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The Effects of Thirdhand Smoke on Biomarkers of Exposure, Inflammation and Oxidative Stress

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The Effects of Thirdhand Smoke on Biomarkers of Exposure, Inflammation and Oxidative Stress

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THESIS

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Dedication and Acknowledgments

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The Effects of Thirdhand Smoke on Biomarkers of Exposure, Inflammation and Oxidative Stress

Kelly Pratt

Abstract

Background. Second hand smoke changes physically and chemically after it is released into the environment. Some of the resulting chemicals and particulate matter are toxic and persist in the indoor environment as thirdhand smoke.

Objectives. The aims of this study were to measure the effects of thirdhand smoke on biomarkers inflammation and oxidative stress and biomarkers of tobacco smoke exposure.

Methods. After generation of thirdhand smoke with a smoke generating machine and smoke aging chamber, 17 healthy nonsmokers were given a three-hour respiratory exposure to thirdhand smoke. The systemic effects of respiratory exposure to thirdhand smoke were tested by measuring biomarkers of inflammation, vascular endothelial growth factor and interleukin-6, and a biomarker of oxidative stress, 8-isoprostane, using enzyme-linked immunosorbent assay kits. Biomarkers of cigarette exposure, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone and cotinine, were measured with liquid chromatography–atmospheric pressure chemical ionization tandem mass spectrometry.

Results. The results show significant increases in 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone and cotinine after three hours of exposure to thirdhand smoke and no significant increases in interleukin-6, vascular endothelial growth factor or 8-isoprostane.

Conclusion. These results have implications for stricter indoor smoking regulations as well as health education around smoking. Additionally, the significant increase in levels of biomarkers, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone and cotinine, with thirdhand smoke exposure corroborate the reliability of using these biomarkers for thirdhand smoke exposure screening.

Table of Contents

List of Tables

List of Figures

The Study Problem

Thirdhand smoke (THS) is a new term for the third major route of exposure to cigarette smoke, in addition to active smoking and second hand smoke (SHS) exposure (Winickoff et al., 2009). Recent studies found that certain cigarette smoke chemicals, such as nicotine, nitrosamines and polycyclic aromatic hydrocarbons (PAHs), stick to surfaces and persist in the indoor environment for hours, days, months and years (Matt et al., 2011). These chemicals can be perceived by their smell, or as a yellow-brown stain on light colored walls and surfaces. The chemicals in THS can react, at any time, to form new chemicals, such as formaldehyde and tobacco-specific N-nitrosamines (TSNA), 4-(methylnitrosamino)-4-(3-pyridyl) butanal (NNA) (Sleiman, Gundel, et al., 2010) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) (Schick, Farraro, et al., 2013). PAHs, formaldehyde, NNA and NNK are oxidants (Destaillats, Singer, Lee, & Gundel, 2006). NNK, formaldehyde and some PAHs, such as benzo[a]pyrene, are known human carcinogens (Matt et al., 2011).

The chemicals found in THS can be inhaled, ingested or dermally absorbed, and especially put children, who spend most of their time exploring their environment, at increased risk of THS exposure. As THS is a novel concept, little is known about the health effects of THS toxins lingering in a room.

While THS is a new area of research, there is strong scientific evidence of the harmful health effects of SHS. SHS is defined as the combination of exhaled mainstream and sidestream smoke. Mainstream smoke is the smoke that is inhaled and exhaled by the smoker, and makes up ~15% of SHS, whereas sidestream smoke, the smoke that is emitted from the end of a burning cigarette, composes the other ~85% of SHS (Centers for Disease Control (CDC), 2014; US Surgeon General's report [USSGR], 2006). SHS is an indoor pollutant (California

Environmental Protection Agency [Cal/EPA], 2005) and is classified as a Group 1 carcinogen (International Agency on Research on Cancer [IARC], 2004), meaning the chemicals in SHS are known to cause cancer in humans. Each year in the United States, SHS accounts for about 34,000 premature deaths from heart disease, 7,300 deaths from lung cancer and 8,000 deaths from stroke (Centers for Disease Control [CDC], 2014). SHS increases the risk of developing coronary heart disease by 25-35%, the risk of stroke by 20-30% and the risk of lung cancer by 20-30% (USSGR, 2006).

Systemic inflammation and oxidative stress, terms that describe an overall imbalance in inflammatory regulation, have been linked with SHS exposure and a variety of disease states. This linkage suggests that the measurement of inflammation and oxidative stress during and after exposure to THS may be a useful indication of the potential health effects caused by THS. Inflammation and oxidative stress play important roles in the pathogenesis of diabetes, autoimmune diseases and cancer (Khansari et al, 2014), cardiovascular disease (Papaharalambus et al., 2007), asthma (Zhang et al., 2009) and chronic obstructive pulmonary disease (Rahman, 2005).

There have been no human studies on the effects of exposure to THS on systemic inflammation and oxidative stress. Information is only available from animal studies. The first published study of the effects of THS exposure on systemic inflammation used a mouse model (Martins-Green et al., 2014). In this study, mice lived in cages that contained bedding and cloth that had been exposed to cigarette smoke for 24-26 weeks (at 6 hours/day and 5 days/week at 30 mg/m^3 total particulate material (TPM)). The mice were exposed via inhalation, ingestion and dermal absorption. The study showed that exposure of mice to THS produced significant

elevations of systemic inflammatory cytokines and excess collagen production in the lungs of mice. This study was one of the first studies to test the effects of THS on humans, which can have vast implications for public health policy, research and education.

Definition of Terms

Systemic Inflammation

The result of prolonged inflammation that involves chronic activation of the innate immune system (Newton & Dixit, 2012), a shift in the types of inflammatory cells present, and changes in the systemic levels of cytokines and other signaling molecules. (Dinarello, 2000). Systemic inflammation differs from tissue-specific inflammation in that the affected areas include the vascular endothelium and whole organ systems as opposed to a localized area. Biomarkers of inflammation include vascular endothelial growth factor (VEGF), interleukin-6 (IL-6), interleukin-8, interleukin-18, tumor necrosis factor-alpha and C-reactive protein.

Oxidative Stress

The state in which antioxidants cannot completely neutralize free radicals therefore leaving the body vulnerable to damage by reactive oxygen species (Betteridge, 2000; Dalle-Donne, Rossi, Colombo, Giustarini, & Milzani, 2006).

Particulate Matter

Particulate matter (PM) is the sum of all solid and liquid particles suspended in air that vary greatly in size, composition, and origin. Particles can be directly emitted, for instance, when combustion occurs or when dust is entrained by wind. They can also be formed when gaseous pollutants condense into PM. Particles can be categorized by size, which provides information about the chemical composition, source and aerodynamic properties of the particles.

The aerodynamic properties of particles determine how they move through air and how far they travel into respiratory passages. The coarse fraction ranges from 2.5 to 10 μm in diameter (PM10 - PM2.5). The fine fraction contains the particles 2.5 μm and less (PM2.5). The particles below 0.1 μm in diameter are called ultrafine particles (Environmental Protection Agency (EPA), 2013).

Aerosol

A suspension of tiny particles or droplets and gases in the air, such as dusts, mists, or fumes (CDC, 2010).

Secondary organic aerosols (SOAs)

The aerosol mass arising from the oxidation reactions of gas-phase organic species (Kroll & Seinfeld, 2008).

Adsorption

An increase in the concentration of a dissolved substance at the interface of a condensed and a liquid and gaseous phase due to the operation of surface forces (International Union of Pure and Applied Chemistry (IUPAC), 2006).

Absorption

The process of one material being retained by another; this may be the physical solution of a gas, liquid, or solid in a liquid, attachment of molecules of a gas, vapor, liquid, or dissolved substance to a solid surface by physical forces (IUPAC, 2006).

Desorption

The decrease in the amount of an adsorbed substance (IUPAC, 2006).

Deposition

The adhesion of particles to surfaces by settling and impaction (Schick et al, 2014).

Literature Review

Particulate and Chemical Composition of THS

Respiratory SHS exposure is the involuntary inhalation of the combination of sidestream smoke, from the end of a burning cigarette and mainstream smoke exhaled from the lungs of smokers (Matt et al., 2011). SHS contains more than 4,000 chemicals of which 250 are known to be toxic. Many of these substances are eye and respiratory irritants, mutagens, cardiovascular and reproductive toxicants, or carcinogens (USSGR, 2006). SHS has 50 known or suspected

Table 1 n

Major Components of THS as Classified by their Physicochemical Characteristics

Category	Vapor pressure range	Examples
Very volatile organic compounds	>7 to 13 kPa	Formaldehyde, acrolein, 1,3-butadiene, acetaldehyde
Volatile organic compounds	-.01 to 10 kPa	Benzene, toluene, styrene, 2-butanone, phenol, pyridine, styrene, 3-ethenylpyridine, N-nitrosodimethylamine,
Semivolatile organic compounds	10^{-2} to 10^{-8} kPa	Nicotine, naphthalene, chrysene, fluoranthene, pyrene, N-nitrosonomicotine, NNK
Particulate organic compounds	$<10^{-8}$ kPa	Benzo(α)pyrene, benzo(b)fluoranthene, benzo(k)fluoranthene, solanesol
Gas-phase inorganic compounds	>13 kPa	CO_2, H_20, CO, NH_3

Note. Adapted from Thirdhand tobacco smoke: emerging evidence and arguments for a multidisciplinary research agenda by Matt et al., 2011, *Environmental Health Perspectives*, *119*(9), 1218-1226

The behavior of individual chemicals in SHS and their likelihood to persist in the indoor environment as THS can be predicted based on their physicochemical properties (see Table 1). The substances released in SHS are broken into the following five categories: gas phase inorganic, very volatile organic compounds (VVOCs), volatile organic compounds (VOCs), semi volatile organic compounds (SVOCs), or particulate organic compounds (Van Loy, Riley, Daisey, & Nazaroff, 2001). To understand the transformation of SHS into THS, the differences between these components of SHS need to be explored.

Gas-phase inorganics. By mass, gas phase inorganics make up approximately 85% of SHS (IARC, 2004). Gas phase inorganics have the highest vapor pressures (>13 kPa) among chemicals in SHS. Gas phase inorganics present in SHS include carbon dioxide, water, carbon monoxide, nitrogen oxides and ammonia (IARC, 2004). As these compounds are not adsorbed to surfaces to an appreciable degree and are removed by ventilation, gas phase inorganics contribute little to THS.

VVOCs. By mass, VVOCs make up approximately 2% of SHS (IARC, 2004) and have the second highest vapor pressures (>7-13 kPa) in SHS. Due to their high volatility, VVOCs remain in the gas phase at room temperature and their concentrations are largely determined by emission rates, indoor volumes, and ventilation. VVOCs in SHS are usually lost to ventilation within a couple of hours after the cigarette is burned. Formaldehyde, acrolein, acrylonitrile, N,N dimethylnitrosamine, 1,3-butadiene and acetaldehyde are identified in THS (Daisey, 1999). VVOCs in THS can either be released by combustion during smoking or by the reactions of adsorbed THS chemicals with ambient oxidants (Destaillats et al., 2006). However, little research has yet been done to measure VVOCs in THS.

VOCs. By mass, VOCs make up about 0.4% of SHS (IARC, 2004) and have lower vapor pressures (.01 to 10 kPa) than VVOCs. If not removed by ventilation, VOCs are capable of adsorbing to surfaces. Sleiman et al. (2014) performed a series of experiments to chemically characterize THS aerosol. The VOCs identified 18 hours after the smoking session included furans, carbonyls, aldehydes and nitriles (Sleiman, 2014). However, it is not known whether the VOCs identified were the result of direct combustion during smoking or the chemicals reactions that occurred between the products of the combustion process.

SVOCs. By mass, SVOCs make up approximately 1% of SHS (IARC, 2004). SVOCs have higher molecular weights, lower vapor pressures (10^{-2} to 10^{-8} kPa) and higher boiling point temperatures than VOCs (Daisey, 1999). SVOCs exist in gas, liquid or solid forms in indoor environments, and partition between indoor air and surfaces. Combustion from a smoking cigarette forces SVOCs into the gas phase. As they cool, they condense into droplets and are adsorbed into particles and indoor surfaces. Once adsorbed, they can also re-emit into the gas phase.

The tendency of SVOCs to adsorb to dust and surfaces reduces their removal by ventilation and increases their persistency indoors long after active smoking ceases. SVOCs found in THS include nicotine, naphthalene, phenols, cresols and TSNAs, NNK and NNN (Destaillats et al., 2006; Singer, Hodgson, Guevarra, Hawley, & Nazaroff, 2002; Singer, Hodgson, & Nazaroff, 2003; Sleiman, Destaillats, et al., 2010).

Although SVOCs constitute only a small fraction of the total mass produced by a cigarette, smoking can lead to a significant amount of SVOCs accumulating over time. Schick and her colleagues conducted experimental studies using a SHS generation system, as described

in Schick et al. (2012), and found that 62% of the PAHs,, 70% TSNAs and 80% of the nicotine in SHS deposited on indoor surfaces within 60 minutes (Schick et al., 2013). This data suggests that a majority of the PAHs, TSNAs and nicotine remain on indoor surfaces after a cigarette has been smoked, under normal residential ventilation conditions.

PM. By mass, approximately 11% of SHS is composed of PM (IARC, 2004). PM has vapor pressures ($<10^{-8}$ kPa) lower than those of SVOCs. PM derived from cigarette smoke contain a mixture of solid particles and liquid droplets. Fine particles are those less than 2.5 microns in diameter. The mass median aerodynamic diameters of SHS PM is approximately 0.2 microns, thereby classifying them as fine particles (Klepeis, Apte, Gundel, Sextro, & Nazaroff, 2003; Nazaroff & Cass, 1989). The composition of PM identified includes benzo(a)pyrene (Schick, 2014), solanesol and certain SVOCs, such as PAHs, (Benner et al., 1989). Because SVOCs can move between vapor phase and particulate phase, vapor phase SVOCs may evaporate from PM over time to deposit on surfaces (Daisey, 1999).

SOAs. Reactions between SHS and common indoor air pollutants, like ozone and nitrous acid, can generate ultrafine particles called secondary organic particles (SOAs) (Sleiman, Destaillats, et al., 2010). Unpublished data from Schick et al. (2013) shows that THS compounds emitted from surfaces, back into air, can form what appears to be SOA particles. In this experiment chemically characterizing THS, Schick et al. (2013) found that although 50% of the total particles from a smoking session were lost from the SHS aerosol within one hour, there were still detectable particle levels 18 hours after the last cigarette was smoked.

Sleiman and his colleagues were also able to identify the formation of SOAs from THS (2010). Most of the aerosols identified by Sleiman et al. (2010) were a result of nicotine reacting

with ozone. Prior literature has also demonstrated emissions of SOAs from nicotine-ozone reactions (Petrick, Svidovsky, & Dubowski, 2011; Sleiman, Destaillats, et al., 2010). Nicotine can also react with nitrous acid to form TSNAs: NNK (Schick, Farraro, et al., 2013) and NNN (Sleiman, Gundel, et al., 2010). One study found the presence of NNK and NNN in dust samples from all the smokers' homes tested (N=22), as compared with occasional detection in the nonsmokers' homes (N=24) (Thomas et al., 2014).

Routes of Exposure to THS

Because of the dynamic nature of THS chemicals, there are multiple routes of exposure possible, including inhalation, ingestion, and dermal absorption (Matt et al., 2011). Inhalation exposure includes both the gas and particle phase of THS aerosol. The particle phase of THS contains ultrafine SOA particles and larger dust particles that have adsorbed THS chemicals from the gas phase. Ingestion exposure occurs with the consumption of an item that THS has adsorbed to, such as dust particles, or with mouthing THS-contaminated surfaces. Mouthing behaviors are common among children and place them at an increased risk of ingestion exposure to THS. Lastly, dermal exposure occurs when THS chemicals absorb through the skin with dermal contact to pollutants that have adsorbed or settled on surfaces, materials, or dust.

THS Components and Oxidative Stress and Inflammation

SVOCs. Nicotine, the most abundant SVOC in THS, poses a significant risk to those exposed to THS. Various studies have found correlations between exposure to nicotine and increased systemic inflammation (Aicher et al., 2003; Benowitz, Fitzgerald, Wilson, & Zhang, 1993), endothelial cell function (Cucina et al., 1999; Mayhan & Patel, 1997; Neunteufl et al.,

2002), lipid accumulation and insulin resistance (Cluette-Brown et al., 1986; Hellerstein et al., 1994; Stefanovich, Gore, Kajiyama, & Iwanaga, 1969).

PAHs found in THS are also associated with negative health outcomes. PAHs are neutral, nonpolar hydrocarbons composed of multiple aromatic rings. They are a byproduct of the incomplete combustion that occurs when a cigarette is smoked (U.S. Department of Health and Human Services, 1995). PAHs are well known for their carcinogenic and mutagenic properties (Luch, 2005). Animal studies show that PAHs cause significant immune, neurologic and developmental effects (Dasgupta & Lahiri, 1992; Szczeklik et al., 1994; Zhao, 1990).

VOCs. Wang et al. exposed male mice to filtered air and VOCs: formaldehyde, benzene, toluene, and xylene (2012). Blood analysis showed significant increases in IL-6 concentrations, indicating an effect on systemic inflammation. The VOCs also significantly increase nitric oxide synthase and glutathione (Wang, Li, Liu, & Jin, 2012), both of which are indicators of oxidative stress (Schiffrin, 2008). In a study by Koren et al., 14 healthy human subjects were exposed to a mixture of VOCs, including 2-butanone, at concentrations found in new homes and office buildings. They found statistically significant increases in polymorphic nuclear cells, an indicator of early inflammation (Wright, Moots, Bucknall, & Edwards, 2010), immediately after a 4 hour exposure session to VOCs and 18 hours later (Koren, Graham, & Devlin, 1992).

Quinones, a group of VOCs found in THS, are reaction products of polyaromatic compounds (Daisey, 1999; Maskos & Dellinger, 2007). Quinones induce oxidative stress in oxidized cellular macromolecules, such as DNA, protein and lipids, by leading to the formation of reactive oxygen species (Bolton, Trush, Penning, Dryhurst, & Monks, 2000; Gurbani et al., 2013; Kelly, 2003).

PM. Studies show that both acute and chronic exposure to PM can increase inflammation and oxidative stress. Particulate air pollution, such as wood smoke, diesel exhaust and atmospheric pollution (K. J. Chuang, Chan, Su, Lee, & Tang, 2007; Kampfrath et al., 2011; Mutlu et al., 2007; Ruckerl et al., 2007), have been linked to oxidative stress and inflammation. Tornkvist et al.'s study (2007) revealed that a one-hour exposure session to diesel exhaust at 300 g/m^3 PM led to increases in plasma TNF-a and IL-6 in healthy volunteers. Another study examined plasma IL-6 concentrations in children from an urban area with high ambient PM concentrations children from a rural area with low ambient PM concentrations (Nazariah, Juliana, & Abdah, 2013). They found significance differences between the levels of IL-6 between the children living in urban areas and those living in rural areas. Li et al. (2003) studied the dose-dependent effects of PM on oxidative stress in alveolar macrophage-derived cell lines and found increased levels of heme oxygenase-1 expression, a strong indicator of oxidative stress.

SOAs. In vitro studies have confirmed lung epithelial damage with exposure to SOAs (Baltensperger et al., 2008). NNK, compound found in THS, is carcinogenic (Hang et al., 2013; Schick, Farraro, et al., 2013; Sleiman, Gundel, et al., 2010). The carcinogenic activity of NNK is directed primarily at lung tissue (Akopyan & Bonavida, 2006; S. S. Hecht & Hoffmann, 1988). NNK, combined with NNN, has also been linked with the development of oral cavity tumors (S. S. Hecht & Hoffmann, 1988). Oxidative stress has been cited as a possible underlying mechanism of the carcinogenicity of SOAs (C. H. Chuang & Hu, 2006; Demizu et al., 2008).

Common Biomarkers of Oxidative Stress and Inflammation

Oxidative stress. 8-isoprostane is a prostaglandin like compound formed by the free radical-catalyzed peroxidation of essential fatty acids (Morris et al, 1990) and has been a validated measure of in-vivo oxidative stress (Morrow, 2005). Studies show that 8-isoprostane is elevated in smokers (Morrow et al., 1995) and healthy nonsmokers after acute second hand smoke exposure (Ahmadzadehfar, Oguogho, Efthimiou, Kritz, & Sinzinger, 2006). 8-isoprostane has been the most studied class of isoprostanes because of its stability and accuracy (Dalle-Donne et al., 2006).

Inflammation. Vascular endothelial growth factor (VEGF) is a vascular growth factor that has been associated with endothelial dysfunction. It has been considered a valid measure of inflammation and an indicator of cardiovascular disease (Schmidt-Lucke et al., 2005). Subjects who were briefly exposed to SHS consistently have increased levels of circulating VEGF (Heiss et al., 2008; Suzuki et al., 2008). IL-6, a chief stimulator of most acute inflammatory phase proteins (Gabay & Kushner, 1999), is a good measure of inflammation because of its rapid rise with pro-inflammatory stimuli (Gabay & Kushner, 1999). Preliminary studies in the Schick laboratory suggested that SHS exposure increases circulating levels of IL-6. Smokers had consistently higher levels of IL-6 that nonsmokers in a study by Tousoulis et al. (2013).

Biomarkers of exposure. Cotinine is a metabolite of nicotine and a widely used, validated biomarker of mainstream and SHS exposure (Benowitz, 1996; Petersen, Leite, Chatkin, & Thiesen, 2010). On average, 70-80 % of nicotine is converted to cotinine and 10-15% of cotinine is excreted into the urine (Benowitz & Jacob, 1994; Benowitz, Jacob, Fong, & Gupta, 1994), allowing cotinine to be effectively measured in urine samples. Cotinine has recently been

used for developing risk estimates for lung cancer related to THS exposure (Lee, 1991; Repace & Lowrey, 1993).

4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNAL) is a metabolite of NNK (IARC, 2004). NNK belongs to a group of chemicals called TSNAs that are formed by nitrosation of nicotine and related alkaloids found in tobacco. NNAL is a validated biomarker for exposure to NNK and, like many other metabolites, it is most concentrated in urine (Anderson et al., 2003; Carmella, Han, Fristad, Yang, & Hecht, 2003; Stephen S. Hecht et al., 2001; Parsons, Carmella, Akerkar, Bonilla, & Hecht, 1998; Thomas et al., 2014).

However, the exposure times in these studies are longer than the 3-hour exposure time stipulated in this study. Additionally, prior research has demonstrated that larger size, high vapor pressure chemicals such as NNK and nicotelline are less likely to desorb from surfaces into THS aerosol than smaller size, lower vapor pressure chemicals such as nicotine (Schick, Farraro, et al., 2013). Because of these reasons, significant increases in urinary NNAL after exposure to THS are not expected.

Study Aims and Biological Model

The purpose of this pilot study was to investigate the effects of acute THS respiratory exposure on systemic inflammation and oxidative stress in healthy human volunteers by measuring biomarkers of IL-6, VEGF and 8-isoprostane. Investigating the effects of respiratory exposure to THS particles on circulating levels of inflammatory factors will increase our knowledge of the health risks posed by THS. The second aim of this study was to evaluate the response of cotinine and NNAL to THS exposure. Demonstrating statistically significant increases in these biomarkers with exposure to THS corroborates the reliability of using these

biomarkers as indication of exposure to tobacco smoke. The conceptual framework of this study is presented in Figure 1.

The hypotheses of this study were categorized according to the aims of the study and were as follows:

Aim 1: This study tested the effects of THS on oxidative stress and inflammation.

1. THS will cause increases in circulating levels of IL-6
2. THS will cause increases in circulating levels of VEGF
3. THS will cause increases in urinary levels of 8-isoprostane

Aim 2: This study tested the effects of THS on potential biomarkers of exposure.

4. THS will cause increases in urinary cotinine levels
5. THS will not cause increases in NNAL

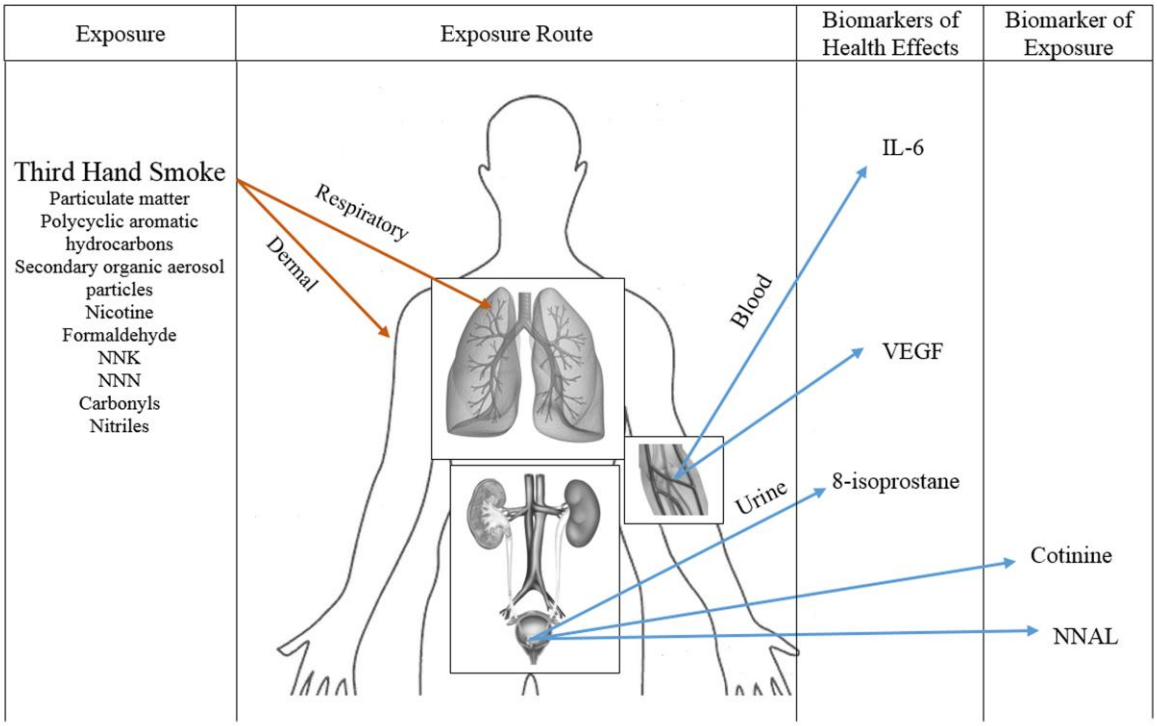

Figure 1. Conceptual framework.

Methods

Study Design

This study was a randomized, crossover study design using a convenience sample of healthy, nonsmokers (see Figure 2). Each subject was exposed to the respirable aerosol fraction of THS (THS exposure) and to conditioned, filtered air (control exposure) for 3 hours on separate study visits. The sequence of exposures was randomized and the two study visits were separated by a minimum of 21 days to avoid carry-over effects. For examining health effects, this study measured the levels of VEGF and IL-6 in blood samples and 8-isoprostane in urinary samples. This study consisted of two controlled human exposure studies of the respirable fraction of THS (THS01 and THS02). This study also used data from a previous study of SHS exposure (SHS01) to determine the most appropriate plasma analysis procedures.

1. THS01, a three arm crossover comparison of the health effect of three-hour exposures to SHS at 350 $\mu g/m^3$, THS at 350 $\mu g/m^3$ and conditioned, filtered air.
2. THS02, a two arm, crossover comparison of 3 hour exposures to THS at 350 $\mu g/m^3$, and conditioned, filtered air.
3. SHS01, a two arm crossover comparison of the health effects of 30 minute exposures to SHS at 1000 $\mu g/m^3$ TPM and to conditioned, filtered air.

Although the studies varied in the number of test conditions and the type of smoke, they all employed similar recruiting methods, inclusion and exclusion criteria, washout periods, exposure equipment and questionnaires. The description of the THS02 study, which is enrolling now, is generalizable to all three studies.

This study required five visits to the Schick laboratory at San Francisco General Hospital. The total time that each patient spent in the laboratory was 16 hours, spread over a period of three months. Each exposure session took approximately 6 hours, follow up visit took 1 hour, and screening visit took 3 hours.

This study was approved by University of California, San Francisco's (UCSF) institutional review board, the Committee on Human Research.

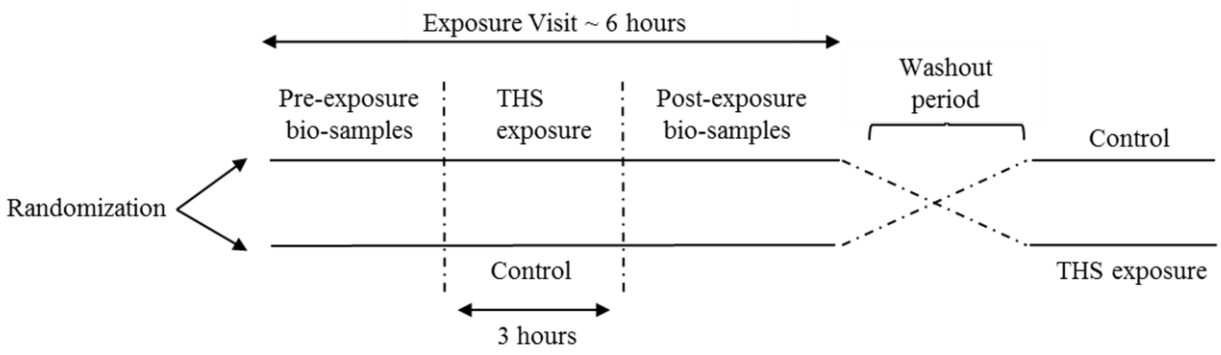

Figure 2. Crossover study design.

Experimental Setting and Systems

The SHS/THS generation system used in this study was designed to create an aged cigarette smoke aerosol as a model for SHS for human exposure studies. It uses a "flow cell" design where smoke is created, aged by transit through a smoke aging chamber and delivered continuously to human subjects through a modified respirator hood (Figure 3). The rate at which the smoke moves through the system can be controlled to match normal indoor ventilation rates of 0.5-1 air changes per hour. The smoke aging chamber and the ducting used to convey the smoke are made of stainless steel.

In preliminary studies, paper and cloth were added to the aging chamber, to test their effects on particle deposition in the aging chamber and smoke chemistry. The addition of terrycloth, an absorbent material with a complex surface profile, increased the deposition of PM, nicotine and TSNAs (Schick, Farraro, et al., 2013). Over time, more materials, including wallboard, carpet and other types of cloth, were added to the smoke aging chamber to test chemical deposition. As the smoke began to build up on these materials, the Schick laboratory researchers began to notice that a wave of particles came out of the smoke aging chamber when it was flushed with clean air the morning after a smoke experiment. Unpublished research shows that the particles in this 18-24 hour old aerosol are larger than the SHS particles aged only 30 to 60 minutes (see Figure 4). These particles were used for the THS exposures in this study.

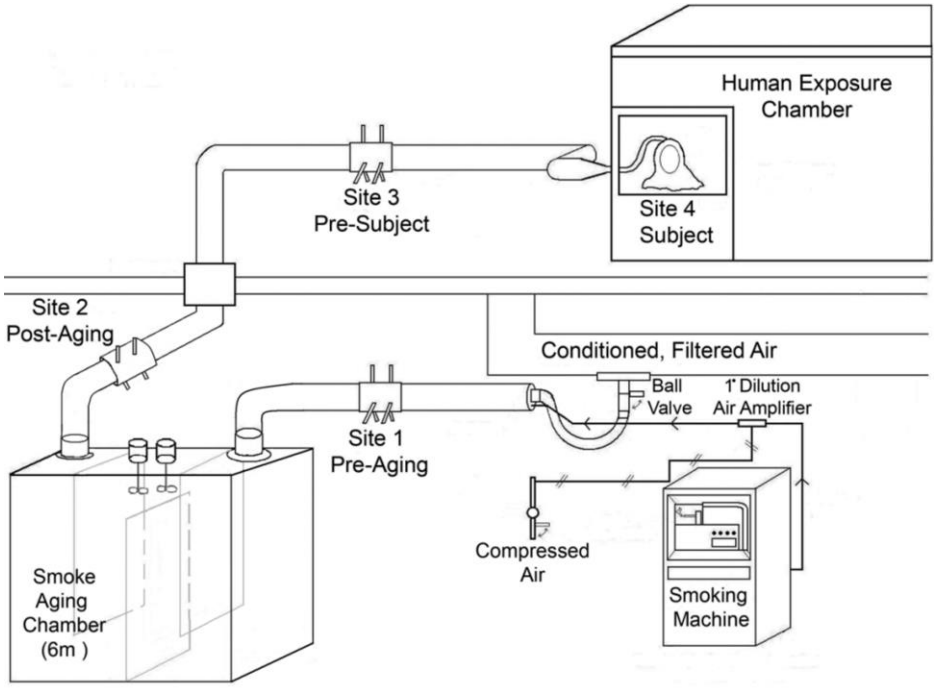

Figure 3. SHS/THS generation and conduction system.

SHS generation. A day before the scheduled exposure session with a subject, Marlboro Red cigarettes (hard pack), purchased at retail, were conditioned and smoked by an automatic cigarette smoking machine (model TE-10z, Teague Enterprises, Woodland, California, USA) according to International Standards Organization (ISO) standard 3308, with a two second puff every minute (ISO, 2012) (see Appendix D for image of smoking machine).

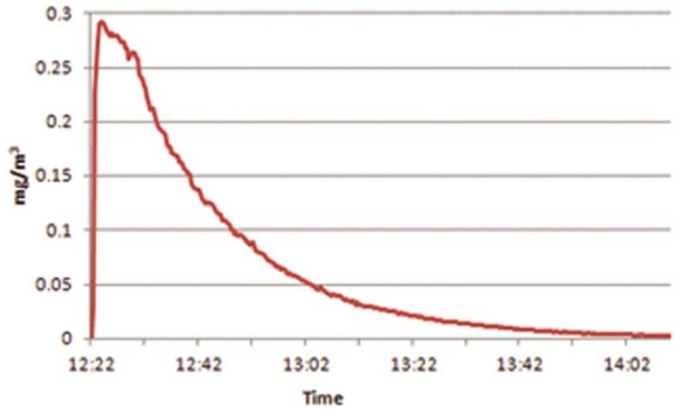

Figure 4. THS particulate matter as measured by a laser photometer calibrated to gravimetric measurements.

The smoke was then mixed with filtered, conditioned air to dilute it to achieve the target particle concentration of 300 μg m³. The concentration of the smoke was controlled by allowing smoke to enter the air amplifier. The primary dilution was accomplished using an air amplifier (model CDF-200h, Vaccon Co. Medway, MA). The secondary dilution was accomplished by injecting the diluted smoke into a stream of conditioned filtered air from the HEC. This air was moved by the inline blower fans in the HEC HVAC system and directed to the aging chamber.

The aging chamber. The stainless steel aging chamber is 6 m^3 with three vertical baffles and two fans for air mixing (see Figure 5). The velocity of the aerosol through the smoke aging system and exposure hood was set at 200 liters per minute and was controlled by the ball valve,

which allows for the input of conditioned air. This gave a transit time through the aging chamber of 30 minutes. The air velocity was monitored via two air velocity transmitters: one down-stream and the other upstream from the aging chamber.

Particle concentration, before and after transit through the smoke aging chamber, was measured using a laser photometer calibrated using the average concentration determined by the mass of PM collected. The mass was then divided by the volume passing through the filter to obtain an average concentration. The % mass deposited was determined with the following equation: M dep = [1 – C2/C1] * 100 (C1 for pre-aging and C2 for post aging).

The aging chamber contains common indoor furnishing such as carpet, painted wall board, vinyl flooring and various materials (cotton, polyester cloth, wool and chromatography paper). The environment in the aging chamber is meant to model typical household conditions.

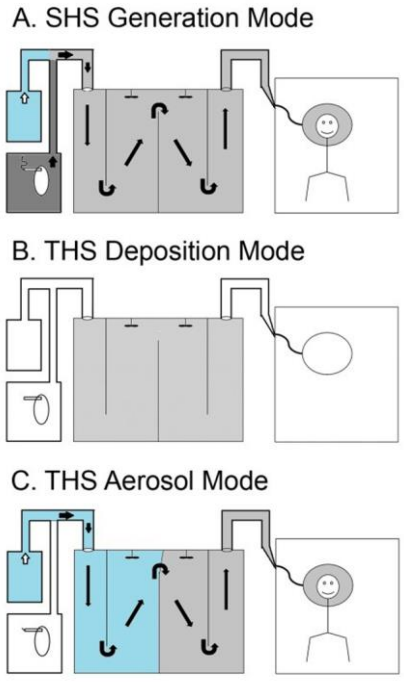

Figure 5. Overview of how THS is generated in the Schick laboratory.

THS generation. After SHS was generated and conducted into the aging chamber, the system was shut down and the smoke was allowed to age the night before an exposure session for a total of 18-24 hours. During this aging period, the smoke changed chemically and physically, as described in the introduction, including sorbing and depositing onto surfaces, reacting and re-emitting back into the aerosol phase, creating THS.

Prior unpublished studies from Schick laboratory observed a linear relationship between SHS particle output and THS particle output with 30-50% of the original particle concentration present in the aging chamber the day after the smoking session (see Figure 6). In this study, the concentration of SHS generated the day before an exposure was compared to the peak concentration of THS during exposure in order to assure consistent and accurate THS generation.

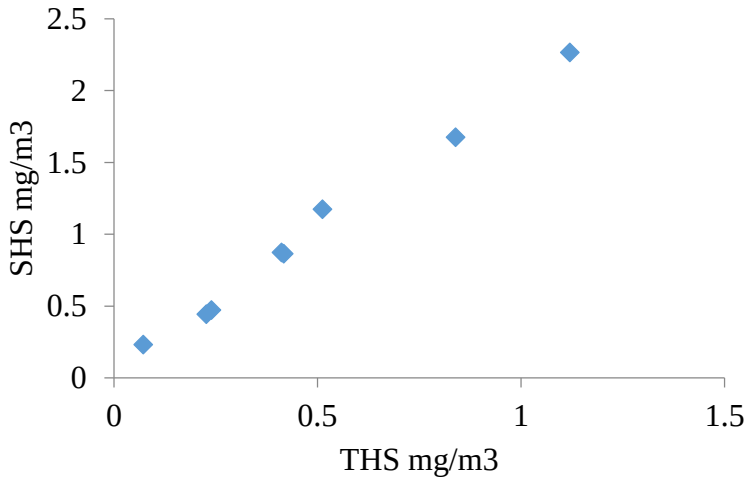

Figure 6. The ratio of SHS output to THS output in prior experiments.

The human exposure chamber. The human exposure chamber is a stainless steel chamber (9 x 9 x 9 feet) equipped with automatic climate control, reclining chair for the subject to sit on and monitoring equipment (see Appendix D for image of human exposure chamber).

There is a small window in the human exposure chamber where the research assistant conducting the study can observe the subject during the exposure period. The air exchange rate in the chamber is about 0.85 per hour.

When the subject was ready for exposure to THS, clean air was flushed through the aging chamber into a duct leading to the human exposure chamber into the Tyvek hood (Airmate # BE-10–3, 3M, Inc., St. Paul, MN) of the subject (see Appendix D for image of a subject undergoing exposure). When a subject was exposed to the conditioned, filtered air as a control exposure, clean air was produced by a powered air purifying respirator system (GVP-100. 3M, Inc., St. Paul, MN) with a high-efficiency particulate air filter (GVP-440, 3M, Inc., St. Paul, MN) that is worn around the waist and routed directly into the subjects' hood. Both the THS and clean air exposure took place in the human exposure chamber (see images in Appendix D of subject undergoing exposure to THS versus clean air).

Sample

Based on previous studies with SHS and preliminary data from the THS02 study (Schick, 2013; Pratt, 2015), a sample size of at least 15 subjects was chosen. This calculation provided an 80% chance of detecting an effect size of 20% +/- between THS and conditioned, filtered air exposures**.**

The study sample included 17 subjects from San Francisco Bay Area (10 women and 7 men) ranging in age from 23 to 49 years (M= 38.6 years, SD= 10.4 years). The study began with 254 potential participants and 237 dropped from the study for various reasons (see Figure 7 for subject dropout information). The inclusion criteria included an age range of 18-50, ability to moderately exercise and no history of chronic diseases. The exclusion criteria for this study

included smoking and ongoing or recent exposure to secondhand smoke, occupational exposure to smoke, dust or fumes, allergies, pregnancy, recreational drug use, and use of medications for high blood sugar, blood pressure, cholesterol, autoimmune disorders, tendonitis and arthritis.

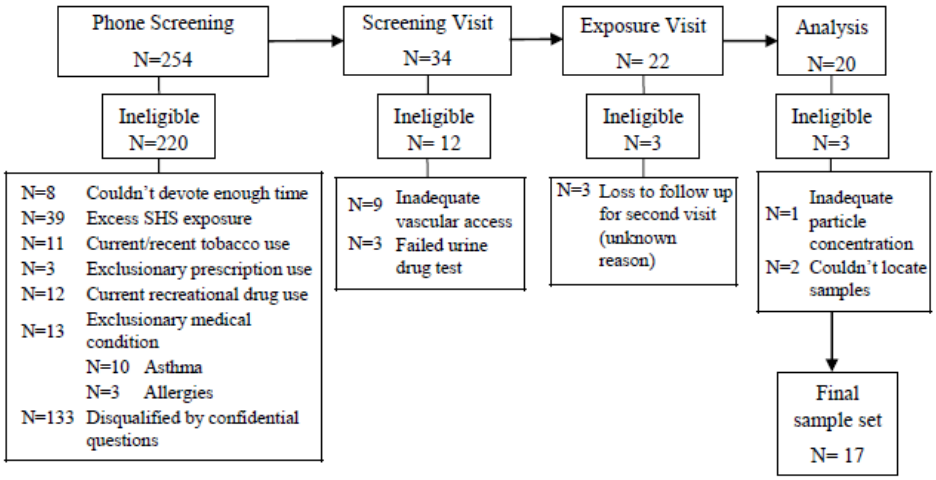

Figure 7. Subject dropout information.

Nonsmokers were determined as having smoked no more than 50 packs of cigarettes in their life, not smoking cigarettes in the past year. Marijuana smokers were excluded if they had ever smoked daily and if they had smoked even once in the preceding 3 months (Schick, van den Vossenberg, et al., 2013). A complete list of the exclusion criteria is provided in Appendix A.

Initial recruitment began with an advertisement on Craigslist calling for healthy human volunteers with a short description of the study and the contact information of the laboratory. After interested subjects sent an email, the research team then requested a time with the subject to deliver a phone questionnaire. The initial screening was carried out with a questionnaire delivered over the phone (see Appendix B for copy of screening questionnaire). If the subjects were initially deemed eligible, an in-person screening visit was then scheduled. All subjects were paid $300 with the completion of the study for their time, effort and travel expenses.

Study Procedures

Screening visit. As part of the consent process, an informed consent form was reviewed with the subjects, where the risks and benefits of the study procedures were explained and then a signature was obtained from the subject to confirm their understanding. During the consent process, the Experimental Subject's Bill of Rights and Health Insurance Portability and Accountability Act (HIPAA) document were reviewed with the subjects. After consent was obtained, each subject underwent a screening process to determine their eligibility to enroll in the study. A medical questionnaire was administered to gain a detailed medical history of the subjects (see Appendix C). A urine sample was then collected to screen for pregnancy and urinary tract infection. The urine sample was also used to perform a drug screening for recent use of tobacco, benzodiazepine, opiates and marijuana exposure. The blood sample in the screening visit was only collected in order to determine whether a subject had dependable vascular access.

An allergy skin prick test was performed to determine the subject's responsiveness to 12 common allergens in the Bay Area. The allergen test is not relevant to the current study, but will be used to characterize banked samples. A subject is classified as atopic when both a wheal and a flare are present in response to two allergens. All screening tests were performed in the laboratory with the exception of blood collection and administration of the skin allergy test, which were performed in UCSF's CTSI Clinical Research Center at the San Francisco General Hospital. Blood collection was performed by trained nurses or phlebotomists on the research medical unit. All other screening tests were performed by trained laboratory staff.

Exposure visit. The subjects were asked to stop taking the following medications before the experiment in order to avoid possible effects of medications on measured biomarkers (see Table 2). The patients were also asked to fast for 12 hours before the study visits so that the chemistry of the blood samples wasn't affected by metabolism.

On the day of the exposure visit, the subject was seated in the exposure chamber and donned a Tyvek hood with transparent, full-face shield. The hood allowed the subject to breathe either smoke or filtered air. Air flow through the exposure hood was set to 200 liters/minute +/- 10 liters. Each exposure session started with 30 minutes of exposure to THS through the hood and then the subject left the exposure chamber to provide a blood sample. Then they returned for another 2.5 hours of exposure. During the first 30 minutes of exposure, the subject reclined quietly. After the blood draw at 30 minutes, the subject was free to use electronic devices, read, watch movies or listen to music.

Blood samples were drawn by nurses at UCSF's CTSI Clinical Research Center before exposure, three hours after start of exposure and the next day on follow up visit. The blood was then centrifuged by a research associate at the laboratory to pellet red cells and the plasma supernatant was aliquoted and stored at -80° C. Urine samples were collected in the laboratory before exposure, 3 hours after exposure and at the follow up visit in the laboratory the next day. Two urine cups were provided to the subjects to take home between the exposure visit and the next day follow up visit: one urine cup for a sample before bed and another urine cup for a sample upon waking.

Table 2

Medications Withheld Before the Study

Drug or Nutrient	Duration of hold
Steroid nasal spray (Flonase, Nasonex, etc)	2 Weeks
Aspirin	7 days
Other nonsteroidal anti-inflammatory drugs: Acetominophen (Tylenol), Ibuprofen (Motrin) and Naproxen Sodium (Naprosyn)	5 days
Erectile dysfunction drugs (Viagra, Cialis, Levitra)	5 days
Oral antihistamines (Claritin, Zyrtec, Allegra etc.)	5 days
Mulitvitamins and vitamin supplements	5 days
Decongestants (Sudafed)	3 days
Caffeinated beverages and foods	12 hours
Alcohol	12 hours

Biospecimen analysis procedures. Banked plasma and urine samples, stored at -80 degrees C, were thawed and assayed with commercially available enzyme-linked immunosorbent assay (ELISA) kits for VEGF, IL-6 and 8-isoprostane. Banked urine samples, stored at -20 degrees C, were thawed and assayed for measurement of cotinine and NNAL with the help of our collaborators at the UCSF Benowitz laboratory.

In order to determine the most appropriate brands of ELISA kits to use and the best dilution factors for the samples, a series of pilot ELISAs were first performed on a set of samples from the SHS01 study that had been previously tested for IL-6 and VEGF via Luminex bead-based assays (EMD Millipore, Inc., Billerica, MA). The following ELISA assay kit brands were tested: R&D sciences and eBiosciences for IL-6, Thermo Scientific and Invitrogen for VEGF and Oxford Biomedical and Detroit R&D for 8-isoprostane. Each kit was tested according to the manufacturer's instructions. The following ELISA kits were chosen: Invitrogen VEGF ELISA kit (Camarillo, CA), R&D systems IL-6 ELISA kit (Minneapolis, MN) and Detroit R&D systems 8-isoprostane ELISA kit (Detroit, MI). These brands were chosen based on how closely the

concentrations of the SHS01 samples obtained via the ELISA kit matched with previously confirmed concentrations via Luminex bead assays- a substantially more reliable method (Dupont, Wang, Wadhwa, Culhane, & Nelson, 2005). If two kits had comparable concentrations, the kit with the least complex protocol was chosen.

According to the manufacturer's instructions, the minimum detectable concentration was 0.70 pg/mL for IL-6, 5 pg/mL for VEGF and 10 pg/ml for 8-isoprostane. Intra- and inter-plate coefficients of variance (CV) are 4.2% and 6.4%, respectively, for IL-6 and 5.5% and 9.3%, respectively, for VEGF. The coefficients of variance for the 8-isoprostane ELISA kit were not available.

The ELISAs used are colorimetric assays where the concentrations of the biomarkers were determined by measuring the amount of light absorbed by each sample using a spectrophotometer (SpectraMax 190, Molecular Devices, Sunnyvale, CA). All samples were run in triplicate and the values were averaged. Each plate had a purified bovine antibody as a positive internal control (Bio-Rad, Hercules, CA), blank wells as negative controls and a set of standard samples with known concentrations of the biomarker of interest. The concentrations of the standards were plotted on a graph to create a standard curve. An equation was then created from the standard curve and used to calculate the concentrations of each sample based on the optical density (O.D.) values obtained from the spectrophotometer (Invitrogen, 2010; R&D systems, 2013; Detroit R&D, 2015). Each measurement was corrected for non-specific binding and false positive results by subtracting blank values from the final values. The final concentrations were expressed as picograms per microliter (pg/ml).

Cotinine and NNAL in urine specimens were both analyzed by the UCSF Benowitz laboratory. Cotinine and total NNAL were measured by liquid chromatography–atmospheric pressure chemical ionization tandem mass spectrometry (Goniewicz et al., 2011), following the methods by Bernert et al. (2005) and Jacob et al. (2008). The minimum detectable doses were 0.05 ng/ml and 0.25 pg/ml for cotinine and NNAL, respectively. The values of cotinine, NNAL and 8-isoprostane obtained from urine samples were normalized to the levels of creatinine, as measured by the SFGH Clinical Laboratory, to account for differences in urine dilution. Values obtained from these samples were also normalized to PM concentrations for each individual exposure session.

Statistical Analysis

Data analysis was performed with the SAS software package (2014, Cary, NC). A univariate analysis of variance with a fixed-effects model for repeated measures was used to determine the differences between the mean concentrations of IL-6, VEGF and 8-isoprostane from THS exposures versus conditioned, filtered air exposures. Statistical significance was determined at $p < 0.05$. A one-tailed test was used in the analysis because an effect in one direction was the focus of the hypotheses of this study. The models were estimated using maximum likelihood estimation. The models include effects for time, order of exposure and clean or smoke exposure. Logarithmic transformation was applied when the data was skewed.

Results

Sample. The study sample included 17 subjects from San Francisco Bay Area (10 women and 7 men) ranging in age from 23 to 49 years (M= 38.6 years, SD= 10.4 years). Education levels and ethnicity of the subjects are presented in Table 3.

Table 3

Subjects' Demographics

SubjectID	Age	Gender	Race	Education
2	47	Female	Caucasian	PhD
5	23	Male	African-American	some college
6	49	Female	Caucasian	some college
18	40	Male	Caucasian	college
27	47	Male	Caucasian	college
35	44	Female	Caucasian	college
133	27	Female	API	in college
134	23	Male	Caucasian	some college
136	25	Female	API	college
142	48	Female	African-American	high school
144	44	Female	Caucasian	college
146	47	Male	Caucasian	masters
169	37	Female	Caucasian	college
171	49	Male	Hispanic	college
183	49	Female	Caucasian	some college
182	25	Male	Hispanic	high school
192	32	Female	Caucasian	college

Note. API= Asian Pacific Islander

Biomarker. Results for biomarkers are summarized in Tables 4 and 5 and presented as mean concentrations of each biomarker per time point.

No time dependent effects of THS on biomarkers of inflammation and oxidative stress were found in this study. The analysis revealed no statistically significant effect of THS exposure on VEGF levels (p= 0.406)(see Figure 8). IL-6 slightly increased at 30 minutes and 3 hours and then leveled off by the next day's measurement but the changes were not statistically significant (p=0.333) (see Figure 9). 8-isoprostane levels dropped 30 minutes after exposure and then slightly increases the first day and decreased by the next day's measurements (see Figure 10). However the trends in 8 isoprostane elevations were similar in both clean air and THS exposure and the changes were not statistically significant (p=0.113).

For cotinine and NNAL, there were significant changes over time 3 hours after THS exposure. Cotinine increased steadily across all time points, as compared to the control exposure and levels off with the next day visit (see Figure 11) and the findings were statistically significant ($p < .0001$). The increase in NNAL with exposure to THS (see Figure 12) was stastically significant ($p=0.034$) after logarithmic transformation of the orginally skewed NNAL data.

Table 4

Summary of data on effects of clean air on biomarkers of exposure, inflammation and oxidative stress

	Timepoints	Cotinine n=12	NNAL n=11	8-isoprostane n= 15
Urine	Baseline	0.06 ± 0.07	0.56 ± 0.75	706.92 ± 679.28
	30 min	0.11 ± 0.17	0.65 ± 0.71	256.50 ± 258.71
	3 hours	0.16 ± 0.31	0.25 ± 0.29	627.67 ± 568.68
	Before bed	0.16 ± 0.31	0.25 ± 0.29	627.67 ± 568.68
	First a.m.	0.12 ± 0.24	0.27 ± 0.31	679.90 ± 622.26
	Next day	0.15 ± 0.25	0.48 ± 0.55	759.35 ± 972.49
		IL-6 N= 17	VEGF N=17	
Plasma	Baseline	0.67 ± 0.80	47.42 ± 3.51	
	30 min	0.81 ± 0.36	77.57 ± 62.55	
	3 hours	1.17 ± 1	66.55 ± 25.78	
	Next day	0.78 ± 0.54	60.85 ± 17.96	

Note. Means and standard deviation expressed as pg/ml

Table 5

Summary of data on effects of THS on biomarkers of exposure, inflammation and oxidative stress

	Timepoints	Cotinine n=12	NNAL n=11	8-isoprostane n= 15
Urine	Baseline	0.44 ± 1.13	0.78 ± 0.80	529.04 ± 377.50
	30 min	0.86 ± 0.83	0.61 ± 0.59	291.50 ± 282.62
	3 hours	1.22 ± 0.89	0.41 ± 0.69	633.18 ± 514.80
	Before bed	1.22 ± 0.89	0.41 ± 0.40	633.18 ± 514.80
	First a.m.	1.14 ± 0.92	0.61 ± 0.59	649.31 ± 397.08
	Next day	1.15 ± 1.05	0.71 ± 1.08	478.75 ± 463
		IL-6 N= 17	VEGF N=17	
Plasma	Baseline	0.84 ± 0.48	57.52 ± 26.19	
	30 min	0.90 ± 0.52	60.82 ± 32.74	
	3 hours	1.49 ± 1.06	76.66 ± 47.86	
	Next day	0.80 ± 0.37	61.91 ± 35.45	

Note. Means and standard deviation expressed as pg/ml

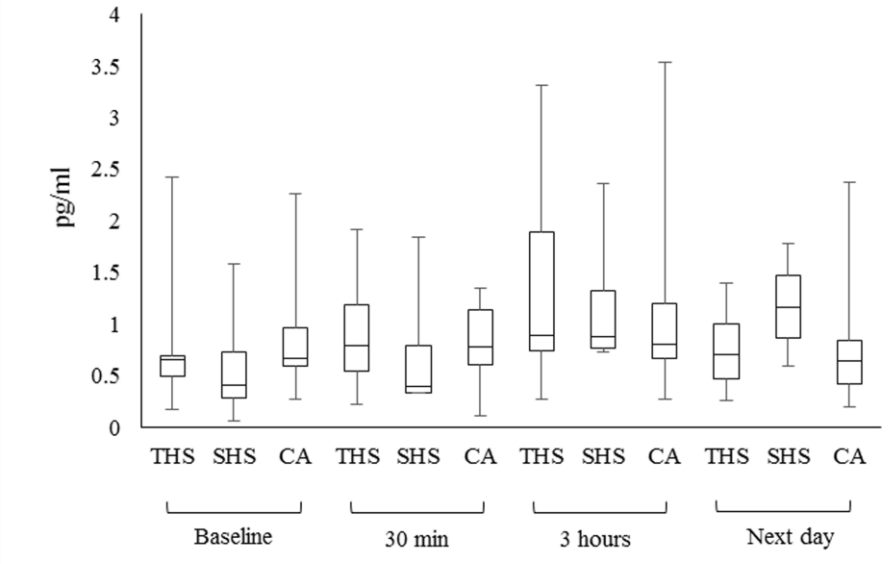

Figure 8. Effect of THS on VEGF levels

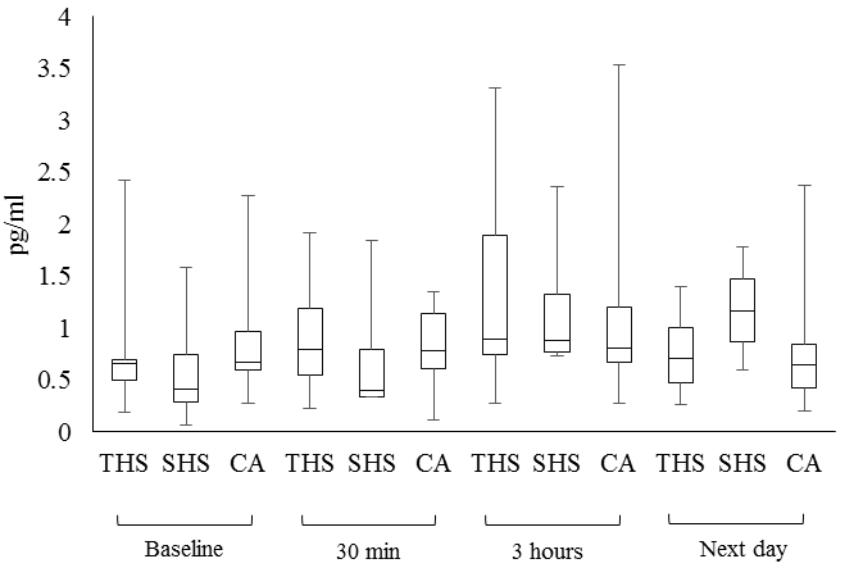

Figure 9. Effect of THS on IL6 levels

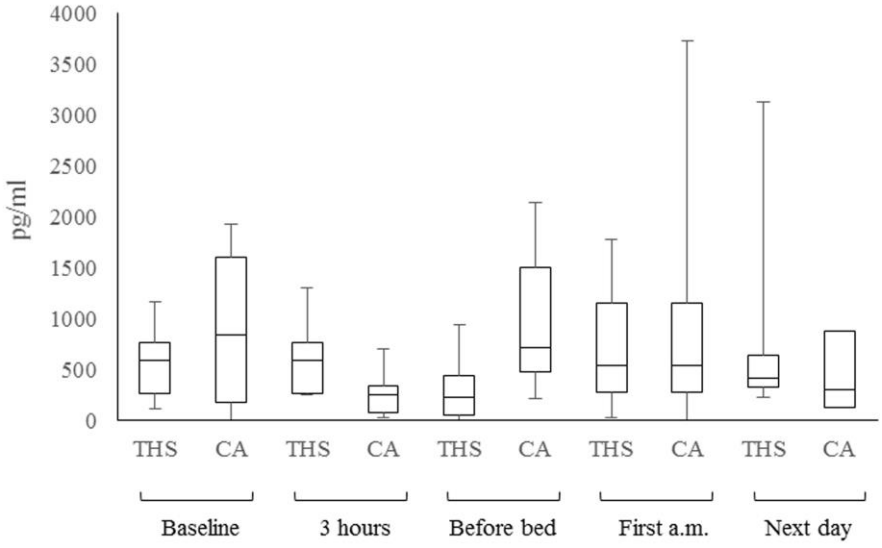

Figure 10. Effect of THS on 8-isoprostane

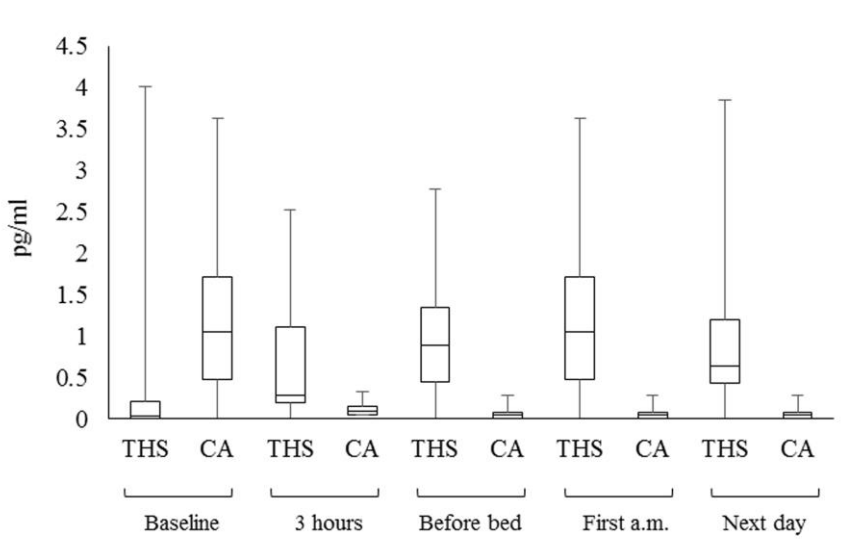

Figure 11. Effect of THS on Cotinine

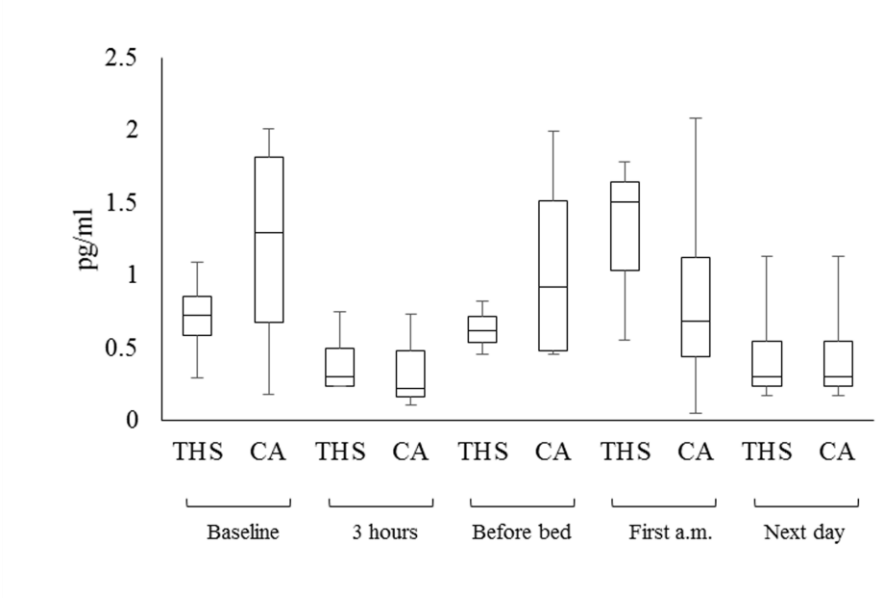

Figure 12. Effect of THS on NNAL

Discussion

This study was the first study to measure inflammatory factors and markers of oxidative stress during respiratory exposure to THS and CA. Additionally, this study measured biomarkers of smoke exposure, cotinine and NNAL, during respiratory exposure to THS and CA. The data from this study revealed significant increases in cotinine and NNAL,

corraborating the utility of these biomarkers in screening and characterization of environmental exposure to tobacco smoke. There was no significant impact of THS on levels of IL-6, VEGF and 8-isoprostane, suggesting acute exposure to THS didn't significantly increase systemic inflammation and oxidative stress.

This study found a positive association between THS and IL-6, indicating inflammation. The findings of IL-6 raising at 30 minutes and dropping off 24 hours later were consistent with the biologic mechanism of this protein (Gabay & Kushner, 1999). On the other hand, VEGF had no association with THS exposure in this study. Although SHS exposure has been associated with higher levels of circulating VEGF and IL-6 (Heiss et al., 2008; Suzuki et al., 2008), this study did not find a statistically significant increase in levels of circulating VEGF and IL-6 with THS exposure. This finding suggests that IL-6 and VEGF may respond to higher concentrations of smoke particles, such as in mainstream and SHS, than those found in THS.

For 8-isoprostane, this study observed a slight increase in circulating 8-isoprostane levels but the finding was not significant. Without any specific exposure or disease state, the level of endogenous isoprostanes vary widely throughout the day due to physiologic factors such as age, gender, ethnicity and hormones (Helmersson & Basu, 1999; Roberts & Morrow, 2000). It is possible that this observation with due to this normal daily variability. It is also possible that this study was not adequately powered enough to detect a true association between THS exposure and elevated 8-isoprostane levels.

This study also observed significant increases in cotinine. Since cotinine, a metabolite of nicotine, is a reliable marker for recent nicotine exposure (Benowitz, 1996; Petersen et al., 2010),

it is not surprising to observe increases in urinary cotinine with episodic exposure to THS, especially given that nicotine comprises the majority of chemicals in THS.

Unexpectedly, this study observed a statistically significant increase in NNAL in urine with THS exposure. Prior literature that has explored NNAL levels with acute exposure to tobacco smoke did not show significant results (Anderson et al., 2003; Carmella et al., 2003; Stephen S. Hecht et al., 2001; Parsons et al., 1998; Thomas et al., 2014). However, our findings are consistent with a study by Anderson et al. (2003) that reported significant increases in NNAL with 4-hour exposure to THS in 18 nonsmokers. Further studies are necessary to test the effect of various conditions, such as smoke concentration, on levels of NNAL.

Strengths. This study used a randomized control trial design, which is considered the gold standard for clinical research because it provides the strongest evidence of causation, minimizes bias and has high internal validity. The strict control of exposure conditions and the extensive chemical characterization of the exposure aerosol could help establish causality between the exposure to THS and changes in biomarkers of inflammation and oxidative stress. Moreover, the use of a crossover design reduced the influence of confounding covariates and increases statistical efficiency so less subjects were required to determine stastistical significance.

Limitations. This study was a pilot study with a small sample size and it is possible that the data was not highly powered enough to reveal significant relationships. There may be differences in response of IL-6, VEGF and 8-isoprostane by gender and age, but the study was not adequately powered to detect these differences. Lastly, a power analysis was not performed to determine the sample size needed to reveal stastistically significant relationships.

Implications for further research. This study highlights the need for further research exploring the effects of THS exposure on human health. Since a power analysis was not performed, the nonsignificant results of Il-6, VEGF and 8-isoprostane mean that additional studies with a power analysis are needed in order to more definitively determine whether Il-6, VEGF and 8-isoprostane increases significantly with THS exposure. Since literature suggests 8-isoprostanes vary greatly throughout the day and with various health conditions, future studies with 8-isoprostane should take this variability into account. The significant results of the increase of NNAL with THS exposure suggest that these biomarkers may play important roles in future biomontoring. However, since the sample size for the NNAL analysis was small (N=11), higher powered studies are needed to confirm or reject these findings. Given that this study only explored respiratory exposure of THS, further studies should also examine dermal exposure to THS, especially since the major components of THS are SVOCs, readily absorbed through the skin.

Major implications to field of nursing. Establishing negative health effects of smoke beyond the life of the burning cigarette increases our knowledge of nonsmoker exposure drastically, making THS a major public health issue. Determining that THS provides a low level, persistent exposure to documented hazardous compounds would bring heightened awareness of the need for further regulation regarding places cigarettes can be smoked. In addition to impacts that further research into THS could have on policy and regulation, establishing THS as a health hazard may influence healthcare's approach to environmental health education and smoking cessation efforts.

Nurses particularly play a significant role in health promotion and prevention surrounding environmental health. The importance of nurses in promoting environmental health stems back to Florence Nightingale and her work linking clean air and water with increased survival rates in wounded soldiers of the Crimean War in the 1800s (Butterfield, 2002). The nursing process recognizes all determinants of health and disease as playing a role in an individual's overall health, include environmental conditions. In 1995 the Institute of Medicine report, "Nursing, Health and the Environment," called upon nurses to integrate environmental health into their nursing practice, research, education, and advocacy (Pope, Snyder, & Mood, 1995).

Additionally, establishing danger with exposure to THS can benefit occupational health nurses in their pursuit to identify overlooked workplace hazards, such as THS, in professions such as housekeeping and bartending. The Occupational Safety & Health Administration's (OSHA) General Duty Clause, a mandate stating that employers have a responsibility to maintain a safe environment for their employees, cannot be applied to THS in the work environment (OSHA Act of 1970). Although there are indoor air regulations applicable to some of the major components found in THS, none of these exposures normally exceed the permissable exposure limits (PELs) (OSHA, 2006). When the danger of THS is further substantiated via research studies, occupational health nursing professionals are better able to advocate for their worker patients either via upstream efforts, such as policywork, or downstream efforts, such as case management.

This study's significant findings regarding increased levels of cotinine and NNAL with acute exposure to THS have implications for possibily utilizing these biomarkers in the future for

monitoring THS exposure in a variety of settings, ranging from workplaces to homes. Environmental biomonitoring is a substantially important for assessing exposure population-wide (CDC, 2015). Monitoring for THS exposure allows for the collection of the necessary information to influence policy, regulation and education regarding the hazards of THS to minimize and eliminate the harms posed by this novel, ubiquitous toxin.

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Appendix A

Exclusion Criteria

1 of the following criteria	Subjects <18 years or >50 years
	Physician diagnosis of asthma, heart disease, hypertension, thyroid disease, diabetes, renal or liver impairment or glaucoma
	Unstable psychiatric condition (such as current major depression, history of schizophrenia or bipolar disorder) or current use of more than two psychiatric medications
	Pregnancy or breastfeeding (by history)
	Alcohol or illicit drug dependence within the past 5 years
	BMI > 35 and < 18
	Current illicit drug use (by history or urine test)
	More than 1/2 pack year smoking history
	Ever a daily marijuana smoker
	Smoked anything within the last 3 months
	Unable to hold allergy or other OTC medicines
	Occupational exposure to smoke, dusts and fumes
	Concurrent participation in another clinical trial
	Unable to communicate in English
2 or more of the following criteria	Systolic blood pressure > 150
	Diastolic blood pressure > 100
	Fasting Blood glucose > 110
	LDL >130

Appendix B

Phone Questionnaire

Initial Screening Questionnaire
THS02 Study

Hello, is the (person you are trying to call)? My name is ____. I'm calling from the UCSF Human Exposure Lab because you responded to an ad we posted on Craigslist for a study on thirdhand cigarette smoke. Are you still interested in participating in our study? Do you have about 10 minutes to answer some questions? (If no, ask when to call). Some of the questions I'll ask will be personal in nature. Do you have some privacy now? (If no, ask when would be a better time to call.)

This questionnaire is designed to quickly see if you may be eligible for this study without sacrificing your privacy. First, I will ask you 4 questions. Please do not answer these questions out loud, but remember if your answer to any of the questions is "no".

1. Could you come to the San Francisco General Hospital for a total of 5 half-days Monday – Friday, over the next three months?
2. Can you walk briskly on a treadmill or ride an exercise bike for 30-minutes at a time?
3. Are you between the ages of 18 & 50?
4. Do you have a USA social security number?

Without giving any other details, did you answer "no" to any of the questions?
If "no": Thank you for your time and interest; however, you are not eligible for this study.

If "yes": I would like to ask you 9 more questions to see if you are eligible. Please do not answer these questions out loud, but remember if your answer to any of the questions is "yes".

1. Do you have any serious health problems other than allergies?
2. Are you currently taking medication for arthritis, tendonitis, high blood sugar, high cholesterol, high blood pressure, heart problems, acne, or auto-immune disease?
3. Do you normally breathe through your mouth instead of your nose?
4. Have you ever had nasal polyps or sinus surgery?
5. Do you currently smoke?
6. Have you smoked more than 50 packs of cigarettes in your life?
7. Have you ever smoked or injected heroin, methamphetamine or crack cocaine?
8. Has a doctor ever told you that you have asthma?
9. (if female) Is there any chance that you are pregnant?

Without giving any other details, did you answer "yes" to any of the questions?

If "yes": Thank you for your time and interest; however, you are not eligible for this study.

If "no": Next I have a block of questions about your smoking history. Many of the people who

participate in our studies have smoked something at some point in their lives. Please just answer my questions honestly, so I can determine whether you are a good fit for this study. Please do not answer these questions out loud, but remember if your answer to any of the questions is "yes".

1. Have you smoked marijuana in the past 3 months?
2. Have you smoked marijuana more than 50 times in your life?
3. Have you ever smoked marijuana daily?
4. Have you ever worked as a cook in a restaurant that served pit-smoked barbecue?

If "yes": Thank you for your time and interest; however, you are not eligible for this study.
If "no": You may be eligible for this study. May I ask you some more questions? Please list all the medications you are taking at this time

Please list all the vitamins, supplements and herbal medicines you are taking at this time.

Describe your exercise activity: type of exercise, how often and how long do you do it in a week.

Do you have allergies?

If allergies, what allergic to?

Smoking :
Please list all the tobacco products you've smoked.

The last time you smoked anything?
What was it?
Age/year started?
Age/year stopped?
How many have you smoked total?

For more than one smoking period or more than one substance smoked

What do/did you smoke?
Age/year started?
Age/year stopped?
How many have you smoked total?

What do/did you smoke?
Age/year started?
Age/year stopped?
How many have you smoked total?

What do/did you smoke?
Age/year started?
Age/year stopped?
How many have you smoked total?

What do/did you smoke?
Age/year started?
Age/year stopped?
How many have you smoked total?

Please describe where and how often you're exposed to secondhand smoke in your daily life.

Do you have any friends or family members who smoke around you?

What are the smoking rules in your household?

Do you have any neighbors who smoke close enough to your home that you can smell the smoke?

If ineligible: Thank you for your time and interest; however, you are not eligible for this study.

If eligible: Thank you for your patience in answering all of these questions. Do you think you are still interested in participating in our study? If yes, proceed to gather identifying data. (Write at top of page!)

What is your schedule and when can you come to our laboratory for screening? We will contact you in the next two weeks to schedule your first visit.

If you decide not to participate in this study, I would appreciate your letting me know the reason for your decision. I can be reached by email at Schick.Lab@ucsf.edu.

E-mail: _____ Phone:_____ Today's Date:

First Name:
Last Name:

DOB: Age: Gender

Appendix C

Medical Questionnaire

Subject ID # _____

PERSONAL INFORMATION:

First name: _____

Last name: _____

DOB: Age: Gender:

Height: Weight:

Are you planning to move within the next six months? Yes No

Has any of your contact information changed?

Are you Hispanic, [Latino I Latina], or of Spanish origin?
Choose all that apply.

1 No, not of Hispanic, Latino or Latina, or Spanish origin
2 Yes, Mexican, Mexican American, Chicano or Chicana
3 Yes, Puerto Rican
4 Yes, Cuban
5 Yes, Another Hispanic, Latino or Latina, or Spanish origin
6 DON'T KNOW
7 REFUSED

What is your race? Choose all that apply.	
1	White
2	Black or African American
3	American Indian or Alaska Native
4	Asian Indian
5	Chinese
6	Filipino
7	Japanese
8	Korean
9	Vietnamese

10	Other Asian
11	Native Hawaiian
12	Guamanian or Chamorro
13	Samoan
14	Other Pacific Islander
15	DON'T KNOW
16	REFUSE

17. Where were you born?

☐ In the U.S. ☐ Outside the U.S.

16. Where were your parents born?

	Mother	**Father**	
	☐ In the U.S.	☐ In the U.S.	
	☐ Outside the U.S.	☐ Outside the U.S.	

18. What is your level of education?

☐ Some high school

☐ High school graduate/GED

☐ Some college

☐ 2-year college degree

☐ 4-year college degree

☐ Graduate degree (masters or doctorate)

19. What is the highest level of education obtained by your parents?

	Mother	**Father**
	☐ Unknown	☐ Unknown
	☐ Some high school	☐ Some high school
	☐ High school graduate/GED	☐ High school graduate/GED
	☐ Some college	☐ Some college
	☐ 2-year college degree	☐ 2-year college degree
	☐ 4-year college degree	☐ 4-year college degree
	☐ Graduate degree (masters or doctorate)	☐ Graduate degree (masters or doctorate)

EXERCISE:

1. Do you have any medical or physical condition that would limit you from doing moderate exercise such as biking or jogging for several 1/2 hour periods separated by 1/2 hour rest periods?

1-YES* 2-NO 3-DK

2. How often do you exercise? _____

3. What type(s) of exercise? _____

ALLERGIES:

4. Have you EVER had a problem with frequent sneezing or a runny or blocked nose when you did NOT have a cold or the flu?

1-YES 2-NO 3-DK

5. Over the past 12 months, have you had a problem with frequent sneezing, or a runny or blocked nose when you did not have a cold or the flu?

1-YES 2-NO 3-DK

If NO proceed to question 12, if YES:

6. Have these nose symptoms been accompanied by itchy or red watery eyes?

1-YES 2-NO 3-DK

7. Are these symptoms seasonal?

1-YES 2-NO 3-DK

If NO proceed to question 9, if YES:

8. What season? (circle all that apply)

1-FALL 3-WINTER 3- WINTER 4-SPRING 5-DK

9. Over the past 12 months, do these nose or eye symptoms interrupt your sleep or daily activities?

1-YES 2-NO 3-DK

10. Over the past 12 months, do you have these symptoms occur for more than 4 consecutive days a week AND more than 4 consecutive weeks?

1-YES 2-NO 3-DK 11. Have you ever been told by any doctor that you had "hay fever" or "allergic rhinitis"?
1-YES 2-NO 3-DK

Do you experience a runny nose or nasal congestion when you (Questions 12 to 19):
12. Eat hot or spicy foods?
1-YES 2-NO 3-DK

13. Are exposed to bright lights?
1-YES 2-NO 3-DK

14. Use household cleaning products?
1-YES 2-NO 3-DK

15. Smell strong perfumes, colognes or after-shaves?

1-YES 2-NO 3-DK

16. Experience sudden changes in air temperature or humidity?
1-YES 2-NO 3-DK

17. Exercise?
1-YES 2-NO 3-DK

18. Consume alcohol?
1-YES 2-NO 3-DK

19 Other factors? (Describe_____ _____)

20. Are you allergic to insect stings?
1-YES 2-NO 3-DK

21. Are you allergic to any foods?
1-YES 2-NO 3-DK

If NO proceed to question 24, if YES:

22. List the types of foods:_____ _____

23. Have you ever received allergy shots? 1-YES 2-NO 3-DK

If NO proceed to question 26, if YES:

24. Are you currently receiving allergy shots?
1-YES 2-NO 3-DK

25. When was the most recent treatment?(date)_____ _ _

Has a doctor ever told you that you had the following conditions (Questions 26-28)?

26. Otitis media (middle ear infection)?
1-YES 2-NO 3-DK

27. Sinusitis (sinus infection)?
1-YES 2-NO 3-DK

28. Nasal polyps?
1-YES 2-NO 3-DK

29. Have you ever had a broken nose ("deviated septum")?
1-YES 2-NO 3-DK

30. Have you ever had a severe head injury (concussion, skull fracture)?
1-YES 2- NO 3-DK

ECZEMA:

31. Have you EVER had eczema?
1-YES 2-NO 3-DK

If NO proceed to question 33, if YES:

32. Do you still have eczema?
1-YES 2-NO 3-DK

33. Have you ever had an itchy rash that was coming and going for at least 6 months?
1-YES 2-NO 3-DK

If NO proceed to question 35, if YES:

34. Has this itchy rash at any time affected any of the following places: the folds of the elbows, behind he knees, in front of the ankles, under the buttocks or around the neck, ears or eyes.
1-YES 2-NO 3-DK

ASTHMA:

35. Have you **ever** been told by any doctor that you have asthma?
1-YES** 2-NO 3-DK (28)

If NO proceed to question 52, if YES:

36. Have you **ever** been hospitalized for your asthma?

1-YES** 2-NO 3-DK

If NO proceed to question 38, if YES:

37. Over the past **2 years**, how often have you been hospitalized?

1-**NONE** 2- **1-2** 3- **3-5** 4- **more than 6** 5-**DK**

38. Have you ever required intubation (a breathing tube) for your asthma?

If NO proceed to question 40, if YES:

39. Over the past **2 years**, how often have you been intubated?

1-**NONE** 2- **1-2** 3- **3-5** 4- **more than 6** 5- **DK**

40. Have you **ever** been given a course of Prednisone (steroids) for your asthma?

If NO proceed to question 42, if YES:

41. Over the past **2 years**, how many courses of steroids have you taken?

1-**NONE** 2- **1-2** 3- **3-5** 4- **more than 6** 5-**DK**

42. Have you **ever** visited the emergency room due to your asthma?

1-YES 2-NO 3-DK

If NO proceed to question 44, if YES:

43. Over the past **2 years**, how many times have you visited the emergency room?

1-**NONE** 2- **1-2** 3- **3-5** 4- **more than 6** 5-**DK**

44. In the past **2 weeks**, how many times per week have you had problems with cough, wheezing, chest tightness, or shortness of breath ? (circle)

1- **NONE** 2- **1-2** 3- **3-6** 4- **≥7** 5- **DK**

45. Over the past **2 weeks,** , how many times have you been awakened at night due to your asthma symptoms?

1- **NONE** 2- **1-2** 3- **3-4** 4- **≥5** 5- **DK**

46. Over the past **2 weeks**, how many asthma attacks have you had?

1- **NONE** 2- **1-2** 3- **3-4** 4- **≥5** 5- **DK**

If NONE then proceed to 48, if more than one:

47. How long have your typical asthma attacks lasted?

1-**hours** 2- **1-2 days** 3-**≥3 days** 4-**DK**

48. How many days have you had to miss work or school due to your asthma?

1- **NONE** 2- **1-2** 3- **3-6** 4- **≥7** 5- **DK**

49. Have you ever used a peak flow meter?
1-YES 2-NO 3-DK

If NO proceed to question 52, if YES:

50. Are you currently using one? 1-YES 2-NO 3-DK
51. What is your usual peak flow value when you are feeling well?
_____

TUBERCULOSIS:

1. Do you currently have tuberculosis?
2. Have you ever had a positive skin test for tuberculosis (TB)?
3. Have you ever taken medicine to cure tuberculosis?

4. Have you ever had a chest X-ray for tuberculosis?

MEDICATIONS

Now get your medications!

52. Name all your medications and dosages:(Taken past month?)

Name_____ Dosage_____ Current
Name_____ Dosage_____ Current
Name_____ Dosage_____ Current
Name_____ Dosage_____ Current
Name_____ Dosage_____ Current
Name_____ Dosage_____ Current

Could you tolerate stopping (Questions 53 to 56):

53. Long term antihistamines : (Claritin for a week and Allegra and Zyrtec for 3 days prior to testing)

1-YES 2-NO 3-DK

54. Short term antihistamines (see list) for at least 12 hours prior to testing?

1-YES 2-NO 3-DK

55. Steroid nose spray for 2 weeks?

1-YES 2-NO 3-DK

56. Mucinex or other mucus thinner for 3 days prior to testing?

1- YES 2-NO 3-DK

57. Are you currently taking any other drugs (i.e. vitamins, over the counter, allergy, non-prescription, asthma, or other non-asthma prescriptions)?

1-YES 2-NO 3-DK

If NO proceed to question 59, if YES:

58. What are they (list all)?_____

59. Are you currently taking any herbal remedies?

1-YES 2-NO 3-DK

60. Are you allergic to any medications?

1-YES 2-NO 3-DK

If NO proceed to question 64, if YES:

61. Name of medicine _____

62. Reaction type? (circle all that apply)

1- HIVES 2-ANAPHYLAXIS 3-OTHER

63. If OTHER for question 62, please specify:

SMOKING AND TOBACCO USE

These questions are about your use of tobacco products. This includes cigarettes, chewing tobacco, snuff, cigars, and pipe tobacco. The first questions are about cigarettes only.

CIGARETTES

Have you ever smoked part or all of a cigarette? o Yes o No

How old were you when you first smoked all or part of a cigarette? o Age or year: _____

o Month (if been smoking less than 2 years): _____

How long has it been since you last smoked all or part of a cigarette? o Within the last 30 days o More than 30 days ago but within the past 12 months

o More than 12 months ago but within the past 3 years

o More than 3 years ago

Now, think about the past 30 days, up to and including today. During the past 30 days, have you smoked part or all of a cigarette?
Yes No

During the past 30 days, on how many days did you smoke part or all of a cigarette?
# of days (estimate): _____

On the day(s) you smoked cigarettes during the past 30 days, how many cigarettes did you smoke (per day, on average)?
# of cigarettes/day (estimate): _____

Has there ever been a period in your life when you smoked cigarettes every day for at least 30 days?

On average, how many individual cigarettes OR packs per day did you smoke when you smoked daily? (choose 1 to answer)

Individual cigarettes per day _____
Packs per day ___

How many years have you smoked cigarettes every day? o # of years (estimate): _____

The next questions are about the brand of cigarettes you smoke -- the brand is the name that is on the pack.

During the past 30 days, what brand of cigarettes did you smoke most often?
_____

During the past 30 days, what type of cigarettes did you smoke most often?

Lights Ultra Lights Mediums Full Flavor

During the past 30 days, were the cigarettes you smoked menthol?
Yes No

During the past 30 days, have you smoked part or all of a roll-your-own tobacco cigarette?

Yes No

CIGAR

The next questions are about smoking cigars. By cigars we mean any kind, including big cigars, cigarillos, and even little cigars that look like cigarettes. We do not mean blunts or cigars that you add other things to.

Have you ever smoked part or all of any type of cigar?

Yes No

How old were you when you first smoked all or part of a cigar?

Age/year: _____
Month (if smoking less than 2 years): _____

How long has it been since you last smoked all or part of a cigar? More than 30 days ago but within the past 12 months More than 12 months ago but within the past 3 years o More than 3 years ago

Now think about the past 30 days including today. During the past 30 days, have you smoked all or part of a cigar?

Yes No

During the past 30 days, on how many days did you smoke a cigar?

# of days (estimate):_____

During the past 30 days, how many cigars did you smoke?
o # of cigars (estimate): ____

Has there ever been a period in your life when you smoked cigars every day for at least 30 days?

On average, how many cigars per day did you smoke when you smoked daily?

cigars per day: _____

How many years have you smoked cigars every day?

# of years (estimate): _____

The next question is about the brand of cigars you smoke -- the brand is the name that is on the pack.

During the past 30 days, what brand of cigars did you smoke most often?
_____

TOBACCO PIPE

The following questions are about using a pipe to smoke tobacco.

Have you ever smoked tobacco in a pipe?

Yes No

How old were you when you first smoked from a tobacco pipe?

Age/year: _____
Month (if been smoking less than 2 years): _____

Now think about the past 30 days including today. During the past 30 days, have you smoked from a pipe?

Yes No

During the past 30 days, on how many days did you smoke tobacco from a pipe?

# of days (estimate):_____

During the past 30 days, how many times did you smoke tobacco from a pipe?

# of times (estimate): ____

Has there ever been a period in your life when you smoked tobacco from a pipe every day for at least 30 days?

On average, how many bowls of tobacco per day did you smoke when you smoked daily?

cigars per day: _____

How many years have you smoked a pipe every day?

# of years (estimate): _____

The next question is about the brand of tobacco you smoke -- the brand is the name that is on the pack.

During the past 30 days, what brand of tobacco did you smoke most often?
_____

SNUFF/DIP

These next questions are about your use of snuff, sometimes called dip. Snuff is a finely ground form of tobacco that usually comes in a container called a tin. You can use snuff by placing a pinch or dip in your mouth between your lip and gum or between your cheek and gum. Snuff can also be inhaled through the nose. Snuff is sold in both loose form and in ready-to-use packets.

Have you ever used snuff?

Yes No

How old were you when you first started using snuff?

Age/year: _____
Month (if been using for less than 2 years): _____

Now think about the past 30 days including today. During the past 30 days, have you used snuff, even once?

Yes No

If yes

During the past 30 days, how many days did you use snuff?

# of days (estimate):_____

During the past 30 days, what brand of snuff did you use most often?

If no

If haven't used in the past 30 days, how long has it been since you last used snuff?

CHEWING TOBACCO

The next questions are only about chewing tobacco. Chewing tobacco is coarsely shredded tobacco that is sold in pouches of loose tobacco leaves or in a "plug" or "twist" form. To use chewing tobacco, you either chew it or hold it in your cheek or inside your lower lip.

Have you ever used chewing tobacco, even once?

Yes No

How old were you when you first chewed tobacco? OR What is your best guess of how long it has been since you have chewed tobacco?

Age/year: _____

Month (if been using for less than 2 years): _____

Now think about the past 30 days including today. During the past 30 days, have you used chewing tobacco, even once?
Yes No

If yes

During the past 30 days, on how many days did you use chewing tobacco?

# of days (estimate):_____

During the past 30 days, what brand of chewing tobacco did you use most often?

If no

If haven't used in the past 30 days, how long has it been since you last used chewing tobacco?

SNUS POUCHES

The next questions are only about snus pouches. Snus pouches are moist powder tobacco products that are consumed by placing it under the upper lip for extended periods of time.

Have you ever used snus pouches?

Yes No

How old were you when you first used snus pouches?

Age/year: _____
Month (if been using for less than 2 years): _____

Now think about the past 30 days including today. During the past 30 days, have you used snus pouches?

Yes No

If yes

During the past 30 days, on how many days did you use snus pouches?

# of days (estimate):_____

During the past 30 days, what brand of snus pouches did you use most often?
_____

If no

If haven't used in the past 30 days, how long has it been since you last used snus pouches? _____

DISSOLVABLE TOBACCO

The next questions are only about dissolvable tobacco. Dissolvable tobacco are tobacco products that dissolve in the mouth and can come in the form of "strips", "sticks" or "orbs".

Have you ever used any dissolvable tobacco product? Yes No

How old were you when you first dissolvable tobacco products?

Age/year: _____
Month (if been using for less than 2 years): _____

Now think about the past 30 days including today. During the past 30 days, have you used dissolvable tobacco?

Yes No

If yes

During the past 30 days, on how many days did you use dissolvable tobacco?

# of days (estimate):___

During the past 30 days, what brand of dissolvable tobacco did you use most often?

If no

If haven't used in the past 30 days, how long has it been since you last used dissolvable tobacco? _____

SUMMARY OF ORAL TOBACCO USE

Has there ever been a period in your life where you used snuff, snus or chewing tobacco every day for at least 30 days?

How many years have you used snus or chewing tobacco every day?_____

E-CIGARETTES

The next questions are only about E-cigarettes. E-cigarettes are battery powered devices that use a heating element to vaporize a liquid solution most commonly containing nicotine and other flavorings. We include E-hookahs under this definition.

Have you ever used an e-cigarette?

Yes No

How old were you when you first used an e-cigarette?

Age or year: _____

Month (if been smoking less than 2 years): _____

How long has it been since you last used an e-cigarette?

o Within the last 30 days
o More than 30 days ago but within the past 12 months
o More than 12 months ago but within the past 3 years
o More than 3 years ago

Now, think about the past 30 days, up to and including today. During the past 30 days, have you smoked an e-cigarette?

Yes No

If yes

During the past 30 days, how many days did you used an e-cigarette?
# of days (estimate): _____

If you use a cartridge e-cigarette, during the past 30 days, how many cartridges have you used total? o # of cartridges (estimate): _____

If you use a refillable e-cigarette, during the past 30 days, how much juice have you used each week?
# of ml/bottles (estimate): _____

How many coils do you go through each month? _____

What brand of e-cigarette did you use? _____

What brand of e-cigarette juice/cartridge do you use? _____

What is the concentration or strength of nicotine in the liquid you used?

o 0 mg/ml
o 4-8 mg/ml
o 9-12 mg/ml
o 18-24 mg/ml

o 36 mg/ml
o Not sure
Has there ever been a period in your life when you used an e-cigarette every day for at least 30 days?

On average, how many times did you use an e-cigarette per day when you vaped daily?

times per day: _____

How many years/months have you used an e-cigarette every day?

# of years (estimate): _____

# of months (estimate):_____

If no

If haven't used in the past 30 days, how long has it been since you last used e-cigarettes?
_____

HOOKAH

The next questions are about smoking flavored tobacco out of a hookah. Hookah is single or multi stemmed water pipe for vaporizing and smoking flavored tobacco.

Have you ever smoked hookah?

Yes No

How old were you the first time you smoked hookah?

Age or year: _____ Month (if been smoking less than 2 years): _____

Now think about the past 30 days including today. During the past 30 days, have you smoked hookah?
Yes No

During the past 30 days, on how many days did you smoke hookah? o # of days (estimate): _____

During the past 30 days, how many times did you smoke hookah? o # of times (estimate): _____

How long has it been since you last smoked hookah? _____

TOBACCO QUIT ATTEMPTS

Have you ever tried to quit using tobacco products?
Yes No

What tobacco product(s) did you try to quit? _____

How many quit attempts have you made over your lifetime?

# of attempts (estimate): _____

How many quit attempts have you made last year?

# of attempts (estimate): _____

Are you currently trying to quit using tobacco products?
Yes No

If yes

Are you currently using medication to aid your efforts to quit? (such as nicotine patches or prescription drugs such as Wellbutrin, varenicline, Wellbutrin, Zyban, Chantix, or bupropion)

Yes Which ones?
No

MARIJUANA USE

MARIJUANA

The next questions are about marijuana. Marijuana is also called cannabis, weed, pot, bud, green, trees, grass, and herb. This also includes resin, concentrates, or extracts made from marijuana, such as hash (hashish), dabs, hash oil, wax, and BHO (butane hash oil). Marijuana is usually smoked, either in cigarettes (joints) or in a pipe, but it can also be vaporized or cooked in food.

Have you ever, even once, used marijuana?

Yes No

How old were you when you first used marijuana?
o Age or year: o Month (if been using less than 2 years):

How long has it been since you last used marijuana?
o Within the last 30 days
o More than 30 days ago but within the past 12 months
o More than 12 months ago but within the past 3 years
o More than 3 years ago

During the past 30 days, how many days did you use marijuana? o # of days (estimate): _____

On the day(s) you used marijuana during the past 30 days, how many times did you use it (per day, on average)?
o # of days (estimate): _____

Now think about the past 12 months, including today. We want to know how many days you've used marijuana during the past 12 months. What would be the easiest way for you to tell us how many days you've used it?

Please pick one of these 3 questions to answer.

Average number of days per week during the past 12 months: _____
Average number of days per month during the past 12 months: _____
Total number of days during the past 12 months: _____

Has there ever been a period in your life when you used marijuana every day for at least 30 days? _____

On average, how many times a day did you use it when you used it daily? _____

How many years did you use it every day? _____

How do you most often use marijuana?
•Smoking
•Vaporizing

•In food

What form of marijuana do you most often use?
•Bud/whole marijuana
•Marijuana concentrates (hash, dabs, hash oil, wax, and BHO)
•Marijuana tinctures

MARIJUANA & TOBACCO

The next questions are about marijuana and tobacco smoked together. This includes 'spliffs' (marijuana and tobacco mixed in a cigarette), 'moles' (marijuana and tobacco mixed in a pipe), and 'blunts' (marijuana rolled in a tobacco cigar wrapper).

Have you ever, even once, smoked marijuana and tobacco together?

Yes No

How old were you when you first smoked marijuana and tobacco together?

Age or year: _____
Month (if been using less than 2 years): _____

How long has it been since you last smoked marijuana and tobacco together?

Within the last 30 days
o More than 30 days ago but within the past 12 months
o More than 12 months ago but within the past 3 years
o More than 3 years ago

During the past 30 days, on how many days did you smoke marijuana and tobacco together? o # of days (estimate): _____

On the day(s) you smoked marijuana and tobacco together during the past 30 days, how many times did you smoke them (per day, on average)? o # of days (estimate): _____

Now think about the past 12 months, from through today. We want to know how many days you've smoked marijuana and tobacco together during the past 12 months. What would be the easiest way for you to tell us how many days you've used it?

Please pick one of these 3 questions to answer.

Average number of days per week during the past 12 months: _____
Average number of days per month during the past 12 months: _____
Total number of days during the past 12 months: _____

OTHER SUBSTANCE USE

The next sets of questions are going to address all other forms of drug use.

ALCOHOL

Have you ever, even once, had alcohol?

Yes No

How old were you when you had your first drink of alcohol? o Age or year: _____ o Month (if been using less than 2 years): _____

How long has it been since you last had a drink of alcohol?
o Within the last 30 days
o More than 30 days ago but within the past 12 months
o More than 12 months ago but within the past 3 years
o More than 3 years ago

During the past 30 days, how many days did drink alcohol?

# of days (estimate): _____

On the day(s) you had a drink of alcohol during the past 30 days, how many drinks did you have (per day, on average)?

# of days (estimate): _____

1 shot of liquor OR 1 five ounce glass of wine OR 1 bottle of beer = 1 drink

Now think about the past 12 months, including today. We want to know how many days you had a drink of alcohol during the past 12 months. What would be the easiest way for you to tell us how many days you've used it?

Please pick one of these 3 questions to answer.

Average number of days per week during the past 12 months: _____
Average number of days per month during the past 12 months: _____
Total number of days during the past 12 months: _____

Has there ever been a period in your life when you had a drink of alcohol every day for at least 30 days? _____

On average, how many drinks of alcohol did you every day? _____

How many years did you drink alcohol every day? _____

In the past 30 days, have you had more than 5 drinks in one day? _____

STIMULANT DRUGS

The next questions are only about stimulant drug use. Stimulants are a class of psychoactive drugs that may increase alertness, movement and wakefulness. Common forms are cocaine, 'crack', meth, amphetamine ('speed') and MDMA ('ecstasy' or 'molly').

Have you ever used a stimulant?

Yes No

How long has it been since you last used a stimulant? o Within the last 30 days

o More than 30 days ago but within the past 12 months o
More than 12 months ago but within the past 3 years o More
than 3 years ago

Now think about the past 12 months, including today. We want to know how many days you've used a stimulant during the past 12 months. What would be the easiest way for you to tell us how many days you've used it?

Please pick one of these 3 questions to answer.

Average number of days per week during the past 12 months: _____
Average number of days per month during the past 12 months: _____
Total number of days during the past 12 months: _____

DEPRESSANT DRUGS

The next questions are only about depressant drug use. Drugs in the class are known to reduce arousal and stimulation in the brain. Common types of depressants include codeine, hydrocodone, oxycodone, heroin, morphine and methadone.

Have you ever used a
depressant? o Yes o No

How long has it been since you last used a depressant?

o Within the last 30 days
o More than 30 days ago but within the past 12 months
o More than 12 months ago but within the past 3 years
o More than 3 years ago

Now think about the past 12 months, including today. We want to know how many days you've used a depressant during the past 12 months. What would be the easiest way for you to tell us how many days you've used it?

Please pick one of these 3 questions to answer.

Average number of days per week during the past 12 months: _____
Average number of days per month during the past 12 months: _____
Total number of days during the past 12 months: _____

PSYCHEDELIC DRUGS

The next questions are only about psychedelic drug use. Psychedelics are psychoactive drugs, also known as hallucinogens, which produce experiences outside of familiar states of consciousness. Common forms of psychedelic drugs include LSD ('acid'), mescaline, psilocybin mushrooms ('shrooms'), salvia divinorum and DMT.

Have you ever used psychedelic?

Yes No

How long has it been since you last used a psychedelic?

o Within the last 30 days
o More than 30 days ago but within the past 12 months
o More than 12 months ago but within the past 3 years
o More than 3 years ago

Now think about the past 12 months, including today. We want to know how many days you've used a psychedelic during the past 12 months. What would be the easiest way for you to tell us how many days you've used it?

Please pick one of these 3 questions to answer.

Average number of days per week during the past 12 months: _____
Average number of days per month during the past 12 months: _____
Total number of days during the past 12 months: _____

DISSOCIATIVE DRUGS

The next questions are only about dissociative drug use. Dissociative drugs are a class of hallucinogens which produce feelings of detachment-dissociation. Examples include dextromethorphan (DXM), ketamine, PCP ('angel dust').

Have you ever used a dissociative drug? o Yes o No

How long has it been since you last used a dissociative drug? o Within the last 30 days o More than 30 days ago but

within the past 12 months o More than 12 months ago but
within the past 3 years o More than 3 years ago

Now think about the past 12 months, including today. We want to know how many days you've used a dissociative drug during the past 12 months. What would be the easiest way for you to tell us how many days you've used it?
Please pick one of these 3 questions to answer.

Average number of days per week during the past 12 months: _____

Average number of days per month during the past 12 months: _____

Total number of days during the past 12 months: _____

SECONDHAND SMOKE EXPOSURE

87. What are the smoking rules in your household, if any? Would you say…
1-Smoking is completely banned for everyone…
2-Smoking is generally banned for everyone with few exceptions…
3-Smoking is allowed in some rooms only, or… 4-
There are no restrictions on smoking?

88. Is smoking allowed outside near the entrance or windows to the house?
1-YES 2-NO 3-DK

89. Do you currently work for money in an indoor setting such as an office, plant or store outside of your home?
1-YES 2-NO 3-DK

90. Is smoking allowed anywhere inside the building where you work?
1-YES 2-NO 3-DK

91. Do people smoke near the entrances to the building where you work?
1-YES 2-NO 3-DK

92. Do you spend time near people who are smoking outdoors?
1-YES 2-NO 3-DK

93. Do you drive or ride in a car with anyone who smokes?
1-YES 2-NO 3-DK

94. When you mother was pregnant with you, did either of your parents smoke?
1-MOTHER 2-FATHER 3-BOTH 4-NEITHER

95. When you were a child, did anyone regularly smoke inside your home?
1-YES 2-NO 3-DK

MEDICAL CONDITIONS

96. How many times were you sick last year?
1- 0-1 Times 2- 2-4 Times 3- More than 5 times.

97. Has a doctor ever told you that you had a problem with your heart that would limit your activity or ability to exercise?

1-YES(MD) 2-NO 3-DK

98. In the past 12 months, have you taken any medication for your heart?

1-YES(MD) 2-NO 3-DK

If NO proceed to question 100, if YES:

99. List all medication(s),_____

100. Have you ever had any angina or chest pain with exertion?

1-YES(MD) 2-NO3-DK

If NO proceed to question 102, if YES:

101. Have you had angina or chest pain with exertion in the past 6 months?

1-YES(MD) 2-NO 3-DK

102. Have you ever been told that you had high blood pressure?

1-YES(MD) 2-NO 3-DK

103. In the past 12 months, have you taken medication for your blood pressure?

1-YES(MD) 2-NO 3-DK

If NO proceed to question 105, if YES:

104. List all medication (s)._____

105. Do you have any other major medical illnesses (i.e. cancer, etc.).

1-YES(MD) 2-NO 3-DK

If NO proceed to question 107, if YES:

106. List all illness(es). _____

OCCUPATIONAL HISTORY:

107. Have you ever worked in a job that exposed you to vapors, dust or fumes?

1-YES 2-NO 3-DK Please list job titles and descriptions

going back 5 years:

Date Job Title Job Description Exposures (smoke, dust, metals, fumes or vapors):

108. _____

109. _____

110. _____

111. _____

112. _____

113. Do you have hobbies or recreational activities that expose you to smoke, dust, fumes or vapors?

1-YES (MD) 2-NO 3-DK

If NO proceed to question 112, if YES:

114. What is the hobby or activity? (Describe) _____

115. Describe the exposure? _____

Appendix D

Images of SHS/THS Generation and Exposure System

Figure D1. Human Exposure Chamber

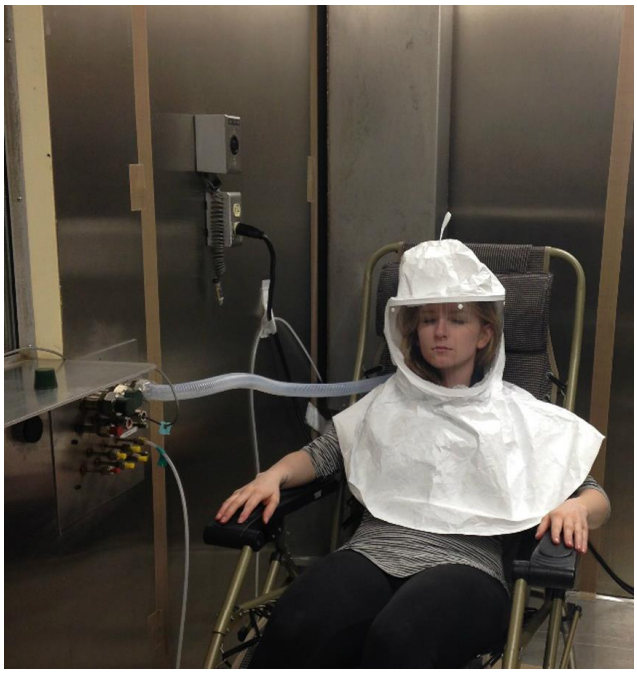

Figure D2. Subject undergoing THS exposure.

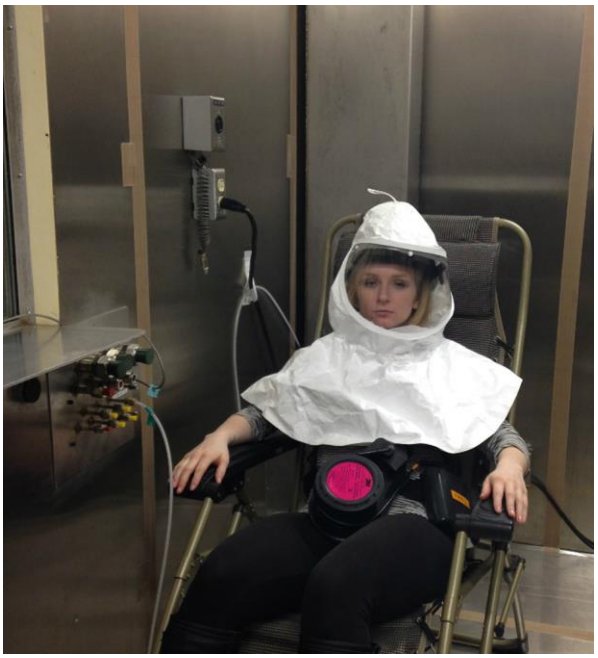

Figure D3. Subject undergoing clean air exposure.

Figure D3. Smoking Machine

Figure D4. Aging Chamber

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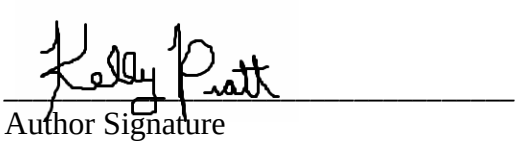

Author Signature

09/08/2015

Date