SBMA) in all urine samples 3 hours after the drill while benzene and toluene were also detected in air samples[18]. Structural fire training has been shown to contribute to

firefighters' select chemical exposure as shown by urine analyzed for PAH metabolites and breath analyzed for VOCs including benzene[8]. The study found an increase in nearly all urinary PAH metabolites from pre- to 3-hour post training. Instructors that supervised three trainings per day also had an increased concentration at each collection with a 30-fold increase in 1-hydroxypyrene. Moreover, breath concentrations of benzene also increased 2- to 7-fold immediately following training.

With increasing awareness of the occupational exposure to hazardous chemicals during firefighting, advances in personal protective equipment (PPE) with the addition of particulate-blocking hoods as well as fire department protocols to include routine laundering of PPE after fire exposure are being developed[19]. Mayer et al. studied three PPE ensembles including a new knit hood, turnout jacket and pants, new particulate-blocking hood, turnout jacket and pants, and laundered particulate-blocking hood, with laundered turnout jacket and pants. Pre- and post-fire training exposure exhaled breath samples and personal air sampling outside and inside the turnout jacket was collected in 24 firefighters to measure exposure to VOCs and naphthalene. The study found that benzene, toluene, and naphthalene concentrations inside the turnout jackets were similar to that of outside the turnout jacket concentrations. Additionally, benzene concentrations in breath samples were significantly elevated from pre- to post-fire training irrespective of PPE ensemble. This highlights the importance of developing alternative strategies for risk reduction.

The commonly exposed chemicals such as benzene and PAHs are activated by Phase I metabolism to reactive metabolites and are detoxified through Phase II metabolism[20]. Cytochrome P450-dependent oxidation of benzene produces benzene oxide which is further either excreted as spontaneously rearranged phenol or further metabolized to hydroquinone and other metabolites[21]. On a cellular level, benzene oxide disrupts cellular function by reacting with peptides and proteins[21, 22] Benzene oxide can be detoxified by glutathione S transferases (GST) to produce (SPMA). Similarly, the carcinogenic effect of PAH is dependent on oxidative metabolism with production of dihydrodiol epoxide metabolites and PAH quinones resulting in DNA damage leading to mutations in oncogenes and tumor suppressor genes[23]. PAH quinones and hydroxylated PAHs also modulate cell proliferation, DNA damage response via mitogen-activated protein kinases, Akt kinase, and calcium-dependent signaling pathways[24]. Glucuronidation of these active PAH metabolites represents a detoxification pathway of PAHs. Enhancing the detoxification of the commonly exposed chemicals is an arena that warrants further investigation.

2.2 Broccoli Seed and Sprout Products

Broccoli extract potently induces phase II detoxification enzymes[25] including GST, aldehyde dehydrogenase (ALDH), and NAD(P)H:quinone oxidoreductase (NQ01) which mitigate the effects of environmental carcinogens including benzene, aldehydes, and PAHs by enhancing their detoxification [26]. More than 80% of inducer activity is driven by the phytochemical sulforaphane (SF) which is produced when its precursor glucoraphanin (GR) is hydrolyzed by myrosinase during food preparation or chewing [27]. Mechanistically, SF disrupts the polyubiquitination of the NRF2 transcription factor mediated by its inhibitory protein, KEAP1. This frees NRF2 to translocate to the nucleus and bind to antioxidant response elements (AREs) in the promoter regions of target genes, inducing their transcription [28, 29]. As GR is 20-50 times more concentrated in broccoli seeds relative to mature plants [27, 30], broccoli seed products (BSPs) were developed as a chemopreventive agent against carcinogen-induced cancers, and the safety and pharmacokinetics (PK) of various BSPs in humans has been established [29, 31, 32].

Proof-of-concept clinical trials in healthy volunteers have shown that BSPs rich in GR and/or SF are well-tolerated and promote rapid, sustained detoxification of the airborne pollutants acrolein and benzene [29, 31, 33]. In preclinical studies, SF has exhibited chemopreventive activity against carcinogen-induced stomach, skin, and breast cancers [34, 35]. Studies in *Nrf2*$^{-/-}$ mice have shown that the chemopreventive effect of SF against benzo[a]pyrene-induced gastric cancer and 7,12-dimethylbenz(*a*)anthracene (DMBA)-induced skin cancer depends on the NRF2 signaling pathway [34]. The relevance of NRF2 to oral cancer chemoprevention is highlighted by the enhanced susceptibility of *Nrf2*$^{-/-}$ mice to oral cancer induced by the carcinogen 4-nitroquinoline-1-oxide (4NQO), and the reduced susceptibility of *Keap1*$^{-/-}$ mice[36]. We have shown that SF reduces both the incidence and total burden of oral tumors in 4NQO-treated mice[32].

Avmacol (Broccoli Seed and Sprout Extract): The regular and extra strength Avmacol products (Avmacol and Avmacol ES, manufactured under GMP standards by Nutramax Laboratories, Inc.) are commercially available dietary supplements that contain GR plus the fully active enzyme myrosinase, thus yielding a higher and more consistent dose of SF upon ingestion. The regular Avmacol tablets contained GR-rich broccoli seed extract, freeze-dried broccoli sprouts for the myrosinase source, and the inert excipients required to form a tablet. Avmacol ES is the current, commercially available BSSE. Each tablet contains double the quantity of GR and myrosinase activity as the regular formulation. In addition, Avmacol ES contains extract of maitake mushroom, hypothesized to augment innate immunity[37].

2.3 Rationale

We have completed a series of healthy volunteer studies to confirm the bioavailability of SF when delivered as Avmacol, hereafter referred to as broccoli seed and sprout extract (BSSE) with results summarized below.

- NCT02800265. In this pilot study, 10 healthy volunteers were treated with the regular strength BSSE, 8 tablets daily for 3 days. This represented an exposure of 100 mg GR per day (SF equivalent of 140 µmol per day if 100% converted). Overnight urine (12 hours) was collected at baseline and after the third dose then evaluated by liquid chromatography tandem mass spectrometry for SF and its glutathione conjugates, SF-cysteine, and SF-N-acetylcysteine. Urinary excretion of SF and its metabolites was negligible for all participants at baseline. Relative to multiple historical studies administering GR, where mean bioavailability as SF metabolites was 5% over 24 hours, mean bioavailability as SF metabolites for BSSE was 20% over 12 hours (Bauman, unpublished data). In prior studies of GR, 60% of the bioavailable dose of GR is excreted in the urine as SF metabolites during the first 12 hours, and 40% in the subsequent 12 hours. The estimated total mean bioavailability of BSSE was therefore estimated at 35%. This hypothesis was generally confirmed in a pure bioavailability study conducted by collaborators at Johns Hopkins, where urine was collected for 24 hours following a single dose of BSSE. In this study, total excretion of SF metabolites was 34.3% of dose (SD 9.7%) in the pilot phase and 32.8% of dose in the proton pump inhibitor phase[38].
- NCT03182959. In a recently completed pilot study, 11 subjects who had completed curative-intent treatment for tobacco-related head and neck squamous cell cancer were treated with two doses of BSSE in a randomized, crossover design: 4 tablets and 8 tablets for 4 weeks each, separated by a 4-week washout. The primary endpoint is NRF2 upregulation in buccal cells as measured by upregulation of mRNA of the target gene *NQO1* and secondary endpoints include bioavailability of BSSE as SF metabolites and modulation of mucosal signatures of inflammation and immunity (NanoString nCounter® PanCancer panel). Analyses of bioavailability and mucosal bioactivity are ongoing.

- NCT03402230. We recently published the results of this randomized, crossover trial evaluating the detoxification of benzene and other tobacco carcinogens by BSSE in otherwise healthy current smokers (≥ 20 pack-years) [33, 39]. Each participant was treated for 2 weeks with both low and higher-dose BSSE (148 µmol vs. 296 µmol of GR daily), separated by a 2-week washout, with randomization to low-high vs. high-low sequence.

Table 1. Urinary excretion of carcinogen metabolites in NCT03402230

Carcinogen Metabolite	BSSE Dose Level	Pre (95% CI) pmol/mgCr	Post (95% CI) pmol/mgCr	Post/Pre (95% CI)	*p*-Value
Benzene (SPMA)	Low	7.8 (6.3, 9.6)	9.0 (7.2, 11.3)	1.2 (1.0, 1.3)	**0.05**
	High	7.6 (6.2, 9.4)	9.1 (7.3, 11.3)	1.2 (1.0, 1.4)	**0.04**
Acrolein (3-HPMA)	Low	9934.5 (8008.0, 12,324.4)	11,032.1 (9161.1, 13,285.2)	1.1 (1.0, 1.3)	**0.11**
	High	9750.0 (7903.7, 12,027.6)	12,450.1 (10,658.8, 14,542.5)	1.3 (1.1, 1.5)	**<0.01**
Croton-aldehyde (3-HMPMA)	Low	10,962.6 (9026.8, 13,313.6)	11,482.0 (9677.5, 13,622.9)	1.1 (0.9, 1.2)	**0.56**
	High	**10,808.6 (8947.3, 13,057.0)**	**12,795.1 (10,922.9, 14,988.1)**	**1.2 (1.02,1.37)**	**0.02**

The primary endpoint of NCT03402230 was detoxification of benzene, measured by urinary excretion of its mercapturic acid, SPMA. Secondary endpoints included bioavailability, detoxification of acrolein and crotonaldehyde, modulation by GST genotype, and toxicity. Forty-nine participants enrolled, including 26 (53%) females with median use of 20 cigarettes/day. Low and higher-dose BSSE showed a mean bioavailability of 11% and 10%, respectively. As shown in Table 1 above, higher-dose BSSE significantly upregulated urinary excretion of the mercapturic acids of benzene ($p = 0.04$), acrolein ($p < 0.01$), and crotonaldehyde ($p = 0.02$), independent of GST genotype. Low dose BSSE also significantly upregulated urinary excretion of SPMA ($p = 0.05$).

We have completed pilot studies to assess the metabolomic changes in firefighters pre- and post- fire. Several urinary metabolites that are associated with cancer risk or are known carcinogens were identified in the post-fire urine[58]. We plan to explore the modulatory effects of BSSE on metabolomic changes. To facilitate the metabolomic analysis, we are collecting additional information on beverage consumption history, over-the-counter and prescription medication use, consumption of supplements/vitamins, pre-workouts and protein powders/shakes, blood and/or plasma donation history and turnout gear fit, which can impact the abundance of various metabolites.

Our overall hypothesis is that BSSE intervention can lead to enhanced detoxification of the commonly exposed carcinogens in firefighters, which could ultimately reduce their cancer risk. We propose a phase II, randomized,

double-blind, placebo-controlled trial to evaluate if BSSE intervention can enhance Phase II detoxification pathways in firefighters. The trial was initially designed to determine the effects of BSSE on detoxification of carcinogens in the flashover training setting because this training activity provides a controlled exposure environment. Commonly exposed chemicals such as benzene and PAHs were consistently detected in firefighters' urine collected following the training fire[9,27].

The first cohort of six participants completed the study under protocol versions 8.0 and 8.1. The urine samples were analyzed for levels of the benzene metabolite, SPMA (the primary study endpoint). Contrary to expectations, SPMA levels were not consistently elevated in post-flashover samples compared to baseline levels (see Table 2). It is likely that exposure to benzene during flashover is minimal.

Table 2. Total amount of SPMA excreted over 24 hours (pmol).

Participant ID – intervention prior to flashover training	Baseline	Post flashover training	Post burn room training[1]
315 - placebo	848.25	1660.27	385.33
316 – BSSE	776.54	1089.17	2374.18
317- BSSE	1082.33	650.58	767.52
318 - placebo	342.52	605.96	310.36
319 - BSSE	755.12	650.83	503.92
320 - placebo	794.85	724.70	Missed collection

[1] no intervention prior to burn room training

Based on these preliminary data, the study was temporarily closed to accrual to determine if any changes were needed in the study design and to optimize the study design if necessary. During the temporary closure, we unblinded the treatment assignment of this first cohort of six participants and found that the inconsistent elevation of benzene metabolite levels post-flashover could not be explained by the intervention (BSSE vs. placebo) received prior to flashover (see **Table 2**).

We also worked in parallel through research collaboration to assess benzene exposure during another type of firefighter training (burn room training) and found that the SPMA levels were also not consistently elevated after the burn room training fire compared to baseline levels (see **Table 2**). The collective information suggests that exposure to benzene during the current fire training protocols is minimal, possibly due to procedures implemented by the fire department to minimize carcinogen exposure during training fires. These preliminary data suggest that measuring the benzene metabolite after the training fire is not a viable endpoint to evaluate the effects of BSSE on the detoxification of carcinogens due to the low and variable benzene exposure during the training fire.

Based on the available information, we selected to assess the intervention effects on Phase II detoxification pathways by assessing changes in acetaminophen metabolism as a surrogate for detoxification of benzene and PAHs. Metabolism of acetaminophen occurs through three detoxification pathways: glucuronidation, sulfation, and glutathione conjugation. This makes acetaminophen metabolism a good model to study the detoxification pathways involved in protection against exposure to a wide range of exogenous and endogenous toxins. Assessing acetaminophen metabolism has been used previously as a relatively non-invasive approach to determine the effects of Spanish black radish, a cruciferous vegetable, on Phase II detoxification pathways[40]. Changes in urinary acetaminophen mercapturate after acetaminophen administration will be used as a surrogate biomarker for the upregulation of glutathione conjugation, the primary pathway responsible for the detoxification of benzene to

SPMA. Changes in urinary acetaminophen glucuronide after acetaminophen administration will be used as a surrogate biomarker for the upregulation of glucuronidation, the primary pathway responsible for the detoxification of PAHs.

We also propose a longer, 10-week intervention period to assess the modulatory effects of BSSE on epigenetic modifications, including dysregulated miRNA and DNA methylation, observed in incumbent firefighters. The longer intervention period provides the unique opportunity to explore the effects of BSSE on the detoxification of carcinogens, including benzene and PAHs, which firefighters are exposed to during real world firefighting activities, such as actual fire and/or fuel leaks. Because of the unpredictability of occurrence of the real world fire and/or fuel leaks and uncontrolled exposure in actual fire and fuel leaks, we have moved the assessment of the detoxification of benzene and PAHs to exploratory objectives. For these exploratory endpoints, we plan to collect documentation of fire/fuel leak activities throughout the intervention period and adjust for these potential confounders.

Increasing evidence suggests that tobacco and alcohol use are risk factors in the development of intraepithelial neoplasia and cancer. In addition, tobacco and alcohol use may adversely affect agent intervention, for example by altering the safety profile or metabolism of a drug. Standardized assessments of tobacco and alcohol use during clinical trials will aid in understanding the potential relationship between the use of these products and clinical endpoints or cancer prevention biomarkers. Therefore, NCI, DCP is including assessment of tobacco and alcohol use at baseline and end of treatment, to determine the potential impact of tobacco and alcohol use on 1) treatment toxicity and symptom burden, and 2) the efficacy of treatment intervention.

The NCI, DCP Assessment of COVID-19 exposure and vaccine status at baseline and end of study will be used to determine the potential impact on 1) treatment toxicity and symptom burden, and 2) the efficacy of treatment intervention.

3. SUMMARY OF STUDY PLAN

Study Design: This is a phase II, randomized, double-blinded, placebo-controlled study to evaluate whether BSSE can upregulate Phase II detoxification pathways in firefighters. We plan to randomize a total of 66 participants (1:1 randomization) to receive BSSE or matched placebo for 12-weeks intervention, with an allowable window of 14 days before or 7 days after the scheduled 12-week time point. Based on an estimated attrition rate of < 10%, we expect to have a minimum of 60 evaluable participants (a minimum of 30 participants per intervention assignment).

Treatment Plan and Primary Assessments: After consent and confirmation of eligibility, participants will be randomized to BSSE or placebo in a double-blinded fashion. During their baseline visit, all participants will be asked to provide a spot urine sample and to participate in a short single-dose acetaminophen study with urine collection to assess their baseline metabolism of acetaminophen. Prior to starting, participants will be asked to complete a Pre-Acetaminophen Spot Urine Questionnaire (Appendix E) and to provide a spot urine. Afterward, they will be asked to take two 500 mg acetaminophen tablets (i.e., a single extra strength dose of Tylenol) and collect all urine over the next 8 (-1 and + 2) hours (i.e., cumulative urine collection). After completing the pre and post-acetaminophen urine study, participants will take four tablets BSSE (Avmacol ES) or placebo daily for a 12-week intervention period, with an allowable window of 14 days before to 7 days after the scheduled 12-week time point.

To assess the activity of BSSE to detoxify real-world exposures to carcinogens benzene and PAHs, during the intervention period, all participants will be asked to collect a spot urine within 8 ± 2 hours of their post-fire/fuel leak activity and to complete a Post-Fire/Fuel Leak Spot Urine Questionnaire (Appendix F). This post-fire/fuel leak spot urine and the Post-Fire/Fuel Leak Spot Urine Questionnaire (Appendix F) will be collected by the Tucson Fire Department (TFD) research liaisons or study staff within 24 hours of completion.

At the end of intervention, participants will repeat the acetaminophen metabolism study. Participants must complete the end-of-intervention assessment prior to stopping the study agent to ensure that the acute effects of BSSE on acetaminophen metabolism can be accurately evaluated. During their end-of-study visit, all participants will again be asked to provide a spot urine sample and complete the Pre-Acetaminophen Spot Urine Questionnaire (Appendix E) before being instructed to take two 500 mg acetaminophen tablets and collect all urine over the next 8 (-1 and + 2) hours. With the baseline data, the end-of-intervention acetaminophen study will be used to assess the change in the metabolism of acetaminophen with intervention. Participants will discontinue the study agent only after completing their end-of-intervention pre- and post-acetaminophen urine collections.

Additional Study Assessments: Blood collection will occur at screening and on the last visit for safety labs and assessment of epigenetic modifications. Blood collected at screening will be used for GST genotyping. Since up to 12 weeks may occur between Visit 1 (screening) and Visit 2 (baseline), participants will be asked at Visit 2 about any changes in their health status since screening. If a change is reported, the participant will be re-evaluated for eligibility as clinically indicated, including repeat clinical laboratory testing as needed. Participants will be contacted by telephone or email (per their telecommunications preference) at 1 week + 7 days, 4 weeks ± 7 days and 8 weeks ± 7 days for adverse events (AEs), adherence to the study agent, and assessment of adequate completion of Adverse Event (Symptom)/New Medication Diary and Intake Calendar. Intake Calendar and Adverse Event (Symptom)/New Medication Diary will be collected during the end of intervention visit. Participants will be contacted by telephone or email (per their telecommunications preference) 2 weeks ± 7 days after the end of the treatment period for AE assessment.

4. PARTICIPANT SELECTION

The Protocol Risk for Auditing for this study is low.

High Risk studies require 100% review and confirmation of eligibility of **all participants** by the LAO **prior to enrollment**

Low and Intermediate risk studies require confirmation of eligibility of the first two participants enrolled at each accruing LAO or AO. This review should take place as soon as possible but not more than two weeks following enrollment/randomization. Continuing eligibility review can be determined based on the LAO assessment of AO performance and protocol complexity.

For those international sites where data sharing is prohibited, this policy does not apply.

4.1 Inclusion Criteria

4.1.1 Male or female incumbent firefighters who are current non-smokers.

4.1.2 Age ≥18 years.

4.1.3 Karnofsky performance scale ≥ 70% (see Appendix A).

4.1.4 Participants must have adequate and stable end organ and bone marrow function as defined below:

Absolute neutrophil count	≥ 1,000/microliter
Platelets	≥ 100,000/microliter
Total bilirubin	≤ 2 × institutional upper limit of normal (ULN)
AST (SGOT)/ALT (SGPT)	≤ 2 × ULN
Creatinine	≤ 1.5 × ULN

4.1.5 Participants on chronic suppressive antiviral therapy for herpes simplex virus (HSV) are eligible.

4.1.6 The effects of BSSE on the developing human fetus at the recommended therapeutic dose are unknown. For this reason, women of child-bearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation. Should a woman become pregnant or suspect she is pregnant while participating in this study, she should inform her study physician immediately.

4.1.7 Ability to understand and the willingness to sign a written informed consent document.

4.2 Exclusion Criteria

4.2.1 History of invasive cancer within the past 2 years, except for excised and cured non-melanoma skin cancer or carcinoma in situ of the cervix. Participants who continue adjuvant treatment for an index cancer occurring > 2 years ago, such as adjuvant hormonal therapy for breast cancer, are excluded. Participants who are on anti-neoplastic treatment for a chronic malignancy, such as multiple myeloma or chronic myelogenous leukemia, are excluded.

4.2.2 Chronic, current or recent (within the past 2 weeks) use of systemic steroid doses equivalent to prednisone > 5 mg daily for continued use > 14 days. Use of inhaled steroids, nasal sprays, and topical creams for small body areas (< 10% body surface area) is allowed.

4.2.3 Participants may not be receiving any other investigational agents.

4.2.4 History of allergic reactions attributed to compounds of similar chemical or biologic composition to Avmacol ES (BSSE).

4.2.5 Uncontrolled intercurrent illness including, but not limited to, serious ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, or cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.

4.2.6 Pregnant or lactating women. Pregnant women are excluded from this study because the effects of BSSE on the developing human fetus are unknown. Because there is an unknown but potential risk for AEs in nursing infants secondary to treatment of the mother with BSSE, breastfeeding should be discontinued if the mother is treated with BSSE.

4.2.7. Participants with known human immunodeficiency virus (HIV), chronic hepatitis B virus (HBV), or hepatitis C virus (HCV) infection. Participants with HIV, HBV and HCV are excluded from this study because there is no information regarding the impact of anti-viral drugs on the bioavailability of Avmacol ES. SF is known to modulate certain phase 1 and phase 2 enzymes involved in drug metabolism. The potential for SF to alter the metabolism (either by increasing or decreasing) of antiviral therapy could have an effect on the efficacy of the pharmaceuticals to keep viral titers low and the disease under control. Since many of the drugs used in therapies of these viral infections have extensive CYP450 enzymatic impact and BSSE has its own enzymatic properties, there is concern for drug-to-drug interactions.

4.2.8. Ongoing use of any supplements containing active compounds in cruciferous vegetables such as SF and GR. The use of supplements related to the study agent may confound the study endpoints. Participant will be eligible if they agree to stop the SF or GR product at least 14 days prior to the Baseline Visit.

4.2.9. History of allergic reactions to acetaminophen or the formulation ingredients or any other contraindication to acetaminophen use.

4.2.10 Unwilling or unable to refrain from the use of non-study acetaminophen (or acetmainphen containing products) for 72 hours prior to the baseline evaluation of acetaminophen metabolism and for 72 hours prior to the end-of-intervention evaluation of acetaminophen metabolism.

4.3 Participant Consideration Regarding Alcohol Consumption

There is no safety concern associated with moderate or even heavy alcohol consumption in this study, provided participants do not exhibit underlying liver dysfunction. All participants will undergo screening for liver function, and those with clinically significant abnormalities—defined as AST or ALT >2× the upper limit of normal (ULN)—will be excluded to ensure participant safety. Regular alcohol use will neither limit eligibility nor require restriction during the study, with the exception of a 24-hour abstinence period immediately before and after administration of the acetaminophen probe. This temporary restriction is necessary to minimize any short-term impact of alcohol on the metabolism readouts. Outside of this window, participants will be encouraged to maintain their usual alcohol consumption habits in order to evaluate the intervention under real-world conditions.

There is no concern that alcohol use will compromise the safety of participants or the integrity of the metabolic data collected to assess individual response to the intervention. While regular alcohol intake may affect steady-state metabolism and detoxification pathways, this variability is not considered a risk. Alcohol use will be systematically recorded and treated as a covariate in statistical analyses to assess whether it modifies the effect of the intervention.

4.4 Inclusion of Women and Minorities

Both men and women (as applicable) and members of all races and ethnic groups are eligible for this trial.

4.5 Recruitment

Study participants will be recruited from Tucson and surrounding areas. Dr. Burgess has worked with the Tucson firefighters for 30 years, and on cancer risks in firefighters since 2015. This has included a study of exposures and cancer risk in Tucson firefighters funded by Federal Emergency Management Agency which enrolled 525 participants, including 28 women and 55 Hispanic firefighters. The study team has been meeting with the long-

term partners of Dr. Burgess, including Health and Safety Captain, Mr. John Gulotta from the TFD to discuss the current proposal concept and trial design. Mr. Gulotta has been instrumental to the recruitment of firefighters to Dr. Burgess's prior study and has provided valuable input on the design of the proposed trial and is committed to support the recruitment of the proposed trial.

4.6 Planned Accrual

The planned enrollment tables below match the sex, race and ethnicity demographic data for firefighters within southern Arizona.

DOMESTIC PLANNED ENROLLMENT REPORT

Racial Categories	Not Hispanic or Latino: Female	Not Hispanic or Latino: Male	Hispanic or Latino: Female	Hispanic or Latino: Male	Total
American Indian/Alaska Native		2			2
Asian		3			3
Native Hawaiian or Other Pacific Islander					
Black or African American	1	5			6
White	5	37	1	10	53
More Than One Race				2	2
Total	6	47	1	12	66

5. REGISTRATION PROCEDURES

5.1 Investigator and Research Associate Registration with CTEP

Food and Drug Administration (FDA) regulations require IND sponsors to select qualified investigators. NCI policy requires all persons participating in any NCI-sponsored clinical trial to register and renew their registration annually which is done via the Registration and Credential Repository (RCR).

To register, all individuals must obtain a Cancer Therapy Evaluation Program (CTEP) Identity and Access Management (IAM) account (https://ctepcore.nci.nih.gov/iam). In addition, persons with a registration type of Investigator (IVR), Non-Physician Investigator (NPIVR), or Associate Plus (AP) (*i.e.*, clinical site staff requiring write access to Rave or acting as a primary site contact) must complete their annual registration using CTEP's web-based Registration and Credential Repository (RCR) (https://ctepcore.nci.nih.gov/rcr). Documentation requirements per registration type are outlined in the table below.

Documentation Required	IVR (Investigator)	NPIVR (Non-physician Investigator)	AP (Associate Plus)	A (Associate)	AB (Associate Basic)
FDA Form 1572	✔	✔			

Financial Disclosure Form	✔	✔	✔		
NCI Biosketch (education, training, employment, license, and certification)	✔	✔	✔		
GCP training	✔	✔	✔		
CV (optional)	✔	✔	✔		

An active CTEP-IAM user account and appropriate RCR registration is required to participate in all CP-CTNet clinical trials.

All personnel directly involved in the performance of procedures required by the protocol and the collection of data should be registered in the RCR.

Personnel associated with the five registration types include, but is not limited to, the following:

- **Investigator (IVR)** — MD, DO, or international equivalent
- **Non-Physician Investigator (NPIVR)** — advanced practice providers (e.g., NP or PA) or graduate level researchers (e.g., PhD)
- **Associate Plus (AP)** — clinical site staff (e.g., RN or CRA) with data entry access to RAVE. Also includes site administrator, data administrator, and consenting person. Individuals with an auditing role should register as an AP.
- **Associate (A)** — other clinical site staff involved in the conduct of NCI-sponsored trials.
- **Associate Basic (AB)** — individuals (e.g., pharmaceutical company employees) with limited access to NCI-supported systems

In addition, the site-protocol Principal Investigator (PI) must meet the following criterion:
- Active registration status
- The IRB number of the CIRB (IRB of record) listed on their Form FDA 1572

Additional information can be found on the CTEP website at https://ctep.cancer.gov/investigatorResources/default.htm. For questions, please contact the RCR ***Help Desk*** by email at RCRHelpDesk@nih.gov.

6. NCI CENTRAL INSTITUTIONAL REVIEW BOARD

The NIH policy on the Use of a Single Institutional Review Board for Multi-Site Research https://grants.nih.gov/grants/guide/notice-files/not-od-16-094.html became effective on January 25, 2018. In compliance with this policy, NCI Central IRB (NCI CIRB) is the sole IRB of record for all accruing sites conducting clinical trials through the CP-CTNet, all CP-CTNet U.S.-based sites must be members of the NCI CIRB, and utilize the Cancer Prevention and Control CIRB as their IRB of record. International sites should submit Research Ethics Board (REB) approval to the DCP Regulatory contractor following country-specific regulations.

Signatory Institutions must submit a Study Specific Worksheet (SSW) to the CIRB via IRBManager to indicate their intent to open the study locally. The CIRB's approval of the SSW is then communicated to the PIs at the Signatory Institution and the Regulatory Contractor. In order for the SSW approval to be processed, the Signatory Institution must inform which CIRB-approved institutions aligned with the Signatory Institution are participating in the study.

Should changes to the study become necessary, protocol amendments will be submitted to the DCP PIO according to DCP Amendment Guidelines. The DCP-approved amended protocol must be approved by the CIRB prior to implementation.

7. AGENT ADMINISTRATION

Intervention will be administered on an outpatient basis. Reported AEs and potential risks are described in Section 8.2.

7.1 Dose Regimen and Dose Groups

The study agent is BSSE (administered as Avmacol ES) vs. matched placebo, 4 tablets once daily for 12 weeks.

7.2 BSSE Administration

- Study agent will be dispensed at Baseline Visit. Each bottle will contain 182 tablets. Participants will receive two bottles, sufficient for 12 week intervention period with an allowable window of 14 days before to 7 days after the scheduled 12-week time point.
- The participant will self-administer the study agent.
- Participants will be instructed to take 4 tablets of the assigned study agent once daily with food around the same time each day until the day before the end of intervention visit.

7.3 Run-in Procedures

Not applicable.

7.4 Contraindications

Participants will be instructed to avoid alcohol consumption for 24 hours prior to, and 24 hours after, study-specified acetaminophen administration.

- Rationale for Alcohol Restriction Window:
  The 24-hour window before and after acetaminophen administration is a precautionary measure to minimize potential confounding effects of recent alcohol consumption on hepatic metabolism, specifically cytochrome P450 2E1 (CYP2E1) activity. Acute alcohol intake can transiently induce CYP2E1 or competitively inhibit it, depending on timing and dose, which may unpredictably alter the formation of N-acetyl-p-benzoquinone imine (NAPQI), a hepatotoxic metabolite of acetaminophen. While this risk is primarily of concern at supratherapeutic doses or with chronic alcohol use, we

included this conservative restriction to reduce variability in metabolic response that could compromise the study's ability to detect treatment-related effects on detoxification pathways.

7.5 Concomitant Medications

- Use of supraphysiologic, systemic steroid doses equivalent to prednisone > 5 mg daily for more than 14 consecutive days is to be avoided, if medically possible, while on intervention. Should a participant require supraphysiologic, systemic steroid doses for > 14 days, they will be taken off study.
- Participants are to avoid any herbs, herbal supplements, or nutraceuticals containing active compounds in cruciferous vegetables such as SF and GR while on study. Should a participant initiate any herbs, herbal supplements, or nutraceuticals containing SF and GR while on study, they will be taken off study. A daily multivitamin and/or mineral supplements are acceptable.
- Participants will refrain from taking non-study acetaminophen (or any products containing acetaminophen) for 72 hours prior to and for 24 hours after study-specified acetaminophen administration.

Participants will be asked about all medications (prescription and over-the-counter), vitamin and mineral supplements, and/or herbs taken and documented by study staff during each study visit and during phone contacts 1 week (+ 7 days), 4 weeks ± 7 days and 8 weeks ± 7 days after initiating study agent. Staff will record start and stop date, dose and route of administration, and indication. Medications taken for a procedure (*e.g.*, biopsy) should also be included.

7.6 Dose Modification

If a participant misses a dose of BSSE or placebo, the dose is considered skipped and should not be replaced. If a participant vomits following a dose, the dose should not be retaken. Missed or vomited doses reported during phone contacts will be documented, along with symptoms.

No dose modification will be made for Grade 1 AE. If a participant experiences a related Grade 2 AE (possibly, probably, or definitely attributable to study agent), no dose holding or modification of BSSE or placebo is required. If the related Grade 2 AE is unacceptable to the physician or participant, study agent may be held for up to 7 days at the discretion of the investigator. When the AE has resolved to acceptable Grade 2 or better, study agent will be restarted at the same dose. If the AE has not resolved to acceptable Grade 2 or better within 7 days, study agent will be permanently withdrawn, and the participant followed for resolution of AEs.

If a participant experiences a related Grade 3 AE (possibly, probably, or definitely attributable to study agent) or any Grade 4 AE (regardless of relationship to study agent), study agent will be permanently withdrawn. Participants will be followed at least weekly for resolution of AEs.

When possible and appropriate, participants withdrawn early will be encouraged to return for sample collection and clinical labs for safety.

7.7 Adherence/Compliance

7.7.1 Participants will be considered compliant for statistical analysis if they have taken ≥80% of their assigned study doses based on pill count.

7.7.2 The primary measure of medication compliance is based on pill count and supplemented with the Participant Diary.

8. PHARMACEUTICAL INFORMATION

8.1 BSSE (IND # [redacted] IND NCI, Division of Cancer Prevention)

BSSE will be administered as Avmacol ES, provided by Nutramax Laboratories under IND # [redacted] and sponsored by NCI, DCP.

The regular and extra strength (ES) Avmacol products (Avmacol and Avmacol ES, respectively, manufactured in tablet form under GMP standards by Nutramax Laboratories, Inc.) are commercially available dietary supplements that contain GR and the fully active enzyme myrosinase (MYROSIMAX). The combination of myrosinase and GR results in a higher and more consistent dose of SF upon ingestion of Avmacol or Avmacol ES. SF has been shown to mitigate the effects of environmental carcinogens including benzene, aldehydes, and PAHs found in tobacco smoke by enhancing their detoxification. Mechanistically, SF disrupts the polyubiquitination of the NRF2 transcription factor mediated by its inhibitory protein, KEAP1. This frees NRF2 to translocate to the nucleus and bind to AREs in the promoter regions of target genes. Avmacol ES tablets, which contain approximately 2 times the amount of GR and myrosinase as the regular strength Avmacol tablets, also contain maitake mushroom extract. The maitake mushroom extract contains beta-glucans which are hypothesized to enhance the activity of SF and to augment innate immunity, providing an additional potential mechanism through which Avmacol ES can help support the body's natural detoxification process and prevent cancer progression. A previous proof-of-concept study (NCT03402230) in which otherwise healthy current smokers (≥20 pack-years) were administered a low or high daily dose of regular strength Avmacol for 2 weeks in a crossover design with a 2-week washout period between Avmacol treatment periods, showed a dose-dependent increase in the urinary excretion of the detoxification metabolites of benzene and acrolein, 2 carcinogens in cigarette smoke, indicating that Avmacol treatment could enhance the detoxification of at least some carcinogens in cigarette smoke. In the current study, the investigators propose to evaluate whether a dose of Avmacol ES at least equivalent to the higher daily dose of Avmacol from the previous study can upregulate Phase II detoxification pathways in firefighters. Demonstration of a sustained effect in a rigorous, placebo-controlled study would justify moving Avmacol forward in a primary or secondary chemoprevention setting in this at-risk cohort.

The bioavailability of SF was determined to be 34.2% in healthy volunteers administered Avmacol based on quantitation of SF metabolites in the urine of individuals. The pharmacodynamic effects of Avmacol dosing was evaluated in peripheral blood mononuclear cells (PBMCs) isolated from study participants at baseline and 24 hours following Avmacol administration. When compared with samples obtained prior to dosing, a significant increase in the expression of genes associated with cytoprotective, detoxification, and antioxidative functions (HO-1, HSP27, HSP70) and a decrease in the expression of genes associated with inflammation was observed in the samples obtained 24 hours after Avmacol administration[38]

Avmacol ES manufactured under cGMP by Nutramax Laboratories, Inc., is the current, commercially available BSSE dietary supplement for use in this study. Each Avmacol ES tablet contains 490 mg of active GR and myrosinase blend. It also contains maitake mushroom extract; the manufacturing specifications requires that each Avmacol ES tablet contains at least 30 mg of GR per tablet, double the quantity of GR activity relative to the Avmacol regular strength formulation. In addition to these active ingredients, Avmacol ES contains inactive excipients, i.e., microcrystalline cellulose, maltodextrin, hydroxypropyl cellulose, starch, and < 2% of croscarmellose sodium, silicon dioxide, and magnesium stearate. The GR content is 48 mg/tablet and myrosinase activity (assessed by conversion of GR to SF) is 13 mg/tablet for the specific Avmacol ES lot utilized in the study. Stability testing will be performed on the lot of Avmacol ES utilized for the study, and all clinical supplies will be used within expiry. Matching placebo tablets manufactured under cGMP by Nutramax Laboratories, Inc. will also be used in this study.

8.2 Acetaminophen

Acetaminophen (500 mg tablets) for the study will be locally sourced by the University of Arizona study staff and dispensed by study personnel. Storage and handling of acetaminophen will be conducted in accordance with the manufacturer's instructions.

8.3 Reported Adverse Events and Potential Risks

Broccoli seed and sprout products including Avmacol have been studied extensively in more than 500 subjects in multiple phase 1 and phase 2 clinical studies. In most of these studies the BSP was administered as beverages prepared from broccoli sprouts or crude broccoli seed extracts normalized for GR or SF content to identify dose. No grade 3 or higher AE were reported. Reported grade 1 or 2 AEs have included poor taste of the study agent, most common during ingestion of SF-rich broccoli sprout extract beverages, eructation, flatulence, loose stool not meeting CTCAE criteria for diarrhea, diarrhea, abdominal pain or cramping associated with loose stool or diarrhea. During the first healthy volunteer study with Avmacol, the taste of the study agent was not a source of toxicity – likely because the taste is masked by encapsulation.

The toxicities from NCT03402230, our completed, randomized, crossover trial evaluating the detoxification of benzene and other tobacco carcinogens by BSSE in otherwise healthy current smokers[39], are summarized in **Table 3**. Twenty-nine of 49 (59%) of participants reported at least one AE attributable to BSSE; all 29 (100%) were gastrointestinal AE. The most common AEs were loose stool not meeting CTCAE v.4 criteria for diarrhea (24%) or diarrhea (22%), all Grade 1. Ten of 49 (20%) participants experienced mild abdominal pain typically associated with loose stool or diarrhea. Despite the prevalence of mild gastrointestinal AEs, compliance with treatment was 98%. These symptoms are consistent with other studies and expected due to the high fiber content of BSSE. The dose of BSSE in the current trial is identical to the high dose arm of NCT03402230.

Table 3. Treatment-Emergent Adverse Events in Clinical Study of Avmacol for Detoxification of Tobacco Carcinogens in Heavy Smokers (NCT03402230**)**

Toxicity	**Low Dose (4 Avmacol Regular Strength Tablets)[a] N=49**	**High Dose (8 Avmacol Regular Strength Tablets)[b] N=49**	**Both Arms N=49[c]**
Abdominal Pain	1 (2%)	10 (20%)	10 (20%)

Bloating	0 (0%)	1 (2%)	1 (2%)
Diarrhea	3 (6%)	10 (20%)	11 (22%)
Loose Stool	7 (14%)	9 (18%)	12 (24%)
Flatulence	3 (6%)	7 (14%)	10 (20%)
Nausea	1 (2%)	0 (0%)	1 (2%)
Vomiting	0 (0%)	1 (2%)	1 (2%)
Weight Loss	0 (0%)	1 (2%)	1 (2%)

[a]Low dose: Subjects were given 4 Avmacol regular strength tablets per day.
[b]High dose: Subjects were given 8 Avmacol regular strength tablets per day.
[c]Participants who had AE in either or both treatment arms.

Acetaminophen is an FDA-approved medication that is available over the counter without a prescription. It is commonly used to reduce fever and relieve pain. The dosage used in this study is well within the FDA's recommended daily limits. While side effects from taking study-specified acetaminophen in this study are unlikely, it is important to note that no medication, including FDA-approved over-the-counter drugs, is entirely free from risk. Possible side effects of acetaminophen may include:

Liver warning: Severe liver damage may occur if:
- adult takes more than 4,000 mg of acetaminophen in 24 hours
- acetaminophen is taken with other drugs containing acetaminophen
- adult has 3 or more alcoholic drinks every day while using acetaminophen

Allergy alert: acetaminophen may cause severe skin reactions. Symptoms may include:
- skin reddening
- blisters
- rash

Acetaminophen (500 mg tablets) for the study will be locally sourced by the University of Arizona study staff and dispensed by study personnel. Storage and handling of acetaminophen will be conducted in accordance with the manufacturer's instructions.

8.4 Availability

Avmacol ES (490 mg of proprietary GR and myrosinase blend per tablet) is a commercially available dietary supplement manufactured and marketed by Nutramax Laboratories, Inc. Matching placebo tablets will also be manufactured by Nutramax Laboratories, Inc. Avmacol ES and matching placebo tablets will be supplied to investigators by the Division of Cancer Prevention (DCP), NCI.

Avmacol ES and matched placebo are provided to the NCI under a Clinical Trials Agreement (CTA) between Nutramax, Inc. and the DCP, NCI (see §14.7).

Acetaminophen is a commercially available antipyretic manufactured by several pharmaceutical companies. Acetaminophen will be purchased locally over the counter for the study by the University of Arizona study staff and dispensed by study personnel. Storage and handling of acetaminophen will be conducted in accordance with the manufacturer's instructions.and will be provided to participants by study personnel.

8.5 Agent Distribution

Agents will only be released by NCI, DCP after documentation of CIRB approval of the DCP-approved protocol and consent is provided to DCP and the collection of all Essential Documents is complete (see DCP website for description of Essential Documents).

NCI, DCP-supplied agents may be requested by the Investigator (or his/her authorized designees) at each Organization. DCP guidelines require that the agent be shipped directly to the institution or site where the agent will be prepared and administered. DCP does not permit the transfer of agents between institutions (unless prior approval from DCP is obtained). DCP does not automatically ship agents; the site must make a request. Agents are requested by completing the DCP Clinical Drug Request form: https://prevention.cancer.gov/sites/default/files/uploads/clinical_trial/Investigational-Agent-Request.docx (to include complete shipping contact information) and e-mailing the form to the DCP agent repository contractor:

John Cookinham
MRIGlobal
DCP Repository
1222 Ozark Street
North Kansas City, MO 64116
Phone: (816) 360-3805
E-mail: NCI.DCP@mriglobal.org

Acetaminophen (500 mg tablets) for the study will be locally sourced by the University of Arizona study staff and dispensed by study personnel. Storage and handling of acetaminophen will be conducted in accordance with the manufacturer's instructions.

8.6 Agent Accountability

The Investigator, or a responsible party designated by the Investigator, must maintain a careful record of the inventory and disposition of all agents received from DCP. The Investigator is required to maintain adequate records of receipt, dispensing and final disposition of study agent. This responsibility has been delegated to study site PI and the institution's Research Pharmacy or study coordinator. Include on receipt record from whom the agent was received and to whom study agent was shipped, date, quantity and batch or lot number. On dispensing record, note quantities and dates study agent was dispensed to and returned by each participant.

Acetaminophen (500 mg tablets) for the study will be locally sourced by the University of Arizona study staff and dispensed by study personnel. Storage and handling of acetaminophen will be conducted in accordance with the manufacturer's instructions.

8.7 Packaging and Labeling

BSSE tablets and matching placebo will be manufactured and packaged by Nutramax. Nutramax will ship the packaged BSSE (Avmacol ES) and matched placebo bottles to the NCI DCP Drug Repository, MRIGlobal, in Kansas City, MO. MRIGlobal will label bottles according to the blinded randomization schema, and ship to study site.

Each bottle will be labeled with a three-part label identifying study specific information including study title, DCP protocol number, dosing instructions, recommended storage conditions, the name and address of the distributor, and treatment ID.

8.8 Storage

BSSE and placebo tablets should be stored at room temperature in a secure, locked area of the research facility, with temperature excursions permitted between 15–30°C (59–86°F). The CP-CTNet Lead Academic Organization, the DCP agent repository contractor, DCP Medical Monitor and Nurse Consultant should be notified in the event of a Temperature Excursion.

Storage and handling of acetaminophen will be conducted in accordance with the manufacturer's instructions.

8.9 Registration/Randomization

This trial will use a web-based Registration/Randomization System, developed and maintained by the CP-CTNet Data Management, Auditing, and Coordinating Center (DMACC). The Help Desk includes technical personnel and administrators of the registration programs at the Data Management Center in Amherst, NY, USA. The Help Desk is available round the clock 7 days per week, except for New Year's Eve, Memorial Day, Independence Day, Thanksgiving Day, and Christmas Day.

Frontier Science Randomization Help Desk
4033 Maple Rd, Amherst, NY 14226 USA
Phone: +1 716 834 0900 Extension 7301
Email: UserSupport_CP-CTNet@frontierscience.org

Participants will be considered registered on the date they sign the approved informed consent document with a member of the study staff. Following registration, eligibility will be confirmed. After determination of eligibility and completion of informed consent document, eligible participants will be randomized (1:1) to either BSSE or placebo in double-blind fashion.

Note: The Registration and Randomization process is documented in the "Stars User Guide".

8.10 Blinding and Unblinding Methods

This is a randomized, double-blind (participants and investigators), placebo-controlled trial. Blinding and unblinding methods are specified as follows:

- Investigators and clinical research coordinators will avoid speculating with the participant regarding the participant's treatment group.
- The individual authorized to break the blind, if conditions are met, is the Study PI in consultation with the DCP medical monitor.
- The blind may be broken in the following two circumstances:
    - Medical necessity: The participant is experiencing a serious adverse event (SAE) AND knowledge of treatment assignment would alter the clinical management of the SAE

OR

- Safety and informed consent for current and future participants: The participant is experiencing an SAE AND knowledge of treatment assignment would change understanding of the toxicity profile of BSSE, therefore altering safety and informed consent for current and future participants

- Procedure for breaking the blind:
  - The SAE is reported according to procedures described in Section 13.0.
  - The treating investigator consults with the Study PI regarding the specific circumstances, and whether knowledge of treatment assignment would alter the clinical management of the SAE OR would change the understanding of the toxicity profile of BSSE such that safety and informed consent would be impacted for current and future participants.
  - If the SAE meets criteria for unblinding, the Study PI then authorizes DMACC to unblind treatment assignment for that participant.
  - DMACC unblinds the participant and informs the Study PI and the treating investigator: 1) unblinding has occurred; and 2) the treatment assignment of the unblinded participant.
- The CIRB will be notified of the unblinding event.

The NCI DCP Medical Monitor must be notified that the blind has been broken:

Malgorzata Wojtowicz, MD, Medical Monitor
NCI/Division of Cancer Prevention
NIH National Cancer Institute
9609 Medical Center Drive
Room 5E-104, MSC 9781
Bethesda, MD 20892
PHONE: 240-276-7012
FAX: (240) 276-7848
malgorzata.wojtowicz@nih.gov

8.11 Agent Destruction/Disposal

DCP-supplied agents: at the completion of investigation, all unused study agent will be returned to NCI, DCP Repository according to the DCP "Guidelines for AGENT RETURNS" and using he DCP form "DCP Returned Agents List".

9. CLINICAL EVALUATIONS AND PROCEDURES

9.1 Schedule of Events

Evaluation/ Procedure	Visit 1 Consenting/ Screening	Randomization	Visit 2/ Baseline[2]	Intervention Period[11]	Visit 3/ End of Intervention[11]	Follow-up
Informed Consent	X					
Assess Eligibility	X					
Medical History[1]	X					

Baseline Signs/Symptoms[2]	X		X			
Review for any change in health status since Visit 1/screening[2a]			X			
Tobacco and Alcohol Use Assessment (DCP Baseline)	X					
Concomitant Medications[3]	X		X	X	X	
Baseline COVID Assessment	X					
Vital Signs	X		X		X	
Height, and Weight[4]	X				X[4]	
Karnofsky Performance Status	X				X	
Blood Draw: Clinical Labs (CBC/Diff, CMP) and Research Tests[5]	X				X	
Urine Pregnancy Test[6]	X		X			
Randomization[7]		X				
Pre-Acetaminophen: Collect Spot Urine and questionairre[8, 9]			X		X	
Administer Acetaminophen			X		X	
Post-acetaminophen cumulative 8-hr Urine Collection[10]			X		X	
Provide Intake Calendar and Adverse Event (Symptom)/New Medication Diary			X			

Dispense Study Agent[11]			X			
Spot Urine Collection after Each Fire/Fuel Leak Activity[12]				X		
Post-Fire/Fuel Leak Spot Urine Questionnaire[12] and Fire/Fuel Incident Report Appendix H[12]				X		
Participant Contact and review of Adverse Events, new/change in medication and pill intake[13]				X	X	X
Collect Study Agent/Pill Count					X	
Review Intake Calendar and Adverse Event (Symptom)/New Medication Diary[14]					X	
Tobacco and Alcohol Use Assessment (DCP Follow-Up)					X	
Follow-up COVID-19 Assessment					X	

[1] Participants will be asked the day of the last physical exam (month and year required). For those who do not recall the actual day of the month, the 15th of the month will be substituted.
[2] Baseline Visit should occur within 12 weeks of screening/consenting. Vital signs, conmeds, pregnancy test (if applicable), and any change in health status will be re-assessed at visit 2.
[2a]If a change in health status is reported at Visit 2, participant will be re-evaluated for eligibility as clinically indicated.
[3] Participants using any supplements containing active compounds in cruciferous vegetables such as SF and GR may be considered eligible if they agree to stop the SF or GR product at least 2 weeks prior to the Baseline Visit.
[4] Weight only to be collected at post screening visit.
[5] Research tests during Visit 1 include screening for GST genotypes and assessment of epigenetic modification (miRNA and DNA methylation). Research tests during Visit 3 include assessment of epigenetic modifications (miRNA and DNA methylation).
[6]Urine pregnancy test is required only in women of child-bearing potential.
[7] Randomization will be completed after the subject is registered and confirmed eligible, after the Screening Visit and prior to Visit 2.

[8] Participants will be instructed to avoid alcohol consumption for 24 hours prior to and 24 hours after study-specified acetaminophen administration. Participants will also be instructed to refrain from taking non-study acetaminophen (or any products containing acetaminophen) for 72 hours prior to and for 24 hours after study-specified acetaminophen administration.
[9] Over-the-counter and prescription medications, supplements use including pre-workout and protein beverages, other beverage consumption aside from water during the 24 hours prior to pre-acetaminophen dose spot urine collection, and blood and/or plasma donation history will be collected as part of the Pre-Acetaminophen Spot Urine Questionnaire.
[10]The 8-hr (-1 and + 2 hr) post acetaminophen urine will be collected by the TFD research liaisons or study staff within 24 hours after completion.
[11] Participants will be instructed to begin taking the study agent within two weeks of the Baseline Visit. The intervention period will last a minimum of 12 weeks, with an allowable window of 14 days before to 7 days after the scheduled 12-week time point. Participants will stop taking the study agent only after completing the End-of-Intervention Visit (Visit 3), which includes the end-of-study acetaminophen dose and the associated 8-hour post-dose urine collection conducted within this same window. This design ensures that the effects of the study intervention on acetaminophen metabolism are assessed while participants are actively taking the study agent.
[12] The spot urine and the Post-Fire/Fuel Leak Spot Urine Questionnaire will be collected by the TFD research liaisons or study staff within 24 hours of completion. TFD research liaison will complete Fire/Fuel Incident Report Appendix H.
[13] Participants will be contacted by phone or email per their preferred method of telecommunication at week 1 + (7 days), week 4 ± 7 days and week 8 ± 7 days for evaluation and documentation of AEs, compliance, and proper completion of Adverse Event (Symptom)/New Medication Diary and Intake Calendar.
[14] Intake Calendar and Adverse Event (Symptom)/New Medication Diary will be collected during Visit 3/End of Intervention.

9.2 Schedule of Urine Samples and Spot Urine Questionnaire Collection

Evaluation/Procedure	Visit 1 Consenting & Screening	Visit 2 Baseline	Intervention 12 weeks	Visit 3 End of Intervention
Pre-acetaminophen Spot Urine Collection		X		X
Pre-Acetaminophen Spot Urine Questionnaire[1]		X		X
Post-acetaminophen 8-hour Cumulative Urine Collection[2]		X		X
Post-fire/fuel Leak Spot Urine Collection [3]			X	
Post-Fire/Fuel Leak Spot Urine Questionnaire[4]			X	

[1] Over the counter and prescription medications, supplements use including pre-workout and protein beverages, other beverage consumption aside from water during the 24 hours prior to pre-acetaminophen dose spot urine collection, and blood and/or plasma donation history will be collected as part of the Pre-Acetaminophen Spot Urine Questionnaire.
[2] The End-of-Intervention Visit (Visit 3), when participants stop taking the study agent, occurs at 12 weeks with an allowable window of 14 days before to 7 days after the scheduled time point. Participants must complete the end-of-study acetaminophen dose and the associated 8-hour post-dose urine collection while remaining on the study agent. Study agent is discontinued only after these assessments are completed. Remaining on the agent during the end-of-study acetaminophen collection ensures that the acute effects of the intervention on acetaminophen metabolism are accurately evaluated. The post acetaminophen 8 (-1 and + 2) hour urine will be collected by the TFD research liaisons or study staff within 24 hours after completion.

[3]The post-fire/fuel leak 8 (± 2) hour spot urine will be collected by the TFD research liaisons or study staff within 24 hours after completion.
[4]Time of urine collection will be recorded along with a brief questionnaire on exposure, including type of fire and firefighter activity, along with post-fire washdown activity, will be collected as part of the Post Fire Urine Collection Questionnaire completed by the participant electronically and supplemented with a Fire Incident report to be completed by the TFD research liaison. The post-fire/fuel leak spot urine will be collected by the TFD research liaisons or study staff within 24 hours of completion.

9.3 Baseline Testing/Pre-Study Evaluation

Visit 1 (Screening Evaluation): Participants will undergo a screening evaluation in which the informed consent form will be signed. To minimize participant burden, remote consent may be implemented in compliance to the instructions in Appendix G. Participants will be assessed for study eligibility. Detailed inclusion and exclusion criteria are listed in sections 4.1 and 4.2. All participants will be evaluated for medical history including date (month and year required) of last physical exam, baseline signs/symptoms, tobacco/alcohol use (DCP baseline forms), COVID baseline assessment (DCP baseline form), and concurrent medication (conmed) and supplement use. In the event, the participant cannot recall the actual day of the month of the last physical exam, the 15th of the month will be collected as the date. All participants will undergo evaluation to obtain height, weight, vital signs (blood pressure, pulse, temperature), and Karnofsky performance status assessment. Blood will be collected for laboratory analysis with complete blood count with differential (CBC-diff) and comprehensive metabolic panel (CMP) as well as research testsincluding GST genotypes and assessment of epigenetic modification (miRNA and DNA methylation). Women of childbearing capacity will complete a urine pregnancy test. The participant's preferred telecommunications contact method will be determined (telephone or email).

If a participant is using any supplements containing active compounds in cruciferous vegetables such as SF and GR and agrees to stop the SF or GR product at least 2 weeks prior to the Baseline Visit, he/she will undergo screening for eligibility to participate in this study.

Randomization: Once determined eligible, participants will be randomized (1:1) to BSSE or matched placebo.

9.4 Evaluation During Study Intervention

Visit 2 (Baseline): During this visit, participants will undergo assessment for vital signs and concomitant medications/supplements. They will be asked about any change in health status since Visit 1 (screening). If change in health status is reported, participant will be re-evaluated for eligibility as clinically indicated, including repeat clinical laboratory testing if needed. Urine pregnancy test will be repeated, if applicable. Two tablets of 500 mg acetaminophen will be administered to the participant and the participant will be instructed to collect a spot urine prior to acetaminophen dosing and to collect all urine for 8 (-1 and + 2) hours following the acetaminophen administration. Coordinators will administer the questions from the Pre-Acetaminophen Spot Urine Questionnaire (Appendix E) at the time of the pre-acetaminophen dose spot urine collection. Participants will be instructed to keep the post-acetaminophen dose urine in a cooler with ice packs, provided by the study staff, during the 8-hr. post-dose urine collection. Upon completion of the 8-hr post-dose urine collection, the sample will be picked up by the study staff or TFD research liaisons and processed within 24 hours of collection. Participants will be provided with BSSE or matched placebo and will be instructed to take 4 tablets of the assigned study agent each day with food until the day before the end of intervention visit. Participants will be instructed to start taking the study agent within 2 weeks of the Baseline Visit and notify the study staff by telephone or e-mail when they start the agent intervention. They will also be provided with, and shown how to use, an Intake Calendar

for recording study agent intake and Adverse Event (Symptom)/New Medication Diary for new medications and side effects.

Participants will also be instructed to collect a spot urine within 8 (± 2) hours post each of their fire/fuel leak activity, when feasible. Participants will be provided with the post-fire urine collection supplies. Participants will be instructed to complete the Post-Fire/Fuel Leak Spot Urine Questionnaire (Appendix F) when post-fire urine is collected. The collected urine will be kept in a cooler with ice packs and picked up by the study staff or TFD research liaisons and processed within 24 hours of collection, when feasible. TFD research liaison will complete Fire/Fuel Incident Report (Appendix H) to document exposures.

Participants will be contacted by study staff 1 week (+ 7 days), 4 weeks ± 7 days and 8 weeks ± 7 days after starting agent intervention to assess and document any AEs, compliance with study intervention including any missed pills, and appropriate completion of Adverse Event (Symptom)/New Medication Diary and Intake Calendar.

9.5 Evaluation at Completion of Study Intervention

Visit 3 (End-of-Intervention): For the end-of-intervention evaluation, participants will be assessed for AEs, conmeds, and agent compliance. Intake Calendar and Adverse Event (Symptom)/New Medication Diary and any remaining study agent will be collected during the visit. Blood will be collected for laboratory analysis with CBC-diff and CMP and for research tests including assessment of epigenetic modification (miRNA and DNA methylation). All participants will undergo evaluation to obtain weight, blood pressure, pulse, and temperature. Tobacco/alcohol use (DCP follow-up form), Karnofsky Performance Status, and COVID follow-up assessment will be performed during this visit. Two tablets of 500 mg acetaminophen will be administered to the participant and the participant will be instructed to collect a spot urine prior to acetaminophen dosing and to collect all urine for 8 (-1 and + 2) hours following the acetaminophen administration. Participants will be instructed to complete the Pre-Acetaminophen Spot Urine Questionnaire (Appendix E) for the pre-acetaminophen dose spot urine collection. Participants will be instructed to keep the post-acetaminophen dose urine in a cooler with ice packs, provided by the study staff, during the 8-hr post-dose urine collection. Upon completion of the 8-hr post-dose urine collection, the sample will be picked up by the study staff or TFD research liaisons and processed within 24 hours of collection.

9.6 Post-intervention Follow-up Period

End of Study Assessment: Participants will be contacted by telephone or email 2 weeks ± 7 days after completing study treatment per their preference to assess AEs.

Participant's documentation of fire/fuel leak activities that occurred during the study agent intervention will be collected from the records of the TFD via the TFD research liaison to minimize participant burden.

9.7 Methods for Clinical Procedures

Not Applicable.

10. CRITERIA FOR EVALUATION AND ENDPOINT DEFINITION

10.1 Primary Endpoint

Primary endpoint. Compare the change (from baseline to end-of-intervention) in total amount of acetaminophen mercapturate excreted in the urine after acetaminophen dosing between BSSE and placebo arms.

10.2 Secondary Endpoints

The secondary endpoints are:

1) Compare the change (from baseline to end-of-intervention) in total amount of acetaminophen glucuronide excreted in the urine after acetaminophen dosing between BSSE and placebo arms.
2) The safety and tolerability of BSSE, as measured by NCI CTCAE version 5.

Exploratory objectives include the following analysis: 1) to assess whether the *GSTM1* and *GSTT1* genotypes are important genetic modulators of BSSE-induced detoxification of carcinogens in the setting of exposure to BSSE; 2) to explore the effects of BSSE on urinary metabolome; 3) to evaluate the effects of BSSE on the urinary excretion of the mercapturic acid of benzene after fire/fuel leak activities, 4) to assess the effects of BSSE on urinary excretion of the metabolites of PAHs after fire/fuel leak activities, 5) to evaluate the effects of BSSE on epigenetic modifications (blood microRNA and DNA methylation).

10.3 Off-Agent Criteria

Participants may stop taking study agent for the following reasons: completed the protocol-prescribed intervention, AE or SAE, inadequate agent supply, noncompliance, concomitant medications, medical contraindication. Participants will continue to be followed, if possible, for safety reasons and in order to collect endpoint data according to the schedule of events.

10.4 Off-Study Criteria

Participants may go ‘off-study’ for the following reasons: the protocol intervention and any protocol-required follow-up period is completed, AE/SAE, any AE occurring in association with first administration of acetaminophen at Visit 2, lost to follow-up, non-compliance, concomitant medication, medical contraindication, withdraw consent, death, determination of ineligibility (including screen failure), pregnancy.

10.5 Study Termination

NCI, DCP as the study sponsor has the right to discontinue the study at any time.

11. CORRELATIVE/SPECIAL STUDIES

11.1 Rationale for Methodology Selection

Integral Biomarkers

- Urinary Excretion of Acetaminophen Metabolites: Urinary levels of acetaminophen and its metabolites (acetaminophen mercapture, acetaminophen glucuronide, and acetaminophen sulfate) will be quantified by liquid chromatography tandem mass spectrometry, as previously described [41].

Exploratory Biomarkers

- Urinary Excretion of the Mercapturic Acid of the Carcinogen, Benzene: Urinary levels of the mercapturic acid of benzene will be quantified by liquid chromatography tandem mass spectrometry, as previously described[33, 42]. Specific analytes to be quantified include S-phenyl mercapturic acid (SPMA), a metabolite of benzene.

- Urinary Excretion of PAH metabolites: Urinary excretion of PAH metabolites, including 1-hydroxypyrene, will be evaluated to assess detoxification of PAHs. PAH metabolites will be measured by liquid chromatography tandem mass spectrometry, as previously described[43].

- *GSTM1* and *GSTT1* Genotypes: Genomic DNA will be isolated from blood collected at the baseline clinic visit (week 1). *GSTM1* and *GSTT1* will be genotyped using standard methods as previously described [44]. Briefly, two separate PCR reactions will be used to determine *GSTM1* and *GSTT1* homozygous deletion. Each amplification will include an internal PCA control (CYP1A1 fragment) to ensure null results are attributable to GST gene deletion but not due to PCR failure. PCR products will be analyzed by electrophoresis (1.5% agarose) and ethidium bromide staining. The presence or absence of *GSTM1* and *GSTT1* genes will be detected by the presence or absence of a band at 480 base-pairs (corresponding to *GSTT1*) and a band at 215 base-pairs (corresponding to *GSTM1*).

- Untargeted Urinary Metabolomics: Untargeted metabolomics will be performed as described previously [45]. Briefly, pre-acetaminophen spot urine collected at both baseline and at the end of intervention as well as spot urine collected after each fire/fuel leak activity will be analyzed using an Exploris Orbitrap 480 for ultra-high performance liquid chromatography in two modes: ESI C18 (reverse phase) positive and HILIC (hydrophilic interaction liquid chromatography) negative, with standard quality control, filtering, and imputation procedures. We plan to use Compound Discoverer for peak identification and annotation. Mass spectra will be matched against our in-house library, and against external libraries including ChemSpider and Metabolika.

- miRNA: RNA will be isolated from blood and miRNA expression will be measured using the nCounter Human v3 miRNA expression panel (NanoString Technology Inc., Seattle, Washington) with 800 miRNAs from miRBase v21 as well as 5 housekeeping genes and 20 assay controls (six positive, eight negative, and six ligation controls)[12-14]. The panel includes greater than 95% of human miRBase reads.

- DNA methylation: DNA will be isolated from blood and analyzed using Infinium MethylationEPIC array as described previously[64]. This is a genome-wide methylation analysis tool targeting ~930K unique methylation sites in the most biologically significant regions of the human methylome.

11.2 Comparable Methods

The methods proposed for integral and exploratory biomarkers are standard methodologies used in other research studies, including our recently completed, randomized crossover study of low and high dose BSSE in otherwise healthy smokers (NCT03402230)[33, 39] and our studies in firefighters[12-15, 46]. The resulting data will be able to be compared to existing data.

The methods proposed for untargeted urinary metabolomics are also standard methodologies used in our prior research studies of untargeted metabolomics in municipal and wildland-urban interface firefighters[45]. The results should be comparable to our prior studies, after correcting for batch effects. The quality control and extraction methods were also developed in line with other environmental studies in the United States using untargeted metabolomic approaches.

12. SPECIMEN MANAGEMENT

12.1 Laboratories

Clinical chemistry and hematology panels will be outsourced to a contracted commercial diagnostic laboratory service (i.e., Sonora Quest).

The urinary biomarker analyses, including the acetaminophen metabolites and carcinogen metabolites, and untargeted metabolomics will be conducted in the University of Arizona Cancer Center Analytical Chemistry Shared Resource.

miRNA analysis will be conducted in the University of Arizona Genetics Core facility.

DNA methylation analysis will be performed in the University of Michigan Advanced Genomics Core.

12.2 Collection and Handling Procedures

Blood for clinical chemistry and hematology panels
Twelve and a half milliliters of blood (1 x 8.5 mL SST tube; 1 x 4 mL lavender-EDTA tube) will be collected at Visits 1 and 3 for CBC with diff and CMP. The SST tube will be held at room temperature for 30 min and then centrifuged. The lavender EDTA tube will be gently inverted to mix for anticoagulation. Blood tubes will be prepped, labeled, and packaged according to the recommendation from the diagnostic laboratory. All samples will be refrigerated prior to transfer to the commercial laboratory and sent for immediate analysis.

Blood for genotyping and epigenetic modifications

Visit 1, Screening:
For genotying and DNA methylation - 1 x 6 mL Lavender EDTA tube will be collected. The lavender-EDTA tube will be inverted gently 5 times and the whole blood will be aliquoted evenly into 2 x 5 mL cryovials.

For miRNA: 1 x 9 mL Tempus^{TM} Blood RNA tube will be collected. Immediately after collection, the tube will be vigorously shaken for 10 seconds and aliquoted into 2 x 5mL cryogenic tubes.

The cryo-vials/tubes will be labeled with the study ID, participant ID, and visit number/date and stored at -70°C or below until analysis.

Visit 3, End of Intervention:
For DNA methylation - 1 x 6 mL Lavender EDTA tube will be collected. The lavender-EDTA tube will be inverted gently 5 times and the whole blood will be aliquoted evenly into 2 x 5 mL cryovials.

For miRNA: 1 x 9 mL Tempus™ Blood RNA tube will be collected. Immediately after collection, the tube will be vigorously shaken for 10 seconds and aliquoted into 2 x 5mL cryogenic tubes.

The cryo-vials/tubes will be labeled with the study ID, participant ID, and visit number/date and stored at -70°C or below until analysis.

Urine for pregnancy test
A single void urine will be collected and tested for pregnancy in the clinic according to package instructions.

Urine collection before and after acetaminophen dosing
A spot urine will be collected before acetaminophen administration and all urine will be collected for 8 (-1 and + 2) hours after acetaminophen administration to assess acetaminophen metabolism. The urine volume will be recorded by study staff to the nearest 10 mL. Five 2 mL aliquots of urine will be retained for testing and the remaining urine discarded. The sample tubes will be labeled with the study ID, participant ID, visit number/date, sample type (pre-dose or post-dose) and stored at -70°C or below until analysis.

Post Acetaminophen Urine Collection Window and Deviation Definition
For the 8-hour post-acetaminophen urine collection, all efforts should be made to complete the cumulative collection within the designated 7–10-hour target window following acetaminophen dosing.

A protocol deviation occurs when the cumulative urine collection is completed outside this window. Collections performed between 4 and 12 hours after acetaminophen dosing will be considered acceptable for analysis if the total urine volume is ≥200 mL, but will be documented as out-of-window.

This allowance provides operational flexibility in field settings while maintaining adequacy for quantitative analysis of acetaminophen metabolites and preserving sample integrity.[47]

Spot urine collection post fire/fuel leak activity
Participants will collect a spot urine within 8 ± 2 hours post each of their fire/fuel leak activity, when feasible. Five 2 mL aliquots of urine will be retained for testing and the remaining urine discarded. The sample tubes will be labeled with the study ID, participant ID, date and time of collection, and sample type (post-fire) and stored at -70°C or below until analysis.

Post-Fire/Fuel Leak Urine Collection Window and Deviation Definition

For post-fire/fuel leak urine collections, participants should collect a single (spot) urine sample within 8 ± 2 hours of completing the fire or fuel leak activity.

A protocol deviation occurs when the sample is collected outside this 6–10-hour window. Collections performed between 4 and 12 hours post-activity will be considered acceptable for analysis if the total urine volume is ≥30 mL, but will be documented as out-of-window.

This range provides operational flexibility in real-world field settings while ensuring adequate sample volume for quantitation of exposure-related metabolites and normalization to creatinine.[48]

12.3 Shipping Instructions

Collected frozen specimens (blood, urine) will be placed on ice packs and hand delivered to UA CP-CTNet research laboratory (University of Arizona Cancer Center, 1515 N Campbell Ave, Room 4971, Tucson, AZ) for storage until analysis.

Frozen blood specimens will be shipped to the University of Michigan Advanced Genomics Core for DNA Methylation testing. The sample manifest and shipping dates will be provided to the University of Michigan Genomics Core and dates will be agreed upon prior to samples being shipped. All samples will be shipped using FedEx priority overnight, with 25-30 lbs. of dry ice per shipping container and shipping comply with IATA guidelines. Once samples are received by the Genomics Core, confirmation will be provided to the University of Arizona.

The shipping address of the University of Michigan Advanced Genomics Core is listed below:
The University of Michigan Advanced Genomics Core
Room 122
NCRC Building 14
University of Michigan
2800 Plymouth Rd.
Ann Arbor, MI 48109-2800

Contact person:
Susan Dagenais
sdagenai@umich.edu

12.4 Tissue Banking

The NCI reserves the right to require the transfer of biologic specimens and data, or true copies of such data, acquired from research supported under this award to an eligible third party. This transfer can occur in order to preserve the specimens and data and/or to continue the research. Third parties supported under this award must be informed of this right.

13. REPORTING ADVERSE EVENTS

DEFINITION: An adverse event (AE) means any untoward medical occurrence associated with the use of a drug in humans, whether or not the untoward occurrence is considered drug related. Thus, an AE can include any unfavorable sign (e.g., an abnormal laboratory finding), symptom, or clinical outcome temporally associated with the use of a test drug, active control, or placebo, regardless of whether the event is thought to be related to the drug. An AE can arise with the use of a drug or biologic (e.g., use for a purpose other than FDA-approved indication or in combination with another drug) and with any route of administration, formulation, or dose, including an overdose.

Please note that all abnormal clinical laboratory values that are determined to be of clinical significance based on a physician's assessment are to be reported as AEs. A clinically significant lab value is one that indicates a new disease process, an exacerbation or worsening of an existing condition, or requires further action(s) to be taken**.** Those labs determined to be of no clinical significance or of unknown clinical significance (per the physician's assessment) should not be reported as AEs. Any lab value of unknown clinical significance should continue to be

investigated/followed-up further for a final determination, if possible. (See the *DCP Baseline and Adverse Event Reporting Guidelines* [https://prevention.cancer.gov/clinical-trials/clinical-trials-management/cp-ctnet-instructions-forms] for more detail on reporting abnormal clinical laboratory values.)

A list of AEs that have occurred or might occur can be found in §8.2 Reported Adverse Events and Potential Risks, as well as the Investigator's Brochure or package insert.

13.1 Adverse Events

13.1.1 Reportable AEs

All AEs that occur after the informed consent is signed and baseline assessments are collected must be recorded on the AE CRF whether or not related to study agent.

13.1.2 AE Data Elements:

The following data elements are required for AE reporting.

- AE verbatim term
- NCI Common Terminology Criteria for Adverse Events version 5.0 (CTCAE v5.0) AE term (MedDRA lowest level term)
- CTCAE (MedDRA) System Organ Class (SOC)
- Event onset date and event ended date
- Treatment assignment code (TAC) at time of AE onset
- Severity grade
- Attribution to study agent (relatedness)
- Whether or not the event was reported as a SAE
- Whether or not the participant dropped due to the event
- Outcome of the event

13.1.3 Severity of AEs

13.1.3.1 Identify the AE using CTCAE v5.0. The CTCAE provides descriptive terminology (MedDRA lowest level term) and a severity grading scale for each AE listed. A copy of the CTCAE can be found at: http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm

AE severity will be assessed according to the grade associated with the CTCAE term. AEs that do not have a corresponding CTCAE term will be assessed according to the general guidelines for grading used in the CTCAE v5.0. as stated below.

CTCAE v5.0 general severity guidelines:

Grade	Severity	Description
1	Mild	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.

2	Moderate	Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living (ADL)*.
3	Severe	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL**.
4	Life-threatening	Life-threatening consequences; urgent intervention indicated.
5	Fatal	Death related to AE.

*Instrumental ADL refers to preparing meals, shopping for groceries or clothes, using the telephone, managing money, *etc*.
**Self-care ADL refers to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

13.1.4 Assessment of relationship of AE to treatment

The possibility that the AE is related to study agent will be classified as one of the following: unrelated, unlikely, possible, probable, definite. Criteria for these classifications are provided in DCP's *Serious Adverse Event Report Form: Instructions for Completion and Submission* **(https://prevention.cancer.gov/clinical-trials/clinical-trials-management/cp-ctnet-instructions-forms).**

13.1.5 Follow-up of AEs

All AEs, including lab abnormalities that in the opinion of the investigator are clinically significant, will be followed according to good medical practices and documented as such. Clinically significant AEs will be followed until resolution to ≤ Grade 1 or baseline, or until 4 weeks after completion of the intervention period, whichever is earlier.

13.1.6 Collection of AEs

AEs will be collected for each participant until the post-intervention follow-up assessment.

13.2 Serious Adverse Events

13.2.1 DEFINITION: Regulations at 21 CFR §312.32 define an SAE as any untoward medical occurrence that at any dose has one or more of the following outcomes:

- Death
- A life-threatening AE
  (According to FDA safety guidance, an AE is considered life-threatening if, in the view of either the investigator or sponsor, its occurrence places the subject at immediate risk of death. It does not include an event that, had it occurred in a more severe form, might have caused death. Example: An allergic reaction resulting in angioedema of the larynx, allergic bronchospasm or anaphylaxis is considered life-threatening; however, an allergic reaction resulting only in a localized rash is not life-threatening.)
- In patient hospitalization or prolongation of existing hospitalization

(NCI, DCP uses admission or stay (including emergency room) equal to or greater than 24 hours as the definition of hospitalization. Exceptions are hospitalization for treatment of a pre-existing condition [unless the condition increased in severity on study], outpatient surgery, planned/elective procedures, and procedures described in the protocol [e.g., pharmacokinetic sampling, surgery] even if the hospital stay is of the described length; however, it does include events resulting from any of these that fulfill other serious outcome criteria, e.g., prolongation of hospitalization or life-threatening.)

- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly or birth defect
- Important medical events that may not be immediately life-threatening or result in death or hospitalization should also be considered serious when, based upon appropriate medical judgment, they may jeopardize the participant and may require intervention to prevent one of the other outcomes listed above.

13.2.2 Reporting SAEs to DCP

13.2.2.1 The accruing LAO and all Affiliated Organizations (AOs) will report SAEs on the DCP SAE Report Form as described in DMACC's CP-CTNet SOP 02-01, found at CP-CTNet SOP 02-01 Reporting Serious Adverse Events (cp-ctnet-dmacc.org) and DCP's *Serious Adverse Event Report Form: Instructions for Completion and Submission* found at https://prevention.cancer.gov/clinical-trials/clinical-trials-management/cp-ctnet-instructions-forms.

13.2.2.2 Contact the DCP Medical Monitor, Protocol PI, and DCP Regulatory Contractor's Safety Department within 24 hours of knowledge of the event. Contact via email is preferred, but phone contact is acceptable.

Malgorzata Wojtowicz, MD
NIH National Cancer Institute
Division of Cancer Prevention
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Bethesda, MD 20892
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The contact information for the DCP Regulatory Contractor's Safety Department is: phone: 650-691-4400 x133; email: safety@ccsainc.com).

Include the following information when contacting the DCP Medical Monitor, Protocol PI, and DCP Regulatory Contractor's Safety Department:

- Participant ID
- Date and time of SAE onset
- Date and time the accruing LAO or AO was notified about the SAE by the study participant or other person(s)
- Name of person reporting the SAE
- Call back phone number and email address
- Accruing LAO or AO at which the subject is enrolled DCP protocol number

- Title of protocol
- Suspected drugs (if any)
- Description of the SAE, including attribution to the Investigational Agent

13.2.2.3 The accruing LAO and AOs will email written SAE reports to the DCP Medical Monitor, Protocol PI, LAO Coordinator, and DCP Regulatory Contractor's Safety Department within 48 hours of learning of the event using the Word SAE Report Form.

13.2.2.4 The DCP Medical Monitor and the DCP Regulatory Contractor will determine which SAEs require submission to FDA or manufacturer as expedited safety reports.

13.2.2.5 The accruing LAO and AOs will comply with applicable regulatory requirements related to reporting SAEs to the CIRB and local IRB/IEC as applicable. Specifically, if an SAE meets the definition of an unanticipated problem (UP; i.e., requires expedited reporting to FDA or the manufacturer as a safety report [serious, unexpected, and related to a study agent]), then it needs to be reported to the CIRB by the Signatory Institution PI at the accruing LAO or AO where the SAE occurred (see SOP 02-02 *Reporting Protocol Deviations* for more information). In addition to CIRB requirements, UPs must be reported to the accruing LAO's or AO's local IRB per local requirements.

13.2.3 Follow-up of SAE

Accruing LAO or AO staff should send follow-up reports as requested when additional information is available. Additional information should be entered on the DCP SAE Report Form in the appropriate format. Follow-up information should be sent to the DCP Medical Monitor, Protocol PI, LAO Coordinator, and DCP Regulatory Contractor's Safety Department as soon as available. SAEs determined to be related to study drug will be followed for 30 days or until resolution of SAE or stabilization, whichever comes sooner. SAEs determined to be unrelated to study drug will be followed for 30 days or until resolution, whichever comes sooner.

14. STUDY MONITORING

14.1 Data Management

This study will report clinical data using Medidata RAVE, a cloud–based clinical trials data management system managed by the DMACC. RAVE will be the database of record for the protocol and subject to NCI and FDA audit. All RAVE users will be trained to use the system and will comply with the instructions in the guidelines provided to the LAO by the DMACC as well as applicable regulatory requirements such as 21 CFR; Part 11.

14.2 Electronic Case Report Forms

The System Variable and Attribute Report (SVAR) template will be used to create the study-specific eCRFs or SVAR workbook. DMACC will contact the LAO to determine if a meeting is needed to discuss the trial and eCRFs before the DMACC creates the draft SVAR. The SVAR template contains NCI Common Data Elements (CDEs) to facilitate data collection and analysis across studies. The SVAR template may require modification to capture the unique data elements (i.e., biomarkers) of each protocol and prepare a protocol-specific SVAR workbook. NCI CDEs, where available, shall be used for the initial SVAR workbooks and all subsequent workbook modifications.

More detailed information about the SVAR development process is available at: Program Resources | CP-CTNet DMACC website (cp-ctnet-dmacc.org)

14.3 Source Documents

Source documentation for this trial will consist of protocol-specific source documents as well as selected clinical records pertinent for eligibility, medical history and physical findings, adverse events and study endpoints. Only those specific records will be copied for the source chart to preserve subject confidentiality.

14.4 Data and Safety Monitoring Plan

The University of Arizona Cancer Center (UACC) Data and Safety Monitoring Board (DSMB) will provide oversight for subject safety for all UA CP-CTNet clinical trials consistent with the National Institutes of Health Policy for Data and Safety Monitoring dated June 10, 1998; further guidance statement issued by the NIH on June 5, 2000, and the policy for Data and Safety Monitoring by Data and Safety Monitoring Boards. The UACC DSMB meets quarterly.

Regular study-specific meetings will be used as a forum to review accrual rates, problematic issues relating to accrual and protocol implementation, adverse events occurrence, follow-up, and reporting; submission of all required study reports; and progress and outcomes of laboratory analyses.

14.5 Sponsor or FDA Monitoring

The NCI, DCP (or their designee), pharmaceutical collaborator (or their designee), or FDA may monitor/audit various aspects of the study. These monitors will be given access to facilities, databases, supplies and records to review and verify data pertinent to the study.

14.6 Record Retention

Clinical records for all participants, including eCRFs, all source documentation (containing evidence of study eligibility, history and physical findings, laboratory data, results of consultations, *etc*.), as well as CIRB records and other regulatory documentation will be retained by the Investigator in a secure storage facility in compliance with Health Insurance Portability and Accountability Act (HIPAA), Office of Human Research Protections (OHRP), Food and Drug Administration (FDA) regulations and guidances, and NCI/DCP requirements, unless the standard at the site is more stringent. The records for all studies performed under an IND will be maintained, at a minimum, for two years after the approval of a New Drug Application (NDA). For NCI/DCP, records will be retained for at least three years after the completion of the research. NCI will be notified prior to the planned destruction of any materials. The records should be accessible for inspection and copying by authorized persons of the Food and Drug Administration. If the study is done outside of the United States, applicable regulatory requirements for the specific country participating in the study also apply.

14.7 Cooperative Research and Development Agreement (CRADA)/Clinical Trials Agreement (CTA)

The agent(s) supplied by DCP, NCI used in this protocol, is/are provided to the NCI under a Collaborative Agreement (CTA) between the Nutraceutical Company, Nutramax, Inc (hereinafter referred to as Collaborator(s))

and the NCI Division of Cancer Prevention. Therefore, the following obligations/guidelines, in addition to the provisions in the "Intellectual Property Option to Collaborator" contained within the terms of award, apply to the use of Agent(s) in this study:

14.7.1 Agent(s) may not be used for any purpose outside the scope of this protocol, nor can Agent(s) be transferred or licensed to any party not participating in the clinical study. Collaborator(s) data for Agent(s) are confidential and proprietary to Collaborator(s) and shall be maintained as such by the investigators. The protocol documents for studies utilizing investigational agents contain confidential information and should not be shared or distributed without the permission of the NCI. If a Participant participating on the study or participant's family member requests a copy of this protocol, the individual should sign a confidentiality agreement. A suitable model agreement can be downloaded from the DCP website.

14.7.2 For a clinical protocol where there is an Investigational Agent used in combination with (an) other Investigational Agent(s), each the subject of different collaborative agreements, the access to and use of data by each Collaborator shall be as follows (data pertaining to such combination use shall hereinafter be referred to as "Multi-party Data").

14.7.3 NCI must provide all Collaborators with prior written notice regarding the existence and nature of any agreements governing their collaboration with NIH, the design of the proposed combination protocol, and the existence of any obligations that would tend to restrict NCI's participation in the proposed combination protocol.

14.7.4 Each Collaborator shall agree to permit use of the Multi-Party Data from the clinical trial by any other Collaborator solely to the extent necessary to allow said other Collaborator to develop, obtain regulatory approval, or commercialize its own investigational agent.

14.7.5 Any Collaborator having the right to use the Multi-Party Data from these trials must agree in writing prior to the commencement of the trials that it will use the Multi-Party Data solely for development, regulatory approval, and commercialization of its own investigational agent.

14.7.6 Clinical Trial Data and Results and Raw Data developed under a collaborative agreement will be made available exclusively to Collaborator(s), the NCI, and the FDA, as appropriate. All data made available will comply with HIPAA regulations.

14.7.7 When a Collaborator wishes to initiate a data request, the request should first be sent to the NCI, who will then notify the appropriate investigators of Collaborator's wish to contact them.

14.7.8 Any manuscripts reporting the results of this clinical trial must be provided to DCP for immediate delivery to Collaborator(s) for advisory review and comment prior to submission for publication. Collaborator(s) will have 30 days (or as specified in the CTA) from the date of receipt for review. Collaborator shall have the right to request that publication be delayed for up to an additional 30 days in order to ensure that Collaborator's confidential and proprietary data, in addition to Collaborator(s)'s intellectual property rights, are protected. Copies of abstracts must be provided to DCP for forwarding to Collaborator(s) for courtesy review as soon as possible and preferably at least three days prior to submission, but in any case, prior to presentation at the meeting or publication in the proceedings. Press releases and other media presentations must also be forwarded to DCP prior to release. Copies of any manuscript, abstract, and/or press release/ media presentation should be sent to the Protocol Information Office at NCI_DCP_PIO@mail.nih.gov.

The Protocol Information Office will forward manuscripts to the DCP Project Officer for distribution to the Collaborator(s). No publication, manuscript or other form of public disclosure shall contain any of Collaborator's confidential/proprietary information.

15. STATISTICAL CONSIDERATIONS

15.1 Study Design/Description

This is a phase II, randomized, double-blinded, placebo-controlled study to evaluate whether BSSE can upregulate Phase II detoxification pathways in firefighters.

15.2 Randomization/Stratification

Each participant will be randomly assigned to BSSE or matched placebo. Block randomization will be used to achieve balance in treatment group allocation.

15.3 Sample Size

We are planning to randomize 33 participants to each group (i.e., BSSE and matched placebo) to achieve an evaluable sample size of 30 participants in each group (assuming loss to follow-up of <10%), where evaluable is defined as the initiation of treatment and the availability of pre-intervention and end-of-intervention urine samples for urinary acetaminophen mercapturate, a surrogate for detoxification of the carcinogen benzene to SPMA. A sample size of 30 per group produces a one-sided 95% CI with a width no wider than 0.432 pooled standard deviations for the difference in change of log-transformed total amount of acetaminophen mercapturate excreted in the urine at the end of intervention from baseline between the BSSE and placebo groups. This sample size (i.e. 30/group) is large enough to reasonably estimate the parameters for use in a future definitive trial and also consistent with the recommended sample size for a future trial designed with 90% power for detecting a small effect size of 0.20 Cohen's d at a two-sided significance level of 5%[65]. In addition, a sample size of 30/group will also help us assess the potential efficacy of BSSE, by giving 80% power to detect an effect size of 0.65 Cohen's d for change in log-transformed total amount of acetaminophen mercapturate based on a two-sample t-test at a one-sided significance level of 5%. The anticipated effect size for changes in acetaminophen mercapturate—a surrogate marker for detoxification of electrophilic environmental toxicants such as benzene, acrolein, and crotonaldehyde—is informed by our prior study of BSSE[48] which showed a consistent and significant increase in the level of conjugated detoxification products of benzene, acrolein and crotonaldhyde in most subjects evaluated.

While metabolism of drugs and toxicants varies widely across individuals, smokers typically exhibit elevated baseline detoxification activity due to chronic exposure-induced enzyme upregulation. As a result, smokers tend to be rapid metabolizers[67]. As such, we anticipate an even greater change in acetaminophen detoxification products among healthy nonsmokers, who lack this background stimulation. However, to ensure a conservative and rigorous design, the current study is powered based on the observed effect size in smokers. A substantial body of literature supports the existence of both low and rapid metabolizers, reinforcing the premise that susceptibility to cancer from environmental exposures is closely tied to an individual's detoxification capacity—an inherently variable and modifiable trait even in healthy populations.PASS 2022 was used to perform sample size and power calculations.

15.4 Primary Objective, Endpoint(s), Analysis Plan

The primary objective of this study is to evaluate the effects of BSSE on the urinary excretion of mercapturic acid of acetaminophen following acetaminophen dosing, a surrogate for detoxification of the carcinogen benzene. The change (from baseline to end-of-intervention) in total amount of acetaminophen mercapturate excreted in the urine after acetaminophen dosing will be compared between BSSE and placebo arms. Log-transformation will be applied to total amount of acetaminophen mercapturate before deriving the changes at the end of intervention from baseline since the total amount of acetaminophen mercapturate may be highly right skewed. Even if the total amount of acetaminophen mercapturate is not highly right skewed, log-transformation will also allow us to compare the relative (%) change in total amount of acetaminophen mercapturate at the end of intervention from baseline between the BSSE and placebo groups through use of the geometric mean of total amount of acetaminophen mercapturate. The relative (%) change and the associated 95% CI by group, as well as the difference in the relative (%) change between BSSE and placebo groups and the associated 95% CI, will be reported. A two-sample t test will be performed to compare changes in the log-transformed total amounts of acetaminophen mercapturate between BSSE and placebo.

15.5 Secondary Objectives, Endpoints, Analysis Plans

The secondary objectives and their respective endpoints are:

1) To evaluate the effects of BSSE on urinary excretion of acetaminophen glucuronide, a surrogate for detoxification of PAHs. Similar to the total amount of acetaminophen mercapturate, log-transformation will be applied to the total amount of acetaminophen glucuronide excreted in the urine before deriving the changes at the end of intervention from baseline. A two-sample t test will be performed to compare changes in the log-transformed total amounts of acetaminophen glucuronide between BSSE and placebo.
2) To evaluate the safety and tolerability of BSSE, as measured by NCI CTCAE version 5. To evaluate the safety of BSSE, toxicity will be measured by adverse events (AE) classified in accordance with NCI CTCAE version 5. The frequency and associated percentage of each specific AE will be tabulated by treatment group and then compared via Fisher's exact test. Tolerability will be measured by adherence rate and will be compared between the BSSE and placebo via Fisher's exact test.

The exploratory objectives and their respective endpoints and analysis plans are:

1) To evaluate whether the GSTM1 and GSTT1 genotypes are important genetic modulators of BSSE-induced detoxification of carcinogens in the setting of exposure to BSSE. The correlation of *GSTM1* and *GSTT1* genotypes with detoxication of acetaminophen mercapturate/acetaminophen glucuronide will be assessed by linear regression for changes from baseline in the log-transformed acetaminophen mercapturate/acetaminophen glucuronide, in which BSSE and genotype indicators and interactions of BSSE, and genotype indicators are the covariates in the model. This will allow us to study whether the BSSE effect is significantly modulated by the genotypes.
2) To explore the effects of BSSE on the urinary metabolome after fire/fuel leak activities. Urinary metabolome will be measured at baseline, post fire exposure, and post study agent intervention. A linear mixed effects model with BSSE and post-intervention indicators and their interaction term as the covariates will be fitted to changes from baseline in each of the log-transformed metabolite levels. This model will allow us to compare the change between BSSE and placebo at post fire exposure and post-intervention, respectively, as well as comparing the changes at post-intervention from post fire exposure between BSSE and placebo.

3) To assess the effects of BSSE on the urinary excretion of mercapturic acid of benzene after fire/fuel leak activities. Urine excretion of mercapturic acid of benzene will be measured at baseline, post fire exposure, and post study agent intervention. A linear mixed effects model with BSSE and post-intervention indicators and their interaction term as the covariates will be fitted to changes from baseline in the log-transformed levels of mercapturic acid of benzene. This model will allow us to compare the change between BSSE and placebo at post fire exposure and post-intervention, respectively, as well as comparing the changes at post-intervention from post fire exposure between BSSE and placebo.
4) To evaluate the effects of BSSE on the urinary excretion of metabolites of PAHs after fire/fuel leak activities. PAH metabolites will be measured at baseline, post fire exposure, and post study agent intervention. A linear mixed effects model with BSSE and post-intervention indicators and their interaction term as the covariates will be fitted to changes from baseline in each of the log-transformed PAH metabolite levels. This model will allow us to compare the change between BSSE and placebo at post fire exposure and post-intervention, respectively, as well as comparing the changes at post-intervention from post fire exposure between BSSE and placebo.
5) To assess the effects of BSSE on pre- to post-intervention modifications of epigenetics (blood microRNA and DNA methylation). Two-sample t tests will be performed to compare changes in log-transformed expression levels between BSSE and placebo.

Due to the exploratory nature of the secondary and exploratory endpoints and multiple comparisons associated with the evaluations, the false discovery rate (FDR) will be controlled at 10% using the Benjamin-Hochberg procedure and the results and findings will be interpreted cautiously. In addition, the total number of comparisons will be reported to allow one to control for FDR at a specific rate, say, ≤10% via the adjusted p-value method. For exploratory endpoints, linear regression may be used to control for variables related to fire/fuel leak exposure when evaluating the effects of BSSE on those endpoints.

15.6 Reporting and Exclusions

The definition of "evaluable" for the primary endpoint analysis is a participant who initiated protocol treatment and provided both pre-study agent intervention and post-study agent intervention urine samples for urinary acetaminophen mercapturate. All of the evaluable participants will be included in the primary analysis.

Participants are considered compliant for secondary "per protocol" statistical analysis if they have **taken ≥ 80% of their assigned study agent doses, as assessed by Study Diary and pill count.**

15.7 Evaluation of Toxicity

All participants will be evaluable for toxicity from the time of their first dose of BSSE vs. placebo. Descriptive statistics of the type and frequency of all AEs will be generated, including 95% confidence intervals.

15.8 Evaluation of Response

All participants with endpoint data will be assessed for response to intervention, based on the endpoints described above in sections 15.4 and 15.5.

15.9 Interim Analysis

None.

15.10 Ancillary Studies

None.

16. REGULATORY AND ETHICAL CONSIDERATIONS

16.1 Required Documents

Besides the regulatory information that will be entered into the Registration and Credential Repository (see Section 5.1), the following documents are also required:

16.1.1 Documentation of Federalwide Assurance (FWA) number for the LAO and all AOs.

16.1.2 Signed Investigator's Brochure/Package Insert acknowledgement form

16.1.3 Delegation of Tasks Log form for the Lead Accruing Organization and all Accruing Sites signed by the PI for each site and initialed by all study personnel listed on the form.

16.2 Informed Consent

All potential study participants will be given a copy of the CIRB-approved Informed Consent to review. The investigator will explain all aspects of the study in lay language and answer all questions regarding the study. If the participant decides to participate in the study, he/she will be asked to sign and date the Informed Consent document. The study agent(s) will not be released to a participant who has not signed the Informed Consent document. Individuals who refuse to participate or who withdraw from the study will be treated without prejudice.

Participants must be provided the option to allow the use of blood samples, other body fluids, and tissues obtained during testing, operative procedures, or other standard medical practices for further research purposes. If applicable, statement of this option should be included within the informed consent document.

Prior to study initiation, the informed consent document must be reviewed and approved by NCI, DCP, and the NCI CIRB. The NCI CIRB approves a model consent for each protocol. Each Signatory Institution inserts their CIRB-approved institutional boilerplate language into the model consent to create the CIRB-approved consent. If the model informed consent document is amended, Signatory Institutions must use the revised model informed consent document and insert their CIRB-approved institutional boilerplate language at the time the change becomes active.

The NCI CIRB is the IRB of record and is the only IRB authorized to approve changes to the protocol or informed consent document. Institutions may require additional oversight that involves the local IRB, but the local IRB is not responsible for any regulatorily-required IRB actions.

16.3 Collection of Regulatory Documents

Regulatory documents will be collected by the DCP regulatory contractor and reviewed for completeness and accuracy.

16.4 Other

This trial will be conducted in compliance with the protocol, the International Conference on Harmonisation's (ICH) Good Clinical Practice (GCP) guidelines, and the applicable regulatory requirements.

17. ROSTER MANAGEMENT

The LAO is responsible for establishing, maintaining, and monitoring all its members that participate in CP-CTNet studies. The LAO must have a "real-time," comprehensive, consolidated roster of all its members with their relevant Cancer Therapy Evaluation Program (CTEP) institution codes, associated investigators, and research staff. This roster information is used for determining compliance with monitoring requirements.

The LAO's organizational rosters will be managed by the CP-CTNet Roster Management System website (https://applications.prevention.cancer.gov/cp-ctnet). Requests to add memberships to a roster will be done via this website. All requests require that the following documents be uploaded:

- Consortium Letter of Commitment
- Site Letter of Commitment
- CV/NIH Biosketch

18. FINANCING, EXPENSES, AND/OR INSURANCE

Study procedures performed during study visits will be covered by the study budget. Research tests, including serum and tissue biomarker evaluations, will not be billed to the subject. Subjects may incur minimal out-of-pocket expenses such as transportation but will not be charged for study agent or any study-related activities. Subjects that complete all required visits and procedures will receive monetary compensation of $350 U.S. dollars which they may use at their discretion for out-of-pocket cost such as transportation. If a subject is unable to complete the entire study, the amount of compensation will be based on how long the subject is in the study. If injury occurs, medical care will be provided and charged to the subject's insurer.

REFERENCES

(1) National Fire Department Registry Quick Facts. **2022**.
(2) Laroche, E.; L'Esperance, S. Cancer Incidence and Mortality among Firefighters: An Overview of Epidemiologic Systematic Reviews. *Int J Environ Res Public Health* **2021**, *18* (5). DOI: 10.3390/ijerph18052519.
(3) Cherry, N.; Galarneau, J. M.; Kinniburgh, D.; Quemerais, B.; Tiu, S.; Zhang, X. Exposure and Absorption of PAHs in Wildland Firefighters: A Field Study with Pilot Interventions. *Ann Work Expo Health* **2021**, *65* (2), 148-161. DOI: 10.1093/annweh/wxaa064. Rossbach, B.; Wollschlager, D.; Letzel, S.; Gottschalk, W.; Muttray, A. Internal exposure of firefighting instructors to polycyclic aromatic hydrocarbons (PAH) during live fire training. *Toxicol Lett* **2020**, *331*, 102-111. DOI: 10.1016/j.toxlet.2020.05.024.
(4) Humans, I. W. G. o. t. E. o. C. R. t. Painting, firefighting, and shiftwork. *IARC Monogr Eval Carcinog Risks Hum* **2010**, *98*, 9-764. Austin, C. C.; Wang, D.; Ecobichon, D. J.; Dussault, G. Characterization of volatile organic compounds in smoke at municipal structural fires. *J Toxicol Environ Health A* **2001**, *63* (6), 437-458. DOI: 10.1080/152873901300343470. Atlas, E. L.; Donnelly, K. C.; Giam, C. S.; McFarland, A. R. Chemical and biological characterization of emissions from a fireperson training facility. *Am Ind Hyg Assoc J* **1985**, *46* (9), 532-540. DOI: 10.1080/15298668591395300.

(5) Burgess, J. L.; Witten, M. L.; Nanson, C. J.; Hysong, T. A.; Sherrill, D. L.; Quan, S. F.; Gerkin, R.; Bernard, A. M. Serum pneumoproteins: a cross-sectional comparison of firefighters and police. *Am J Ind Med* **2003**, *44* (3), 246-253. DOI: 10.1002/ajim.10269.
(6) Fernando, S.; Shaw, L.; Shaw, D.; Gallea, M.; VandenEnden, L.; House, R.; Verma, D. K.; Britz-McKibbin, P.; McCarry, B. E. Evaluation of Firefighter Exposure to Wood Smoke during Training Exercises at Burn Houses. *Environ Sci Technol* **2016**, *50* (3), 1536-1543. DOI: 10.1021/acs.est.5b04752.
(7) Tsai, R. J.; Luckhaupt, S. E.; Schumacher, P.; Cress, R. D.; Deapen, D. M.; Calvert, G. M. Risk of cancer among firefighters in California, 1988-2007. *Am J Ind Med* **2015**, *58* (7), 715-729. DOI: 10.1002/ajim.22466. Jalilian, H.; Ziaei, M.; Weiderpass, E.; Rueegg, C. S.; Khosravi, Y.; Kjaerheim, K. Cancer incidence and mortality among firefighters. *Int J Cancer* **2019**, *145* (10), 2639-2646. DOI: 10.1002/ijc.32199. Baris, D.; Garrity, T. J.; Telles, J. L.; Heineman, E. F.; Olshan, A.; Zahm, S. H. Cohort mortality study of Philadelphia firefighters. *Am J Ind Med* **2001**, *39* (5), 463-476. DOI: 10.1002/ajim.1040. Kang, D.; Davis, L. K.; Hunt, P.; Kriebel, D. Cancer incidence among male Massachusetts firefighters, 1987-2003. *Am J Ind Med* **2008**, *51* (5), 329-335. DOI: 10.1002/ajim.20549. Bates, M. N. Registry-based case-control study of cancer in California firefighters. *Am J Ind Med* **2007**, *50* (5), 339-344. DOI: 10.1002/ajim.20446. Ma, F.; Fleming, L. E.; Lee, D. J.; Trapido, E.; Gerace, T. A.; Lai, H.; Lai, S. Mortality in Florida professional firefighters, 1972 to 1999. *Am J Ind Med* **2005**, *47* (6), 509-517. DOI: 10.1002/ajim.20160. LeMasters, G. K.; Genaidy, A. M.; Succop, P.; Deddens, J.; Sobeih, T.; Barriera-Viruet, H.; Dunning, K.; Lockey, J. Cancer risk among firefighters: a review and meta-analysis of 32 studies. *J Occup Environ Med* **2006**, *48* (11), 1189-1202. DOI: 10.1097/01.jom.0000246229.68697.90.
(8) Fent, K. W.; Toennis, C.; Sammons, D.; Robertson, S.; Bertke, S.; Calafat, A. M.; Pleil, J. D.; Geer Wallace, M. A.; Kerber, S.; Smith, D. L.; et al. Firefighters' and instructors' absorption of PAHs and benzene during training exercises. *Int J Hyg Environ Health* **2019**, *222* (7), 991-1000. DOI: 10.1016/j.ijheh.2019.06.006.
(9) Daniels, R. D.; Kubale, T. L.; Yiin, J. H.; Dahm, M. M.; Hales, T. R.; Baris, D.; Zahm, S. H.; Beaumont, J. J.; Waters, K. M.; Pinkerton, L. E. Mortality and cancer incidence in a pooled cohort of US firefighters from San Francisco, Chicago and Philadelphia (1950-2009). *Occup Environ Med* **2014**, *71* (6), 388-397. DOI: 10.1136/oemed-2013-101662.
(10) Ma, F.; Fleming, L. E.; Lee, D. J.; Trapido, E.; Gerace, T. A. Cancer incidence in Florida professional firefighters, 1981 to 1999. *J Occup Environ Med* **2006**, *48* (9), 883-888. DOI: 10.1097/01.jom.0000235862.12518.04.
(11) Lee, D. J.; Koru-Sengul, T.; Hernandez, M. N.; Caban-Martinez, A. J.; McClure, L. A.; Mackinnon, J. A.; Kobetz, E. N. Cancer risk among career male and female Florida firefighters: Evidence from the Florida Firefighter Cancer Registry (1981-2014). *Am J Ind Med* **2020**, *63* (4), 285-299. DOI: 10.1002/ajim.23086.
(12) Goodrich, J. M.; Jung, A. M.; Furlong, M. A.; Beitel, S.; Littau, S.; Gulotta, J.; Wallentine, D.; Burgess, J. L. Repeat measures of DNA methylation in an inception cohort of firefighters. *Occup Environ Med* **2022**. DOI: 10.1136/oemed-2021-108153.
(13) Jeong, K. S.; Zhou, J.; Griffin, S. C.; Jacobs, E. T.; Dearmon-Moore, D.; Zhai, J.; Littau, S. R.; Gulotta, J.; Moore, P.; Peate, W. F.; et al. MicroRNA Changes in Firefighters. *J Occup Environ Med* **2018**, *60* (5), 469-474. DOI: 10.1097/JOM.0000000000001307.
(14) Jung, A. M.; Zhou, J.; Beitel, S. C.; Littau, S. R.; Gulotta, J. J.; Wallentine, D. D.; Moore, P. K.; Burgess, J. L. Longitudinal evaluation of whole blood miRNA expression in firefighters. *J Expo Sci Environ Epidemiol* **2021**, *31* (5), 900-912. DOI: 10.1038/s41370-021-00306-8.
(15) Zhou, J.; Jenkins, T. G.; Jung, A. M.; Jeong, K. S.; Zhai, J.; Jacobs, E. T.; Griffin, S. C.; Dearmon-Moore, D.; Littau, S. R.; Peate, W. F.; et al. DNA methylation among firefighters. *PLoS One* **2019**, *14* (3), e0214282. DOI: 10.1371/journal.pone.0214282.
(16) Andersen, M. H. G.; Saber, A. T.; Clausen, P. A.; Pedersen, J. E.; Lohr, M.; Kermanizadeh, A.; Loft, S.; Ebbehoj, N.; Hansen, A. M.; Pedersen, P. B.; et al. Association between polycyclic aromatic hydrocarbon exposure and peripheral blood mononuclear cell DNA damage in human volunteers during fire extinction exercises. *Mutagenesis* **2018**, *33* (1), 105-115. DOI: 10.1093/mutage/gex021.
(17) Glass, D. C.; Del Monaco, A.; Pircher, S.; Vander Hoorn, S.; Sim, M. R. Mortality and cancer incidence at a fire training college. *Occup Med (Lond)* **2016**, *66* (7), 536-542. DOI: 10.1093/occmed/kqw079.
(18) Rosting, C.; Olsen, R. Biomonitoring of the benzene metabolite s-phenylmercapturic acid and the toluene metabolite s-benzylmercapturic acid in urine from firefighters. *Toxicol Lett* **2020**, *329*, 20-25. DOI: 10.1016/j.toxlet.2020.04.018.
(19) Mayer, A. C.; Fent, K. W.; Wilkinson, A.; Chen, I. C.; Kerber, S.; Smith, D. L.; Kesler, R. M.; Horn, G. P. Characterizing exposure to benzene, toluene, and naphthalene in firefighters wearing different types of new or laundered PPE. *Int J Hyg Environ Health* **2022**, *240*, 113900. DOI: 10.1016/j.ijheh.2021.113900.
(20) Ross, D. The role of metabolism and specific metabolites in benzene-induced toxicity: evidence and issues. *J Toxicol Environ Health A* **2000**, *61* (5-6), 357-372. DOI: 10.1080/00984100050166361.
(21) Smith, M. T.; Zhang, L.; McHale, C. M.; Skibola, C. F.; Rappaport, S. M. Benzene, the exposome and future investigations of leukemia etiology. *Chem Biol Interact* **2011**, *192* (1-2), 155-159. DOI: 10.1016/j.cbi.2011.02.010.
(22) Smith, M. T. The mechanism of benzene-induced leukemia: a hypothesis and speculations on the causes of leukemia. *Environ Health Perspect* **1996**, *104 Suppl 6*, 1219-1225. DOI: 10.1289/ehp.961041219.

(23) Vondracek, J.; Machala, M. The Role of Metabolism in Toxicity of Polycyclic Aromatic Hydrocarbons and their Non-genotoxic Modes of Action. *Curr Drug Metab* **2021**, *22* (8), 584-595. DOI: 10.2174/1389200221999201125205725. Baird, W. M.; Hooven, L. A.; Mahadevan, B. Carcinogenic polycyclic aromatic hydrocarbon-DNA adducts and mechanism of action. *Environ Mol Mutagen* **2005**, *45* (2-3), 106-114. DOI: 10.1002/em.20095.
(24) Hernandez, L. G.; van Steeg, H.; Luijten, M.; van Benthem, J. Mechanisms of non-genotoxic carcinogens and importance of a weight of evidence approach. *Mutat Res* **2009**, *682* (2-3), 94-109. DOI: 10.1016/j.mrrev.2009.07.002. Nohmi, T. Thresholds of Genotoxic and Non-Genotoxic Carcinogens. *Toxicol Res* **2018**, *34* (4), 281-290. DOI: 10.5487/TR.2018.34.4.281.
(25) Prochaska, H. J.; Santamaria, A. B.; Talalay, P. Rapid detection of inducers of enzymes that protect against carcinogens. *Proceedings of the National Academy of Sciences of the United States of America* **1992**, *89* (6), 2394-2398.
(26) Kensler, T. W. Chemoprevention by inducers of carcinogen detoxication enzymes. *Environmental health perspectives* **1997**, *105 Suppl 4*, 965-970. Itoh, K.; Chiba, T.; Takahashi, S.; Ishii, T.; Igarashi, K.; Katoh, Y.; Oyake, T.; Hayashi, N.; Satoh, K.; Hatayama, I.; et al. An Nrf2/small Maf heterodimer mediates the induction of phase II detoxifying enzyme genes through antioxidant response elements. *Biochemical and biophysical research communications* **1997**, *236* (2), 313-322.
(27) Zhang, Y.; Tang, L. Discovery and development of sulforaphane as a cancer chemopreventive phytochemical. *Acta pharmacologica Sinica* **2007**, *28* (9), 1343-1354. DOI: 10.1111/j.1745-7254.2007.00679.x.
(28) Hong, F.; Freeman, M. L.; Liebler, D. C. Identification of sensor cysteines in human Keap1 modified by the cancer chemopreventive agent sulforaphane. *Chemical research in toxicology* **2005**, *18* (12), 1917-1926. DOI: 10.1021/tx0502138.
(29) Kensler, T. W.; Egner, P. A.; Agyeman, A. S.; Visvanathan, K.; Groopman, J. D.; Chen, J. G.; Chen, T. Y.; Fahey, J. W.; Talalay, P. Keap1-nrf2 signaling: a target for cancer prevention by sulforaphane. *Topics in current chemistry* **2013**, *329*, 163-177. DOI: 10.1007/128_2012_339.
(30) Fahey, J. W.; Zhang, Y.; Talalay, P. Broccoli sprouts: an exceptionally rich source of inducers of enzymes that protect against chemical carcinogens. *Proceedings of the National Academy of Sciences of the United States of America* **1997**, *94* (19), 10367-10372.
(31) Kensler, T. W.; Chen, J. G.; Egner, P. A.; Fahey, J. W.; Jacobson, L. P.; Stephenson, K. K.; Ye, L.; Coady, J. L.; Wang, J. B.; Wu, Y.; et al. Effects of glucosinolate-rich broccoli sprouts on urinary levels of aflatoxin-DNA adducts and phenanthrene tetraols in a randomized clinical trial in He Zuo township, Qidong, People's Republic of China. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology* **2005**, *14* (11 Pt 1), 2605-2613. DOI: 10.1158/1055-9965.EPI-05-0368. Egner, P. A.; Chen, J. G.; Wang, J. B.; Wu, Y.; Sun, Y.; Lu, J. H.; Zhu, J.; Zhang, Y. H.; Chen, Y. S.; Friesen, M. D.; et al. Bioavailability of Sulforaphane from two broccoli sprout beverages: results of a short-term, cross-over clinical trial in Qidong, China. *Cancer prevention research* **2011**, *4* (3), 384-395. DOI: 10.1158/1940-6207.CAPR-10-0296. Kensler, T. W.; Ng, D.; Carmella, S. G.; Chen, M.; Jacobson, L. P.; Munoz, A.; Egner, P. A.; Chen, J. G.; Qian, G. S.; Chen, T. Y.; et al. Modulation of the metabolism of airborne pollutants by glucoraphanin-rich and sulforaphane-rich broccoli sprout beverages in Qidong, China. *Carcinogenesis* **2012**, *33* (1), 101-107. DOI: 10.1093/carcin/bgr229. Egner, P. A.; Chen, J. G.; Zarth, A. T.; Ng, D. K.; Wang, J. B.; Kensler, K. H.; Jacobson, L. P.; Munoz, A.; Johnson, J. L.; Groopman, J. D.; et al. Rapid and sustainable detoxication of airborne pollutants by broccoli sprout beverage: results of a randomized clinical trial in China. *Cancer prevention research* **2014**, *7* (8), 813-823. DOI: 10.1158/1940-6207.CAPR-14-0103.
(32) Bauman, J. E.; Zang, Y.; Sen, M.; Li, C.; Wang, L.; Egner, P. A.; Fahey, J. W.; Normolle, D. P.; Grandis, J. R.; Kensler, T. W.; et al. Prevention of Carcinogen-Induced Oral Cancer by Sulforaphane. *Cancer prevention research* **2016**, *9* (7), 547-557. DOI: 10.1158/1940-6207.CAPR-15-0290.
(33) Bauman, J. E.; Hsu, C. H.; Centuori, S.; Guillen-Rodriguez, J.; Garland, L. L.; Ho, E.; Padi, M.; Bageerathan, V.; Bengtson, L.; Wojtowicz, M.; et al. Randomized Crossover Trial Evaluating Detoxification of Tobacco Carcinogens by Broccoli Seed and Sprout Extract in Current Smokers. *Cancers (Basel)* **2022**, *14* (9). DOI: 10.3390/cancers14092129.
(34) Fahey, J. W.; Haristoy, X.; Dolan, P. M.; Kensler, T. W.; Scholtus, I.; Stephenson, K. K.; Talalay, P.; Lozniewski, A. Sulforaphane inhibits extracellular, intracellular, and antibiotic-resistant strains of Helicobacter pylori and prevents benzo[a]pyrene-induced stomach tumors. *Proceedings of the National Academy of Sciences of the United States of America* **2002**, *99* (11), 7610-7615. DOI: 10.1073/pnas.112203099. Xu, C.; Huang, M. T.; Shen, G.; Yuan, X.; Lin, W.; Khor, T. O.; Conney, A. H.; Kong, A. N. Inhibition of 7,12-dimethylbenz(a)anthracene-induced skin tumorigenesis in C57BL/6 mice by sulforaphane is mediated by nuclear factor E2-related factor 2. *Cancer research* **2006**, *66* (16), 8293-8296. DOI: 10.1158/0008-5472.CAN-06-0300. Ramos-Gomez, M.; Kwak, M. K.; Dolan, P. M.; Itoh, K.; Yamamoto, M.; Talalay, P.; Kensler, T. W. Sensitivity to carcinogenesis is increased and chemoprotective efficacy of enzyme inducers is lost in nrf2 transcription factor-deficient mice. *Proceedings of the National Academy of Sciences of the United States of America* **2001**, *98* (6), 3410-3415. DOI: 10.1073/pnas.051618798.
(35) Cornblatt, B. S.; Ye, L.; Dinkova-Kostova, A. T.; Erb, M.; Fahey, J. W.; Singh, N. K.; Chen, M. S.; Stierer, T.; Garrett-Mayer, E.; Argani, P.; et al. Preclinical and clinical evaluation of sulforaphane for chemoprevention in the breast. *Carcinogenesis* **2007**, *28* (7), 1485-1490. DOI: 10.1093/carcin/bgm049. Zhang, Y.; Kensler, T. W.; Cho, C. G.; Posner, G. H.; Talalay, P. Anticarcinogenic activities of sulforaphane and structurally related synthetic norbornyl isothiocyanates. *Proceedings of the National Academy of Sciences of the United States of America* **1994**, *91* (8), 3147-3150.

(36) Ohkoshi, A.; Suzuki, T.; Ono, M.; Kobayashi, T.; Yamamoto, M. Roles of Keap1-Nrf2 system in upper aerodigestive tract carcinogenesis. *Cancer prevention research* **2013**, *6* (2), 149-159. DOI: 10.1158/1940-6207.CAPR-12-0401-T.
(37) Vetvicka, V.; Vetvickova, J. A Novel Glucan-Sulforaphane Combination Stimulates Immune Response to Influenza in Mouse Model. *American Journal of Immunology* **2016**, *12* (2), 20.28.
(38) Fahey, J. W.; Wade, K. L.; Stephenson, K. K.; Panjwani, A. A.; Liu, H.; Cornblatt, G.; Cornblatt, B. S.; Ownby, S. L.; Fuchs, E.; Holtzclaw, W. D.; et al. Bioavailability of Sulforaphane Following Ingestion of Glucoraphanin-Rich Broccoli Sprout and Seed Extracts with Active Myrosinase: A Pilot Study of the Effects of Proton Pump Inhibitor Administration. *Nutrients* **2019**, *11* (7). DOI: 10.3390/nu11071489.
(39) Bauman, J. E.; Hsu, C.-H.; Centuori, S.; Guillen-Rodriguez, J.; Garland, L.; Ho, E.; Bengston, L.; Wojtowicz, M.; Szabo, E.; Chow, H.-H. S. Randomized crossover trial evaluating detoxication of tobacco carcinogens by broccoli seed and sprout extract in heavy smokers. *Cancer research* **2021**.
(40) Evans, M.; Paterson, E.; Barnes, D. M. An open label pilot study to evaluate the efficacy of Spanish black radish on the induction of phase I and phase II enzymes in healthy male subjects. *BMC Complement Altern Med* **2014**, *14*, 475. DOI: 10.1186/1472-6882-14-475.
(41) Cook, S. F.; King, A. D.; van den Anker, J. N.; Wilkins, D. G. Simultaneous quantification of acetaminophen and five acetaminophen metabolites in human plasma and urine by high-performance liquid chromatography-electrospray ionization-tandem mass spectrometry: Method validation and application to a neonatal pharmacokinetic study. *J Chromatogr B Analyt Technol Biomed Life Sci* **2015**, *1007*, 30-42. DOI: 10.1016/j.jchromb.2015.10.013.
(42) Haiman, C. A.; Patel, Y. M.; Stram, D. O.; Carmella, S. G.; Chen, M.; Wilkens, L. R.; Le Marchand, L.; Hecht, S. S. Benzene Uptake and Glutathione S-transferase T1 Status as Determinants of S-Phenylmercapturic Acid in Cigarette Smokers in the Multiethnic Cohort. *PloS one* **2016**, *11* (3), e0150641. DOI: 10.1371/journal.pone.0150641.
(43) Wang, Y.; Meng, L.; Pittman, E. N.; Etheredge, A.; Hubbard, K.; Trinidad, D. A.; Kato, K.; Ye, X.; Calafat, A. M. Quantification of urinary mono-hydroxylated metabolites of polycyclic aromatic hydrocarbons by on-line solid phase extraction-high performance liquid chromatography-tandem mass spectrometry. *Anal Bioanal Chem* **2017**, *409* (4), 931-937. DOI: 10.1007/s00216-016-9933-x.
(44) Kelsey, K. T.; Nelson, H. H.; Wiencke, J. K.; Smith, C. M.; Levin, S. The glutathione S-transferase theta and mu deletion polymorphisms in asbestosis. *Am J Ind Med* **1997**, *31* (3), 274-279. DOI: 10.1002/(sici)1097-0274(199703)31:3<274::aid-ajim2>3.0.co;2-y.
(45) Furlong, M. A.; Liu, T.; Snider, J. M.; Tfaily, M. M.; Itson, C.; Beitel, S.; Parsawar, K.; Keck, K.; Galligan, J.; Walker, D. I.; et al. Evaluating changes in firefighter urinary metabolomes after structural fires: an untargeted, high resolution approach. *Sci Rep* **2023**, *13* (1), 20872. DOI: 10.1038/s41598-023-47799-x From NLM Medline.
(46) Goodrich, J. M.; Furlong, M. A.; Caban-Martinez, A. J.; Jung, A. M.; Batai, K.; Jenkins, T.; Beitel, S.; Littau, S.; Gulotta, J.; Wallentine, D.; et al. Differential DNA Methylation by Hispanic Ethnicity Among Firefighters in the United States. *Epigenet Insights* **2021**, *14*, 25168657211006159. DOI: 10.1177/25168657211006159 From NLM PubMed-not-MEDLINE. Goodrich, J. M.; Jung, A. M.; Furlong, M. A.; Beitel, S.; Littau, S.; Gulotta, J.; Wallentine, D.; Burgess, J. L. Repeat measures of DNA methylation in an inception cohort of firefighters. *Occup Environ Med* **2022**, *79* (10), 656-663. DOI: 10.1136/oemed-2021-108153 From NLM Medline.
(47) Forrest, J. A.; Clements, J. A.; Prescott, L. F. Clinical pharmacokinetics of paracetamol. *Clin Pharmacokinet* **1982**, *7* (2), 93-107. DOI: 10.2165/00003088-198207020-00001 From NLM.
(48) Barr, D. B.; Wilder, L. C.; Caudill, S. P.; Gonzalez, A. J.; Needham, L. L.; Pirkle, J. L. Urinary Creatinine Concentrations in the U.S. Population: Implications for Urinary Biologic Monitoring Measurements. *Environmental Health Perspectives* **2005**, *113* (2), 192-200. DOI: doi:10.1289/ehp.7337.

APPENDIX A
PERFORMANCE STATUS CRITERIA

Karnofsky Performance Scale

Percent	Description
100	Normal, no complaints, no evidence of disease.
90	Able to carry on normal activity; minor signs or symptoms of disease.
80	Normal activity with effort; some signs or symptoms of disease.
70	Cares for self, unable to carry on normal activity or to do active work.
60	Requires occasional assistance but is able to care for most of his/her needs.
50	Requires considerable assistance and frequent medical care.
40	Disabled, requires special care and assistance.
30	Severely disabled, hospitalization indicated. Death not imminent.
20	Very sick, hospitalization indicated. Death not imminent.
10	Moribund, fatal processes progressing rapidly.
0	Dead.

APPENDIX B

ALCOHOL AND TOBACCO QUESTIONNAIRE INSTRUCTIONS

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- Data collection will be required for all CP-CTNet studies.
    - Data will be collected at baseline and end of every study. Data may also be collected at follow-up visits as determined by each protocol. If you wish to collect additional information beyond these core elements, you may certainly do so. However, all studies need to collect the basic elements in the attached eCRFs.
    - The eCRFs will be completed by the Site Staff or participant at the time of the designated visit.
- Data will be submitted as part of the final clinical data set.

ALCOHOL ASSESSMENT-- BASELINE

REGISTERING INSTITUTION	PARTICIPANT ID	VISIT TYPE	VISIT DATE (MM/DD/YYYY)
_____	_____	_____	__ __ / __ __ / __ __ __ __

Instructions:

For the following questions about drinking alcoholic beverages, a drink means a 12 oz. beer, a 5 oz. glass of wine, or one and a half ounces of liquor.
When a number is requested in the response, please enter a whole number (i.e. "4") and not a range or fraction of a number.

1. In your entire life, have you had at least 12 drinks of any kind of alcoholic beverage?

   ☐ Yes
   ☐ No **(End)**
   ☐ Refuse to answer **(End)**
   ☐ Don't know/Not sure

2. In the past 12 months, on average, how often did you drink any type of alcoholic beverage?

   _____ (Enter the number of days you drank based on the timeframe checked below. Enter 0 if you never drank and skip to Question 6.)
   ☐ Week
   ☐ Month
   ☐ Year
   ☐ Refuse to answer
   ☐ Don't know/Not sure

3. In the past 12 months, on those days that you drank alcoholic beverages, on average, how many drinks did you have per day?

   _____ (Enter the average number of drinks per day)

   ☐ Refuse to answer
   ☐ Don't know/Not sure

4. In the past 12 months, on how many days did you have 5 or more drinks of any alcoholic beverage?

   _____ (Enter the number of days you had 5 or more drinks or enter 0 if none.)

   ☐ Refuse to answer
   ☐ Don't know/Not sure

5. Was there ever a time or times in your life when you drank 5 or more drinks of any kind of alcoholic beverage almost every day?

   ☐ Yes
   ☐ No
   ☐ Refuse to answer
   ☐ Don't know/Not sure

6. If you do not currently drink alcoholic beverages, but did in the past, how long has it been since you last drank regularly?

   ☐ Within the past month (0 to 1 month ago)
   ☐ Between 1 and 3 months (1 to 3 months ago)
   ☐ Between 3 and 6 months (3 to 6 months ago)
   ☐ Between 6 and 12 months (6 to 12 months ago)
   ☐ Between 1 and 5 years (1 to 5 years ago)
   ☐ Between 5 and 15 years (5 to 15 years ago)
   ☐ More than 15 years ago
   ☐ Don't know/Not sure
   ☐ Never drank regularly

7. At the heaviest point, either now or in the past, on the days when you drank, about how many drinks did you drink a day on the average?

   _____ (Enter the number of drinks a day)

   ☐ Refuse to answer
   ☐ Don't know/Not sure

8. How many years have you been drinking (or did drink) regularly?

   _____ years

   ☐ Refuse to answer
   ☐ Don't know/Not sure

9. At what age did you begin drinking regularly?

   _____ years of age

   ☐ Refuse to answer
   ☐ Don't know/Not sure

10. What type(s) of alcohol do you drink? (Mark ALL that apply)

- ☐ Wine
- ☐ Liquor
- ☐ Beer
- ☐ Wine cooler
- ☐ Other _____ (enter other type(s) of alcohol you drink)

CRF completed by (*check one*):

Study participant _____

Study site staff _____ Staff name (*optional*) _____

Date __ __ / __ __ / __ __ __ __
(MM/DD/YYYY)

ALCOHOL ASSESSMENT - FOLLOW-UP

REGISTERING INSTITUTION	PARTICIPANT ID	VISIT TYPE	VISIT DATE *(MM/DD/YYYY)*
_____	_____	_____	__ __ / __ __ / __ __ __ __

Instructions:

For the following questions about drinking alcoholic beverages, a drink means a 12 oz. beer, a 5 oz. glass of wine, or one and a half ounces of liquor.

When a number is requested in the response, please enter a whole number (i.e. "4") and not a range or fraction of a number.

1. During the past 30 days, did you drink any alcoholic beverages?

☐ Yes
☐ No **(End)**
☐ Refuse to answer **(End)**
☐ Don't know/Not sure

2. During the past 30 days, how many days per week or per month did you drink any alcoholic beverages, on the average?

_____ (Enter number of days you drank based on the timeframe checked below. Enter 0 if you did not drink.)

☐ Week
☐ Month
☐ Refuse to answer
☐ Don't know/Not sure

3. On the days when you drank, on average, about how many drinks did you have?

_____ (Enter the average number of drinks you had per day.)

☐ Refuse to answer
☐ Don't know/Not sure

4. In the past 30 days, on how many days did you have 5 or more drinks per day?

_____ (Enter the number of days you had 5 or more drinks or enter 0 if none.)

☐ Refuse to answer
☐ Do not know/Not sure

CRF completed by (*check one*):

Study participant _____

Study site staff _____ Staff name (*optional*) _____

Date __ __ / __ __ / __ __ __ __
(MM/DD/YYYY)

TOBACCO ASSESSMENT – BASELINE

REGISTERING INSTITUTION	PARTICIPANT ID	VISIT TYPE	VISIT DATE (MM/DD/YYYY)
_____	_____	_____	__ __/__ __/__ __ __ __

Instructions:
When a number is requested in the response, please enter a whole number (i.e. "4") and not a range or fraction of a number.

Section A. Basic Cigarette Use Information

1. Have you smoked at least 100 cigarettes (5 packs = 100 cigarettes) in your entire life?

☐ Yes
☐ No → **Skip to Section B**
☐ Don't know/Not sure → **Skip to Section B**

2. How old were you when you first smoked a cigarette (even one or two puffs)?

_____ Years old

3. How old were you when you first began smoking cigarettes regularly?

_____ Years old
☐ Check here if you have never smoked cigarettes regularly.

4. How many total years have you smoked (or did you smoke) cigarettes? Do not count any time you may have stayed off cigarettes.

_____ Years (If you smoked less than one year, write "1.")

5. On average when you have smoked, about how many cigarettes do you (or did you) smoke a day? (A pack usually has 20 cigarettes in it).

_____ Number of cigarettes per day

6. Do you NOW smoke cigarettes?

☐ Everyday
☐ Some days
☐ Not at all → **Skip to question 8**

7. How soon after you wake up do you smoke your first cigarette?

☐ Within 30 minutes
☐ After 30 minutes

8. How long has it been since you last smoked a cigarette (even one or two puffs)?

First check which one of the following choices applies to you. Then, if applicable, write a number on the line for how many days, weeks, months, or years it has been since your last cigarette.

☐ I smoked a cigarette today (at least one puff)
☐ 1-7 days → Number of days since last cigarette _____
☐ Less than 1 month → Number of weeks since last cigarette _____
☐ Less than 1 year → Number of months since last cigarette _____
☐ More than 1 year → Number of years since last cigarette _____
☐ Don't know/Don't remember

Section B. Use of Other Forms of Tobacco

9. Have you ever used other forms of tobacco, not including cigarettes?

☐ Yes
☐ No → **Skip to Section C**

10. How often do you/did you use other forms of tobacco?

☐ Every day → Number of times per day _____
☐ Some days → Number of days _____ per ☐ Week ☐ Month ☐ Year

11. Which of the following products have you ever used regularly?
Check all that apply

☐ Cigarettes
☐ E-cigarettes or other electronic nicotine delivery system
☐ Traditional cigars, cigarillos or filtered cigars
☐ Pipes
☐ Waterpipe
☐ Hookah
☐ Clove cigarettes or kreteks
☐ Bidis
☐ Smokeless tobacco, like dip, chew, or snuff
☐ Snus
☐ Paan with tobacco, gutka, zarda, khaini

☐ Other, Please specify: _____

12. If you do not currently use other forms of tobacco, but did in the past, how long has it been since you last used other forms of tobacco regularly?

☐ Within the past month (0 to 1 month ago)
☐ Between 1 and 3 months (1 to 3 months ago)
☐ Between 3 and 6 months (3 to 6 months ago)
☐ Between 6 and 12 months (6 to 12 months ago)
☐ Between 1 and 5 years (1 to 5 years ago)
☐ Between 5 and 15 years (5 to 15 years ago)
☐ More than 15 years ago
☐ Don't know/Not sure
☐ Never used other forms of tobacco regularly

Section C. Second-Hand Smoke Exposure

13. Are you currently living with a smoker?

☐ Yes
☐ No

14. In the past 30 days, have you lived in a place where other people smoked cigarettes indoors?

☐ Yes
☐ No

15. In the past 30 days, have you worked in a place where other people smoked cigarettes indoors?

☐ Yes
☐ No

16. Thinking of all your childhood and adult years, have you ever lived in a place where other people smoked cigarettes indoors?

☐ Yes In total, for about how many years? _____ If less than 1, write "1."
☐ No

17. Thinking of all the years you have worked, have you ever worked in a place where other people smoked cigarettes indoors?

☐ Yes → In total, for about how many years? _____ If less than 1, write "1."
☐ No

CRF completed by (*check one*):

Study participant _____

Study site staff _____ Staff name (*optional*) _____

Date __ __ / __ __ / __ __ __ __
(MM/DD/YYYY)

TOBACCO ASSESSMENT - FOLLOW-UP

REGISTERING INSTITUTION	PARTICIPANT ID	VISIT TYPE	VISIT DATE *(MM/DD/YYYY)*
_____	_____	_____	__ __ / __ __ / __ __ __ __

Instructions:
When a number is requested in the response, please enter a whole number (i.e. "4") and not a range or fraction of a number.

1. Do you NOW smoke cigarettes?

☐ Everyday
☐ Some days
☐ Not at all → **Skip to Question 3.**
☐ Never smoked → **Skip to Question 4**

2. On average, when you smoked, about how many cigarettes do you (or did you) smoke a day? (A pack usually has 20 cigarettes in it).

_____ Number of cigarettes per day

3. How long has it been since you last smoked a cigarette (even one or two puffs)?

First check which one of the following choices applies to you. Then, if applicable, write a whole number on the line for how many days, weeks, months, or years it has been since your last cigarette.

☐ I smoked a cigarette today (at least one puff)
☐ 1-7 days → Number of days since last cigarette _____
☐ Less than 1 month → Number of weeks since last cigarette _____
☐ Less than 1 year → Number of months since last cigarette _____
☐ More than 1 year → Number of years since last cigarette _____
☐ Don't know/Don't remember

4. Since your last visit, have you used other forms of tobacco, not including cigarettes?

☐ Yes
☐ No (**End)**

5. How often do you/did you use other forms of tobacco?

☐ Every day → Number of times per day _____
☐ Some days → Number of days _____ per ☐ Week ☐ Month ☐ Year

6. Since your last visit, which of the following products have you used? ***Check all that apply***

- ☐ Cigarettes
- ☐ E-cigarettes or other electronic nicotine delivery system
- ☐ Traditional cigars, cigarillos or filtered cigars
- ☐ Pipes
- ☐ Waterpipe
- ☐ Hookah
- ☐ Clove cigarettes or kreteks
- ☐ Bidis
- ☐ Smokeless tobacco, like dip, chew, or snuff
- ☐ Snus
- ☐ Paan with tobacco, gutka, zarda, khaini
- ☐ Other, Specify_____

7. If you do not currently use other forms of tobacco, but did in the past, how long has it been since you last used other forms of tobacco regularly?

- ☐ Within the past month (0 to 1 month ago)
- ☐ Between 1 and 3 months (1 to 3 months ago)
- ☐ Between 3 and 6 months (3 to 6 months ago)
- ☐ Between 6 and 12 months (6 to 12 months ago)
- ☐ Between 1 and 5 years (1 to 5 years ago)
- ☐ Between 5 and 15 years (5 to 15 years ago)
- ☐ More than 15 years ago
- ☐ Don't know/Not sure
- ☐ Never used other forms of tobacco regularly

The following instructions pertain to questions 8 - 10. During each of the following time frames, please indicate whether you smoked cigarettes every day, some days, or not at all.

8. During study treatment

- ☐ Smoked every day
- ☐ Smoked some days
- ☐ Did not smoke at all
- ☐ Don't know/not sure
- ☐ Not applicable

9. After the end of study treatment

- ☐ Smoked every day
- ☐ Smoked some days
- ☐ Did not smoke at all
- ☐ Don't know/not sure
- ☐ Not applicable (I have not completed the study treatment)

10. Since your last visit to this clinic

- ☐ Smoked every day
- ☐ Smoked some days
- ☐ Did not smoke at all
- ☐ Don't know/not sure

CRF completed by (*check one*):

Study participant _____

Study site staff _____ Staff name (*optional*) _____

Date __ __ / __ __ / __ __ __ __
(MM/DD/YYYY)

NATIONAL AND LOCAL RESOURCES TO HELP WITH ALCOHOL ABUSE AND ALCOHOLISM

NIAAA's online guide ***Treatment for Alcohol Problems: Finding and Getting Help*** is written for individuals, and their family and friends, who are looking for options to address alcohol problems. It is intended as a resource to understand what treatment choices are available and what to consider when selecting among them. https://pubs.niaaa.nih.gov/publications/treatment/treatment.htm

Other resources:

National Institute on Alcohol Abuse and Alcoholism www.niaaa.nih.gov
301–443–3860

National Institute on Drug Abuse www.nida.nih.gov
301–443–1124

National Clearinghouse for Alcohol and Drug Information www.samhsa.gov
1–800–729–6686

Substance Abuse Treatment Facility Locator www.findtreatment.samhsa.gov
1–800–662–HELP

Alcoholics Anonymous (AA) www.aa.org
212–870–3400 or check your local phone directory under "Alcoholism"

Moderation Management www.moderation.org
212–871–0974

Secular Organizations for Sobriety www.sossobriety.org
323–666–4295

SMART Recovery www.smartrecovery.org
440–951–5357

Women for Sobriety www.womenforsobriety.org
215–536–8026

Al-Anon Family Groups www.al-anon.alateen.org
1–888–425–2666 for meetings

Adult Children of Alcoholics www.adultchildren.org
310–534–1815

NATIONAL AND LOCAL RESOURCES TO HELP WITH QUITTING SMOKING

NCI's Smokefree.gov offers science-driven tools, information, and support that has helped smokers quit. You will find state and national resources, free materials, and quitting advice from NCI.

Smokefree.gov was established by the Tobacco Control Research Branch of NCI, a component of the National Institutes of Health, in collaboration with the Centers for Disease Control and Prevention and other organizations.

Publications available from the Smokefree.gov website include the following:

- Clearing the Air: Quit Smoking Today for smokers interested in quitting.
- Clear Horizons for smokers over age 50.
- Staying Smoke-Free for Good for smokers who have recently quit.
- Smoke-free for women, including pregnant women.
- Smoke-free information in Spanish
- Pathways to Freedom: Winning the Fight Against Tobacco for African American smokers.

NCI's Smoking Quitline at **1–877–44U–QUIT (1–877–448–7848)** offers a wide range of services, including individualized counseling, printed information, referrals to other resources, and recorded messages. Smoking cessation counselors are available to answer smoking-related questions in English or Spanish, Monday through Friday, 8:00 a.m. to 8:00 p.m., Eastern time. Smoking cessation counselors are also available through LiveHelp, an online instant messaging service. LiveHelp is available Monday through Friday, 8:00 a.m. to 11:00 p.m., Eastern time.

Your state has a toll-free telephone quitline. Call **1–800–QUIT–NOW (1–800–784–8669)** to get one-on-one help with quitting, support and coping strategies, and referrals to resources and local cessation programs. The toll-free number routes callers to state-run quitlines, which provide free cessation assistance and resource information to all tobacco users in the United States. This initiative was created by the Department of Health and Human Services. For more information about quitlines, speak to an expert on the Smokefree.gov website.

APPENDIX C PARTICIPANT CLINICAL TRIAL WALLET CARD

NIH NATIONAL CANCER INSTITUTE

CLINICAL TRIAL WALLET CARD

Show this card to all of your healthcare providers and keep it with you in case you go to the emergency room.

Participant Name:

Diagnosis/Condition:

Study Doctor:

Study Doctor Phone #:

NCI Trial #: UAZ22-11-01

Study Drug(S): Avmacol ES (BSSE)

For more information: 1-800-4-CANCER
cancer.gov | clinicaltrials.gov

APPENDIX D

COVID 19 ASSESSMENT INSTRUCTIONS

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- Data collection will be required for all CP-CTNet studies.
    - Data will be collected at baseline and end of every study. All studies need to collect the elements in the attached eCRFs.
    - The eCRFs will be completed by the Site Staff or participant at the time of the designated visit.
- Data will be submitted as part of the final clinical data set.

CP-CTNET COVID-19 BASELINE ASSESSMENT

REGISTERING INSTITUTION	PARTICIPANT ID	VISIT TYPE	VISIT DATE (MM/DD/YYYY)
_____	_____	_____	__ __ / __ __ / __ __ __ __

Instructions:
The following information is being collected for all Cancer Prevention Clinical Trials Network (CP-CTNet) studies. Only information from before study entry should be reported on this form.

Have you ever had a positive COVID-19 test in the last 3 months?

☐ Yes
☐ No

Have you received a COVID-19 vaccine in the last 3 months?

☐ Yes
☐ No
☐ Prefer not to answer

Comments:

_____

_____

CRF completed by (*check one*):

Study participant _____

Study site staff _____ Staff name (*optional*) _____

Date __ __ / __ __ / __ __ __ __
(MM/DD/YYYY)

CP-CTNET COVID-19 FOLLOW-UP ASSESSMENT

REGISTERING INSTITUTION _____	PARTICIPANT ID _____	VISIT TYPE _____	VISIT DATE (MM/DD/YYYY) __ __ / __ __ / __ __ __ __

Instructions:
The following information is being collected for all Cancer Prevention Clinical Trials Network (CP-CTNet) studies. Only use this form to report information that has become available since the last completion of a CP-CTNet COVID-19 Assessment.

Since your initial study visit, have you had a positive COVID-19 test?

- ☐ Yes
- ☐ No

If Yes, was it in the last 3 months?

- ☐ Yes
- ☐ No
- ☐ Prefer not to answer

Since your initial study visit, have you received a COVID-19 vaccine? (Please Omit this question if study duration is less than 3 months)

- ☐ Yes
- ☐ No
- ☐ Prefer not to answer

If Yes, did was it in the last 3 months? (Please Omit this question if study duration is less than 3 months)

- ☐ Yes
- ☐ No
- ☐ Prefer not to answer

Comments:

_____

_____

CRF completed by (*check one*):

Study participant _____

Study site staff _____ Staff name (*optional*) _____

Date __ __ / __ __ / __ __ __ __
(MM/DD/YYYY)

APPENDIX E
PRE-ACETAMINOPHEN SPOT URINE QUESTIONNAIRE

1. Have you taken any over-the-counter medications during the 24 hours before the urine collection? (Check all that apply)

   ☐ Aspirin or generic aspirin (acetylsalicylic acid)
   ☐ Motrin, Advil, or Ibuprofen
   ☐ Other over-the-counter medications (please list) _____
   ☐ No over the counter medications in the past 24 hours
   ☐ Prefer not to answer

2. Have you taken any prescription medications during the 24 hours before urine collection?

   ☐ Yes, list other prescription medications: _____
   ☐ No
   ☐ Prefer not to answer

3. Please select if you have taken any of the following during the 24 hours before urine collection (Check all that apply)

   ☐ Supplements/Vitamins
   ☐ Pre-workout
   ☐ Protein powder/shake
   ☐ None of the above
   ☐ Prefer not to answer

4. [Question 3 = "Yes"] Please list all supplements/vitamins you have taken during the 24 hours before urine collection:

   _____

5. Have you had anything other than water to drink during the 24 hours before urine collection? Check all that apply.

   ☐ Energy drink (for example Red Bull, Monster, other)
   ☐ Coffee and/or tea
   ☐ Regular soda
   ☐ Diet soda
   ☐ Alcoholic beverage
   ☐ Other, list other: _____
   ☐ None of the above
   ☐ Prefer not to answer

6. Have you ever donated blood and/or plasma?

- ☐ Yes
- ☐ No
- ☐ Prefer not to answer (End of survey)

7. **[Question 6 = "Yes"]** When was the last time you donated blood or plasma? (checkbox)

- ☐ In the last 7 days
- ☐ In the last month
- ☐ In the last 6 months
- ☐ More than 6 months ago
- ☐ Prefer not to answer

APPENDIX F
POST-FIRE/FUEL LEAK SPOT URINE COLLECTION QUESTIONNAIRE

(To be completed by Individual Firefighter)

#1 Name: _____

#2 Time of Urine Collection: _____

#3 Fireground Role Information

- **Primary Role:** ☐ Fire Attack ☐ Ventilation ☐ Search & Rescue ☐ Water Supply
- **Position During Incident:** ☐ Offensive ☐ Defensive

#4 Post-Fire Wash Down Performed On Scene: ☐ Yes ☐ No

#5 Shower Taken Prior to Urine Collection: ☐ Yes ☐ No

QR Code Scanned: ☐ Yes ☐ No (if applicable)

APPENDIX G
REMOTE CONSENT INSTRUCTIONS

1. The participant or their legally-authorized representative (LAR) receives a copy of the informed consent document (e.g., via mail, fax, email, or a web link) ***in advance*** of discussion regarding the study. If mailed, two copies must be mailed so the participant or LAR is able to retain a copy for reference when their signed document is returned to the site and they are waiting to receive the final copy with all necessary signatures back from the site. If an electronic-consent (e-consent) document is provided, the content in the document must be the same as the paper-based consent document.
2. The investigator or designee discusses the study with the potential participant either via telephone or video conferencing. The investigator/designee must have the same consent discussion via telephone/video conferencing that they would have had with the participant or LAR during an in-person meeting. The investigator/designee must also implement a method to ensure the identity of the participant or LAR (e.g., verification of state identification or other identifying documents or use of personal questions or visual methods).
3. If the potential participant or LAR agrees to participation, they sign the consent form and return it to the investigator (e.g., via mail, fax, email, or by signing the e-consent document). If postal mail is used, a pre-paid, self-addressed envelope should be provided to the participant or LAR to mail the signed consent form back to the investigator. The inclusion of a witness in the Remote Consent Procedures is dictated by local institutional policy and must follow FDA and OHRP requirements. When a witness is required, the research record must document the witness' name and that they were present for the informed consent process. The inclusion of the witness' signature on the consent form is dictated by local institutional policy.
4. Once the research team receives the signed informed consent document from the participant or LAR, the investigator/designee who conducted the consent process must sign and date the document using the current date. Under the signature line, the investigator/designee must document whether consent was obtained over the telephone or video conferencing, the date of the telephone/video conference, and the date the signed consent was received. For example, **"Discussed with [participant or LAR name] via [telephone or videoconferencing] on [insert date] and received signed consent form on [insert date]."** Include a brief reason for performing the informed consent discussion over the telephone/videoconferencing.
5. If the site has an informed consent policy that requires the witness to sign the consent document, the witness signs the informed consent. If the site does not have an informed consent policy that requires the signature of the witness on the consent document, then the name of the witness along with the date of the original consenting phone call is recorded in the research records to document the participation of the witness.
6. The date the investigator/designee signs the informed consent document, not the date the consent discussion with the participant or LAR took place, is the official date of informed consent for the participant on the trial.
7. The final informed consent document must be filed in the designated investigator/site regulatory file location. A copy of the final informed consent document, signed by the participant or LAR, the investigator, and the witness (if applicable), must be sent back to the participant via email/scan, fax, or postal mail.

No research activities related to the study can begin until all steps of the informed consent process are complete.

APPENDIX H
FIRE/FUEL INCIDENT REPORT

To be completed by Liaison

#1 Incident Details

- **Date:** _____
- **Time of Call:** _____
- **Time on Scene:** _____
- **Run Number:** _____
- **Unit:** _____
- **Incident Address:** _____

#2 Type of Fire *(check all that apply)*

- **Structure:** ☐ Residential ☐ Commercial ☐ Mobile Home
- **Vehicle:** ☐ Car ☐ Truck ☐ RV/Trailer
- **Fuel Type:** ☐ Gasoline ☐ Diesel ☐ Hybrid ☐ Electric (EV)

#3 Firefighter Activity

- **Primary Role:** ☐ Fire Attack ☐ Ventilation ☐ Search & Rescue ☐ Water Supply
- **Position During Incident:** ☐ Offensive ☐ Defensive

#4 On-Scene Photo Attached: ☐ Yes ☐ No

#5 Additional Notes: