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nRespiratory Exposure to Thirdhand Cigarette Smoke Increases Concentrations of Urinary Metabolites of Nicotine.

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IMPLICATIONS

This study shows that a three-hour inhalational exposure to the tobacco smoke aerosol that forms in a room that has been smoked in and left unventilated overnight causes increases in urinary metabolites of nicotine, but not of the tobacco-specific nitrosamine NNK. This suggests that cleaning personnel and others who live and work in rooms polluted with aged or thirdhand cigarette smoke regularly may have inhalational exposures and potential health effects related to their exposure to nicotine and other smoke toxicants.

ABSTRACT

Introduction. The aims of this study were to characterize particle size in a thirdhand smoke aerosol and measure the effects of controlled inhalational exposure to thirdhand smoke on biomarkers of tobacco smoke exposure, inflammation and oxidative stress in human subjects Secondhand cigarette smoke changes physically and chemically after release into the environment. Some of the resulting chemicals persist indoors as thirdhand cigarette smoke. Thirdhand smoke that is sorbed to surfaces can emit particles back into the air.

Methods. Smoke particle size was measured with a scanning mobility particle sizer/condensation particle counter. Using a crossover study design, 18 healthy nonsmokers received a three-hour inhalational exposure to thirdhand smoke and to filtered, conditioned air. Thirdhand smoke was generated with a smoking machine and aged overnight in a chamber. The chamber was flushed with clean air to create the THS aerosol. The tobacco smoke metabolites cotinine, 3-hydroxycotinine and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) were measured in urine.

Vascular endothelial growth factor and interleukin-6 in plasma, and 8-isoprostane in urine, were measured using enzyme-linked immunosorbent assay kits.

Results. Mean smoke particle size increased with aging (171 nm to 265 nm). We found significant increases in urinary cotinine and 3-hydroxycotinine after three hours of exposure to thirdhand smoke and no significant increases in NNAL, interleukin-6, vascular endothelial growth factor or 8-isoprostane.

Conclusions. Acute inhalational exposure to 22-hour old tobacco smoke aerosol caused increases in the metabolites of nicotine but not the metabolites of the tobacco-specific nitrosamine NNK (4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone). This corroborates the utility of cotinine and NNAL for secondhand and thirdhand smoke exposure screening.

INTRODUCTION

Thirdhand smoke (THS) is a term for a third major route of exposure to cigarette smoke, in addition to active smoking and secondhand smoke (SHS) exposure(1). When tobacco smoke is released into the air, semi-volatile organic compound including nicotine, nitrosamines and polycyclic aromatic hydrocarbons (PAHs), stick to surfaces and persist in the indoor environment for hours, days, months and years after smoking(2, 3). In some cases, 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) (4) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) (5). Many THS components are oxidants(6) and some, such as benzo[a]pyrene and formaldehyde, are known human carcinogens (7).

Research has shown that reaction of THS with ambient concentrations of oxidant gases can form ultrafine particles(8-10), and that cigarette smoke chemicals can sorb to existing particles indoors(11). Our unique secondhand smoke generation system uses a smoking machine to generate cigarette smoke, dilutes it with conditioned, filtered air and ages it for 30 minutes in a stainless steel chamber, to mimic the changes in smoke chemistry that occur in realistic indoor environments(5, 12). We observed particles coming out of our secondhand smoke generation system the morning after the system was used to generate smoke. The smoking machine was not in operation, the smoke aging chamber was sealed overnight, with near zero air exchange, and our previous studies had shown that the SHS particles, nicotine and PAHs sorbed rapidly to surfaces in the chamber(5, 12).

Thus, we hypothesized that the particles that came out of the chamber in the morning were derived from the smoke generated the prior day and would be representative of particulate exposures that people receive when they enter rooms 12-20 hours after smoking has occurred.

The chemicals found in THS can be inhaled, ingested or dermally absorbed. Young children, who spend most of their time exploring their environment, are at increased risk of THS exposure. Little is known about the health effects of THS exposure. 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. Sidestream smoke, the smoke that is emitted from the end of a burning cigarette, composes the other ~85% of SHS (13). SHS is a pollutant indoors and outdoors (14) and is classified as a Group 1 carcinogen (15). SHS exposure 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% (13).

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 (16), cardiovascular disease (17), asthma (18) and chronic obstructive pulmonary disease (19).

The aims of this study were to characterize the THS aeroso and evaluate the acute effects of respiratory exposure to THS on urinary cotinine and NNAL, in healthy human volunteers. 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. We also investigated the effects of respiratory THS exposure on systemic inflammation and oxidative stress by measuring circulating levels of IL-6, VEGF and urinary 8-isoprostane.

METHODS

Study Design

This study used a randomized, crossover study design with a convenience sample of healthy nonsmokers. 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. A sample size of 18 was chosen based on a prior study with similar concentration smoke exposures (350 $\mu g/m^3$ for 3 hours), that showed statistically significant increases in urinary cotinine, 3-hydroxycotinine and NNAL and circulating VEGF with 12 participants (20). The sequence of exposures was randomized and the two study visits were separated by a minimum of 21 days to avoid carry-over effects.

THS Generation and Characterization

One day prior to each THS exposure, Marlboro Red cigarettes (hard pack), were smoked by an automatic cigarette smoking machine (TE-10z, Teague Enterprises, Woodland, California, USA) according to International Standards Organization (ISO) standard 3308, with a two second puff every minute (ISO, 2012). The smoke was conducted through a smoke aging chamber (Figure 1) at the rate of 2 air changes

per hour. The aging chamber contained common indoor furnishing including carpet, painted wall board, cloth and paper. After SHS generation, the system was shut down and the smoke in the chamber was allowed to age for 22 hours. A head-only, respiratory THS exposure was created by flushing the smoke aging chamber with conditioned, filtered air, driving the aerosol from the chamber to a Tyvek hood with transparent, full-face shield (Airmate # BE-10–3, 3M, Inc., St. Paul, MN).

Exposures took place in a stainless steel chamber (9 x 9 x 9 feet) supplied with HEPA and charcoal filtered conditioned air at 0.85 air changes per minute (21). Clean air exposures were produced by attaching the exposure hood to 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). Particle concentration in the exposure aerosol was measured using a laser photometer (Dusttrak II, model 8530, TSI Inc., Shoreview, MN) and gravimetrically (12). We used the area under the laser photometer curve as the measure of particle exposure. Nicotine was measured in six of the experiments using two, stacked filters. The front filter was untreated and collected particles. The rear filter was treated with sodium bisulfate to collect gas/vapor phase nicotine and quantified by GC/MS(5). The total nicotine mass was the sum of gas and particle phase nicotine. Measurements of the particle size distribution in the source SHS, the THS exposure aerosol and in the conditioned, filtered control air were made with a TSI (Shoreview, MN) scanning mobility particle sizer (SMPS, model 3077 differential mobility analyzer, model 3025 condensation particle counter).

Study Participants

Participants were recruited using online advertisements in the San Francisco Bay area. Inclusion criteria included age (18-50), ability to exercise and no history of

chronic diseases. Exclusion criteria included smoking, 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. Nonsmokers were determined as having smoked no more than 50 packs of cigarettes in their life and not smoking cigarettes in the past year. Marijuana smokers were excluded if they had ever smoked daily and if they had smoked in the preceding 3 months (22). This study was approved by the University of California, San Francisco Institutional Review Board.

Study Procedures

The patients were asked to withhold food, alcohol and caffeinated beverages for 12 hours before the study visits. On the day of the exposure visit, the subject was seated in the exposure chamber and donned the exposure hood, which allowed the subject to breathe either smoke or conditioned, filtered air. Air flowed through the exposure hood at 200 liters/minute. Each exposure session started with 30 minutes of exposure through the hood, then the subject left the exposure chamber to provide a blood sample, and returned for another 2.5 hours of exposure. Spot urine samples were collected before exposure, 3 hours after exposure, before bed, at waking and at the follow up visit in the laboratory the next day. The times at which the before bed and at waking samples were collected was set by the participants. The final sample was collected 22 hours after the start of exposure.

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 (#BMS2019, ThermoFisher Scientific

Invitrogen, Camarillo, CA)), IL-6 (#D6050, R&D systems, Minneapolis, MN) and 8-isoprostane (#8iso1, Detroit R&D systems, Detroit, MI) according to the manufacturers' instructions. The level of quantitation was 0.70 pg/mL for IL-6, 5 pg/mL for VEGF and 10 pg/ml for 8-isoprostane. All samples were run in triplicate and the values were averaged. The final concentrations were expressed as picograms per microliter (pg/ml).

Banked urine samples, stored at -20 degrees C, were thawed and assayed for cotinine, 3-hydroxycotinine and NNAL by liquid chromatography–atmospheric pressure chemical ionization tandem mass spectrometry, following the methods of Bernert et al. (23) and Jacob et al. (24)The level of quantitation was 0.05 ng/ml for cotinine, 0.1 ng/ml for 3-hydroxycotinine and 0.25 pg/ml for NNAL.

2.6 Statistical Analysis

Correlations between particle mass input and output and between nicotine exposure and metabolite concentrations, were tested using linear regression in Excel. For metabolite concentration summary statistics and analyses, all participants' data were used. Values below the level of quantitation were set to LOQ/$\sqrt{2}$.: Cotinine = 0.0353 ng/ml, 3-hydroxycotinine = 0.071 ng/ml, NNAL = 0.177 pg/ml. The total excreted moles of the metabolites of nicotine and NNK was estimated by calculating the change in concentration from one time point to the next. Where both values were below the level of quantitation, the difference was recorded as zero. Where a missing value was subtracted from a missing value, the difference was left blank. Where a known value was subtracted from a missing value, the difference was left blank. Where a missing value was subtracted from a known value the difference was recorded as the known value. The change values

were multiplied by the elapsed hours between the time points, and summed to yield the total change in metabolite concentration between baseline and the final timepoint. This change value was divided by the molecular weight of the metabolite to give the change in moles. An estimate of total nicotine metabolites were calculated by adding the masses of cotinine and 3-hydroxycotinine(25). The data were tested for normality with the Shapiro Wilk test. The metabolite change data for cotinine and 3-hydroxycotinine were not normally distributed, so the potential effects of the exposures were tested using the Wilcoxon signed rank test. The NNAL data were normally distributed and a one-tailed, paired t-test was used. Statistical significance was determined at $p < 0.05$. SigmaPlot version 14.0 was used for the Shapiro Wilk, Wilcoxon and t-tests and the linear regressions.

A univariate analysis of variance with a fixed-effects model for repeated measures was performed using SAS software (2014, Cary, NC) to determine the differences between the mean concentrations of cotinine, NNAL, 3-hydroxycotinine, IL-6, VEGF and 8-isoprostane after THS exposures and conditioned, filtered air exposures. Data were log transformed prior to analysis. The models were estimated using maximum likelihood estimation. The models include effects for time, order of exposure and clean or smoke exposure. No data were excluded from these analyses. Statistical significance was determined at $p < 0.05$.

RESULTS

THS Aerosol.

Before the clinical study, we compared the average particle concentration and total particle mass for the SHS input and the THS output on the following day. Using

particle mass data from laser photometers, calibrated to gravimetric measurements, we found a linear relationship between SHS particle input and THS particle output. Approximately 2% of the total input particle mass (area under the photometer data curve) emerged as THS particles and the peak THS output concentration was approximately 50% of the average SHS input concentration. We measured particle size to see how aging affected particle diameter and potential penetration into the respiratory tract. SMPS measurements comparing particle size distribution for the source air, source SHS, the THS aerosol and the ambient laboratory air concentrations (Table 1) show that particle mass and particle number decreased with aging, but particle size increased. Over the course of the clinical study, the average particle exposure mass (area under the photometer curve) was 1.15 +/- 0.95 mg.

In the subset of 6 experiments where nicotine was measured, the majority of the nicotine was in the gas/vapor phase, with an average ratio of gas/vapor phase to particle phase of 15 ± 2. Particle concentration was highest at the start of exposure, declining gradually throughout the exposure. Supplementary Figure 1 shows representative particle concentration data from an exposure. The gap from 10:27 AM to 11:00 AM represents the pause in exposure for the 30-minute blood draw. The last 1.5 hours of exposure were characterized by low concentrations of particles.

Participants. 18 participants completed both exposures (9 women and 9 men). 11 of the participants received their THS exposure first. Two additional participants completed the THS exposure only and one completed the Clean Air exposure only

for a total of 21 participants. Participants ranged in age from 21 to 50 years (median = 37.5). 12 participants identified as Caucasian, 3 as Asian, 2 as African American and 4 identified as 2 or more races and Hispanic.

Biomarkers of Tobacco Smoke Exposure.

*Missing Data***:** From a total of 195 results possible ([18 participants x 5 time points x 2 exposures] + [3 participants x 5 time points x 1 exposure]), there were 176 results available for cotinine, 182 for 3-hydroxycotinine and 162 for NNAL.

Baseline biomarker concentrations: Participants had low concentrations of tobacco smoke metabolites in their urine at baseline indicating that their exposure to secondhand and thirdhand cigarette smoke outside of the study was low. 14 of the 21 participants had cotinine values below the limit of quantitation at one study visit and, of these, five had cotinine values below the limit of quantitation at both study visits. The corresponding numbers of participants with baseline metabolite concentrations below the level of quantitation were six and one for 3-hydroxycotinine and nine and four for NNAL. The tobacco biomarker data were thus skewed toward zero (Table 2). Baseline creatinine data were only slightly skewed.

Metabolites of Nicotine and NNK

Concentrations of urinary cotinine and 3-hydroxy cotinine increased after THS exposure, but not after clean air exposure (Table 3 and Supplementary Table 1). There was a statistically significant difference between THS and clean air exposure for total cotinine and 3-hydroxycotinine ($P < 0.001$), but not for total NNAL ($P = 0.088$). Likewise, there was a statistically significant difference between THS and

clean air exposure for total nicotine metabolites (cotinine + 3-hydroxycotinine) ($P < 0.001$). Peak cotinine and 3-hydroxycotinine concentrations exposure were seen 22 hours (Supplementary Table 1) after THS exposure began.

When analyzed using univariate analysis of variance with a fixed-effects model for repeated measures, cotinine ($P = 0.0011$) and 3-hydroxycotinine ($P = <0.001$) changed significantly with time after exposure and NNAL did not. However, we also saw significant effects for order of exposure ($P = 0.0132$ for cotinine, $P = 0.0217$ for 3-hydroxycotinine).

Correlations to Exposure Metrics

There was a weak correlation between nicotine minute exposure (nicotine concentration x respiratory rate x total exposure time) and total nicotine metabolites ($R^2 = 0.64$) in the subset of exposures with complete nicotine data ($n = 6$). There were no correlations between total particle minute exposure or peak $PM_{2.5}$ concentration and total nicotine metabolites ($R^2 < 0.5$).

Biomarkers of Inflammation and Oxidative Stress. No exposure-dependent effects of THS on plasma IL-6, plasma VEGF or urinary 8-isoprostane were found in this study. We observed a trend toward an increase in VEGF but the finding was not statistically significant.

DISCUSSION

This is the first controlled human exposure study to show that inhalational exposure to THS aerosol causes increases in urinary metabolites of nicotine, but not NNAL. The exposure was designed to mimic the experience of a nonsmoker entering an

unventilated room where smoking occurred on the preceding day. Normally, particle concentration decreases rapidly after smoking. However, in rooms where people smoke regularly and where the ventilation rate is low, particles created by desorbtion of chemicals from surfaces and chemical reactions may prevent airborne particle concentrations from decreasing to the low levels normally associated with clean indoor spaces. Research has shown that THS and many other mixtures of semi-volatile organic compounds can react with ambient concentrations of oxidant gases to create ultrafine particles(8-10, 26). Reaction of surface-sorbed nicotine with ozone formed particles under 50 nm in diameter and displayed surface and humidity-dependent effects(9).

Our data show that a substantial mass of particles can emerge from a closed chamber 20 hours after smoke generation stops (Figure S1) and that the diameter of the particles increases over time (Table 1). We hypothesize that a complex cycle of evaporation of sorbed chemicals from interior surfaces, particle enucleation, particle diameter growth through sorbtion of vapor phase chemicals to existing particles and collisions between particles (coagulation) and particle losses to sorbtion and deposition occurs in environments that are heavily contaminated with thirdhand smoke(27). Further research, in real-world environments, is needed.

We found a positive correlation between the nicotine concentration in the exposure aerosol and the creatinine-corrected total urinary nicotine metabolite concentration (cotinine + 3-hydroxycotinine) with $R^2 = 0.64$. The net increases in cotinine and 3-hydroxycotinine were smaller than those observed in a previous study by our group, after a 30 minute exposure to aged secondhand smoke at 1,000 $\mu g/m^3$. However,

the metabolites showed similar kinetics to the previous study, with cotinine concentrations peaking first, then 3-hydroxycotinine(22). Our findings are also somewhat consistent with a previous THS study by Matt et al., where urinary cotinine and NNAL concentrations increased after nonsmokers slept in smoking rooms in hotels (28). The difference in findings regarding NNAL may be due to the differences in how the participants were exposed. Our exposure was strictly inhalational. Participants inhaled THS through a Tyvek hood while sitting in an exposure chamber that was continuously flushed with conditioned, HEPA-filtered air at 0.85 air changes per minute. Thus, any dermal exposure was through the skin of the head and neck only. Participants in the hotel exposure study had both inhalational and dermal contact as they slept in a room that had been smoked in. NNK is much less volatile than nicotine and resides primarily in the particle phase. During the 22 hour aging period in our study, the NNK present in the input SHS aerosol probably sorbed to surfaces and was removed from the airborne fraction.

Baseline levels of cotinine and 3-hydroxycotinine in this study were similar to those in a previous study performed by the same laboratory between 2010 and 2011(22) and lower than in a previous study performed in the San Francisco Bay area prior to 2005(24). 89% of the baseline urine samples were below 0.2 ng/ml cotinine, 40% were below 0.2 ng/ml 3-hydroxycotinine and 52% were below 0.5 pg/ml NNAL. The maximum baseline tobacco smoke metabolite values were 0.576 ng/ml for cotinine, 3.957 ng/ml for 3-hydroxycotinine and 4.417 pg/ml for NNAL. For perspective, the current, validated cut-point values for discriminating between smokers and nonsmokers are 31 ng/ml for cotinine and 47.3 pg/ml for NNAL(29). Our data show that while many nonsmokers in the San Francisco Bay Area have some exposure to

the chemicals found in tobacco and tobacco smoke, the baseline exposure levels for this group were quite low.

Although SHS exposure has been associated with higher levels of circulating VEGF and IL-6 (30, 31), this study did not find a statistically significant increase in levels of circulating VEGF and IL-6 after THS exposure. This finding suggests that increases in IL-6 and VEGF may be potentiated by higher exposure concentrations. We observed a trend toward an increase in circulating 8-isoprostane levels but the finding was not statistically significant. Earlier studies of inhalational exposure to SHS, with more intensive exposures and similar sample size, have shown significant increases in urinary isoprostanes(32, 33). 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(34, 35). It is likely that this study was not adequately powered enough to detect an association between the THS exposure and elevated VEGF, IL-6 and 8-isoprostane levels.

Limitations.

This study included a small number of participants (18 who completed both exposures), but the crossover study design supported results that are both statistically significant and consistent. We found an order effects in the analysis of variance suggests that the order in which the exposures were given influenced the magnitude of the responses. The two exposures were performed a minimum of 21 days apart so the likelihood of genuine carryover effects was minimal. We used a simple randomization of the participants which led to 15 of the 22 participants receiving their THS exposure first. This may have biased the results and caused the apparent order effect. Another limitation of this study is that the air exchange through our smoke generation system, when it is shut down overnight, is lower than

the air exchange through most homes and businesses. Thus, the exposure in our study may be higher than real-world exposures. However, real THS exposures usually last longer than three hours and include respiratory, dermal and sometimes oral exposure routes. Longer exposures may have greater effects on biomarkers of inflammation and oxidative stress. The absence of significant effects on urinary NNAL in our study, and the fact that NNK is primarily in the particle phase suggest that the skin may be a more important route for NNK exposure.

Conclusions

This study highlights the need for further research exploring the effects of THS exposure on human health and the effects of inhalational exposure in occupational categories like cleaning where there are similar exposures. Given that this study only explored inhalational exposure of THS, further studies should also examine dermal exposure to THS, especially since a major component of THS, nicotine, is readily absorbed through the skin.

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DECLARATION OF INTERESTS

The authors have no conflicts of interest to declare.

DATA AVAILABILITY STATEMENT

The raw urinary tobacco metabolite data for this study are available at Datadryad.org (DOI https://doi.org/10.5061/dryad.rr4xgxdbv.). Limited biometric data on the study participants are available upon request.

BIBLIOGRAPHY

1. Winickoff JP, Friebely J, Tanski SE, et al. Beliefs about the health effects of "thirdhand" smoke and home smoking bans. Pediatrics. 2009;123(1):e74-9.
2. Matt GE, Quintana PJ, Zakarian JM, et al. When smokers move out and non-smokers move in: residential thirdhand smoke pollution and exposure. Tob Control. 2011;20(1):e1. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21037269.
3. Jacob P, 3rd, Benowitz NL, Destaillats H, et al. Thirdhand Smoke: New Evidence, Challenges, and Future Directions. Chem Res Toxicol. 2017;30(1):270-94. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28001376.
4. Sleiman M, Gundel LA, Pankow JF, et al. Formation of carcinogens indoors by surface-mediated reactions of nicotine with nitrous acid, leading to potential thirdhand smoke hazards. Proc Natl Acad Sci U S A. 2010;107(15):6576-81. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20142504.
5. Schick SF, Farraro KF, Perrino C, et al. Thirdhand cigarette smoke in an experimental chamber: evidence of surface deposition of nicotine, nitrosamines and polycyclic aromatic hydrocarbons and de novo formation of NNK. Tob Control. 2013;23(2):152-9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23716171.
6. Destaillats H, Lunden MM, Singer BC, et al. Indoor secondary pollutants from household product emissions in the presence of ozone: A bench-scale chamber study. Environ Sci Technol. 2006;40(14):4421-8. Available from: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=16903280.
7. Matt GE, Quintana PJ, Destaillats H, et al. Thirdhand Tobacco Smoke: Emerging Evidence and Arguments for a Multidisciplinary Research Agenda. Environ Health Perspect. 2011;119(9):1218-26. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21628107.
8. Becquemin MH, Bertholon JF, Bentayeb M, et al. Third-hand smoking: indoor measurements of concentration and sizes of cigarette smoke particles after resuspension. Tob Control. 2010;19(4):347-8. Available from: https://www.ncbi.nlm.nih.gov/pubmed/20530137.
9. Petrick LM, Svidovsky A, Dubowski Y. Thirdhand smoke: heterogeneous oxidation of nicotine and secondary aerosol formation in the indoor environment. Environ Sci Technol. 2011;45(1):328-33. Available from: https://www.ncbi.nlm.nih.gov/pubmed/21141815.
10. Tang X, Gonzalez NR, Russell ML, et al. Chemical changes in thirdhand smoke associated with remediation using an ozone generator. Environmental research. 2021;198:110462. Available from: https://www.ncbi.nlm.nih.gov/pubmed/33217439.
11. DeCarlo PF, Avery AM, Waring MS. Thirdhand smoke uptake to aerosol particles in the indoor environment. Sci Adv. 2018;4(5):eaap8368. Available from: https://www.ncbi.nlm.nih.gov/pubmed/29750194.
12. Schick SF, Farraro KF, Fang J, et al. An apparatus for generating aged cigarette smoke for controlled human exposures studies. Aerosol Sci Technol. 2012;46:1246-55.
13. U.S. Dept. of Health and Human Services, Centers for Disease Control and Prevention, Coordinating Center for Health Promotion, National Center for Chronic Disease Prevention and Health Promotion. The health consequences of involuntary exposure to tobacco smoke : a report of the Surgeon General. Atlanta, GA: US

Centers for Disease Control and Prevention; 2006. Available from: http://www.cdc.gov/tobacco/sgr/sgr2006/index.htm.

14. California Environmental Protection Agency, Office of Environmental Health Hazard Assessment, Office of Air Resources Board. Proposed identification of environmental tobacco smoke as a toxic air contaminant. Oakland California: California Environmental Protection Agency,; 2005 June 24, 2005.

15. International Agency for Research on Cancer (IARC), World Health Organization. Tobacco smoke and involuntary smoking. 2004 ed. International Agency for Research on Cancer, editor2004. 1-1438 p.

16. Khansari N, Shakiba Y, Mahmoudi M. Chronic inflammation and oxidative stress as a major cause of age-related diseases and cancer. Recent Patents on Inflammation & Allergy Drug Discovery. 2009;3(1):73-80.

17. Papaharalambus CA, Griendling KK. Basic mechanisms of oxidative stress and reactive oxygen species in cardiovascular injury. Trends Cardiovasc Med. 2007;17(2):48-54.

18. Zhang L, Wang M, Kang X, et al. Oxidative stress and asthma: proteome analysis of chitinase-like proteins and FIZZ1 in lung tissue and bronchoalveolar lavage fluid. Journal Proteome Research. 2009;8(4):1631-8.

19. Rahman I. The role of oxidative stress in the pathogenesis of COPD: implications for therapy. Treat Respir Med. 2005;4(3):175-200.

20. Frey PF, Ganz P, Hsue PY, et al. The exposure-dependent effects of aged secondhand smoke on endothelial function. J Am Coll Cardiol. 2012;59(21):1908-13. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22595411.

21. Aris RM, Christian D, Hearne PQ, et al. Ozone-induced airway inflammation in human subjects as determined by airway lavage and biopsy. Am Rev Respir Dis. 1993;148(5):1363-72.

22. Schick SF, van den Vossenberg G, Luo A, et al. Thirty minute-exposure to aged cigarette smoke increases nasal congestion in nonsmokers. J Toxicol Environ Health A. 2013;76(10):601-13. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23859154.

23. Bernert JT, Jain RB, Pirkle JL, et al. Urinary Tobacco-Specific Nitrosamines and 4-Aminobiphenyl Hemoglobin Adducts Measured in Smokers of Either Regular or Light Cigarettes. Nicotine & Tobacco Research : Official Journal of the Society for Research on Nicotine and Tobacco. 2005;7(5):729-38. Available from: http://ntr.oxfordjournals.org/content/7/5/729.abstract.

24. Jacob P, 3rd, Yu L, Duan M, et al. Determination of the nicotine metabolites cotinine and trans-3'-hydroxycotinine in biologic fluids of smokers and non-smokers using liquid chromatography-tandem mass spectrometry: biomarkers for tobacco smoke exposure and for phenotyping cytochrome P450 2A6 activity. J Chromatogr B Analyt Technol Biomed Life Sci. 2011;879(3-4):267-76. Available from: https://www.ncbi.nlm.nih.gov/pubmed/21208832.

25. Benowitz NL, Jacob P, 3rd. Metabolism of nicotine to cotinine studied by a dual stable isotope method. Clin Pharmacol Ther. 1994;56(5):483-93. Available from: http://www.ncbi.nlm.nih.gov/pubmed/7955812.

26. Fortenberry C, Walker M, Dang A, et al. Analysis of indoor particles and gases and their evolution with natural ventilation. Indoor Air. 2019;29(5):761-79. Available from: https://www.ncbi.nlm.nih.gov/pubmed/31264732.

27. Jeong SG, Wallace L, Rim D. Contributions of Coagulation, Deposition, and Ventilation to the Removal of Airborne Nanoparticles in Indoor Environments.

Environ Sci Technol. 2021;55(14):9730-9. Available from: https://www.ncbi.nlm.nih.gov/pubmed/34213881.
28. Matt GE, Quintana PJ, Fortmann AL, et al. Thirdhand smoke and exposure in California hotels: non-smoking rooms fail to protect non-smoking hotel guests from tobacco smoke exposure. Tob Control. 2014;23(3):264-72. Available from: https://www.ncbi.nlm.nih.gov/pubmed/23669058.
29. Schick SF, Blount BC, Jacob PR, et al. Biomarkers of exposure to new and emerging tobacco delivery products. Am J Physiol Lung Cell Mol Physiol. 2017;313(3):L425-L52. Available from: https://www.ncbi.nlm.nih.gov/pubmed/28522563.
30. Heiss C, Amabile N, Lee AC, et al. Brief secondhand smoke exposure depresses endothelial progenitor cells activity and endothelial function: sustained vascular injury and blunted nitric oxide production. J Am Coll Cardiol. 2008;51(18):1760-71.
31. Suzuki M, Betsuyaku T, Nagai K, et al. Decreased airway expression of vascular endothelial growth factor in cigarette smoke-induced emphysema in mice and COPD patients. Inhalation Toxicology Journal. 2008;20(3):349-59.
32. Ahmadzadehfar H, Oguogho A, Efthimiou Y, Kritz H, Sinzinger H. Passive cigarette smoking increases isoprostane formation. Life Sci. 2006;78(8):894-7. Available from: https://www.ncbi.nlm.nih.gov/pubmed/16165164.
33. Kato T, Inoue T, Morooka T, Yoshimoto N, Node K. Short-term passive smoking causes endothelial dysfunction via oxidative stress in nonsmokers. Can J Physiol Pharmacol. 2006;84(5):523-9. Available from: https://www.ncbi.nlm.nih.gov/pubmed/16902597.
34. Helmersson J, Basu S. F2-isoprostane excretion rate and diurnal variation in human urine. Prostaglandins, Leukocytes & Essential Fatty Acids. 1999;61(3):203-5.
35. Roberts LJ, Morrow JD. Measurement of F(2)-isoprostanes as an index of oxidative stress in vivo. Free Radic Biol Med. 2000;28(4):505-13.

Table 1: SHS and THS Aerosol Particle Characterization					
	Particle mass (µg/ m^3)	Total particles (#/cm^3)	Count Median Diameter (nm)	Geometric standard deviation	Skew
Filtered Source Air	<10	3,700	55	2.122	0.548
Ambient Air	<10	8,550	45	1.850	0.357
SHS aged 3 minutes	1,396	54,720	171	1.686	0.286
SHS aged 30 minutes	1,310	27,590	190	1.723	0.234
THS aged 22 hours	Initial: 414 Final: 100	11,582	265	2.011	-0.093

1

Table 2: Baseline Urinary Tobacco Metabolite and Creatinine Concentrations (n = 21)				
	Cotinine ng/ml	3-HC ng/ml	NNAL pg/ml	Creatinine mg/ml
Minimum	BLOQ	BLOQ	BLOQ	0.157
25^{th} Percentile	BLOQ	0.112	BLOQ	0.511
Median	0.0433	0.254	0.455	1.067
75^{th} Percentile	0.131	0.613	1.132	1.790
Maximum	0.576	3.957	4.417	3.999
Skewness	2.325	2.280	1.914	1.079
Kurtosis	5.768	4.492	3.0655	0.662
The limits of quantitation (LOQ) were: cotinine = 0.05 ng/ml, 3-hydroxycotinine = 0.1 ng/ml , NNAL = 0.25 pg/ml, creatinine = 0.05 mg/ml. For the statistical calculations, values below the LOQ were set to LOQ/$\sqrt{2}$.				

2

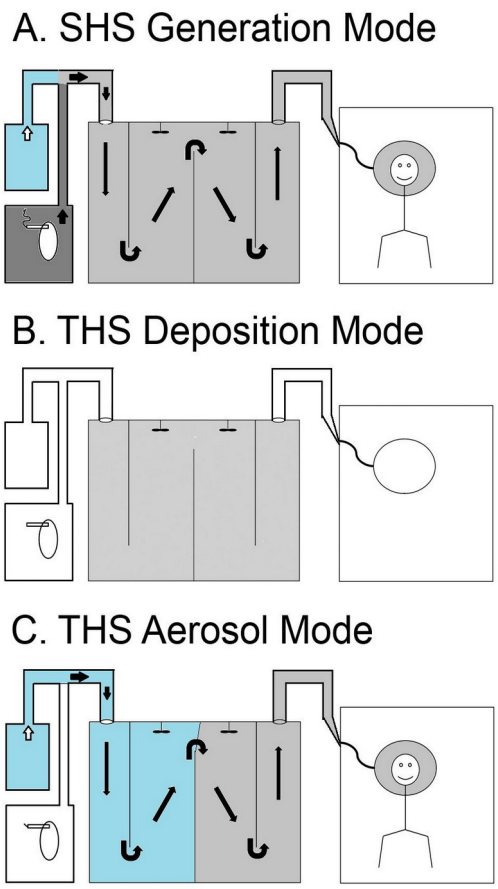
Table 3: Total Change in Metabolites 0-22 h		
	Average (standard deviation)	
	THS	**CA**
Cotinine (nM)	0.280 (0.378)	0.004 (0.011)
3-Hydroxycotinine (nM)	1.796 (3.386)	-0.021 (0.101)
NNAL (pM)	0.055 (0.095)	0.028
The total moles of metabolites excreted were calculated by subtracting the concentration at each time point from the timepoint after it. Each metabolite concentration was multiplied the number of elapsed hours since the prior sample and the totals were summed to generate the total amount of metabolite		

excreted. The total changes in mass were divided by the molecular weight of the molecules to yield the moles.

1

2

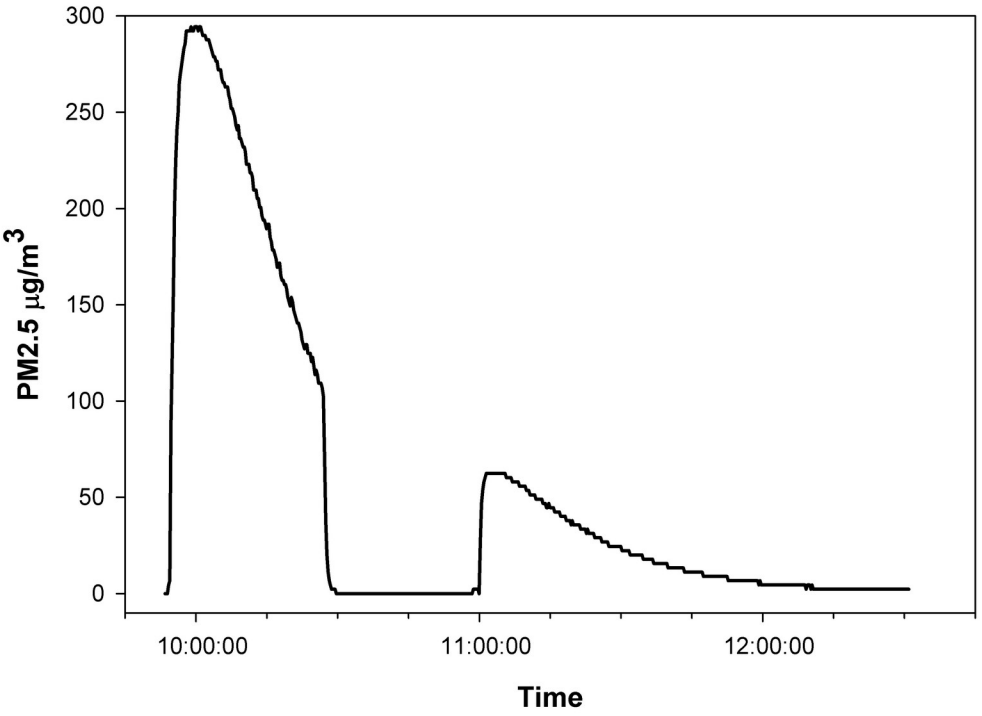
Figure 1: THS Generation



A. One day prior to a THS exposure, Marlboro cigarettes were machine-smoked and diluted smoke was passed through the smoke system.
B. The smoke was held in the system overnight to sorb and react.
C. TheTHS aerosol was created by flushing the smoke system with clean air. Subjects were exposed to the aerosol head-only, while seated.

Supplementary Materials

Supplementary Figure 1: Particle concentration during a THS exposure



Legend:

Decreased particle concentration from 10:27-11:00 is because the airflow was turned off while the participant exited the exposure chamber for the 30-minute blood draw. When they returned, exposure resumed.